Oral presentation at IVAS 2017 (Salamanca, Spain)

FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AGT 1608**

Principal Investigator: **Chen Liang**

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Contents

Abstract

The 10th In Vino Analytica Scientia Symposium was held in Salamanca, Spain, from 17 - 20 July, 2017. More than 200 participants from 24 countries attended this meeting. Topics included:

- Analysis and composition of grapes, wines, spirits and by-products
- Reactions and microbiology
- Omic and chemometric approaches
- Sensory properties and analysis

The travel grant allowed Chen Liang to attend the IVAS 2017 conference and share her research via an invited oral presentation titled 'Controlling unripe characters using magnetic molecularly imprinted polymers to eliminate excessive methoxypyrazines from wines'. The grant also provided Chen with the opportunity to visit the Laboratory for Aroma Analysis and Enology at the University of Zaragoza. The travel has enabled Chen to gain inspiration from the most up to date analytical science in grapes, wines and spirits, and also make connections with other researchers in related areas.

Executive summary

The 10th In Vino Analytica Scientia was held in the beautiful historic city of Salamanca, Spain, from 17 - 20 July, 2017. The travel grant provided Chen Liang, PhD candidate of the University of Adelaide, the opportunity to communicate and share her research through the delivery of an invited oral presentation titled 'Controlling unripe characters using magnetic molecularly imprinted polymers to eliminate excessive methoxypyrazines from wines'.

The conference program consisted of eight keynote lectures, 33 oral presentations and over 200 posters covering different aspects of grapes, wines and derived products. It was a great conference for sharing and discussing the latest developments in analytical chemistry relayed to grape and wine research. A series of social events were also blended into the program, including Salamanca city night tour, wine tasting, gala dinner and wine cellar visits.

Attendance at the conference has provided the opportunity to gain insights for my current PhD project as well as make connections with researchers worldwide for potential collaboration in the future. The lab visit has enabled communications between two

laboratories and to get to know the most cited analytical methods for aroma and flavour analysis developed by the team of Vicente Ferreira.

Background

Chemistry is important in production of all kinds of wines, and understanding the complexity of wine chemistry has been proved to help improve wine quality. Though great progress has been made in understanding the composition of grapes, wines, spirits and by-products, the microbial and chemical reactions that take place during the winemaking process and the sensory properties of the products, the chemistry of wine is still not fully understood and investigated.

Advanced analytical science of grapes and wines is one of the solutions for fully investigating wine chemistry, and further provides advice and solutions for viticultural and oenological practice. The In Vino Analytica Scientia (IVAS) conference brings together international researchers involved in analytical science of wine, brandy and spirits to exchange views, make international collaborations and expand networks.

IVAS 2017 provided a great opportunity to be updated on the most recent developments in analytical wine chemistry and current research emphasis based on industrial issues and requirements. The conference was also a valuable platform to disseminate our research.

The travel grant from AGWA allowed Chen Liang to attend IVAS 2017 and to visit a lab in a related research area.

Project aims

The aim of the project was to attend and present my research at the IVAS 2017 conference in Salamanca, Spain, 17 - 20 July.

Specific aims:

- Deliver the oral presentation ‘Controlling unripe characters using magnetic molecularly imprinted polymers to eliminate excessive methoxypyrazines from wines’.
- Acquire knowledge on the advanced analytical methods in wine chemistry and gain inspiration for my current project.
- Network and make connections via a lab visit.

Conference outcomes

The Abstract (Appendix 5) titled ‘Controlling unripe characters using magnetic molecularly imprinted polymers to eliminate excessive methoxypyrazines from wines’ was accepted as an oral communication by the conference committee. The presentation (Figure 1) took place on 19 July within the ‘Reactions and microbiology’ session. Positive feedback and advice was received through discussions with some of the attendees. Among those, Dr Raquel Garcia from Universidade de Évora, Portugal, who has been leading a team for extraction of pesticides from olive oil using molecularly imprinted polymers, offered strategies for experimental design. Their group has set up successful computational methods for assisting the design of molecularly imprinted polymers, and through our discussions the potential exists for collaboration in the future.

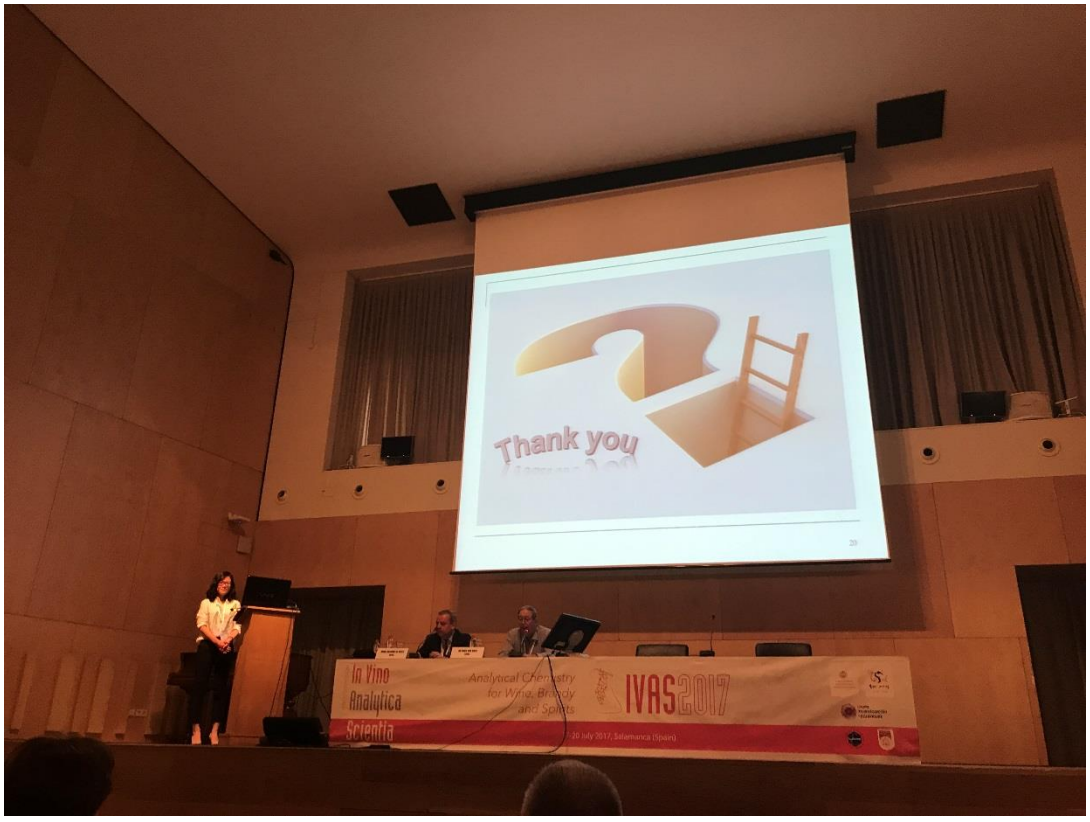


Figure 1. A photograph of Chen Liang presenting results from her PhD project at IVAS 2017.

Chromatography techniques have been widely used as powerful tools in analytical chemistry for analysis of grapes and wine, and they have undergone dramatic developments over the last decades. From one-dimensional chromatography to multidimensional heart-cutting approaches (LC-LC, GC-GC) and to comprehensive two-

dimensional (2-D) chromatography (LC×LC, GC×GC), the applications span from high to very high complexity with more precisions and lower detection limits. The opening lecture given by Luigi Mondello titled 'Two-dimensional comprehensive liquid chromatography techniques for the analysis of bioactive molecules and contaminants in wine' not only demonstrated the high performance of LC×LC technique over one-dimensional LC approaches, but also illustrated the challenges and solutions when adopting this technique. It listed the problems of solvent immiscibility when using on-line LC×LC, and graphically provided solutions. It also indicated methods to enhance the performance of the 2-D technique. The lecture was inspirational and rich with practical guidance.

It was thrilling to find a greatly relevant poster titled 'Determination of ultra-trace levels of alkylmethoxypyrazines in wine by stir bar sorptive extraction combined with multidimensional gas chromatography-mass spectrometry'. In the study, the team of Vincente Ferreira developed a method based on stir bar sorptive extraction (SBSE) following by thermal desorption technique as sample preparation methods. The chromatographic analysis was carried out with heart-cut 2-D gas chromatography-mass spectrometry (2-D GC-MS). The method has pushed the detection limits of alkylmethoxypyrazines down to 0.02-0.07 ng/L in spiked wines, two orders of magnitude lower than some of the headspace solid phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) methods. Their method is robust and convenient with satisfactory precision and linearity. It can be adapted for relatively fast analysis of batches of wine samples. The instruments they are using are the same as the one in our lab, which makes the method more feasible and approachable if lower detection limits are required in the following parts of my PhD project.

Laboratory visit

After the conference, Chen Liang visited Prof. Vicente Ferreira's lab at the University of Zaragoza, Spain. Dr Monica Bueno from Ferreira's team kindly showed Chen around the lab and provided detailed explanations about their instrumental set-up and protocol for aroma analysis. It is well established in both instrumentation and team building.

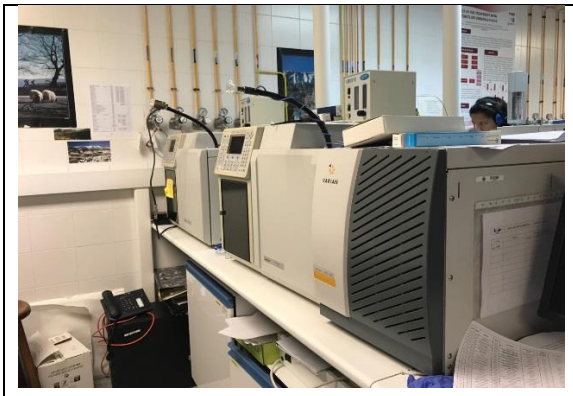
As the lab is noted for aroma and flavour analysis, they have a range of gas chromatographs coupled with various detectors for different specialised usages. Figure 3a shows a dual gas chromatography, olfactory directed compound detection system, coupled

with mass spectrometry. The compounds would elute first on the polar column in the first chromatography oven and different fractions could be eluted together through a second non-polar column. Besides this dual GC system, they also have a 2-D GC (Figure 3b). There is a GC-FPD (flame photometric detector, Figure 3c) for sulfur and phosphorus compound detection, and for better resolution for sulfur compound detection there is also a GC-SCD (sulfur chemiluminescence detector, Figure 3d) with great signal to noise ratio. For ultra-trace compounds, two GC-MS systems (with quadrupole MS, Figure 3e) could be adopted. For better sensitivity and resolution, the lab is planning to couple the GC-MS with another GC as a dual system and also with an on-line solid phase extraction (SPE) apparatus (Figure 3f). They have set up methods for analysing more than 80 aroma compounds in wine for winery requirements. Twenty compounds could be analysed with GC-FID (flame ionisation detector), and the remaining 60 trace compounds could be analysed with GC-MS using an ion-trap (Figure 3g). It is worth noting that when using SPE in the sample preparation process, their lab elutes analytes with nitrogen to avoid air disturbance of trace compounds.

Another main topic they are working on is to investigate the kinetics of oxygen and sulfur dioxide consumption during wine oxidation processes. Sample preparation is carried out in an argon purged, sealed chamber to avoid oxygen (Figure 3h).

Chen was honoured to have a meeting with Prof. Ferreira's group (Figure 2) and did a brief introduction of her project as well as the entire suite of ARC training centre projects. Their team members also briefly explained their own projects and it was really good communication.

Figure 2. Photo of Chen Liang and Prof. Ferreira's group.



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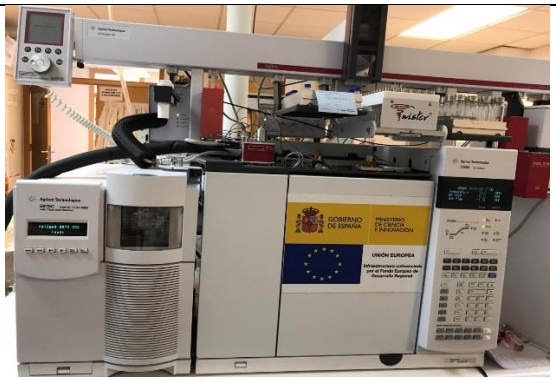

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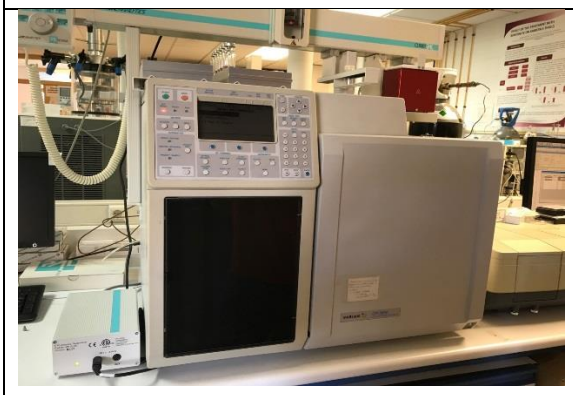

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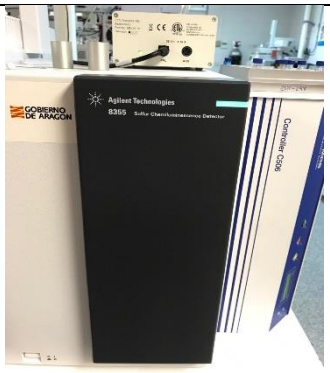

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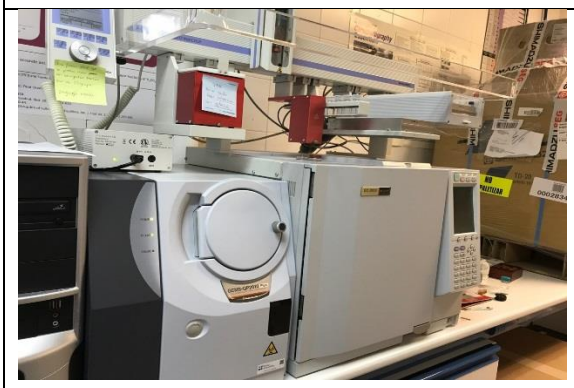

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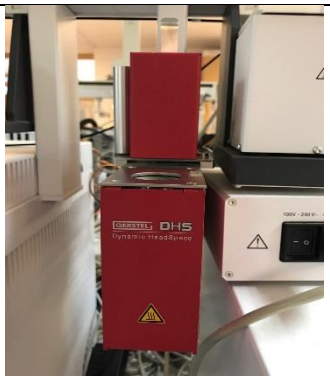

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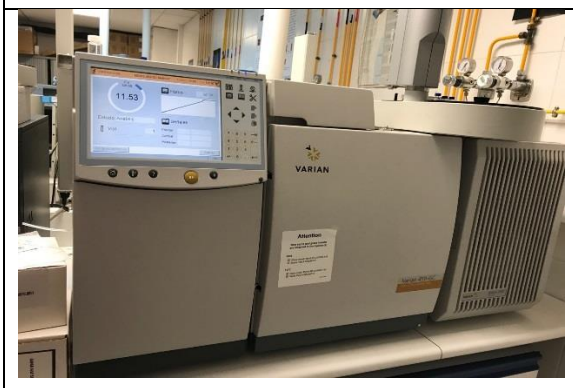

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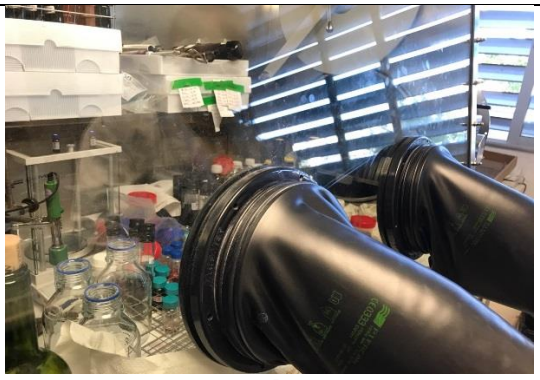

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Figure 3. Photos of laboratory visit.

Appendix 1: Communication

Information gained by attending this symposium and of relevance to the work undertaken in Wine Science at The University of Adelaide will be disseminated to work colleagues in the Wine Research group weekly seminar on 6 September 2017.

Appendix 2: Intellectual Property

Not applicable - This project has no intellectual property attached to it.

Appendix 3: References

Not applicable

Appendix 4: Staff

Chen Liang (PhD candidate, The University of Adelaide)

Appendix 5: Abstract

Controlling unripe characters using magnetic molecularly imprinted polymers to eliminate excessive methoxypyrazines from wines

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Methoxypyrazines (MPs) are known to be responsible for green characters such as vegetative, herbaceous and capsicum-like flavour in grapes and wines [1]. These are potent odorants with extremely low sensory detection thresholds (ng/L), so higher concentrations can be deemed overpowering and undesirable [2]. Early harvest grapes or grapes from cool climate regions may contain higher concentrations of MPs and lead to wines with 'unripe' characters. MPs present in grapes at harvest are largely unaffected by winemaking procedures, so methods for removing excessive MPs post-harvest are warranted.

This project involves synthesis of magnetic molecularly imprinted polymers (MMIPs) to specifically remove MPs from wines. The target molecules or structurally similar compounds are used as templates during polymer synthesis whereupon removal of the template liberates cavities that selectively recognise and bind target molecules [3]. Molecularly imprinted polymers (MIPs) were also synthesised to compare with their magnetic counterparts [4, 5]. Non-imprinted polymers were made exactly the same but without the template molecule added, to act as controls. Microwave synthesis was adopted to compare with conventional synthesis under reflux. 3-isobutyl-2-methoxypyrazine (IBMP) was chosen as a target molecule and physical characterisations and adsorption tests were carried out to evaluate the polymers.

Separation of the magnetic polymer is realised by adding an external magnetic field (or decanting/using in a packed column for other polymers). Using GC-MS analysis, magnetic polymers were found to have removed 40-60% of IBMP (initial concentration of 30 ng/L) from model wine and white wine within ten minutes. The addition of magnetic nanoparticles and microwave induced polymerisation did not affect cavity size and adsorption properties compared to regular imprinted polymers, and the polymer can be regenerated by washing in diethyl ether. Adsorption isotherm tests showed both Langmuir and Freundlich isotherms would fit with MMIPs whereas only Freundlich would fit with MNIPs. However, imprinted polymers could not be differentiated from non-imprinted controls under current adsorption tests, so different monomers and reaction solvents have been trialled to improve polymer specificity for IBMP. Furthermore, MMIPs will be tested in winemaking trials and their effect on the concentrations of MPs and other wine aroma volatiles will be reported.

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