

**PREPARATION AND APPLICATION OF NEW SORPTIVE  
POLYMERS APPLIED TO WINE WITH A FOCUS ON  
REMOVAL OF 3-ISOBUTYL-2-METHOXYPYRAZINE**

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## THESIS SUMMARY

Synthetic polymers have been used as solid phase extraction (SPE) sorbents for analysis of food and wine components due to their versatile and stable sorptive properties. Moreover, polymers such as polyvinylpolypyrrolidone (PVPP) can be used as fining agents in the winemaking process and polymeric materials have been investigated as a remedial treatment to remove undesirable compounds from wines. In order to decrease the loss of desirable components and improve selectivity, tailor-made sorbents known as molecularly imprinted polymers (MIPs) have also been produced and broadly studied.

MIPs have been widely used in a range of areas where selective binding is of importance, such as immunoassays, sensors, and analytical chemistry and biochemistry (sample clean-up and pre-concentration). MIPs, also known as *plastic antibodies*, can selectively recognise and bind with target molecules by binding at recognition sites formed with a template molecule during preparation of polymer. Based on the selective binding character, it would be beneficial to apply MIPs in winemaking to help remove off-flavours including elevated methoxypyrazines (MPs) without affecting other wine components. Excessive MPs can lead to unbalanced aroma, yielding wines with strong vegetative and herbaceous notes that suppress the pleasant fruity and floral bouquets. Early harvest grapes to make lower alcohol wines, or grapes from cool climate regions, may contain higher levels of MPs and could produce wines with 'unripe' characters. Since grape-derived MPs are relatively stable during general winemaking procedures, investigation of MIPs as a post-harvest treatment to specifically remove MPs from grape juice or wine is warranted.

3-Isobutyl-2-methoxypyrazine (IBMP), reminiscent of green capsicum aroma, has been chosen as a target compound in this project. A range of putative imprinted polymers along with non-imprinted polymers (NIPs) were synthesised through bulk polymerisation. Additionally, putative imprinted magnetic polymers (PIMPs) were prepared analogously to the putative imprinted polymers with the addition of magnetic nanoparticles. In this way, PIMPs could be separated by an external magnet compared to non-magnetic polymers that require separation through filtration or used in a packed column. Adsorption tests and physical characterisations were carried out to assess the various polymers. Several fundamental challenges in the molecular imprinting process that could lead to experimental artefacts were observed during trials and relative adjustments were made to avoid the pitfalls. Adsorption tests were first carried out in

model wine solution, and the adsorption of IBMP on putative imprinted polymers (magnetic/non-magnetic) could not be differentiated from non-imprinted counterparts (magnetic/non-magnetic), and neither could their isotherms. Further adsorption test in original porogen solvent showed that there was no difference between the imprinted and the non-imprinted counterparts, meaning the adsorption was dominated by hydrophobic interactions without specific binding in model wine solution. During the preliminary investigation of imprinting methods, microwave-assisted polymerisation was adopted as comparison. Results showed that microwave synthesis did not introduce differences to the polymers regarding adsorption isotherms and physical characters (determined by scanning electron microscopy and Fourier-transform infrared spectroscopy) compared to the thermal synthesis.

The practical usage of magnetic polymers in winemaking was investigated with putative imprinted magnetic polymer (PIMP) and non-imprinted magnetic polymer (NIMP) added to IBMP-spiked Cabernet Sauvignon grape must, pre- and post-fermentation. Polylactic acid-based (PLA) film was adopted as a post-fermentation treatment to compare. Olfactory sensory analysis revealed that wines treated with PIMP and NIMP were found to have trace 'green' characters, whereas, PLA treatments turned out to have noticeable 'green' characters and were most similar to the untreated control wines. The sensory results were consistent with the results from chemical analysis of volatiles. IBMP concentrations were decreased from 21 ng/L (in the control wines) to 7 ng/L and 5 ng/L (i.e., below the detection threshold of IBMP in red wine) by PIMP and NIMP post-fermentation addition, respectively, in comparison to the PLA wine having 17 ng/L. The difference between PIMP and NIMP arose with the timing of addition, where pre-fermentation treatments had less sorption of IBMP and other wine volatiles than the post-fermentation treatments, with the same trend observed for colour properties. In spite of the sorption of volatile compounds other than IBMP, the overall aroma intensity and fruity characters were not different according to sensory analysis. The character impact role of IBMP in wine aroma matrix was observed as well.

As the key for imprinting relies on the reaction between templates and monomers in the proper porogen environment, a series of newly prepared polymers (non-magnetic) with various template, functional monomer and porogen solvent combinations were trialled to achieve specific binding. Although further study is still required to improve specificity, polymers (non-imprinted)

with different functional monomer and porogen solvent combinations were found to have affinities towards different groups of compounds in wine, and could potentially be used as sorbents for fining of wine, SPE or as stationary phases for liquid chromatography. Physicochemical properties (surface area, pore size distribution, and polarity) of polymeric sorbents could be affected by the compositions and production methods and the relation between physicochemical properties and sorption properties in wines was investigated, along with that of four commercial sorbents (C<sub>18</sub>, Oasis HLB, Strata SDB-L, and PVPP). In terms of production methods, thermally synthesised polymers were found to have larger surface area, on the other hand, microwave synthesised polymers were observed to have narrower pore size distributions with abundance of micro- and mesopores. Polarities of the sorbents could be differentiated by copolymerising various hydrophilic monomers. Thermally synthesised sorbent with double the amount of methacrylic acid as co-monomer in acetonitrile may be more polar and has the potential for carbonyl compounds extraction. Microwave-produced sorbent with 4-vinylbenzoic acid in dichloromethane was found to have higher retention of non-polar, small molecular weight compounds (e.g., various volatiles) in white and red wines. Sorbents prepared by microwave in dichloromethane with methacrylic acid and acrylic acid as co-monomers, respectively, were observed to have higher affinity towards moderately polar, small to large molecular weight compounds (e.g., pigments and other phenolics) in wines. As for the four commercial sorbents, C<sub>18</sub> was found to have high affinity towards volatiles and low affinity towards phenolics in white and red wines; PVPP, on the contrary, was observed to have high sorption of phenolics and low retention of volatile compounds. Oasis HLB and Strata SDB-L were found to have higher sorptive ability among the commercial sorbents, with Oasis HLB having higher affinity towards moderately polar compounds than that of Strata SDB-L. With similarities to the commercial sorbents regarding hydrophobic and hydrophilic characters, our synthesised polymers showed potential use as selective sorbents.

A series of polymers (magnetic, putative imprinted) were successfully synthesised and characterised. Results from this project revealed some fundamental challenges faced with the molecular imprinting process and some pitfalls that could be avoided in future work. The usage of magnetic polymers in winemaking provided a feasible post-harvest option to remediate wines with elevated levels of MPs. The possibilities of applying selective polymeric sorbents in wine for analytical and remedial purposes have been investigated. Improvement of the specificity of the imprinted polymers applied in wine should also be the focus of future work.

## DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Chen Liang

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\_\_\_\_\_ 25/10/2018

Date

## PUBLICATIONS

This thesis is a collection of manuscripts that were published in *Molecules* and *Journal of Agricultural and Food Chemistry* (JAFC) during candidature. According to the 2017 InCites Journal Citation Reports by Thomson Reuters, the impact factor of JAFC was 3.412 and the impact factor of *Molecules* was 3.098.

The text and figures in Chapters 2-4 are presented in different formats based on each journal's specific requirements. A signed statement of authorship by all of the authors, listing individual contributions to the work, is included at the beginning of each chapter.

The thesis is based on the following publications.

Chapter 2. Liang, C; Jeffery, D. W.; Taylor, D. K. Preparation of magnetic polymers for the elimination of 3-isobutyl-2-methoxypyrazine from wine. *Molecules* 2018, 23, 1140-1154.

Chapter 3. Liang, C; Ristic, R; Jiranek, V; Jeffery, D.W. Chemical and sensory evaluation of magnetic polymers as a remedial treatment for elevated concentrations of 3-isobutyl-2-methoxypyrazine in Cabernet Sauvignon grape must and wine. *Journal of Agricultural and Food Chemistry* 2018, 66, 27, 7121–7130

Chapter 4. Liang, C; Boss, P.K.; Jeffery, D.W. Extraction properties of new polymeric sorbents applied to wine. *Journal of Agricultural and Food Chemistry* 2018, 66, 38, 10086-10096



## CONFERENCES AND WORKSHOPS

### Oral presentations

- Crush Grape and Wine Science Symposium, Adelaide, November 2017
- 10th In Vino Analytica Scientia - Analytical chemistry for wine, brandy and spirits, Salamanca, Spain, July 2017
- Australian Society of Viticulture and Oenology awards dinner, Adelaide, October 2016
- ARC Industrial Transformation Training Centre workshop, Coonawarra, October 2016
- The University of Adelaide, School of Agriculture, Food and Wine, Postgraduate Symposium, Adelaide, September 2016
- 16th Australian Wine Industry Technical Conference, Adelaide, student forum session, winner of the People's Choice award, July 2016
- ARC Industrial Transformation Training Centre workshop, Wagga Wagga, May 2016
- ARC Industrial Transformation Training Centre workshop, Tasmania, May 2015

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# **CHAPTER 1**

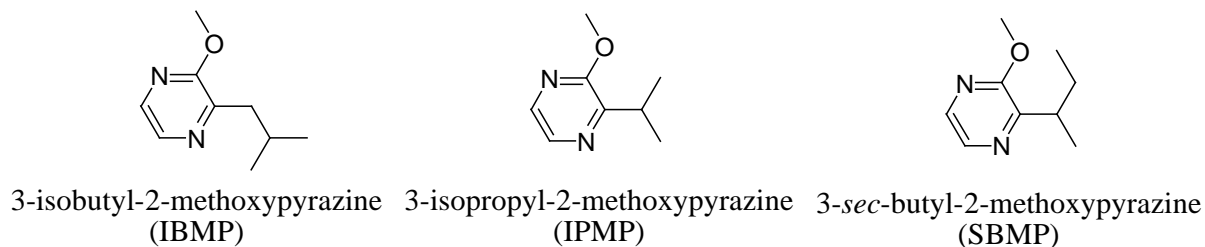
## **Literature Review**

Most of the literature review was prepared in the first 6 months of candidature and it mainly covers the literature up to March 2015. The state of the art literature beyond this review has been included in the introduction sections of publications in Chapters 2–4.

## 1.1 Introduction to Methoxypyrazines in Grapes and Wines

### 1.1.1 General introduction to methoxypyrazines

Methoxypyrazines (MPs) have been found to be responsible for numerous green characters detected in wine (Allen, et al. 1991), including the fresh green aromas such as green bell pepper, grass, green bean, dusty and herbal, as well as cooked green vegetal aromas such as asparagus and cooked/steamed green vegetables (King, et al. 2011). Low concentrations of methoxypyrazines can add varietal flavour, however, high concentrations ( $> 20$  ng/L) are undesirable and impart overpowering unripe characters. Compounding the problem of high levels of MPs in wines is that some grape derived thiol compounds such as 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) can also contribute to the overall green character of a wine (King, et al. 2011), although they are more noted for their ability to impart sensory characters of grapefruit, passionfruit and tropical fruit to wine (Jouanneau, et al. 2012). Three main methoxypyrazines have been found in grapes and wines and have been determined to be 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-sec-butyl-2-methoxypyrazine (SBMP) (Figure 1).



**Figure 1.** Chemical structures of the three main methoxypyrazines found in grapes and wines.

Methoxypyrazines can be measured and detected at ng/L levels and have a very low sensory threshold. It is reported that the threshold of MPs can be as low as 320 pg/L (Botezatu and Pickering 2012). When the bell pepper aroma contributed by methoxypyrazines in wines is abundant, the overall aroma of the wine is driven by bell pepper characters instead of the typical fruit berry and floral characters that are appealing to most customers (Hein, et al. 2009). High levels of MPs can also add bitterness to wines. Since high concentrations of methoxypyrazines are considered undesirable, taking measures to lower excessive methoxypyrazines in wines is warranted. The quantity of IBMP in grapes and wines is much higher than that of IPMP and SBMP. Consequently, the level of IBMP may be utilised as a representative compound among the methoxypyrazines and its measurement can be used as an

indicator of the overall green character potential of grapes and wines. The detection threshold of IBMP is 0.5 ng/L in water and 10 ng/L in red wine, and the recognition threshold is 15 ng/L in red wine (Lopez, et al. 2011). The concentration range of IBMP found in wines is 0.4 - 44 ng/L (van Wyngaard, et al. 2014).

### 1.1.2 Multicoloured Asian lady beetles (MALB) as a source of methoxypyrazines

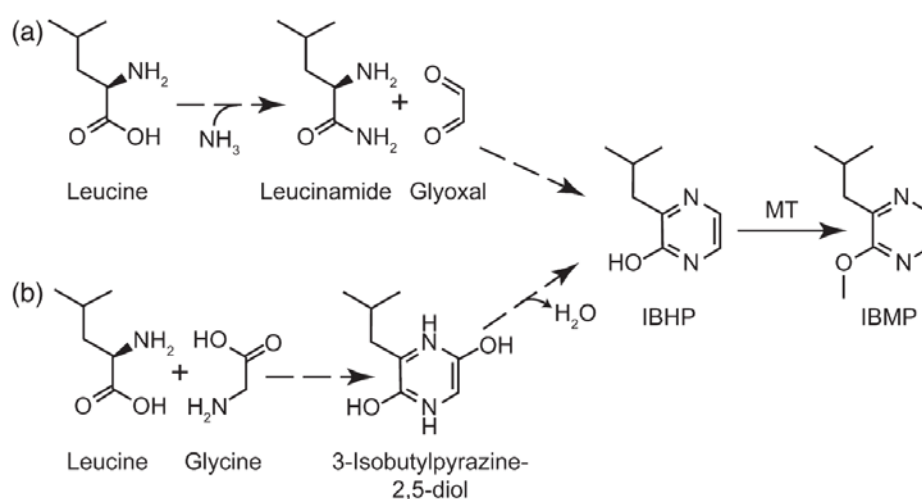
Another source of methoxypyrazines in grapes and wines originates from insect origin. Grapes and wines contaminated by *Harmonia axyridis* (Pallas) (MALB) have been recorded to lead to an IPMP concentration increase in wines. Due to the extremely low threshold of IPMP, 0.3 ng/L in white wine or 2.3 ng/L in red (Lopez, et al. 2011), MALB infection can be a problem in wine making process. It has been shown that the MALB are introduced into the winemaking process when the grapes are harvested as they attach themselves to 'injured' ripe berries (Pickering, et al. 2006). As the beetles are incorporated with the grapes at harvest, they are therefore present during the crushing and other normal operations employed during the winemaking process. The beetles therefore get crushed and undergo a process called 'reflex bleeding' where they secrete a yellow fluid containing the MPs into the must (Galvan, et al. 2008). Sensory properties of MALB affected wines indicate as peanut, bell pepper and asparagus aromas in white wines and peanut, bell pepper/asparagus, earthy/herbaceous aromas and flavour in red wines. In addition, fruity and floral aroma intensities are again lowered in both wine types (Pickering, et al. 2004). This kind of off-flavour caused by MALB is called 'lady bug taint' (LBT). According to Pickering et al. (2005), IPMP is primarily responsible for the taint, although some IBMP is also present. A survey indicates that the presence of these beetles is now having an influence over a large number of wine making regions, including those in the north-eastern United States, eastern Canada, parts of the western United States and Europe (Pickering, et al. 2006). Consequently, viticulturists and winemakers are trying different methods to eliminate them from wines.

### 1.1.3 Cultivar diversities and IBMP concentrations in grapes and wines

In terms of IBMP concentrations in grapes, there are cultivar diversities reflecting that the synthesis and accumulation of IBMP are controlled by fruit genotype (Koch, et al. 2010). High levels of IBMP have been detected in the mature berries of Cabernet Sauvignon, Cabernet Franc, Sauvignon Blanc, Merlot, Semillon and Carmenere, however only small amounts are detected in the unripe berries of Chardonnay and Riesling. Among the group displaying high levels of IBMP accumulation, Cabernet Sauvignon usually contains the most of the red varieties and Sauvignon Blanc for the whites.

### 1.1.4 Biosynthetic and degradation of IBMP in grapes

Two biosynthetic pathways to IBMP have been elucidated and are depicted in Figure 2 (Dunlevy, et al. 2013). 3-Isobutyl-2-hydroxypyrazine (IBHP) is the precursor of IBMP from both pathways. IBMP's formation involves the methylation of the non-volatile precursor IBHP with an essential methyltransferase. The enzyme is encoded by the gene *VvOMT*, which includes *VvOMT1*, *VvOMT2*, *VvOMT3* and *VvOMT4* (Guillaumie, et al. 2013, Vallarino, et al. 2011). *VvOMT3* is more efficient in producing IBMP compared with the other three, due to its high affinity and specificity to IBHP. The gene is expressed in Cabernet Sauvignon at the time of accumulation of IBMP, but not expressed in the Pinot variety, which lacks the presence of IBMP. Thus, *VvOMT3* is believed to be a key factor in the final step of methoxypyrazine formation (Dunlevy, et al. 2013).



**Figure 2.** Two proposed pathways for 3-isobutyl-2-methoxypyrazine (IBMP) biosynthesis in living organisms. (a) Biosynthesis from leucine and glyoxal proposed by Murray et al. (1970). (b) Biosynthesis from leucine and glycine proposed by Cheng et al. (1991). Dashed arrows indicate reactions for which biochemical evidence does not exist. MT, methyltransferase, IBHP, 3-isobutyl-2-hydroxypyrazine. From Dunlevy, et al, A methyltransferase essential for the methoxypyrazine-derived flavour of wine. Copyright © 2013 by John Wiley Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.

IBMP accumulation occurs mainly before veraison and goes through significant change during ripening. The amount of IBMP decreases largely after veraison and reduces with increasing grape maturity. The mode of degradation of IBMP is still not clear, however, IBHP was proposed to be a key intermediate in the degradation process as there is a stage after veraison where the concentration of IBHP and IBMP are inversely correlated in the ripening

wine grapes. Consequently it was proposed that the concentration of IBHP could be used as a proxy for IBMP at veraison (Ryona, et al. 2010). However, recent research revealed that IBHP peaks and begins to decline 1-2 weeks after IBMP detection, suggesting that the previous hypothesis may be wrong (Harris, et al. 2012).

#### 1.1.5 Factors affecting methoxypyrazine levels in grapes and wines

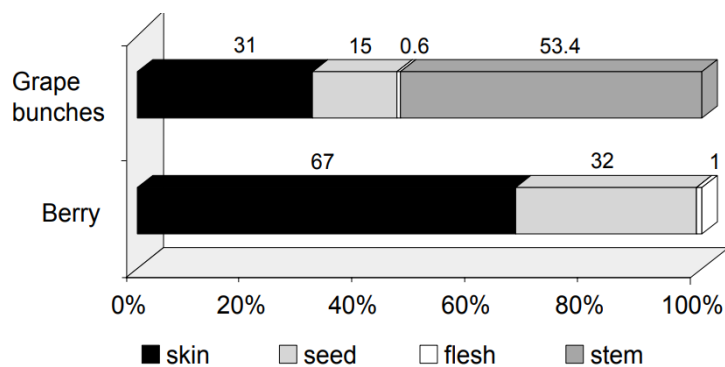
##### 1.1.5.1 Viticultural factors

It has been found that the IBMP concentration in grapes can be influenced by many factors including grape maturity, sunlight exposure, water status, temperature, vine vigour and yield. Thus, many viticultural changes can be made to alter the potential content of IBMP. Increased light exposure from basal leaf removal leads to reduced IBMP concentrations (Ryona, et al. 2008, Sala, et al. 2004, Scheiner, et al. 2010). Conditions that stimulate vine vigour such as increased irrigation, additional nitrogen fertilisation and low bud numbers result in increased IBMP concentration, due to the fact that they promote canopy growth and accordingly reduce sunlight exposure (Dunlevy, et al. 2013, Mendez-Costabel, et al. 2014). Higher temperature is thought to promote degradation of IBMP, and thereby leads to a lower content at harvest. Recent research focusing on the mechanism of these effects revealed that light exposure reduces the expression of the methyltransferase gene *VvOMT3* and therefore decreases the concentration of the precursor IBHP (Vallarino, et al. 2014). It has been suggested that light exposure is the main factor affecting the synthesis and accumulation of IBMP before veraison rather than the degradation process post-veraison. Thus, in practice, it appears that more light exposure before veraison is more effective in reducing IBMP concentrations.

##### 1.1.5.2 Pre-fermentation factors

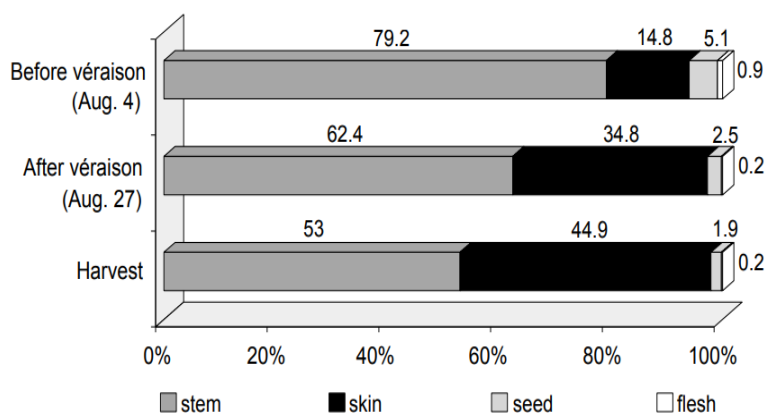
The content of IBMP in wines depends primarily on the concentration found in the grapes at harvest as it has been reported that the concentration of IBMP in wine is  $67 \pm 13\%$  of that found in the berries (Ryona, et al. 2009). Considering this strong correlation, a method of rapid measurement of IBMP in grapes has also been developed to predict the IBMP concentration in wines. The location of IBMP is mainly concentrated in the stems, followed by the skins and seeds, with little in the flesh, Figure 3 (de Boubée, et al. 2002).



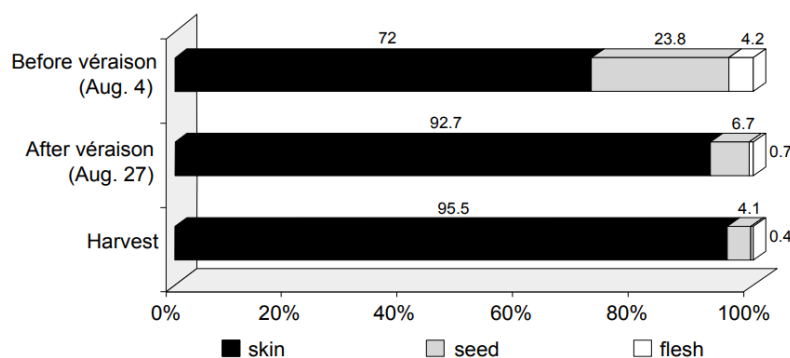


**Figure 3.** Distribution (in %) of IBMP in the various parts of Cabernet Sauvignon grape bunches during ripening in 1998 (de Boubée, et al. 2002).

Furthermore, the proportion of IBMP in the various parts of the bunch varies during ripening, with a reduction in the stems and seeds found, and a concomitant increase in the skins, Figure 4 (de Boubée, et al. 2002). Thus, avoiding contact of the stems during the vinification process is an effective way to avoid unripe characters in a finished wine (Hashizume, et al. 1998). The extraction of IBMP from grapes into wine depends mainly on the maceration time with the IBMP levels increasing during the first twenty-four hours of maceration in the aqueous phase before fermentation. This discovery highlights that neither ethanol nor certain yeast strains are responsible for extraction of IBMP from grapes into the juice as the extraction primarily takes place prior to fermentation.



(a)



(b)

**Figure 4.** Distribution (in %) of IBMP in the various parts of Cabernet Sauvignon grape bunches and grapes during ripening in 1999 (a); Distribution (in %) of IBMP in the various parts of Cabernet Sauvignon grape during ripening in 1999 (b) (de Boubée, et al. 2002).

#### 1.1.5.3 Microbial factors

A number of bacteria such as *Pseudomonas* sp. can degrade certain pyrazine compounds as source of carbon and energy (Muller and Rappert 2010). Thus, selection of a certain yeast strain may have an influence on the concentration of methoxypyrazines in wine. For example, Lalvin BM45, a wine yeast strain, has been found to increase IPMP by 11 ng/L (29%) in wine during fermentation (Pickering, et al. 2008). However, currently no wine yeast strains, *Lactobacillus* strains or *Oenococcus oeni* strains used for malolactic fermentation have been found to decrease IBMP in wine.

#### 1.1.5.4 Bottle-aging factors

The concentration of IBMP during bottle ageing varies with closure and packaging type. Among Tetra Pak Prisma (Tpk) aseptic cartons, molded synthetic cork closures, screw caps, and natural cork closures, IBMP decreased most when Tpk was employed followed by synthetic cork closures, screw caps, and natural cork closures. The authors concluded that gas permeability and closure absorption account for these differences (Blake, et al. 2009). Moreover, the concentrations of IBMP decreased by approx. 30% over 12 months of bottle ageing under different light, temperature and bottle hue conditions (Blake, et al. 2010). It may be possible that non-covalent bonds formed between polyphenols and IBMP, which lower its volatility and concentration over headspace (Lund, et al. 2009).

### 1.1.6 Methods for lowering excessive methoxypyrazines levels in wines

Several pre- or post-fermentation treatments may be applied to remediate excessive methoxypyrazine levels in wine caused by utilising unripe fruit at harvest or as a result of lady bug contamination (Table 1). These methods generally lack selectivity and their practical use is quite limited. Clarification of must prior to fermentation decreases IBMP by about 50%, but this method is not suitable for red wine making and is incapable of erasing the trait completely (de Boubée, et al. 2002). Thermovinification has been adopted to decrease methoxypyrazines in wine by evaporation, but it produces cooked aromas to the finished wine. Wines tainted by *Harmonia axyridis* can be treated with bentonite, activated charcoal, oak chips, deodorised oak chips and either ultraviolet or visible light. With the exception of activated charcoal and deodorised oak chips, applications have no influence on IPMP concentrations, and the absorption ability of activated charcoal and deodorised oak chips lacks in selectivity, and may cause loss of other compounds in wine as well. Oak chips, however, do lower the sensory intensity of LBT attributes by masking with the strong oak aroma (Pickering, et al. 2006). Treatment of grape juice and must with food-grade silicone before fermentation can effectively decrease methoxypyrazine levels without affecting fermentation-derived flavour in finished wines. However, this method is also lacking in selectivity as it leads to a lowering of the natural grape varietal characters of wine (Ryona, et al. 2012).

Recent research has led to the development of three novel approaches to reduce methoxypyrazine levels in wine. The first method is based on protein binding technology incorporating the lipocalin mouse major urinary protein 1 (mMUP) combined with a 10 KDa molecular weight cut-off polyethersulfone (PES) membrane filtration system. It has been found to effectively decrease IBMP and IPMP levels by up to 98%. However, using this method to remove methoxypyrazines from wine is limited since the structure of mMUP changes in the presence of ethanol, and the 10 KDa pore size PES membrane is too small for industrial juice and wine processing. The second approach employs the absorptive properties of a range of food-grade polyethylene, polypropylene and silicon based polymers to remove methoxypyrazines in wine, however, their effects have been found to only be moderate (Gary Pickering, et al. 2014). The final approach involves the use of molecularly imprinted polymers (MIPs), which forms the main focus of this project and is detailed further below.

**Table 1.** Summary of pre- or post-fermentation treatments applied in an attempt to remediate excessive methoxy-pyrazine levels in wines.

Matrix	MPs studied	Technique	% reduction	Caveats	Ref.
Grape juice	IBMP	24 h Must clarification (200 NTU)	50	Not suitable for skin-fermented wine	(de Boubée, et al. 2002)
Grape juice	IBMP	Thermovinification (60 °C)	29-67	Produces cooked aromas	
Wine	IPMP	Bentonite	n.s.		(Pickering, et al. 2006)
		Oak chips	n.s.	Masks sensory intensity with oak aroma	
		Activated charcoal	34	Lacks selectivity	
		Deodorised oak	6	Lacks selectivity	
Wine	IBMP,SBMP,IPMP	Ultraviolet or visible light	n.s.		(Blake, et al. 2010)
Grape juice	IBMP,IPMP	Silicone added to juice/must	50-90	Lower grape varietal character	(Ryona, et al. 2012)
Wine	IBMP,IPMP	Protein (mMUP) binding	98	Protein changes structure under ethanol conditions	(Pickering, et al. 2014)
		Polymer absorption	40	Moderately effective	
Wine	IBMP	Molecularly imprinted polymers	85	Promising practical use	(Belbruno 2014)

n.s. denotes no selectivity

## 1.2. Introduction to Molecularly Imprinted Polymers (MIPs)

### 1.2.1 General introduction to MIPs

Molecularly imprinted polymers (MIPs) are now one of the most promising highly selective materials that may be used as an alternative to traditional solid phase extraction (SPE) sorbents such as silica-gel, polyamide, ion-exchange resins and reverse-phase materials. MIPs possess structural complementary cavities for target molecules, which are also used as template substrates). Put simply, the polymers gain memories of the template compound by association and disassociation during the synthetic process. The molecular imprinting procedure is based on interactions between the polymerisable monomers with certain functional groups and template molecules. Furthermore, in the presence of a porogenic solvent and the cross-linking agent, polymerisation occurs to form a stable matrix surrounding the templated molecule of interest. Removal of the template by washing/leaching produces a polymer that possesses a molecular memory enabling the selective rebinding of the target molecule from within a solution, as depicted in Figure 5 (Haupt, et al. 2012). Consequently, MIPs exhibit recognition and binding abilities similar with antibodies for their antigens.



**Figure 5.** General principle of molecular imprinting. A molecular template (T) is mixed with functional monomers (M) and a cross-linker (CL) resulting in the formation of a self-assembled complex (1). The polymerization of the resulting system produces a rigid structure bearing imprinted sites (2). Finally removal of the template liberates cavities that can specifically recognize and bind the target molecule (3). Reprinted with permission from (Haupt 2003). Copyright 2003 American Chemical Society

The binding sites between the template and monomer molecules can be formed by covalent, non-covalent, semi-covalent, metal-binding and metal-mediated methods (Alexander, et al. 2006). For covalent imprinting, templates and functional monomers bind covalently prior to polymerisation, after which the covalent bond is cleaved and the template

is removed from the polymers. The same covalent bond is formed during the rebinding process of MIPs in the presence of the target molecule. The non-covalent approach uses templates and functional monomers in the presence of cross linking agents, porogenic agents and reaction initiators. The linkage between the templates and functional monomers is formed through hydrophobic interactions, van der Waals forces, hydrogen bonding, and ion pairing. Rebinding of the MIPs and the target molecule is through the same non-covalent interactions. Compared to the non-covalent approach, covalent binding may present higher selectivity, with less unwanted interactions and better extraction ability. However, this method is considered less versatile and as such, the non-covalent approach is more prevalently used.

Another protocol that combines the advantages of both covalent and non-covalent binding is termed the semi-covalent approach, in which covalent bonds are formed in the polymer making process and non-covalent bonds formed during the extraction process. Metal binding strategies are also used for imprinting of metal ions that can bind to a wide range of functional groups. The strength of interaction varies enormously from weak, readily exchangeable to strong due to the metal oxidation state and ligand affinities. Finally, the metal mediated method for the production of MIPs utilises the fact that metal ions act as an alternative means of association between the template and functional monomer. The complex consists of functional monomers linked to metal ions (generally a transition metal), which in turn coordinates to the template.

As the non-covalent method is most widely used, the stability of the non-covalent complex is crucial to increasing the strength of the specific binding sites and lowering of non-specific interactions. Consequently, the formation of non-covalent imprinted polymers generally relates to the types of functional monomers, template molecules, cross linkers, porogenic solvents and radical initiators employed during manufacture, and all contribute to adduct stability and selectivity. Pardo has extensively discussed these factors in an excellent review article, and the following section highlights some of the key factors influencing the stability and selectivity of MIPs (Pardo, et al. 2012).

## 1.2.2 Factors influencing stability and selectivity of MIPs

### 1.2.2.1 Functional monomer

Functional monomers must have the ability to form strong complexes with the template substrate to ensure that the polymers produced have optimal recognition properties. Commonly used monomers include methacrylic acid (MAA), acrylamide (AA), and 4-

vinylpyridine (4-VP), with MAA being the most commonly used. Its broad application appears to be due to the presence of the carboxylate group that can act as both a proton donor and hydrogen bond acceptor. It should be noted that MAA and other polar monomers are not suitable for poorly polar or non-polar template substrates that cannot form hydrogen bonds. In this situation, suitable interactions between templates and monomers are based on hydrophobic effects. An example of such a situation was demonstrated with the use of  $\beta$ -cyclodextrin for cholesterol imprinting (Asanuma, et al. 1997). On the other hand, the commonly used monomers (MAA, AA, and 4-VP) are not strongly hydrophilic and as such, are not appropriate to be used to extract water-soluble compounds (e.g., water-soluble acid dyes) from an aqueous environment. Consequently, many researchers have focused on developing new monomers for water soluble template substrates (Qin, et al. 2008, Luo, et al. 2011). Finally, computational modelling can also be utilised for ideal monomer selection (Puzio, et al. 2013, Basozabal, et al. 2013).

#### 1.2.2.2 Template

The functional groups attached to the template molecule play an important role in determining polymer selectivity. The presence of abundant functional groups provides the possibility to maximise binding interactions within the active sites of the polymers, although excellent selectivity has also been found for templates with two or less functional groups. The pre-organisation of functional groups does not appear to work in concert with during the imprinting or rebinding process due to shape selectivity, which may account for the observation of lowered performance for some templates containing three or more functional groups. In certain circumstances, such as when the template molecule is expensive or difficult to synthesise, an analogue molecule called a ‘dummy template’ may be utilised as a substitute (Puzio, et al. 2013). The use of dummy templates can also prevent template ‘bleeding’, which is caused by difficult removal of some of the template at the washing stage after polymerisation. Another aspect of a template’s effect on the design of these polymers is the template/monomer ratio (T/M). For the non-covalent process, typical ratios of the T/M are from 1/4 to 1/8, with the optimum ratio being obtained from trials or predicted from modelling. Excess amounts of templates or monomers will lead to undesirable self-association.

### 1.2.2.3 Cross-linker

Cross-linkers are utilised in the copolymerisation of the monomers around the template substrate and provide the polymers with rigid matrices. Commonly used cross-linkers are divinylbenzene (DVB), ethylene glycol dimethacrylate (EGDMA), pentaerythritol triacrylate (PTA) and *N,N*-methylenebisacrylamide (BisAA), with EGDMA being the most prevalent. A series of pure isomers of DVB and EGDMA were compared in MIPs studies and in their system EGDMA showed better performance than DVB in terms of separation factor. The responsible factors may be that EGDMA showed less non-specific hydrophobic interaction with the template and this crosslinking agent led to more flexible polymer chains (Wulff, et al. 1987). The choice of cross-linker is polymer network dependent, that the reactivity of cross-linker shall be similar to that of functional monomer in the reaction mixture. The ratio of cross-linkers and template monomer complexes again affects the recognition ability of the MIPs. It has been suggested that a higher ratio of cross-linker will enhance stability of the polymers, but an excess of cross-linker will result in covering some of the binding sites and overly interact with the solvent. Some researchers have developed functional cross-linkers that directly link with the template substrate, thus no monomers are needed during preparation (Sibrian-Vazquez and Spivak, 2004) and the possible binding sites may be increased as well.

### 1.2.2.4 Porogen solvent

It has been revealed that polarity of the solvent plays the leading role rather than its porogenic attributes when aiding polymer construction. The selection of solvent to be employed should be based on the desired characters of the template monomer complex. Thus, for non-covalent complex polymers, non-polar or apolar solvents such as acetonitrile, chloroform, dichloromethane and toluene, are usually used, as they aid in maximising hydrogen bonding between the template and monomers/cross-linkers. The desired binding solvent should also be considered as this will influence the MIP performance. Typically, the ideal binding solvent is the same as the porogen.

### 1.2.2.5 Initiator

The polymerisation can be initiated thermally or photochemically. The most commonly used initiators for molecular imprinting are azoinitiators (e.g. 2,2'-azobisisobutyronitrile, AIBN), which decompose under both thermal and photochemical



conditions to produce free radical initiators that begin the free radical propagation cycle to produce the polymers.

### 1.2.3 Synthetic protocol

There are a range of polymerisation protocols that have been developed to synthesise MIPs. Bulk polymerisation is widely used, because it is simple and versatile, however, polymer yield may be affected by grinding and processing, and the polymers produced may have cavities varying in shape and size. In order to obtain spherical and polymers of certain sizes, in situ polymerisation, precipitation polymerisation, suspension polymerisation and multi-swelling polymerisation have been developed (Haupt and Mosbach 2000).

### 1.2.4 Characterisation of MIPs

The synthesised MIPs are often characterised utilising Fourier transform infrared (FTIR), scanning electron microscopy (SEM) and gas adsorption measurement ( $N_2$ ). The use of FTIR provides information on how the template molecules bind within the active sites of the MIPs. In addition, differences between the MIPs and NIPs can be clearly seen from key absorbance differences. Images obtained by SEM aid to reveal the surface morphology of the MIPs. It is generally believed that the more uniform and open structure the MIPs have results in better template substrate embedding (Song, et al. 2009). Consequently, such SEM images can help aid in optimisation of MIPs. Gas adsorption measurement provides information on surface area and porosity (e.g., pore size distribution) of polymers, which may help evaluate the adsorption performance of MIPs.

### 1.2.5 Adsorption analysis of MIPs

To assess the effectiveness of MIPs, non-imprinted polymers (NIPs) are used as a control. Control imprinted polymers prepared with an unrelated template could be included as comparisons to verify that the higher affinity is from molecular imprinting rather than physical differences (Cederfur, et al. 2003). Adsorption analysis of MIPs often involves batch binding assays in the beginning and further solid phase extraction (SPE), given that MIPs are used as sorbents. Binding data gathered after equilibrium between the template and the polymers could be analysed to characterise binding sites of the polymers. Adsorption models (e.g., Langmuir isotherms, Freundlich isotherm, Scatchard isotherm, etc.) may be applied to fit with the equilibrium binding data to evaluate binding properties (e.g., monolayer or multilayer adsorption, homogeneous or heterogeneous binding surface, etc.).

Moreover, structurally similar compounds to the template are often employed to test the specificity of MIPs. Batch binding tests have been applied to characterise binding properties of MIPs synthesised for vanillin and resveratrol analysis in wine (Puzio, et al. 2013, Schwarz, et al. 2006).

### 1.2.6 Applications of MIPs

Molecularly imprinted polymers are mainly used for separation in fields of chromatography, capillary electrochromatography and SPE. The most frequent use of MIPs in wine is either as fining agents for removal of undesirable compounds or SPE sorbents for wine components analysis. They can be used to absorb undesirable compounds from wine, such as separating ochratoxin A out of wine (Cao, et al. 2013, Giovannoli, et al. 2014, Maier, et al. 2004), eliminating 2,4,6-trichloroanisole (TCA taint) from aged red wines (Garde-Cerdán, et al. 2008, Schwarz, et al. 2006), extracting fungicides from wine samples (Baggiani, et al. 2007, Bitar, et al. 2014), inspecting histamine levels in wine (Basozabal, et al. 2013). Active compounds such as polyphenols in wine can be enriched through SPE, and MIPs show better enrichment effects than normal sorbent materials. Many researchers have used MIP-SPE to help determine trace amount of polyphenol concentrations in wine (Denderz and Lehotay 2014, Euterpio, et al. 2013, Hashim, et al. 2013, Molinelli, et al. 2002, Puzio, et al. 2013, Theodoridis, et al. 2006). Table 2 contains examples of MIPs synthesised for separation of compounds from wine.

Several recent patents detail two general experimental protocols for making molecularly imprinted polymers for the extraction of IBMP from wine. The first prepared a MIP by phase inversion production, and the other is based on monomer MIP production. Phase inversion MIP production does not require a cross-linking agent. The template directly reacts with a certain polymer, which is called 'a host polymer' (Belbruno 2014). Poly(4-vinylphenol) (P4-VP), poly(methylmethacrylate) (PMMA), poly(4-vinylphenol)-co-poly(methylmethacrylate) (P4-VP-PMMA) can be utilised as the host polymer. Solvents for reaction vary depending on the host polymer. A common solvent for P4-VP and P4-VP-PMMA MIPs is ethanol whilst toluene is used for PMMA MIPs. The monomer MIP production process follows the common bulk polymerisation routine. That is, the template along with the functional monomer is added to the solvent, stirred for a period of time, then the cross-linking agent and initiator are added. The mixture is then purged with nitrogen, sealed and placed at 60 °C for 24 h. The template used in these patents was 2-

methoxypyrazine (e.g., a dummy template) instead of 3-isobutyl-2-methoxypyrazine, as it was more easily washed out of the polymer (Belbruno and Kelm 2013).

MIPs are also used for protein, amino acid, DNA and RNA, peptide, hormone and carbohydrate separations. Electrochemical and chemiluminescence sensors based on molecularly imprinted polymers have been developed to detect and quantify polyphenols, thus avoiding the traditional high performance liquid chromatography method (Leite, et al. 2014, Wang and Zhang 2007, Xiang and Li 2009).

### 1.2.7 Challenges and progress – molecular imprinting in water

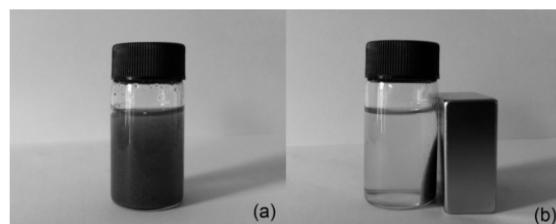
Despite the progress and broad application of imprinted polymers, challenges still need to be addressed. One of the main barriers is that most of the reported MIPs only function in an organic solvent matrix and hardly show specificities in aqueous solutions. As the pre-organisation of templates and functional monomers relies on hydrogen bonds, it could be destabilised easily by bulk water. Water-soluble crosslinking agents are employed to build water-compatible MIPs, however, they are not sufficient enough to reinforce the polymers. Efficient water-compatible MIPs have been reported recently, in which the polymers were obtained by grafting with hydrophilic macromolecules by facile reversible addition/fragmentation chain-transfer (RAFT) precipitation polymerisation (Pan, et al. 2011). RAFT is one of the powerful controlled radical polymerisation (CRP) techniques. Compared to the conventional free radical polymerisation, CRP is more versatile in providing well-defined polymers with predetermined molecular weights and low molar-mass dispersions. Moreover, polymers obtained through CRP have “living” end groups, which could be further modified and extended (Zhang 2014). Such water-compatible MIPs with hydrophilic brushes have been applied in real biological samples for direct drug quantification (Niu, et al. 2015).

### 1.2.8 Introduction to magnetic molecularly imprinted polymers (MMIPs)

Magnetic molecularly imprinted polymers (MMIPs) are fabricated MIPs attached to magnetic substrates. The major advantage of utilising magnetic molecularly imprinted polymers is that they can be directly separated from solution by adding an external magnetic field as demonstrated in Figure 6, instead of employing the tedious solid phase extraction process.

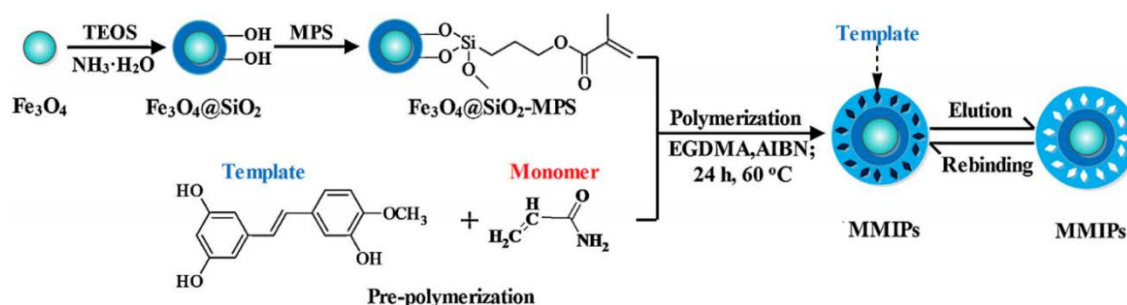
**Table 2.** Examples of MIPs synthesised for separation of compounds from wine.

Target	Template	Monomer	Cross-linker	Initiator	Solvent	Reaction condition	Washing solvent	Ref.
Ochratoxin A	OTA mimics	synthesised monomer	EGDMA	AIBN	chloroform	65 °C 24 h	acetone: acetic acid (96:4)	(Jodlbauer, et al. 2002)
2,4,6-Trichloroanisole	pentachlorophenol (PCP)	4-VP	DVB-80	AIBN	acetonitrile: toluene (75:25)	60 °C 24 h	methanol: acetic acid (1:1)	(Garde-Cerdán, et al. 2008)
Histamine	histamine	itaconic acid	trimethylolpropane trimethacrylate (TRIM)	1,1-azobis(cyclohexane-carbonitrile)	<i>N,N'</i> -dimethyl formamide (DMF)	UV lamp (100W) 30min	methanol: acetic acid (95:5)	(Basozabal, et al. 2013)
Quercetin	quercetin	4-VP	EGDMA	AIBN	acetone	60 °C overnight	methanol: acetic acid (85:15)	(Molinelli, et al. 2002)
Resveratrol	( <i>E</i> )-resveratrol	4-VP	EGDMA	AIBN	acetonitrile: ethanol (5:1)	50 °C 24 h further 60 °C 24 h	methanol: acetic acid (9:1)	(Euterpio, et al. 2013)
Gallic acid	gallic acid	MAA	EGDMA	AIBN	methanol	60 °C 24 h	methanol: acetic acid (9:1)	(Denderz and Lehotay 2014)
Protocatechuic acids	protocatechuic acids	MAA	EGDMA	AIBN	methanol	60 °C 24 h	methanol: acetic acid (9:1)	(Denderz and Lehotay 2014)



**Figure 6.** Separation process of MMIPs in the absence (a) and presence (b) of an external magnetic field. Reprinted from Applied Surface Science, 320, Liu, et al, Preparation of a magnetic molecularly imprinted polymer for selective recognition of rhodamine B, 138-145, Copyright (2014), with permission from Elsevier

MMIPs have been used to detect fluoroquinolone antibiotics in environmental water samples (Chen, et al. 2010), extract chloramphenicol and sulfonamides from honey (Chen and Li 2013, Chen, et al. 2009), separate sudan dyes from chilli powder (Piao and Chen 2012), and separate vanillin from food samples (Ning, et al. 2014). MMIPs have also been used in wine to recognise rhodamine B and resveratrol. In both experiments,  $\text{Fe}_3\text{O}_4$  was used as a magnetic support attached to the polymers. During the preparation protocol for the separation of resveratrol (Figure 7), tetraethoxysilane (TEOS) and 3-methacryloxypropyltrimethoxysilane (MPS) were used to initially coat  $\text{Fe}_3\text{O}_4$ . For rhodamine B, polyvinylpyrrolidone (PVP) and oleic acid were utilised (Liu, et al. 2014). The purpose of coating is to protect the metal particles from oxidation and also provide the link with the polymers. Besides FTIR and SEM, the produced MMIPs may also be characterised by X-ray diffraction (XRD) and vibrating sample magnetometer (VSM) before absorption analysis.



**Figure 7.** Schematic representation of preparation for MMIPs. Reprinted from Journal of Chromatography A, 1300, Chen, et al, Preparation of magnetic molecularly imprinted polymer for selective recognition of resveratrol in wine, 112-118, Copyright (2013), with permission from Elsevier

### Summary of research aims

The aim of this project is to control the excessive amount of 3-isobutyl-2-methoxypyrazine (IBMP) related ‘unripe’ characters using synthetic polymers (magnetic, imprinted) as post-harvest treatments. Grapes of some varieties grown in cool climate regions or those harvested early to make lower alcohol wines may contain elevated IBMP levels that impart potent capsicum-like, vegetative and herbaceous aromas to the finished wines. Current post-harvest remedial treatments are generally lacking in specificity and some may lead to undesirable aroma and flavour changes in the finished wines. To improve specificities and provide alternative post-harvest treatments, the project has the following detailed objectives.

- 1) Prepare imprinted polymers (magnetic and non-magnetic) specific for IBMP sorption, characterise their physical attributes and evaluate their sorption properties in model wine matrix.
- 2) Evaluate, via chemical and sensory analyses, the practical usage of magnetic polymers in wine-making as pre- and post-fermentation treatments to remove excessive IBMP.
- 3) Prepare various polymers and investigate the relation between their physicochemical properties and sorption properties of wine components.

#### Objective 1: Preparation of magnetic polymers for elimination of IBMP.

Molecularly imprinted polymers have been widely used in areas where specific binding and separation is of great importance, thus, it would be beneficial to apply imprinted polymers in wines for specific removal of IBMP. Furthermore, these polymers could gain magnetic properties by incorporating magnetic nanoparticles and this may aid in the separation of polymer particles from liquids by applying an external magnet rather than filtration or passing through a packed column. Therefore, magnetic molecularly imprinted polymers were prepared along with their non-magnetic counterpart as comparison. Microwave-assisted synthesis was adopted and compared to the conventional thermal synthesis. Physical characterisations and sorption tests in model wine were carried out, including adsorption isotherms and kinetic binding studies. Results and details of this study are reported in the publication in Chapter 2.

#### Objective 2: Chemical and sensory evaluation of magnetic polymers for IBMP removal from wine.

Wine aroma is determined by groups of volatile compounds that may have synergistic or masking effect with each other. The effect of putative imprinted magnetic polymer on other volatile compounds besides IBMP was investigated by chemical and sensory analyses. Magnetic

polymers (putative imprinted and non-imprinted) were applied to IBMP-spiked Cabernet Sauvignon grape must and wine as pre- and post-fermentation treatments in the winemaking trial. Practical usage of magnetic polymers in winemaking was evaluated and the character impact role of IBMP in wine aroma was assessed. Further details are described in the publication in Chapter 3.

Objective 3: Extraction properties of new polymers in wine.

Synthesised polymers have specificities towards certain group of compounds rather than specific single compound as for molecularly imprinted polymers. Accordingly, they could be applied as solid phase extraction sorbents or polymeric sorbents for removal of undesirable compounds. The composition and production method of polymers have a great impact on their physicochemical properties and further on sorption properties. New polymers produced with different components and production methods were applied in wines as new polymeric sorbents, and the relation between their physicochemical properties and sorption properties in wines was studied, along with four commercial sorbents (C<sub>18</sub>, Oasis HLB, Strata SDB-L, and PVPP) as comparison. The results are explained in the publication in Chapter 4.

## References

- Alexander, C., H.S. Andersson, L.I. Andersson, R.J. Ansell, N. Kirsch, I.A. Nicholls, J. O'Mahony, and M.J. Whitcombe (2006) Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003. *J. Mol. Recognit.* 19, 106-180.
- Allen, M.S., M.J. Lacey, R.L.N. Harris, and W.V. Brown (1991) Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* 42, 109-112.
- Asanuma, H., M. Kakazu, M. Shibata, and T. Hishiya (1997) Molecularly imprinted polymer of  $\beta$ -cyclodextrin for the efficient recognition of cholesterol. *Chem. Commun.* 1971-1972.
- Baggiani, C., P. Baravalle, G. Giraudi, and C. Tozzi (2007) Molecularly imprinted solid-phase extraction method for the high-performance liquid chromatographic analysis of fungicide pyrimethanil in wine. *J. Chromatogr. A* 1141, 158-164.
- Basozabal, I., A. Gomez-Caballero, G. Diaz-Diaz, A. Guerreiro, S. Gilby, M.A. Goicolea, and R.J. Barrio (2013) Rational design and chromatographic evaluation of histamine imprinted polymers optimised for solid-phase extraction of wine samples. *J. Chromatogr. A* 1308, 45-51.
- Belbruno, J.J (2014) Methods for preparation of molecularly imprinted polymers for wine extraction. U.S. Patent WO2014062632A1.
- Belbruno, J.J. and M. Kelm (2013) Molecularly imprinted polymers for wine extraction and method for production of molecularly imprinted polymers. U.S. Patent WO2013056226A1.
- Belbruno., J.J. and M. kelm (2014) Molecularly imprinted polymers for wine extraction. U.S. Patent 2014/0242237 A1.
- Bitar, M., P. Cayot, and E. Bou-Maroun (2014) Molecularly imprinted polymer solid phase extraction of fungicides from wine samples. *Anal. Methods* 6, 6467.
- Blake, A., Y. Kotseridis, I. Brindle, D. Inglis, and G. Pickering (2010) Effect of light and temperature on 3-alkyl-2-methoxypyrazine concentration and other impact odourants of Riesling and Cabernet Franc wine during bottle ageing. *Food Chem.* 119, 935-944.
- Blake, A., Y. Kotseridis, I.D. Brindle, D. Inglis, M. Sears, and G.J. Pickering (2009) Effect of closure and packaging type on 3-alkyl-2-methoxypyrazines and other impact odorants of Riesling and Cabernet Franc wines. *J. Agric. Food Chem.* 57, 4680-4690.



- Botezatu, A. and G.J. Pickering (2012) Determination of ortho-and retronasal detection thresholds and odor impact of 2,5-dimethyl-3-methoxypyrazine in wine. *J. Food Sci.* 77, S394-S398.
- Cao, J., W. Kong, S. Zhou, L. Yin, L. Wan, and M. Yang (2013) Molecularly imprinted polymer-based solid phase clean-up for analysis of ochratoxin A in beer, red wine, and grape juice. *J. Sep. Sci.* 36, 1291-1297.
- Cederfur, J., Y.X. Pei, Z.H. Meng, M. Kempe (2003) Synthesis and screening of a molecularly imprinted polymer library targeted for penicillin G. *J. Comb. Chem.* 5, 1, 67-72
- Chapman, D.M., J.H. Thorngate, M.A. Matthews, J.-X. Guinard, and S.E. Ebeler (2004) Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. *J. Agric. Food Chem.* 52, 5431-5435.
- Chen, F.F., X.Y. Xie, and Y.P. Shi (2013) Preparation of magnetic molecularly imprinted polymer for selective recognition of resveratrol in wine. *J. Chromatogr. A* 1300, 112-118.
- Chen, L. and B. Li (2013) Magnetic molecularly imprinted polymer extraction of chloramphenicol from honey. *Food Chem.* 141, 23-28.
- Chen, L., X. Zhang, L. Sun, Y. Xu, Q. Zeng, H. Wang, H. Xu, A. Yu, H. Zhang, and L. Ding (2009) Fast and selective extraction of sulfonamides from honey based on magnetic molecularly imprinted polymer. *J. Agric. Food Chem.* 57, 10073-10080.
- Chen, L., X. Zhang, Y. Xu, X. Du, X. Sun, L. Sun, H. Wang, Q. Zhao, A. Yu, H. Zhang, and L. Ding (2010) Determination of fluoroquinolone antibiotics in environmental water samples based on magnetic molecularly imprinted polymer extraction followed by liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* 662, 31-38.
- de Boubée, D.R., A.M. Cumsille, M. Pons, and D. Dubourdieu (2002) Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractability during vinification. *Am. J. Enol. Vitic.* 53, 1-5.
- Denderz, N. and J. Lehotay (2014) Using of molecularly imprinted polymers for determination of gallic and protocatechuic acids in red wines by high performance liquid chromatography. *J. Chromatogr. A* 1372, 72-80.
- Dunlevy, J.D., E.G. Dennis, K.L. Soole, M.V. Perkins, C. Davies, and P.K. Boss (2013) A methyltransferase essential for the methoxypyrazine-derived flavour of wine. *Plant J* 75, 606-617.

- Dunlevy, J.D., K.L. Soole, M.V. Perkins, E.L. Nicholson, S.M. Maffei, and P.K. Boss (2013) Determining the methoxypyrazine biosynthesis variables affected by light exposure and crop level in Cabernet Sauvignon. *Am. J. Enol. Vitic.* 64, 450-458.
- Euterpio, M.A., I. Pagano, A.L. Piccinelli, L. Rastrelli, and C. Crescenzi (2013) Development and validation of a method for the determination of (*E*)-resveratrol and related phenolic compounds in beverages using molecularly imprinted solid phase extraction. *J. Agric. Food Chem.* 61, 1640-1645.
- Galvan, T.L., S. Kells, and W.D. Hutchinson (2008) Determination of 3-alkyl-2-methoxypyrazines in lady beetle-infested wine by solid-phase microextraction headspace sampling. *J. Agric. Food Chem.* 56, 1065-1071.
- Garde-Cerdán, T., A. Zalacain, C. Lorenzo, J.L. Alonso, and M.R. Salinas (2008) Molecularly imprinted polymer-assisted simple clean-up of 2,4,6-trichloroanisole and ethylphenols from aged red wines. *Am. J. Enol. Vitic.* 59, 396-400.
- Giovannoli, C., C. Passini, F. Di Nardo, L. Anfossi, and C. Baggiani (2014) Determination of ochratoxin A in Italian red wines by molecularly imprinted solid phase extraction and HPLC analysis. *J. Agric. Food Chem.* 62, 5220-5225.
- Guillaumie, S., A. Ilg, S. Réty, M. Brette, C. Trossat-Magnin, S. Decroocq, C. Léon, C. Keime, T. Ye, R. Baltenweck-Guyot, P. Claudel, L. Bordenave, S. Vanbrabant, E. Duchêne, S. Delrot, P. Darriet, P. Hugueney, and E. Gomès (2013) Genetic analysis of the biosynthesis of 2-methoxy-3-isobutylpyrazine, a major grape-derived aroma compound impacting wine quality. *Plant Physiol* 162, 604-615.
- Harris, S.A., I. Ryona, and G.L. Sacks (2012) Behavior of 3-isobutyl-2-hydroxypyrazine (IBHP), a key intermediate in 3-isobutyl-2-methoxypyrazine (IBMP) metabolism, in ripening wine grapes. *J. Agric. Food Chem.* 60, 11901-11908.
- Hashim, S.N.N.S., L.J. Schwarz, R.I. Boysen, Y. Yang, B. Danylec, and M.T.W. Hearn (2013) Rapid solid-phase extraction and analysis of resveratrol and other polyphenols in red wine. *J. Chromatogr. A* 1313, 284-290.
- Hashizume, K., S. Kida, and T. Samuta (1998) Effect of steam treatment of grape cluster stems on the methoxypyrazine, phenolic, acid, and mineral content of red wines fermented with stems. *J. Agric. Food Chem.* 46, 4382-4386.

- Haupt, K., A.V. Linares, M. Bompert, and B.T. Bui (2012) Molecularly imprinted polymers. *Top Curr Chem* 325, 1-28.
- Haupt, K (2003) Peer reviewed: Molecularly imprinted polymers: The next generation. *Anal. Chem.* 75, 17, 376 A-383 A
- Haupt, K. and K. Mosbach (2000) Molecularly imprinted polymers and their use in biomimetic sensors. *Chem. Rev.* 100, 2495-2504.
- Hein, K., S.E. Ebeler, and H. Heymann (2009) Perception of fruity and vegetative aromas in red wine. *J. Sens. Stud.* 24, 441-455.
- Jodlbauer, J., N.M. Maier, and W. Lindner (2002) Towards ochratoxin A selective molecularly imprinted polymers for solid-phase extraction. *J. Chromatogr. A* 945, 45-63.
- Jouanneau, S., R.J. Weaver, L. Nicolau, M. Herbst-Johnstone, F. Benkwitz, and P.A. Kilmartin (2012) Subregional survey of aroma compounds in Marlborough Sauvignon Blanc wines. *Aust. J. Grape Wine Res.* 18, 329-343.
- King, E.S., P. Osidacz, C. Curtin, S.E.P. Bastian, and I.L. Francis (2011) Assessing desirable levels of sensory properties in Sauvignon Blanc wines - consumer preferences and contribution of key aroma compounds. *Aust. J. Grape Wine Res.* 17, 169-180.
- Koch, A., C.L. Doyle, M.A. Matthews, L.E. Williams, and S.E. Ebeler (2010) 2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. *Phytochemistry* 71, 2190-2198.
- Leite, F.R.F., W. de Jesus Rodrigues Santos, and L.T. Kubota (2014) Selective determination of caffeic acid in wines with electrochemical sensor based on molecularly imprinted siloxanes. *Sens. Actuators, B* 193, 238-246.
- Liu, X., D. Yu, Y. Yu, and S. Ji (2014) Preparation of a magnetic molecularly imprinted polymer for selective recognition of rhodamine B. *Appl. Surf. Sci.* 320, 138-145.
- Lopez, R., E. Gracia-Moreno, J. Cacho, and V. Ferrreira (2011) Development of a mixed-mode solid phase extraction method and further gas chromatography mass spectrometry for the analysis of 3-alkyl-2-methoxypyrazines in wine. *J. Chromatogr. A* 1218, 842-848.
- Lund, C.M., L. Nicolau, R.C. Gardner, and P.A. Kilmartin (2009) Effect of polyphenols on the perception of key aroma compounds from Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* 15, 18-26.

- Luo, X., Y. Zhan, Y. Huang, L. Yang, X. Tu, and S. Luo (2011) Removal of water-soluble acid dyes from water environment using a novel magnetic molecularly imprinted polymer. *J. Hazard Mater.* 187, 274-282.
- Maier, N.M., G. Buttinger, S. Welhartizki, E. Gavioli, and W. Lindner (2004) Molecularly imprinted polymer-assisted sample clean-up of ochratoxin A from red wine: merits and limitations. *J. Chromatogr. B* 804, 103-111.
- Mendez-Costabel, M.P., K.L. Wilkinson, S.E.P. Bastian, C. Jordans, M. McCarthy, C.M. Ford, and N.K. Dokoozlian (2014) Effect of increased irrigation and additional nitrogen fertilisation on the concentration of green aroma compounds in *Vitis vinifera L.* Merlot fruit and wine. *Aust. J. Grape Wine Res.* 20, 80-90.
- Molinelli, A., R. Weiss, and B. Mizaikoff (2002) Advanced solid phase extraction using molecularly imprinted polymers for the determination of quercetin in red wine. *J. Agric. Food Chem.* 50, 1804-1808.
- Muller, R. and S. Rappert (2010) Pyrazines: occurrence, formation and biodegradation. *Appl Microbiol Biotechnol* 85, 1315-1320.
- Ning, F., H. Peng, L.L. Dong, Z. Zhang, J. Li, L. Chen, and H. Xiong (2014) Preparation and characterization of superparamagnetic molecularly imprinted polymers for selective adsorption and separation of vanillin in food samples. *J. Agric. Food Chem.* 62, 46, 11138-11145
- Niu, H., Y.Q. Yang, H.Q. Zhang (2015) Efficient one-pot synthesis of hydrophilic and fluorescent molecularly imprinted polymer nanoparticles for direct drug quantification in real biological samples. *Biosens. Bioelectron.* 74, 440-446.
- Pan, G., Y. Zhang, Y. Ma, C. Li, & H. Q. Zhang (2011) Efficient one-pot synthesis of water-compatible molecularly imprinted polymer microspheres by facile RAFT precipitation polymerization. *Angew. Chem. Int. Ed.* 50, 49, 11731-11734.
- Pardo, A., L. Mespouille, P. Dubois, P. Duez, and B. Blankert (2012) Targeted extraction of active compounds from natural products by molecularly imprinted polymers. *Cent. Eur. J. Chem.* 10, 751-765.
- Piao, C. and L. Chen (2012) Separation of Sudan dyes from chilli powder by magnetic molecularly imprinted polymer. *J. Chromatogr. A* 1268, 185-190.

- Pickering, G., J. Lin, A. Reynolds, G. Soleas, and R. Riesen (2006) The evaluation of remedial treatments for wine affected by *Harmonia axyridis*. *Int. J. Food Sci. Technol.* 41, 77-86.
- Pickering, G., D. Inglis, A. Botezatu, A. Beh, E. Humes, and I. Brindle (2014) New approaches to removing alkyl-methoxypyrazines from grape juice and wine. *Scientific Bulletin. Series F. Biotechnologies*, XVIII, 130-134.
- Pickering, G., J. Lin, R. Riesen, A. Reynolds, I. Brindle, and G. Soleas (2004) Influence of *Harmonia axyridis* on the sensory properties of white and red wine. *Am. J. Enol. Vitic.* 55, 153-159.
- Pickering, G.J., Y. Lin, A. Reynolds, G. Soleas, R. Riesen, and I. Brindle (2005) The influence of *Harmonia axyridis* on wine composition and aging. *J. Food Sci.* 70, S128-S135.
- Pickering, G.J., M. Spink, Y. Kotseridis, D. Inglis, I.D. Brindle, M. Sears, and A.L. Beh (2008) Yeast strain affects 3-isopropyl-2-methoxypyrazine concentration and sensory profile in Cabernet Sauvignon wine. *Aust. J. Grape Wine Res.* 14, 230-237.
- Puzio, K., R. Delepee, R. Vidal, and L.A. Agrofoglio (2013) Combination of computational methods, adsorption isotherms and selectivity tests for the conception of a mixed non-covalent-semi-covalent molecularly imprinted polymer of vanillin. *Anal. Chim. Acta.* 790, 47-55.
- Qin, L., X.-W. He, W.-Y. Li, and Y.-K. Zhang (2008) Molecularly imprinted polymer prepared with bonded  $\beta$ -cyclodextrin and acrylamide on functionalized silica gel for selective recognition of tryptophan in aqueous media. *J. Chromatogr. A* 1187, 94-102.
- Ryona, I., S. Leclerc, and G.L. Sacks (2010) Correlation of 3-isobutyl-2-methoxypyrazine to 3-isobutyl-2-hydroxypyrazine during maturation of bell pepper and wine grapes (*Vitis vinifera*). *J. Agric. Food Chem.* 58, 9723-9730.
- Ryona, I., B.S. Pan, D.S. Intrigliolo, A.N. Lakso, and G.L. Sacks (2008) Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet Franc). *J. Agric. Food Chem.* 56, 10838-10846.
- Ryona, I., B.S. Pan, and G.L. Sacks (2009) Rapid measurement of 3-alkyl-2-methoxypyrazine content of winegrapes to predict levels in resultant wines. *J. Agric. Food Chem.* 57, 8250-8257.

- Ryona, I., J. Reinhardt, and G.L. Sacks (2012) Treatment of grape juice or must with silicone reduces 3-alkyl-2-methoxypyrazine concentrations in resulting wines without altering fermentation volatiles. *Food Res. Int.* 47, 70-79.
- Sala, C., O. Busto, J. Guasch, and F. Zamora (2004) Influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazines content in musts and wines from the *Vitis vinifera* variety Cabernet Sauvignon. *J. Agric. Food Chem.* 52, 3492-3497.
- Scheiner, J.J., G.L. Sacks, B. Pan, S. Ennahli, L. Tarlton, A. Wise, S.D. Lerch, and J.E. Vanden Heuvel (2010) Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red winegrapes. *Am. J. Enol. Vitic.* 61, 358-364.
- Schwarz, L., M.C. Bowyer, C.I. Holdsworth, and A. McCluskey (2006) Synthesis and evaluation of a molecularly imprinted polymer selective to 2,4,6-trichloroanisole. *Aust. J. Chem.* 59, 129-134.
- Sibrian-Vazquez, M. and D.A. Spivak (2004) Molecular imprinting made easy. *J. Am. Chem. Soc.* 126, 25, 7827-7833.
- Song, X., J. Li, J. Wang, and L. Chen (2009) Quercetin molecularly imprinted polymers: Preparation, recognition characteristics and properties as sorbent for solid-phase extraction. *Talanta* 80, 694-702.
- Theodoridis, G., M. Lasáková, V. Škeříková, A. Tegou, N. Giantsiou, and P. Jandera (2006) Molecular imprinting of natural flavonoid antioxidants: Application in solid-phase extraction for the sample pretreatment of natural products prior to HPLC analysis. *J. Sep. Sci.* 29, 2310-2321.
- Vallarino, J.G., F. Gainza-Cortes, C. Verdugo-Alegria, E. Gonzalez, and Y.M. Moreno (2014) Abiotic stresses differentially affect the expression of O-methyltransferase genes related to methoxypyrazine biosynthesis in seeded and parthenocarpic fruits of *Vitis vinifera* (L.). *Food Chem.* 154, 117-126.
- Vallarino, J.G., X.A. Lopez-Cortes, J.D. Dunlevy, P.K. Boss, F.D. Gonzalez-Nilo, and Y.M. Moreno (2011) Biosynthesis of methoxypyrazines: elucidating the structural/functional relationship of two *Vitis vinifera* O-methyltransferases capable of catalyzing the putative final step of the biosynthesis of 3-alkyl-2-methoxypyrazine. *J. Agric. Food Chem.* 59, 7310-7316.

- van Wyngaard, E., J. Brand, D. Jacobson, and W.J. du Toit (2014) Sensory interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatised Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* 20, 178-185.
- Wang, L. and Z. Zhang (2007) Molecular imprinted polymer-based chemiluminescence imaging sensor for the detection of trans-resveratrol. *Anal. Chim. Acta* 592, 115-120.
- Wulff G, J. Vietmeier, H. G. Poll (1987) Enzyme-analog built polymers—22: influence of the nature of the cross-linking agent on the performance of imprinted polymers in racemic-resolution. *Makromol. Chem.* 188: 731–740.
- Xiang, H.Y. and W.G. Li (2009) Electrochemical sensor for trans-resveratrol determination based on indium tin oxide electrode modified with molecularly imprinted self-assembled films. *Electroanalysis* 21, 1207-1210.
- Zhang, H.Q (2014) Water-compatible molecularly imprinted polymers: Promising synthetic substitutes for biological receptors. *Polymer* 55, 699-714.

## CHAPTER 2

### **Preparation of Magnetic Polymers for the Elimination of 3-Isobutyl-2-methoxypyrazine from Wine**

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Contribution to the Paper	Contributed to design of the experiments. Synthesised and analysed the polymers (physical characterisations and adsorption tests). Performed the GC-MS analysis of IBMP, interpreted data, and carried out statistical analysis. Completed first draft of the manuscript and revised based on co-author input. Helped address reviewer comments and made corrections to the manuscript based on the reviews.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4/6/2018

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- the candidate's stated contribution to the publication is accurate (as detailed above);
- permission is granted for the candidate to include the publication in the thesis; and
- the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.



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Signature		Date	4/6/2018.

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Article

# Preparation of Magnetic Polymers for the Elimination of 3-Isobutyl-2-methoxypyrazine from Wine

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**Abstract:** 3-Isobutyl-2-methoxypyrazine (IBMP), the most prevalent grape-derived methoxypyrazine, can contribute green bell pepper, vegetative and herbaceous aromas and flavours to wines. At elevated concentrations, this potent odorant may mask desirable fruity and floral aromas in wine and may be considered as a fault. A new remediation method for wines with elevated IBMP levels has been trialled using magnetic polymers, prepared in the same way as ordinary polymers but with the incorporation of iron oxide nanoparticles as magnetic substrates. Characterisation by Fourier transform-infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) showed no difference between thermally synthesised and microwave synthesised polymers. Magnetic polymers were found to have removed over 40% of the IBMP present in spiked model wine and white wine within ten minutes. The addition of magnetic nanoparticles and microwave-induced polymerisation did not affect the adsorption properties of the polymer in model wine and the polymer could be regenerated at least five times. Both Langmuir and Freundlich isotherms were found to fit the data for both types of polymer. However, attempts to produce imprinted polymers were not achieved, as they were found not to be differentiated from non-imprinted counterparts via adsorption tests.

**Keywords:** polymer synthesis; wine flavour; adsorption isotherm; adsorption kinetics; reusability

## 1. Introduction

Alkylmethoxypyrazines (MPs) are aroma volatiles noted for their potency and ability to impart sensory characters of green bell pepper, grass, and vegetables to wine [1]. Three grape-derived MPs have been uncovered in recent decades—3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-*sec*-butyl-2-methoxypyrazine (SBMP) [1]—that are mainly located in grape stems, followed by skins and seeds [2]. Another source of MPs in wines originates from the contamination of grapes by Coccinellidae beetles, leading to a wine fault known as ‘ladybug taint’ (LBT) [3,4]. More recently, 2,5-dimethyl-3-methoxypyrazine (DMMP) was identified as another MP compound released by Coccinellidae that contributes to LBT odour [5]. MPs can contribute to the so-called varietal flavours of certain grape varieties including Cabernet Sauvignon, Cabernet Franc, Sauvignon Blanc, and Carmenere [6]; however, high levels ( $\geq 20$  ng/L) can be overpowering and cause undesirable ‘green’ and ‘unripe’ characters [7] due to the extremely low sensory thresholds of MPs. The detection and recognition thresholds of IBMP determined in red wine are 10 ng/L and 15 ng/L, respectively [8]. The quantity of IBMP is constantly found to be higher than IPMP and SBMP in grapes and wines, and ranges in wines from below 2 ng/L to around 50 ng/L [9]. Consequently, the level of IBMP may be utilised as an indicator of the overall green character potential of grapes and wines.

Grape IBMP concentrations can be affected by grape maturity, sunlight exposure, water status, temperature, vine vigour, and yield [10–13]. It has been confirmed that greater light exposure for the

berries before veraison can decrease IBMP accumulation [12], but changes during ripening have not been explained entirely. The decrease in IBMP concentration during berry maturation may be mainly driven by dilution due to an increase in berry weight [14], with no clear degradation pathway of IBMP thus far being elucidated.

Since IBMP is relatively stable during fermentation and ageing [15,16], remedial methods are necessary when there are highly elevated IBMP levels, for example, in grapes from cool climate regions or when grapes are picked early to make lower alcohol wines. Several pre- or post-fermentation treatments to remove excessive MPs from juice or wine have been investigated. Must clarification is reported to remove 50% of IBMP in grape juice [2], but this is not suitable for skin-fermented wines. Fining agents such as bentonite and activated charcoal have little effect on the concentration of MPs in wine and lack selectivity [17]. In recent years, several kinds of polymers have been used to remediate juice or wine with excessive MPs [18,19], and silicone and polylactic acid polymers have been reported to be able to remove grape-derived MPs in wine while causing little change in most non-targeted volatile aroma compounds and colour parameters [20]. Addressing the issue of selectivity, molecularly imprinted polymers (MIPs) have been utilised for the extraction of MPs from wine [21–23].

MIPs offer a promising alternative to traditional solid-phase sorbents by possessing complementary cavities for target molecules such as IBMP. By association and then disassociation of a template (target molecule or target analogue) during synthesis, this group of polymers gains “memories” of the target molecule and can therefore bind specifically with that compound [24]. Furthermore, MIPs can be made into magnetic forms, termed magnetic molecularly imprinted polymers (MMIPs), through the attachment of magnetic substrates [25]. The major advantage of using MMIPs is they can be directly separated by an external magnetic field instead of by filtration. Magnetic polymers have been applied to extract inorganic food components such as heavy metals and organic components including veterinary drugs, pesticides, and hormones [26]. Magnetic particles coated with MIPs have been used to extract resveratrol from red wine [25], Sudan dyes from chili powder [27], bisphenol A in milk [28], and vanillin in food samples [29].

In this study, attempts were made to produce synthetic MMIPs for IBMP extraction from wine, with a comparison to magnetic non-imprinted polymers (MNIPs), and to non-magnetic counterparts for what appears to be the first time. In addition, microwave-induced polymerisation versus conventional thermal synthesis was evaluated. Physical characterisation and adsorption analysis were carried out to evaluate the different polymers.

## 2. Results and Discussion

### 2.1. Preparation of Polymers

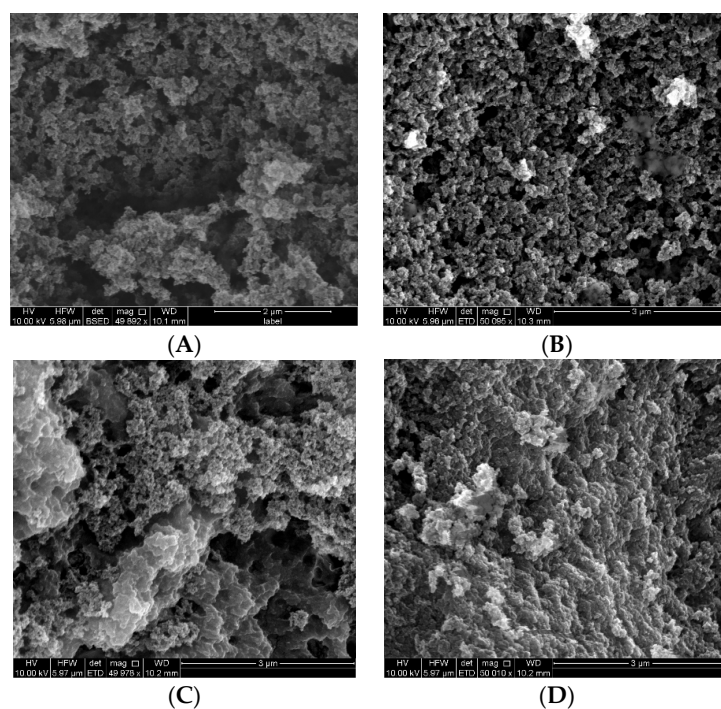
Polymers were prepared as outlined in Section 3.2. In an attempt to produce molecularly imprinted polymers (referred to as MMIPs throughout to differentiate from polymers produced without the use of a template), 2-methoxypyrazine was employed as a template to overcome the ‘template bleeding’ problem that occurs when IBMP has been utilised as the template in previous trials [23]. That said, even with continuous multiple solvent extraction, an equilibrium may be reached where there is always residual template left in the polymer, which would leak into the solutions during adsorption tests [30]. In other cases,  $\mu\text{g}$  or  $\text{ng}$  levels of residual template would be an acceptable level of bleeding for the analysis of compounds in the  $\text{mg}$  range. However, in the case of IBMP and the ultra-trace levels present in grapes and wines,  $\text{ng}$  levels of bleeding of IBMP would be unacceptable and greatly affect adsorption tests conducted within a practical concentration range [31]. Thus, choosing an analogue to IBMP as a template was deemed to be the better way to solve the template bleeding problem [32,33], as ultra-trace levels of 2-methoxypyrazine leaching would not interfere with the adsorption analysis. From a practical consideration, it should be a food-grade chemical with a much higher sensory threshold than IBMP so it would not be detected at trace levels [34]. Microwave synthesis was trialled due to its noticeable time-saving and consistent performance [35]. The synthetic

process went smoothly for both microwave (MW) and conventional methods and the finished products were similar in appearance to bulk polymerisation products.

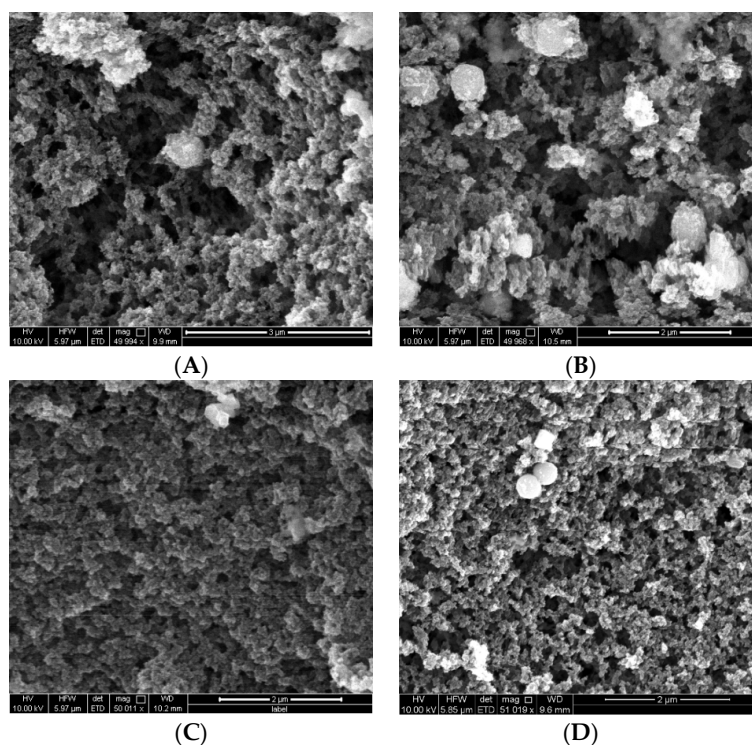
## 2.2. Characterisation of Polymers

Figure 1 shows scanning electron microscopy (SEM) images of the various polymers produced by conventional and MW synthesis. The polymers were deemed to be micro- to meso-porous (<2 nm to 2–50 nm) and no backbone structural difference regarding compactness was found between the imprinted and non-imprinted polymers, nor with the MW synthesised polymers. In comparison to the regular polymers, images of the magnetic polymers implied the presence of metal (bright spheres, Figure 2) due to the incorporation of iron oxide nanoparticles (using commercial iron (II,III) oxide nanoparticles, which may or may not be purely magnetite in the products, so  $\text{Fe}_x\text{O}_y$  has been used). As with the non-magnetic polymers, the backbones of the MMIPs and MNIPs were similar, as were the microwave synthesised magnetic polymers.

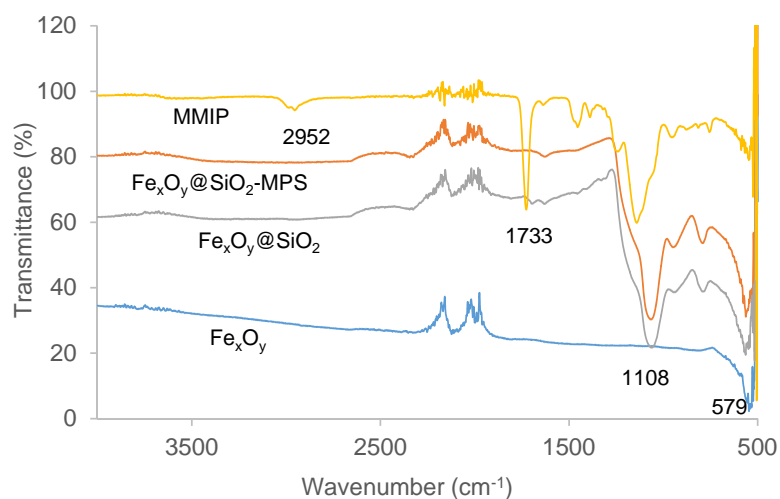
Attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis was performed to further ensure the correct preparation of polymers. Figure 3 shows the FTIR spectra of  $\text{Fe}_x\text{O}_y$ ,  $\text{Fe}_x\text{O}_y@SiO_2$ ,  $\text{Fe}_x\text{O}_y@SiO_2$ -MPS, and MMIP. In line with the results of Chen et al. [25], the adsorption peak at  $579\text{ cm}^{-1}$  found in each spectra was indicative that  $\text{Fe}_x\text{O}_y$  nanoparticles were present in these materials. The peaks around  $1108\text{ cm}^{-1}$  were attributed to Si-O-Si, revealing the formation of the silica shell. The strong peak at  $1733\text{ cm}^{-1}$  associated with the C=O functional group, and the lack of a peak at  $1660\text{ cm}^{-1}$  ordinarily attributable to C=C, indicated the successful formation of MMIP by polymerisation of magnetic nanoparticle-bound 3-(trimethoxysilyl)propyl methacrylate (MPS), ethylene glycol dimethacrylate (EGDMA), and methyl methacrylate (MMA). The peak at  $2952\text{ cm}^{-1}$ , indicative of C-H stretches from methyl and methylene groups, confirmed MMIP coupling with MMA and EGDMA [36]. The FTIR spectra of MNIP, MW MMIP, and MW MNIP coincided with MMIP, and MIP had quite similar FTIR spectra (not shown), except without an adsorption peak of Fe-O at  $579\text{ cm}^{-1}$ . No variations were found between the different batches of polymers.



**Figure 1.** Scanning electron microscopy (SEM) images of polymers prepared by conventional thermal synthesis for (A) molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) (B), and by microwave (MW) synthesis for (C) MW MIP and (D) MW NIP.



**Figure 2.** SEM images of magnetic polymers prepared by conventional thermal synthesis for (A) magnetic molecularly imprinted polymer MMIP and (B) magnetic non-imprinted polymer (MNIP), and by MW synthesis for (C) MW MMIP and (D) MW MNIP.

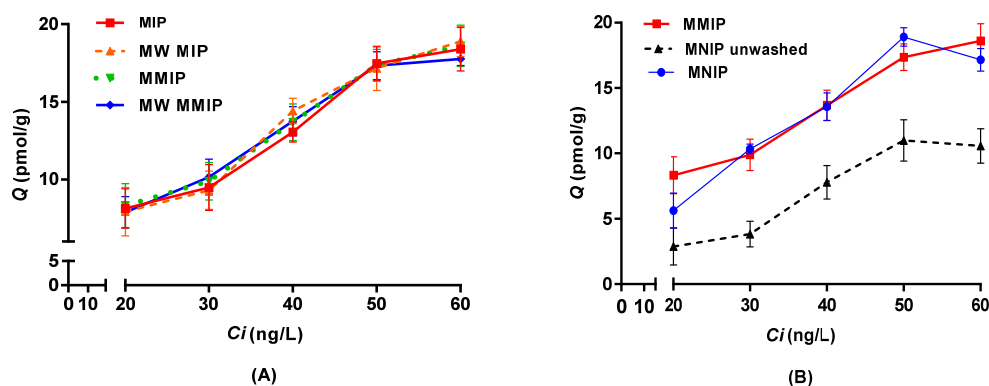


**Figure 3.** Overlaid Fourier transform-infrared (FTIR) spectra of  $\text{Fe}_x\text{O}_y$  nanoparticles,  $\text{Fe}_x\text{O}_y@SiO_2$ ,  $\text{Fe}_x\text{O}_y@SiO_2$ -MPS, and putative MMIP.  $\text{Fe}_x\text{O}_y@SiO_2$ :  $\text{Fe}_x\text{O}_y$  nanoparticles modified with  $SiO_2$ ;  $\text{Fe}_x\text{O}_y@SiO_2$ -MPS: surface-modified magnetic particles; MPS: 3-(trimethoxysilyl)propyl methacrylate.

### 2.3. Adsorption Isotherms

Binding tests were carried out to estimate the adsorption capability of polymers under different initial IBMP concentrations in model wine. The equilibrium isotherms for the adsorption of IBMP onto different imprinted polymers are shown in Figure 4A. The amount of IBMP binding to the polymers increased with increasing initial concentration and no difference was evident among the imprinted polymers according to one-way analysis of variance (ANOVA) and Tukey (HSD) pairwise comparison

( $p < 0.05$ ), including those produced with a microwave or with the inclusion of iron oxide nanoparticles. Thus, under the low concentration range used in this study, the different forms of imprinted polymers could not be differentiated from each other.



**Figure 4.** Adsorption isotherms of 3-isobutyl-2-methoxypyrazine (IBMP) (mean  $\pm$  SD, standard deviation,  $n = 3$ ) for (A) conventional and MW putative imprinted polymers and (B) thermally synthesised MMIP, MNIP, and unwashed MNIP. Q: equilibrium adsorption amount;  $C_i$ : initial IBMP concentration.

The results of binding tests for thermally synthesised MMIP and MNIPs are shown in Figure 4B, where the adsorption amount also increased in line with the initial IBMP concentrations. The adsorption capability of imprinted polymers was not significantly different from the non-imprinted controls (one-way ANOVA, Tukey (HSD) pairwise comparison,  $p < 0.05$ ). However, unwashed MNIP had a much lower binding capacity towards IBMP compared to MMIP, which could be due to cavities occupied by the trapped solvent. It was found that the washing process could not only remove template molecules, but also porogen solvent, from the polymers. This highlighted the importance of treating the non-imprinted polymers in exactly the same way as the imprinted polymers to provide proper controls [37].

Several linear and non-linear adsorption isotherm models were applied to fit the equilibrium data of thermally synthesised MMIP and MNIP. Linear models (Table 1) turned out to have a better fit based on their coefficients of determination ( $R^2$ , Table 2 and Figure S1 of the Supplementary Materials). Polymers were consistently produced using the synthetic procedures outlined in Section 3.2 and batches produced identical results. Both Langmuir and Freundlich isotherms were a good fit for the experimental data. The Langmuir isotherm assumes monolayer adsorption with all sites equivalent to form a homogeneous surface. Once a molecule occupies a binding site, no further adsorption may take place at the same site and a saturation adsorption will be reached, also known as maximum adsorption. On the other hand, the Freundlich isotherm is used to describe surface heterogeneity assuming multilayer adsorption [38]. Though they are based on different theories, both Langmuir and Freundlich models might adequately describe the adsorption at certain concentrations, especially when the concentrations are low and the adsorption capacity of the adsorbent is large enough to make both isotherm equations approach linearity. In the present case, the analytical window is narrow and deliberately limited due to the practical concentration of IBMP in grapes and wines, compared to a concentration range that usually differs by at least two orders-of-magnitude for isotherm determinations. The resultant isotherm may ultimately correspond to only a subset of the sites in MMIP, and while informative, this could be inaccurate and inconsistent for estimating the binding properties in general [39]. Nonetheless, the  $m$  value of the Freundlich isotherm ranging from 0 to 1 indicates surface heterogeneity, where approaching zero means greater heterogeneity. The  $m$  value of 0.5436 for MMIP suggests that some heterogeneity was present; however, a more homogeneous surface could be assumed when the  $m$  value ranges between 0.5 and 0.9 [40]. The  $m$  value of 0.8822 suggested a more

homogeneous surface for MNIP within the tested range, which is in line with the Langmuir assumption. Similar observations were also found in previous studies where the surface of imprinted polymers was more heterogeneous than that of the non-imprinted polymers [39]. In addition, the heterogeneity may also be caused by the addition of templates.  $K$  value (Dubinin-Radushkevich) relates to the free energy  $E$  (kJ/mol) of adsorption per molecule of adsorbate when it is transferred to the surface of the solid from infinity in the solution.  $K < 1$  represents a rough surface with many cavities, and chemisorption can be assumed when the value of  $E$  is over 40 kJ/mol [40]. Thus, chemisorption could be expected for the polymers based on values of 1000 and 316 kJ/mol for MMIP and MNIP, respectively.

**Table 1.** List of linear form adsorption isotherm models.

Isotherm	Equation	Plot
Langmuir Type 2 [41]	$\frac{1}{Q} = \frac{1}{KQ_{max}} \frac{1}{C_f} + \frac{1}{Q_{max}}$	$\frac{1}{Q} V_s \frac{1}{C_f}$ $Q$ (pmol/g): amount of IBMP adsorbed at equilibrium. $C_f$ (ng/L): final equilibrium concentration of IBMP. $Q_{max}$ (pmol/g): maximum adsorption capacity. $K$ (L/nmol): Langmuir adsorption equilibrium constant.
Freundlich [25]	$\log Q = m \log C_f + \log a$	$\log Q V_s \log C_f$ $m$ : adsorption intensity or surface heterogeneity. $a$ (pmol/g): adsorption capacity of IBMP.
Dubinin-Radushkevich [25]	$\ln Q = K \epsilon^2 + \ln Q_{max}$ $\epsilon = RT \ln \left( 1 + \frac{1}{C_f} \right)$ $E = (-2K)^{-1/2}$	$\ln Q V_s \epsilon^2$ $K$ (kJ <sup>2</sup> /mol <sup>2</sup> ): Dubinin-Radushkevich constant. $\epsilon$ : Polanyi potential. $E$ (kJ/mol): mean adsorption energy. $R$ : gas constant (8.314 J/mol/K). $T$ (K): absolute temperature.

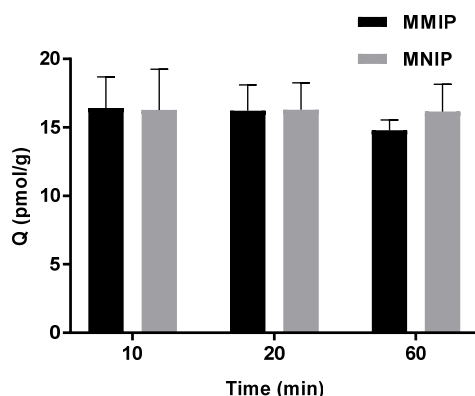
**Table 2.** Langmuir, Freundlich, and Dubinin-Radushkevich isotherm constants for the adsorption of IBMP on thermally synthesised magnetic polymers.

	MMIP	MNIP
<b>Langmuir Type 2</b>		
$Q_{max}$ (pmol/g)	25.19	84.03
$K$ (L/nmol)	0.3028	0.016
$R^2$	0.9688	0.8842
<b>Freundlich</b>		
$a$ (pmol/g)	6.24	1.99
$m$	0.5436	0.8822
$R^2$	0.9598	0.7754
<b>Dubinin-Radushkevich</b>		
$Q_{max}$ (pmol/g)	16.95	21.60
$K$ (kJ <sup>2</sup> /mol <sup>2</sup> )	$5 \times 10^{-7}$	$-5 \times 10^{-6}$
$E$ (kJ/mol)	1000	316
$R^2$	0.8710	0.9556

As shown in the isotherm graph (Figure 4B), specific binding was not observed in the adsorption analysis using model wine. This may be a result of the polymers being synthesised in a non-polar environment (toluene) rather than a wine matrix. A polar environment such as model wine (water, ethanol, tartaric acid) would destabilise the prearranged polymer complex [42] and MIPs should yield a better adsorption performance (relative to NIPs) in the same solvent as they were made [43,44].

#### 2.4. Adsorption Dynamic

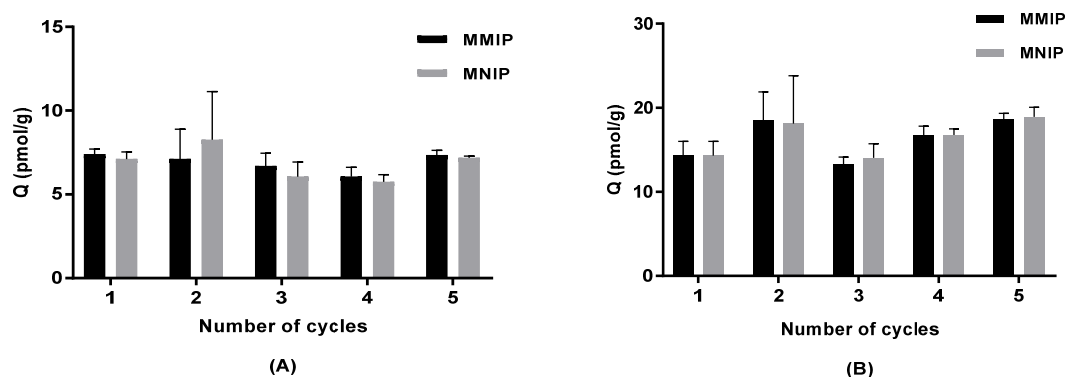
Kinetic adsorption tests were carried out for thermally synthesised MMIP and MNIP in model wine spiked with 30 ng/L of IBMP. Adsorption equilibrium was reached within ten minutes for both polymers (Figure 5), which indicates prompt adsorption. No difference was found for time and polymer type according to one-way ANOVA with Tukey (HSD) pairwise comparison ( $p < 0.05$ ).



**Figure 5.** Kinetics for adsorption of 30 ng/L IBMP in model wine (mean  $\pm$  SD  $n = 3$ ) using thermally synthesised MMIP and MNIP.

### 2.5. Regeneration of Polymers.

Thermally synthesised magnetic polymers were washed (details in Section 3.4) and tested for reusability with low (20 ng/L) and high (50 ng/L) concentrations of IBMP spiked into model wine. MMIP and MNIP remained the same (one-way ANOVA, Tukey (HSD) pairwise comparison,  $p < 0.05$ ) in terms of adsorption ability under both IBMP concentrations after cycling polymers five times (Figure 6).



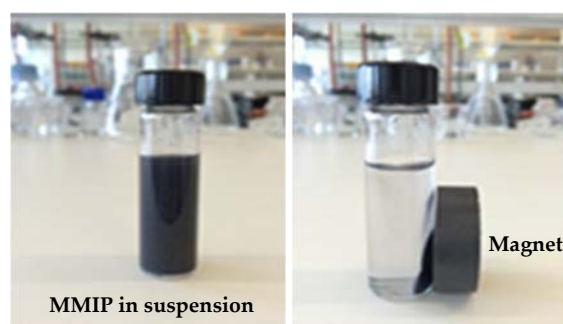
**Figure 6.** Adsorption on thermally synthesised magnetic polymers that were washed and cycled in model wine (mean  $\pm$  SD,  $n = 3$ ) for (A) 20 ng/L IBMP and (B) 50 ng/L IBMP.

### 2.6. Analysis of IBMP Adsorption in Spiked White Wine Samples

Two commercial Sauvignon Blanc wines, one from Australia (Aus, 0.4 ng/L IBMP) and the other from New Zealand (NZ, 14.1 ng/L), were spiked with 30 ng/L of IBMP (yielding IBMP concentrations prior to treatment as shown in Table 3) and used for adsorption testing. Magnetic separation was realised by placing a permanent magnet beside the vial containing the magnetic polymer (Figure 7). There was a lack of significant difference (one-way ANOVA, Tukey (HSD) pairwise comparison,  $p < 0.05$ ) in the equilibrium adsorption amounts ( $Q$ ) and percent adsorption of IBMP by MMIP and MNIP within the same wine. Interestingly, despite the wines being spiked with the same amount of IBMP, a higher amount of adsorption was observed for the NZ wine due to its higher initial IBMP concentration, which matches the adsorption isotherm data (Figure 4). The adsorption of IBMP on MMIP and MNIP in white wine was in line with that of adsorption in model wine solutions, including adsorption amount and binding properties. Overall, for a wine containing elevated IBMP levels (20 ng/L or above), an adsorption ability of up to 45% (using 1%  $w/v$  of polymer) and the reusability



of the polymers described in Section 2.5 indicates they could decrease IBMP to a level below its sensory detection threshold (i.e., <10 ng/L) with perhaps single and certainly multiple treatments.



**Figure 7.** Magnetic separation of putative MMIP in white wine samples.

**Table 3.** Adsorption of IBMP (mean  $\pm$  SD,  $n = 3$ ) by thermally synthesised MMIP and MNIP in commercial Sauvignon Blanc wines spiked with IBMP.

	Australia (Aus)	New Zealand (NZ)
Original IBMP (ng/L)	0.4 $\pm$ 0.1	14.1 $\pm$ 0.4
Spiked with 30 ng/L IBMP	25 $\pm$ 3	38 $\pm$ 2
$Q_{MMIP}$ (pmol/g)	6 $\pm$ 3	10 $\pm$ 3
$Q_{MNIP}$ (pmol/g)	3 $\pm$ 2	9 $\pm$ 2
MMIP adsorption (%)	42 $\pm$ 19	45 $\pm$ 11
MNIP adsorption (%)	19 $\pm$ 10	38 $\pm$ 5

### 3. Materials and Methods

#### 3.1. Chemicals

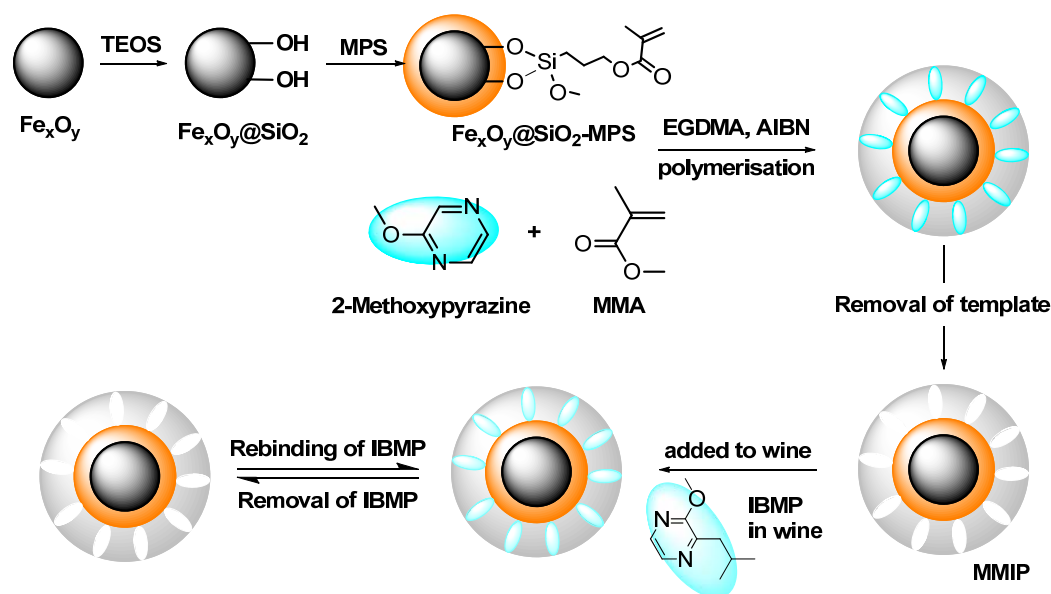
High purity solvents were purchased from Chem-Supply (Adelaide, SA, Australia). Iron (II, III) oxide particles (nanopowder, 50–100 nm particle size, 97% trace metals basis) and analytical reagent grade chemicals were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).  $d_3$ -IBMP (99.9 atom% D) was supplied by C/D/N Isotopes Inc. (Point-Claire, QC, Canada). Water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia).

#### 3.2. Preparation of Polymers

Magnetic polymers were prepared by multi-step polymerisation, as shown in Figure 8.  $Fe_xO_y@SiO_2$ -MPS nanoparticles were prepared first as magnetic bases using commercial iron (II, III) oxide particles instead of preparing through chemical co-precipitation. Surface modification of  $Fe_xO_y$  followed the procedures of Chen et al. [25], with modification of the process of Zhang et al. [45], Zeng et al. [46], and Lu et al. [47]. The attempted MMIPs were then prepared according to Belbruno et al. [23] with some modifications. Briefly, MMA (432  $\mu$ L, 4 mmol) was added to ‘dummy’ template molecule 2-methoxypyrazine (98  $\mu$ L, 1 mmol) in toluene (12 mL) as a functional monomer. The obtained  $Fe_xO_y@SiO_2$ -MPS nanoparticles (1 g) were then added and the mixture was stirred for 2 h at ambient temperature. After this time, cross-linker EGDMA (3.8 mL, 20 mmol) was added along with the initiator 2, 2'-azobisisobutyronitrile (AIBN, 100 mg). The mixture was degassed in an ultrasonic bath for 15 min and purged with nitrogen, sealed, and placed in a 60 °C oil bath for 24 h of polymerisation. The obtained bulk polymers were crushed and separated from the round-bottom flask. The polymers were dried under high vacuum and ground in a ball mill (full-directional planetary ball mill (QXQM-1), Tencan, Changsha, China). Ground polymer was passed through a 150  $\mu$ m sieve (Retsch test sieve, 200 mm  $\times$  50 mm, 150  $\mu$ m, VWR, Tingalpa, QLD, Australia) and the collected

particles were washed with diethyl ether by Soxhlet extraction until no further 2-methoxypyrazine was detected in the washing solvent by gas chromatography-mass spectrometry (GC-MS) analysis [23]. MNIPs were made by the same protocol without addition of the 2-methoxypyrazine as a template. Other putative imprinted and non-imprinted polymers (i.e., MW MMIPs and MW MNIPs) were prepared in the same way as described above, except that the polymerisation process was completed within 1 h at 60 °C in a microwave synthesiser (CEM microwave synthesiser, Discover S, DKSH, Melbourne, VIC, Australia). Reactions were performed in a 35 mL sealed reaction vessel (CEM, DKSH). The microwave power was on in dynamic mode with a pressure limit of 150 psi.

A range of non-magnetic polymers (the MIPs, NIPs, MW MIPs, and MW NIPs) were also prepared in the same way, without the magnetic substrates. All the polymers were prepared in duplicate and gave similar yields of around 92%.



**Figure 8.** Schematic showing the preparation steps used in an attempt to produce MMIPs. TEOS: tetraethoxysilane; MMA: methyl methacrylate; EGDMA: ethylene glycol dimethacrylate; AIBN: 2, 2'-azobisisobutyronitrile.

### 3.3. Characterisation of Polymers

IR spectra of ground polymers were measured by ATR on a PerkinElmer Spectrum 400 FTIR Spectrometer (Scientific Partners, Canning Vale, WA, Australia) in the 4000–500  $\text{cm}^{-1}$  region. SEM images were obtained using an FEI Quanta 450 FEG environmental scanning electron microscope (ThermoFisher, Scoresby, VIC, Australia). The powdered samples were prepared by adhesion to carbon tabs and coated with platinum. SEM images were obtained at an accelerating voltage of 10 kV and magnification of 50,000 $\times$  with a working distance of 10 mm. The images were taken under the same conditions.

### 3.4. Adsorption Equilibrium and Reusability of Polymers

Each polymer (10 mg/mL) was added to model wine solution (12 mL of 12% *v/v* EtOH and 5 g/L tartaric acid in MilliQ water, adjusted to pH 3.4 with 10 M NaOH) containing IBMP (99%, Sigma-Aldrich) at concentrations ranging from 20 ng/L to 60 ng/L. After shaking with an incubator at ambient temperature for 2 h at 120 rpm, polymers were separated by centrifugation (3857 rcf, 20 °C, 10 min) (Hettich, Universal 320/320R, Adela, Adelaide, SA, Australia) and the supernatants (10 mL) were transferred into 20 mL headspace vials and measured by GC-MS as described below. All adsorption tests were conducted in triplicate.

The equilibrium adsorption amounts of IBMP ( $Q$  pmol/g) were calculated according to the following equation:

$$Q = \frac{(C_i - C_f)V}{WM}$$

where  $C_i$  and  $C_f$  (pg/mL) are the initial and final equilibrium concentrations of IBMP, respectively;  $V$  (mL) is the volume of IBMP model wine solution;  $M$  is the molar mass of IBMP; and  $W$  (g) is the amount of polymer added to the model wine solution. The unit of  $Q$  was adjusted in accordance with the unit of  $C$ , so pmol/g was used in accordance with ng/L, where  $\mu\text{mol/g}$  is in accordance with mg/L [25].

For kinetic adsorption tests, thermally synthesised MMIP and MNIP (10 mg/mL) were added into model wine solutions containing 30 ng/L of IBMP. After shaking at 120 rpm at different time intervals (10, 20, 60 min) at ambient temperature, the supernatants were separated by centrifugation and assessed by GC-MS. All adsorption tests were conducted in triplicate.

Thermally synthesised MMIP and MNIP were tested for reusability. Used polymers were immersed in diethyl ether and stirred for several hours to remove IBMP. The high vacuum dried polymers (10 mg/mL) were added to model wine with low (20 ng/L) and high (50 ng/L) IBMP concentrations. After shaking at ambient temperature for 1 h at 120 rpm, polymers were separated by centrifugation and the supernatants were analysed by GC-MS. All reuse tests were conducted in triplicate.

### 3.5. Adsorption of 3-Isobutyl-2-methoxypyrazine in Spiked White Wine Samples

Thermally synthesised MMIP and MNIP (10 mg/mL) were added separately into 10 mL of an Australian Sauvignon Blanc wine (12.5% *v/v* EtOH, Banrock Station, 2016) and a New Zealand Sauvignon Blanc wine (12.5% *v/v* EtOH, Wahu Marlborough, 2016), each spiked with 30 ng/L IBMP. The mixtures were shaken at ambient temperature for 30 min at 120 rpm. A permanent magnet was used to separate the polymers from solutions and supernatants (4 mL) were transferred into 20 mL headspace vials and diluted with 6 mL MilliQ water and adjusted to pH 6 with NaOH [48]. Further GC-MS analysis followed the procedure as detailed below. Selected ion monitoring (SIM) chromatograms are presented in Figure S2 of Supplementary Materials.

### 3.6. Headspace Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry Analysis of 3-Isobutyl-2-methoxypyrazine.

Samples were analysed with an Agilent 6890GC and 5973MSD equipped with a Gerstel MPS2 autosampler. Model wine (10 mL) or diluted wine samples (4 mL wine, 6 mL water) were added to 20 mL headspace vials, with 3 g NaCl and 10  $\mu\text{L}$  of 50  $\mu\text{g/L}$   $\text{d}_3$ -IBMP (in absolute ethanol) as the internal standard. Standard curves were created using model wine or diluted model wine (2.5-fold dilution) spiked with IBMP (solutions in absolute ethanol) ranging from 5–60 ng/L, in addition to 50 ng/L  $\text{d}_3$ -IBMP. A 1 cm 23 gauge DVB/CAR/PDMS (Supelco, Sigma-Aldrich) solid-phase microextraction (SPME) fibre was used for undiluted model wine analysis and a 2 cm SPME fibre was used for white wine analysis. Sampling and instrumental analysis followed the method described by Chapman et al. [49].

### 3.7. Data Analysis

Significant differences between treatments were determined by one-way ANOVA with Tukey's HSD multiple comparison test at  $p < 0.05$  using XLSTAT (version 2014.5.03, Addinsoft, Paris, France). Graphs were processed using GraphPad Prism 7.02 (La Jolla, CA, USA).

## 4. Conclusions

Magnetic polymers were synthesised (including in a microwave), characterised, and found to remove up to 40% or more of the IBMP in model wine solutions and two white wines. Langmuir and

Freundlich isotherm models were used to evaluate polymer binding properties within practical ranges of IBMP concentration in model wine. No difference in comparison to conventional synthesis was observed for the microwave polymers in the adsorption of IBMP from model wine and in physical characters by SEM and FTIR analysis. Furthermore, the addition of the magnetic substrate had no effect on the physical characters and binding properties of the polymers. The easy separation, reasonable adsorption ability towards IBMP, and regeneration ability make magnetic polymers an attractive potential option to remediate wines with elevated MP concentrations. However, the choice of polymer system needs further investigation to improve the specificity of the polymers. Binding of IBMP in model wine and white wine was apparently driven by non-specific hydrophobic interactions and the putatively synthesised molecularly imprinted polymer turned out to be no better at IBMP removal than its non-imprinted counterpart. Two components of the imprinting system could be examined further to improve the specificity. Firstly, the functional monomer could be considered. Compared to MMA, for instance, methacrylic acid has more active carboxylic acid functional groups to interact with the template and reinforce H-bonding [50]. Secondly, the solvent used to prepare the polymers could be optimised. Polymers were prepared using toluene, which acted as a porogen that has a similar size and structure to the target molecule. Thus, it may be that the template did not impart a distinctive enough shape to the polymers [51]. Studies could also include control imprinted polymers prepared with an unrelated template to verify in the event of greater binding that it was from molecular imprinting and not because of physical differences between the polymers [52]. Finally, for the practical usage of MMIPs, examination of the effects of polymers on other wine volatiles, colour parameters, and sensory properties also needs to be further studied.

**Supplementary Materials:** The following are available online, Figure S1: Linear isotherm analysis plots of thermally synthesised magnetic polymers showing (a) Langmuir Type 2 analysis plot of putative magnetic molecularly imprinted polymer (MMIP), (b) Langmuir Type 2 analysis plot of magnetic non-imprinted polymer (MNIP), (c) Freundlich analysis plot of putative MMIP, (d) Freundlich analysis plot of MNIP, (e) Dubinin-Radushkevich analysis plot of putative MMIP, and (f) Dubinin-Radushkevich analysis plot of MNIP; Figure S2: Gas chromatography-mass spectrometry selected ion monitoring chromatograms of white wines showing (a) spiked Australian Sauvignon Blanc, (b) spiked Australian Sauvignon Blanc after putative MMIP treatment, (c) spiked New Zealand Sauvignon Blanc, and (d) spiked New Zealand Sauvignon Blanc after putative MMIP treatment.

**Author Contributions:** D.K.T. conceived and designed the experiments; C.L. performed the experiments, analysed the data, and drafted the manuscript; D.W.J. contributed to the research idea and helped with interpretation of data. All the authors reviewed and edited the manuscript.

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## References

1. Sidhu, D.; Lund, J.; Kotseridis, Y.; Saucier, C. Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 485–502. [[CrossRef](#)] [[PubMed](#)]
2. De Boubée, D.R.; Cumsille, A.M.; Pons, M.; Dubourdiou, D. Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractability during vinification. *Am. J. Enol. Vitic.* **2002**, *53*, 1–5.
3. Pickering, G.J.; Lin, Y.; Reynolds, A.; Soleas, G.; Riesen, R.; Brindle, I. The influence of *Harmonia axyridis* on wine composition and aging. *J. Food Sci.* **2005**, *70*, S128–S135. [[CrossRef](#)]

4. Botezatu, A.; Pickering, G.J. Determination of ortho- and retronasal detection thresholds and odor impact of 2,5-dimethyl-3-methoxypyrazine in wine. *J. Food Sci.* **2012**, *77*, S394–S398. [[CrossRef](#)] [[PubMed](#)]
5. Cai, L.; Koziel, J.A.; O’Neal, M.E. Determination of characteristic odorants from *Harmonia axyridis* beetles using *in vivo* solid-phase microextraction and multidimensional gas chromatography–mass spectrometry–olfactometry. *J. Chromatogr. A* **2007**, *1147*, 66–78. [[CrossRef](#)] [[PubMed](#)]
6. Koch, A.; Doyle, C.L.; Matthews, M.A.; Williams, L.E.; Ebeler, S.E. 2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. *Phytochemistry* **2010**, *71*, 2190–2198. [[CrossRef](#)] [[PubMed](#)]
7. Hein, K.; Ebeler, S.E.; Heymann, H. Perception of fruity and vegetative aromas in red wine. *J. Sens. Stud.* **2009**, *24*, 441–455. [[CrossRef](#)]
8. De Boubée, D.R.; Van Leeuwen, C.; Dubourdiou, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830–4834. [[CrossRef](#)]
9. Botezatu, A.; Kotseridis, Y.; Inglis, D.; Pickering, G. A survey of methoxypyrazines in wine. *J. Food Agric. Environ.* **2016**, *14*, 24–29.
10. Dunlevy, J.D.; Soole, K.L.; Perkins, M.V.; Nicholson, E.L.; Maffei, S.M.; Boss, P.K. Determining the methoxypyrazine biosynthesis variables affected by light exposure and crop level in Cabernet Sauvignon. *Am. J. Enol. Vitic.* **2013**, *64*, 450–458. [[CrossRef](#)]
11. Mendez-Costabel, M.P.; Wilkinson, K.L.; Bastian, S.E.P.; Jordans, C.; McCarthy, M.; Ford, C.M.; Dokoozlian, N.K. Effect of increased irrigation and additional nitrogen fertilisation on the concentration of green aroma compounds in *Vitis vinifera*. Merlot fruit and wine. *Aust. J. Grape Wine Res.* **2014**, *20*, 80–90. [[CrossRef](#)]
12. Ryona, I.; Pan, B.S.; Intrigliolo, D.S.; Lakso, A.N.; Sacks, G.L. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet Franc). *J. Agric. Food Chem.* **2008**, *56*, 10838–10846. [[CrossRef](#)] [[PubMed](#)]
13. Scheiner, J.J.; Sacks, G.L.; Pan, B.; Ennahli, S.; Tarlton, L.; Wise, A.; Lerch, S.D.; Vanden Heuvel, J.E. Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red wine grapes. *Am. J. Enol. Vitic.* **2010**, *61*, 358–364.
14. Gegan, S.M.; Jordan, B. Methoxypyrazine accumulation and O-methyltransferase gene expression in Sauvignon Blanc grapes: The role of leaf removal, light exposure, and berry development. *J. Agric. Food Chem.* **2016**, *64*, 2200–2208. [[CrossRef](#)] [[PubMed](#)]
15. Blake, A.; Kotseridis, Y.; Brindle, I.D.; Inglis, D.; Sears, M.; Pickering, G.J. Effect of closure and packaging type on 3-alkyl-2-methoxypyrazines and other impact odorants of Riesling and Cabernet Franc wines. *J. Agric. Food Chem.* **2009**, *57*, 4680–4690. [[CrossRef](#)] [[PubMed](#)]
16. Pickering, G.J.; Spink, M.; Kotseridis, Y.; Inglis, D.; Brindle, I.D.; Sears, M.; Beh, A.L. Yeast strain affects 3-isopropyl-2-methoxypyrazine concentration and sensory profile in Cabernet Sauvignon wine. *Aust. J. Grape Wine Res.* **2008**, *14*, 230–237. [[CrossRef](#)]
17. Pickering, G.; Lin, J.; Reynolds, A.; Soleas, G.; Riesen, R. The evaluation of remedial treatments for wine affected by *Harmonia axyridis*. *Int. J. Food Sci. Technol.* **2006**, *41*, 77–86. [[CrossRef](#)]
18. Botezatu, A.; Pickering, G.J. Application of plastic polymers in remediating wine with elevated alkyl-methoxypyrazine levels. *Food Addit. Contam. Part A* **2015**, *32*, 1199–1206. [[CrossRef](#)] [[PubMed](#)]
19. Ryona, I.; Reinhardt, J.; Sacks, G.L. Treatment of grape juice or must with silicone reduces 3-alkyl-2-methoxypyrazine concentrations in resulting wines without altering fermentation volatiles. *Food Res. Int.* **2012**, *47*, 70–79. [[CrossRef](#)]
20. Botezatu, A.; Kemp, B.S.; Pickering, G.J. Chemical and sensory evaluation of silicone and polylactic acid-based remedial treatments for elevated methoxypyrazine levels in wine. *Molecules* **2016**, *21*, 1238. [[CrossRef](#)] [[PubMed](#)]
21. Belbruno, J.J. Methods for preparation of molecularly imprinted polymers for wine extraction. U.S. Patent WO2014062632A1, 24 April 2014.
22. Belbruno, J.J.; Kelm, M. Molecularly imprinted polymers for wine extraction and method for production of molecularly imprinted polymers. U.S. Patent WO2013056226A1, 18 April 2013.
23. Belbruno, J.J.; Kelm, M. Molecularly imprinted polymers for wine extraction. U.S. Patent 2014/0242237 A1, 28 August 2014.

24. Haupt, K.; Linares, A.V.; Bompert, M.; Bui, B.T. Molecularly imprinted polymers. *Top. Curr. Chem.* **2012**, *325*, 1–28. [[PubMed](#)]
25. Chen, F.F.; Xie, X.Y.; Shi, Y.P. Preparation of magnetic molecularly imprinted polymer for selective recognition of resveratrol in wine. *J. Chromatogr. A* **2013**, *1300*, 112–118. [[CrossRef](#)] [[PubMed](#)]
26. Hernández-Hernández, A.A.; Álvarez-Romero, G.A.; Contreras-López, E.; Aguilar-Arteaga, K.; Castañeda-Ovando, A. Food analysis by microextraction methods based on the use of magnetic nanoparticles as supports: Recent advances. *Food Anal. Methods* **2017**, *10*, 2974–2993. [[CrossRef](#)]
27. Piao, C.; Chen, L. Separation of Sudan dyes from chilli powder by magnetic molecularly imprinted polymer. *J. Chromatogr. A* **2012**, *1268*, 185–190. [[CrossRef](#)] [[PubMed](#)]
28. Ji, Y.; Yin, J.; Xu, Z.; Zhao, C.; Huang, H.; Zhang, H.; Wang, C. Preparation of magnetic molecularly imprinted polymer for rapid determination of bisphenol A in environmental water and milk samples. *Anal. Bioanal. Chem.* **2009**, *395*, 1125–1133. [[CrossRef](#)] [[PubMed](#)]
29. Ning, F.; Peng, H.; Dong, L.L.; Zhang, Z.; Li, J.; Chen, L.; Xiong, H. Preparation and characterization of superparamagnetic molecularly imprinted polymers for selective adsorption and separation of vanillin in food samples. *J. Agric. Food Chem.* **2014**, *62*, 11138–11145. [[CrossRef](#)] [[PubMed](#)]
30. Ellwanger, A.; Berggren, C.; Bayouhd, S.; Crecenzi, C.; Karlsson, L.; Owens, P.K.; Ensing, K.; Cormack, P.; Sherrington, D.; Sellergren, B. Evaluation of methods aimed at complete removal of template from molecularly imprinted polymers. *Analyst* **2001**, *126*, 784–792. [[CrossRef](#)] [[PubMed](#)]
31. Venn, R.; Goody, R. Synthesis and properties of molecular imprints of Darifenacin: The potential of molecular imprinting for bioanalysis. *Chromatographia* **1999**, *50*, 407–414. [[CrossRef](#)]
32. Theodoridis, G.; Kantifes, A.; Manesiotis, P.; Raikos, N.; Tsoukali-Papadopoulou, H. Preparation of a molecularly imprinted polymer for the solid-phase extraction of scopolamine with hyoscyamine as a dummy template molecule. *J. Chromatogr. A* **2003**, *987*, 103–109. [[CrossRef](#)]
33. Matsui, J.; Fujiwara, K.; Takeuchi, T. Atrazine-selective polymers prepared by molecular imprinting of trialkylmelamines as dummy template species of atrazine. *Anal. Chem.* **2000**, *72*, 1810–1813. [[CrossRef](#)] [[PubMed](#)]
34. Seifert, R.M.; Buttery, R.G.; Guadagni, D.G.; Black, D.R.; Harris, J. Synthesis of some 2-methoxy-3-alkylpyrazines with strong bell pepper-like odors. *J. Agric. Food Chem.* **1970**, *18*, 246–249. [[CrossRef](#)]
35. Zhang, Y.; Liu, R.; Hu, Y.; Li, G. Microwave heating in preparation of magnetic molecularly imprinted polymer beads for trace triazines analysis in complicated samples. *Anal. Chem.* **2009**, *81*, 967–976. [[CrossRef](#)] [[PubMed](#)]
36. Gonzato, C.; Courty, M.; Pasetto, P.; Haupt, K. Magnetic molecularly imprinted polymer nanocomposites via surface-initiated RAFT polymerization. *Adv. Funct. Mater.* **2011**, *21*, 3947–3953. [[CrossRef](#)]
37. Verheyen, E.; Schillemans, J.P.; van Wijk, M.; Demeniex, M.-A.; Hennink, W.E.; van Nostrum, C.F. Challenges for the effective molecular imprinting of proteins. *Biomaterials* **2011**, *32*, 3008–3020. [[CrossRef](#)] [[PubMed](#)]
38. García-Calzón, J.; Díaz-García, M. Characterization of binding sites in molecularly imprinted polymers. *Sens. Actuators B* **2007**, *123*, 1180–1194. [[CrossRef](#)]
39. Rampey, A.M.; Umpleby, R.J.; Rushton, G.T.; Iseman, J.C.; Shah, R.N.; Shimizu, K.D. Characterization of the imprint effect and the influence of imprinting conditions on affinity, capacity, and heterogeneity in molecularly imprinted polymers using the Freundlich isotherm-affinity distribution analysis. *Anal. Chem.* **2004**, *76*, 1123–1133. [[CrossRef](#)] [[PubMed](#)]
40. López, M.D.M.C.; Pérez, M.C.; García, M.S.D.; Vilariño, J.M.L.; Rodríguez, M.V.G.; Losada, L.F.B. Preparation, evaluation and characterization of quercetin-molecularly imprinted polymer for preconcentration and clean-up of catechins. *Anal. Chim. Acta* **2012**, *721*, 68–78. [[CrossRef](#)] [[PubMed](#)]
41. Bolster, C.H.; Hornberger, G.M. On the use of linearized Langmuir equations. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1796–1806. [[CrossRef](#)]
42. Haupt, K.; Dzgoev, A.; Mosbach, K. Assay system for the herbicide 2,4-dichlorophenoxyacetic acid using a molecularly imprinted polymer as an artificial recognition element. *Anal. Chem.* **1998**, *70*, 628–631. [[CrossRef](#)] [[PubMed](#)]
43. Schwarz, L.; Bowyer, M.C.; Holdsworth, C.I.; McCluskey, A. Synthesis and evaluation of a molecularly imprinted polymer selective to 2,4,6-trichloroanisole. *Aust. J. Chem.* **2006**, *59*, 129–134. [[CrossRef](#)]
44. Schwarz, L.; Holdsworth, C.I.; McCluskey, A.; Bowyer, M.C. Synthesis and evaluation of a molecularly imprinted polymer selective to 2,4,6-trichlorophenol. *Aust. J. Chem.* **2004**, *57*, 759–764. [[CrossRef](#)]

45. Zhang, H.-F.; Shi, Y.-P. Magnetic retrieval of chitosan: Extraction of bioactive constituents from green tea beverage samples. *Analyst* **2012**, *137*, 910–916. [[CrossRef](#)] [[PubMed](#)]
46. Zeng, H.; Wang, Y.; Nie, C.; Kong, J.; Liu, X. Preparation of magnetic molecularly imprinted polymers for separating rutin from Chinese medicinal plants. *Analyst* **2012**, *137*, 2503–2512. [[CrossRef](#)] [[PubMed](#)]
47. Lu, F.; Sun, M.; Fan, L.; Qiu, H.; Li, X.; Luo, C. Flow injection chemiluminescence sensor based on core-shell magnetic molecularly imprinted nanoparticles for determination of chrysoidine in food samples. *Sens. Actuators B* **2012**, *173*, 591–598. [[CrossRef](#)]
48. Kotseridis, Y.S.; Spink, M.; Brindle, I.D.; Blake, A.J.; Sears, M.; Chen, X.; Soleas, G.; Inglis, D.; Pickering, G.J. Quantitative analysis of 3-alkyl-2-methoxypyrazines in juice and wine using stable isotope labelled internal standard assay. *J. Chromatogr. A* **2008**, *1190*, 294–301. [[CrossRef](#)] [[PubMed](#)]
49. Chapman, D.M.; Thorngate, J.H.; Matthews, M.A.; Guinard, J.-X.; Ebeler, S.E. Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. *J. Agric. Food Chem.* **2004**, *52*, 5431–5435. [[CrossRef](#)] [[PubMed](#)]
50. Gonzato, C.; Pasetto, P.; Bedoui, F.; Mazeran, P.-E.; Haupt, K. On the effect of using RAFT and FRP for the bulk synthesis of acrylic and methacrylic molecularly imprinted polymers. *Polym. Chem.* **2014**, *5*, 1313–1322. [[CrossRef](#)]
51. Joshi, V.P.; Karmalkar, R.N.; Kulkarni, M.G.; Mashelkar, R.A. Effect of solvents on selectivity in separation using molecularly imprinted adsorbents: Separation of phenol and bisphenol A. *Ind. Eng. Chem. Res.* **1999**, *38*, 4417–4423. [[CrossRef](#)]
52. Cederfur, J.; Pei, Y.; Zihui, M.; Kempe, M. Synthesis and screening of a molecularly imprinted polymer library targeted for penicillin G. *J. Comb. Chem.* **2003**, *5*, 67–72. [[CrossRef](#)] [[PubMed](#)]

**Sample Availability:** Samples of the compounds are not available.



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## Supplementary Information for

# Preparation of magnetic polymers for elimination of 3-isobutyl-2-methoxypyrazine from wine

Chen Liang, David W. Jeffery\* and Dennis K. Taylor

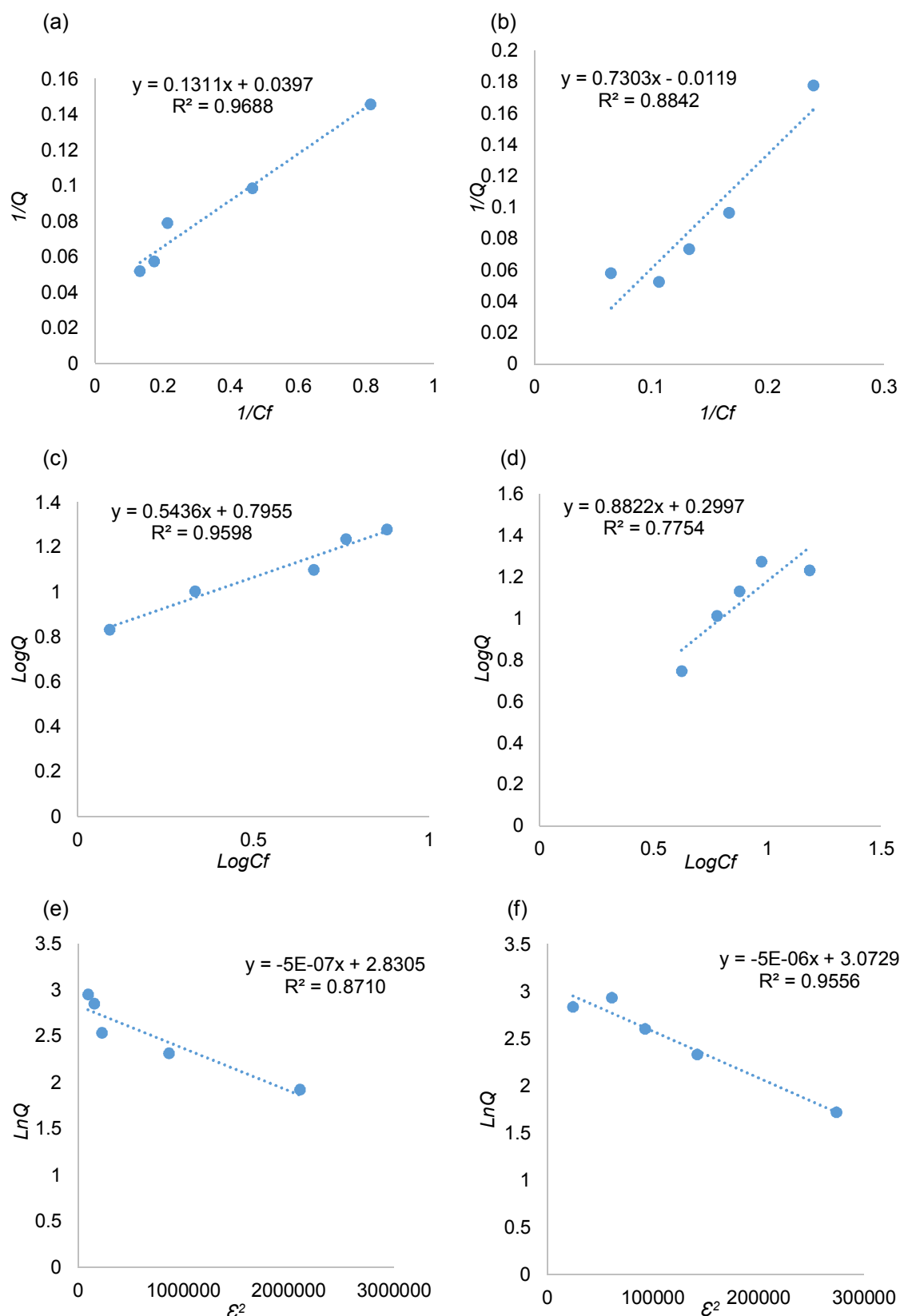
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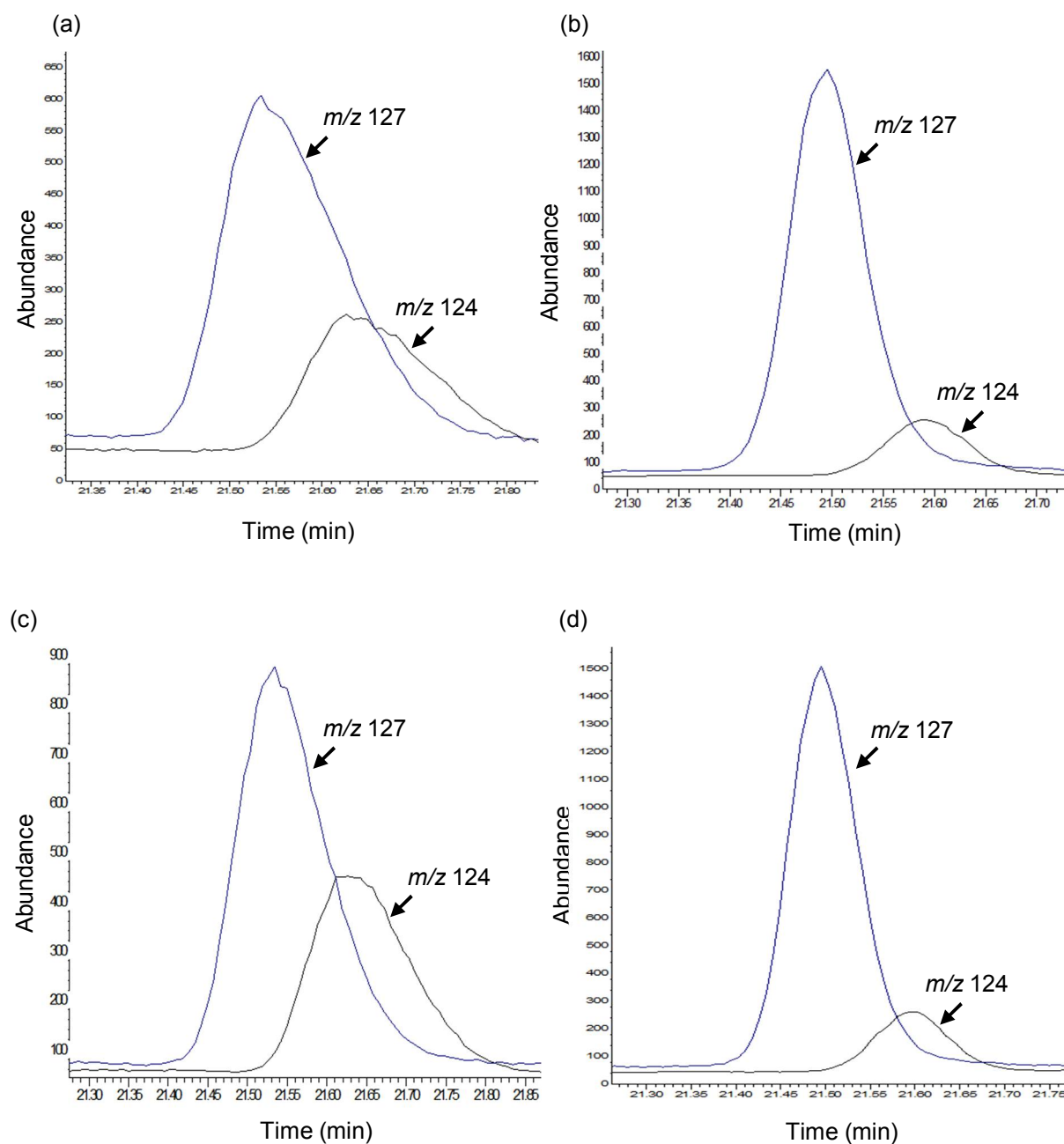
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**Figure S1.** Linear isotherm analysis plots of thermally synthesised magnetic polymers showing (a) Langmuir Type 2 analysis plot of putative magnetic molecularly imprinted polymer (MMIP), (b) Langmuir Type 2 analysis plot of magnetic non-imprinted polymer (MNIP), (c) Freundlich analysis plot of putative MMIP, (d) Freundlich analysis plot of MNIP, (e) Dubinin-Radushkevich analysis plot of putative MMIP, and (f) Dubinin-Radushkevich analysis plot of MNIP.  $Q$ : equilibrium adsorption amount (pmol/g);  $C_f$ : final equilibrium concentration of 3-isobutyl-2-methoxypyrazine (IBMP) (ng/L);  $\epsilon$ : polanyi potential.



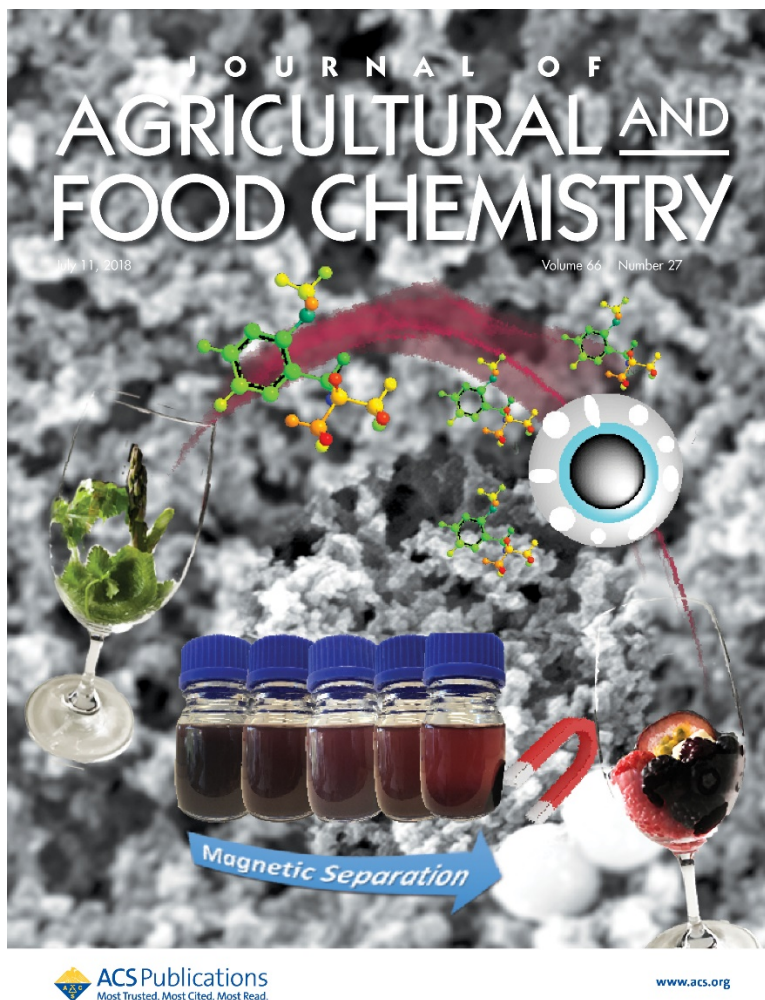
**Figure S2.** Gas chromatography-mass spectrometry selected ion monitoring chromatograms of white wines showing (a) spiked Australian Sauvignon Blanc, (b) spiked Australian Sauvignon Blanc after putative MMIP treatment, (c) spiked New Zealand Sauvignon Blanc, and (d) spiked New Zealand Sauvignon Blanc after putative MMIP treatment. IBMP was quantified using  $m/z = 124$  with  $d_3$ -IBMP at  $m/z = 127$  as the labelled internal standard.

## CHAPTER 3

### Chemical and Sensory Evaluation of Magnetic Polymers as a Remedial Treatment for Elevated Concentrations of 3-Isobutyl-2-methoxypyrazine in Cabernet Sauvignon Grape Must and Wine

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# Statement of Authorship

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Name of Principal Author (Candidate)	Chen Liang		
Contribution to the Paper	Designed and performed polymer synthesis. Carried out wine-making, GC-MS analysis of IBMP and volatile compounds, chemical analysis of wine, and sensory descriptive analysis. Processed and interpreted data and carried out statistical analyses. Completed first draft of the manuscript and revised based on co-author input. Helped address reviewer comments and made corrections to the manuscript based on the reviews.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4.10.2018

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- the candidate's stated contribution to the publication is accurate (as detailed above);
- permission is granted for the candidate to include the publication in the thesis; and
- the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Provided advice on winemaking and assisted with sensory analysis. Helped address reviewer comments. Critically evaluated and revised the manuscript.		
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Name of Co-Author	David W. Jeffery		
Contribution to the Paper	Conceived and designed the experiment. Supervised and directed the project. Discussed the results and provided critical feedback. Critically evaluated and revised the manuscript. Helped address reviewer comments and made corrections to the manuscript based on the reviews. Acted as corresponding author.		
Signature		Date	4/16/18

# Chemical and Sensory Evaluation of Magnetic Polymers as a Remedial Treatment for Elevated Concentrations of 3-Isobutyl-2-methoxy-pyrazine in Cabernet Sauvignon Grape Must and Wine

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## Supporting Information

**ABSTRACT:** 3-Isobutyl-2-methoxy-pyrazine (IBMP) is a potent odorant present in grapes and wines that is reminiscent of green capsicum. Suprathreshold concentrations can lead to obvious vegetative characters and suppress desirable fruity aroma nuances in wines, but options to manage IBMP concentrations are limited. This work investigated pre- and postfermentation addition of a putative imprinted magnetic polymer (PIMP) as a remedial treatment for elevated concentrations of IBMP in Cabernet Sauvignon grape must in comparison to nonimprinted magnetic polymer (NIMP) and to a commercially available polylactic acid (PLA) based film added postfermentation. Chemical and sensory analyses of wines showed that PIMP treatments were more effective than PLA film for decreasing “fresh green” aroma nuances without negatively impacting overall aroma profiles and that postfermentation addition of a magnetic polymer removed up to 74% of the initial IBMP concentration compared to 18% for PLA. Prefermentation addition of magnetic polymers removed 20–30% less IBMP compared to that of postfermentation addition but also had less of an effect on other wine volatiles and color parameters.

**KEYWORDS:** polymer synthesis, sorption properties, character impact compound, aroma matrix, wine, GC-MS

## INTRODUCTION

Wine contains a multitude of volatile compounds that can be classified not only according to certain functional group or structural properties but also based on the role they play in wine. Categories include impact or highly active compounds, impact groups of compounds, subtle compounds or families, compounds forming the base of wine aroma, and off-flavours.<sup>1</sup> Considered as both aroma impact compounds and off-flavors, alkylmethoxy-pyrazines (MPs) are a group of grape-derived compounds that impart vegetative and herbaceous aroma nuances to wines.<sup>2</sup> They are present at trace levels (low ng/L) within a narrow concentration range and contribute varietal characters to some grape cultivars such as Sauvignon Blanc and Cabernet Sauvignon.<sup>3</sup> However, at elevated concentrations, MPs can suppress fruity and floral wine aroma bouquets<sup>4</sup> and can even be considered as off-flavors/taints in some circumstances.<sup>5,6</sup>

Three grape-derived MPs (Table 1) have been identified: 3-isobutyl-2-methoxy-pyrazine (IBMP), 3-isopropyl-2-methoxy-pyrazine (IPMP), and 3-sec-butyl-2-methoxy-pyrazine (SBMP), among which IBMP is consistently found at higher abundances than IPMP or SBMP.<sup>6</sup> With detection and recognition thresholds of 10 and 15 ng/L in red wine, respectively,<sup>7</sup> IBMP imparts a specific strong green capsicum-like aroma to grapes and wines<sup>8</sup> and can be used as an indicator of overall green aroma characters. The content of IBMP in wines largely depends on the concentration in grapes at harvest,<sup>9</sup> and factors related to grape ripening play an integral role in IBMP concentrations. Lower levels of IBMP are found in well-ripened grapes,<sup>10</sup> and several viticultural aspects are deemed to be primary variables affecting IBMP concentrations in wines,

including sunlight exposure and temperature during ripening.<sup>11</sup> On the other hand, the usual winemaking process generally has little effect on final IBMP levels in wine.<sup>5</sup> Therefore, early harvest grapes (e.g., to make lower alcohol wines) or grapes from cool climate regions may contain elevated IBMP concentrations that lead to wines with excessive vegetative/herbaceous aroma characters.

Various postharvest treatments have been trialed to remediate grape juice or wine with elevated concentrations of MPs. Prefermentative maceration/heat treatment can lead to the elimination of substantial amounts of IBMP (around 50% or more in some cases)<sup>13</sup> but might also introduce cooked aromas to the finished wines.<sup>14</sup> Common wine additives such as bentonite, oak chips, deodorized oak chips, and activated charcoal have either no effect or a lack of selectivity that causes loss of other desirable components.<sup>5</sup> Alternatively, plastic polymers widely used in food packaging are known for their “scalping” effects on food flavors,<sup>15</sup> and a range of plastic polymers have recently been used as remedial treatments for grape juice or wine to adsorb excessive MPs.<sup>14,16</sup> Among the polymers, polylactic acid (PLA) based treatments have been reported to remove MPs from wines with no or minimum effects on other volatile compounds according to gas chromatography-mass spectrometry (GC-MS) analysis, although no differences in green aroma characters were perceived by sensory analysis.<sup>17</sup>

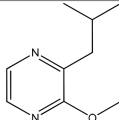
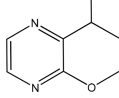
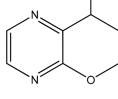
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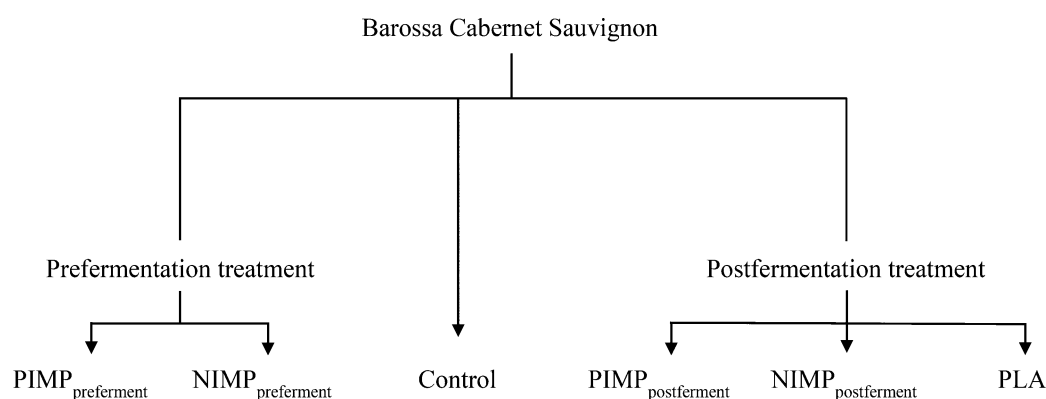
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**Table 1. Structures, Typical Concentration Range in Wines, Olfactory Threshold (ng/L), and Odor Descriptors of Three Grape-Derived Methoxypyrazines**

name	structure	typical range in wines <sup>12</sup> (ng/L)	threshold <sup>8</sup> (ng/L)	odor descriptor <sup>8</sup>
3-isobutyl-2-methoxypyrazine (IBMP)		up to 50	10, 15 in red wine	green bell pepper (capsicum), leafy, vegetative, herbaceous
3-isopropyl-2-methoxypyrazine (IPMP)		up to 11	2 in red wine	earthy, potato, asparagus/pea-like smell
3-sec-butyl-2-methoxypyrazine (SBMP)		up to 5	1-2 in water	peas, bell pepper, ivy leaves

**Figure 1.** Outline of winemaking treatments with putative imprinted magnetic polymer (PIMP) or nonimprinted magnetic polymer (NIMP) added pre- or postfermentation and PLA used postfermentation.

As another option, molecularly imprinted polymers (MIPs), often referred to as *plastic antibodies*, could selectively recognize and bind with target molecules by templating the polymers with such molecules during synthesis. MIPs have been widely used in a range of fields where selective binding is of importance, such as in immunoassays, sensors, analytical chemistry, and biochemistry (for sample cleanup and preconcentration).<sup>18</sup> MIPs can also be functionalized by the addition of magnetic substrates (yielding magnetic MIPs or MMIPs), meaning that they could be separated by an applied magnetic field rather than via filtration or by using them in a packed column, as is the case for conventional MIPs.<sup>19</sup>

On the basis of their selective binding and magnetic separation properties, MMIPs appear to offer an innovative winemaking solution for removing excessive IBMP concentrations (or indeed other off-flavors). As such, the current work aimed to investigate the practical usage of a putative imprinted magnetic polymer (PIMP) in winemaking as a postharvest remedial treatment for elevated grape IBMP levels. PIMP and a nonimprinted magnetic polymer (NIMP) counterpart were used as prefermentation and postfermentation treatments in IBMP-spiked Cabernet Sauvignon grape must and compared with PLA film used as a postfermentation treatment. The impact of these treatments on IBMP concentrations, basic composition, volatile compounds, and olfactory sensory properties of the finished wines was evaluated.

## ■ MATERIALS AND METHODS

**Chemicals.** Analytical reagent (AR) grade solvents were used: acetonitrile was purchased from Merck (Bayswater, VIC, Australia), diethyl ether was purchased from VWR (Tingalpa, QLD, Australia), and ethanol was purchased from Chem-Supply (Adelaide, SA, Australia). Most of the reference compounds (purity of  $\geq 97\%$ ) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia) except for ethyl butanoate, ethyl 2-methylbutanoate, and nonanoic acid, which were obtained from Alfa Aesar (Ward Hill, MA, USA). Deuterated internal standards were obtained from C/D/N Isotopes (Point-Claire, QC, Canada) or synthesized as previously reported for *d*<sub>5</sub>-ethyl nonanoate.<sup>20</sup> Standard solutions were prepared using 100% ethanol.

**Preparation of PIMP and NIMP.** Magnetic polymers were prepared through a multipolymerization process. The magnetic substrate ( $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MPS}$ ) was first prepared based on a modification<sup>21</sup> of the previously described processes.<sup>22–24</sup> Instead of preparing via chemical coprecipitation,  $\text{Fe}_3\text{O}_4$  particles (nanopowder, 50–100 nm particle size, and 97% trace metals basis) were purchased from Sigma-Aldrich. Surface modification of  $\text{Fe}_3\text{O}_4$  particles followed a previous procedure,<sup>23</sup> and the obtained  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  particles were functionalized with polymerizable double bonds.<sup>21</sup> PIMP was prepared according to reports by Belbruno and Kelm<sup>25</sup> and Chen, Xie and Shi<sup>21</sup> with some modifications. Briefly, to 12 mL of acetonitrile/water (9:1 v/v) containing the template molecule 2-methoxypyrazine (1 mmol) ( $\geq 99\%$ , Sigma-Aldrich) was added methacrylic acid (4 mmol) (99%, Sigma-Aldrich) as the functional monomer.  $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MPS}$  particles (1 g) were then added, and the mixture was stirred for 2 h at ambient temperature. Cross-linker ethylene glycol dimethacrylate

(20 mmol) (98%, Sigma-Aldrich) was then added along with initiator 2,2'-azobis(isobutyronitrile) (100 mg, Sigma-Aldrich). The mixture was degassed in an ultrasonic bath for 15 min, purged with nitrogen, sealed in a round-bottomed flask, and heated in an oil bath at 60 °C for 24 h. After polymerization, the mixture had turned into a solid polymer block, which was crushed using a mortar and pestle and ground in a ball mill (full-directional planetary ball mill, QXQM-1, Tencan, ChangSha, China). Particles of selected size were collected by passing through a sieve (Retsch test sieve, 200 mm DIA × 50 mm, 150 μm, VWR).<sup>26</sup> The template molecule was removed through Soxhlet extraction with diethyl ether<sup>25</sup> until no further 2-methoxypyrazine was detected in the washing solvent by GC-MS, and polymers were dried under high vacuum. NIMP was made by the same protocol without adding 2-methoxypyrazine as template.

**Gas Adsorption Measurements.** An ASAP 2020 surface area and pore size analyzer was used. Synthesized polymer samples were degassed overnight in ASAP 2020 analysis tubes at ambient temperature before analysis. Nitrogen (77 K) adsorption analysis was performed in the relative pressure ( $P/P_0$ ) range of  $10^{-6}$  to 1. UHP-grade (99.999%)  $N_2$  was used for all measurements.

**Winemaking Trials.** Cabernet Sauvignon grapes were sourced from a commercial vineyard located in the Barossa Valley region of South Australia. Grapes (21.6 °Brix, pH 3.58) were harvested on first March 2017 with an IBMP concentration of  $5.73 \pm 0.70$  ng/L. Winemaking followed the procedure of Li et al.<sup>27</sup> with modifications. Grapes were randomly distributed and allocated to treatments and control (in triplicate, Figure 1), weighing 10 kg per parcel before crushing. Potassium metabisulfite (PMS, 80 mg/L) was added to the grape must promptly after crushing (based on an assumption of 50% juice yield). The grape must was then spiked with a solution of 50 μg/L IBMP in ethanol (3 mL per treatment) to yield an estimated IBMP concentration of 30 ng/L.

For prefermentation treatments (i.e., PIMP<sub>preferment</sub> and NIMP<sub>preferment</sub>), 10 g/L of the respective polymer was added to grape must and stirred for 2 h before separation by a magnet. After removal of the polymer, the must was inoculated with yeast strain EC 1118 (30 g/hL) (Lallemand, Adelaide, SA, Australia), and wines were fermented at 20 °C, with the cap plunged twice a day. Diammonium phosphate (DAP, 150 mg/L) was added on the first and third days of fermentation. After 6 days, the wines were pressed into 5 L glass demijohns with air locks to complete fermentation (<1 g/L residual sugar determined enzymatically with a D-fructose/D-glucose assay kit, Megazyme, USA) before racking from lees into 5 L glass demijohns and adding 50 mg/L PMS. Wines were sealed and cold-stabilized at 0 °C for 2 months. None of the wines went through malolactic fermentation. Control wine and wine used for postfermentation treatments were prepared in the same manner but without the prefermentation addition of polymer.

Postfermentation treatments (i.e., PIMP<sub>postferment</sub> and NIMP<sub>postferment</sub>) were carried out 1 day before bottling. The polymer (10 g/L) was added to wines and stirred for 2 h before separation with a magnet. Separately, the PLA film (75 μm, 600 cm<sup>2</sup>/L, BI-AX International Inc., Canada)<sup>17</sup> was added to wine postfermentation for 2 h before separation by decanting.

Two kinds of ferrite magnets (permanent magnet) were used to separate the magnetic particles: ferrite ring magnet (100 mm × 60 mm × 17 mm), pull force 3.9 kg, and ferrite pot magnet (90 mm × 12 mm), pull force 30 kg (AMF Magnetics, Rozelle, NSW, Australia). For prefermentation treatments, both types of magnets were used in direct contact with the must to facilitate separation of magnetic polymers. The separation was continued until no more magnetic particles were observed to be attached to a newly applied magnet. For postfermentation treatments, the magnetic particles were separated by externally applying the pot magnet for 15 min and then decanting the wine.

Treatment and control wines were racked from lees and bottled into 375 mL glass wine bottles after the addition of 50 mg/L PMS. Bottled wines were stored at 15 °C for 6 months prior to chemical and sensory analysis.

**Basic Wine Composition.** Wine ethanol concentration (% v/v) was determined with an Alcozyzer Wine ME/DMA 4500 M (Anton Paar, Austria). The pH and titratable acidity (TA, expressed as g/L of tartaric acid) were measured with a combined pH meter and autotitrator (Mettler Toledo, Australia). Wine color parameters were measured using a modified Somers assay.<sup>28</sup>

**IBMP Analysis.** The analysis of IBMP followed the methods of Chapman et al.<sup>29</sup> for sampling and instrumental analysis, and Kotseridis et al.<sup>12</sup> for sample preparation with modifications. Wine (4 mL) and Milli-Q water (6 mL) were added to a 20 mL headspace vial containing 3 g of NaCl, and 10 μL of a solution of 50 μg/L 3-isobutyl-2-methoxy-*d*<sub>3</sub>-pyrazine (99.9 atom % D, C/D/N Isotopes) in ethanol. A standard curve was created with an IBMP-free commercial Pinot Noir wine (13% v/v alcohol) spiked with ethanolic solutions of IBMP (final concentrations of 5, 10, 15, 20, 25, 30, 40, and 50 ng/L) and internal standard solution (10 μL of 50 μg/L solution). All measurements were performed in triplicate.

Samples were analyzed with an Agilent 6890 GC and 5973 mass selective detector (MSD) equipped with a Gerstel MPS2 autosampler. A DVB/CAR/PDMS fiber (23 gauge, 2 cm, Supelco, Sigma-Aldrich) was used for sampling. The sample was incubated at 40 °C for 5 min before extraction for 30 min at 40 °C with agitation. The inlet was operated at 260 °C in splitless mode with the split vent opening after 5 min and a purge flow of 50 mL/min for an additional 5 min. Separation was performed with an HP-5 ms capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA) maintained at 40 °C for 5 min, then increased at 2.5 °C/min to 80 °C, at 5 °C/min to 110 °C, and at 25 °C/min to 230 °C before holding for 5 min at this temperature. Helium was used as carrier gas at a constant flow rate of 1.2 mL/min. The MSD interface was held at 280 °C, and electron ionization at 70 eV was used. Data were collected with Agilent ChemStation software (E.02.02.1431) using selected ion monitoring (SIM) with the selected mass channels for IBMP being  $m/z$  124 and 94, and  $m/z$  127 and 154 for *d*<sub>3</sub>-IBMP, with dwell times of 100 ms. The underlined ions were used for quantification.

**Analysis of Other Volatile Compounds.** Thirty-seven compounds were evaluated and their identity verified by analyzing authentic reference compounds, except for (*Z*)-3-hexen-1-ol, 3-methylbutyl acetate, 2-ethyl-1-hexanol, and 3-methylbutyl octanoate, which were unavailable at the time. Compound identity was also confirmed through mass spectral library matches (NBS 75K) and comparison of mass spectrometric data with those reported in the literature.

Samples were analyzed with an Agilent 7890A GC coupled to a 5975C MSD and fitted with a Gerstel MPS2 autosampler. The headspace sampling procedure, GC column (60 m DB-Wax), and instrumental parameters were as described by Wang et al.<sup>30</sup> except for MS data acquisition. Briefly, the wine sample (0.5 mL) and Milli-Q water (4.5 mL) were added to a 20 mL headspace vial containing 2 g of NaCl, and 10 μL of internal standard mixture in ethanol (2400 μg/L *d*<sub>4</sub>-methyl-1-butanol, 25 μg/L *d*<sub>3</sub>-hexyl acetate, 50 μg/L *d*<sub>13</sub>-1-hexanol, 1.2 μg/L *d*<sub>5</sub>-ethyl nonanoate, 500 μg/L *d*<sub>5</sub>-2-phenylethanol, and 50 μg/L *d*<sub>19</sub>-decanoic acid) was added. Analytes were detected in SIM mode using 2 to 4 selected ions and 35 individual time events with dwell times ranging from 20 to 100 ms. Quantitative data were obtained for 33 compounds by calibrating with the available reference standards, whereas the remaining 4 compounds were semiquantified based on their equivalence to other calibrated compounds. The quantifier/qualifier ions and the internal standard used for calibration of each analyte were based on Wang, Gambetta, and Jeffery.<sup>30</sup> Calibration was undertaken with a mixture of treatment and control wines prepared in this project and spiked with authentic reference standards and internal standard mixture. Each calibration curve contained at least eight points spanning the usual concentration ranges for the analytes, and data were collected with Agilent ChemStation software (E.02.02.1431). Calibration curves were prepared in triplicate, and replicated wines were evaluated.

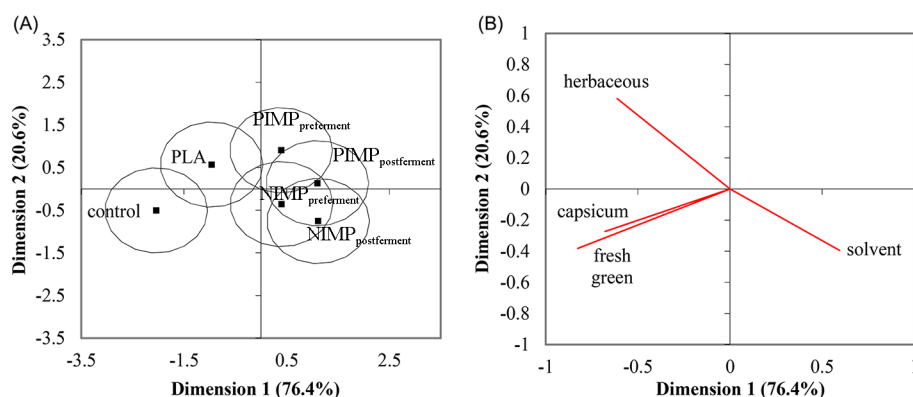
**Thiols Analysis.** 3-Sulfanylhexan-1-ol (3-SH) and 3-sulfanylhexyl acetate (3-SHA) were analyzed according to a chiral high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS)



Table 2. Results of Olfactory Descriptive Analysis (DA) for Different Treated Wines<sup>a</sup>

	control	PLA	PIMP <sub>preferment</sub>	NIMP <sub>preferment</sub>	PIMP <sub>postferment</sub>	NIMP <sub>postferment</sub>	LSD	<i>p</i> -value
overall aroma intensity	59.1	57.5	51.8	60.2	53	56.1	7.7	0.20
berry	38.4	32.1	30.6	34.8	32.2	27.8	8.5	0.20
jammy	22.4	22.3	18.3	20.3	25.5	26.8	7.3	0.20
tropical	11.8	9.5	15.4	23.0	15.5	12.8	10.2	0.16
floral	10.3	7.6	11.3	11.8	7.8	10.1	4.8	0.38
capsicum	16.8 a	11.1 ab	7.1 b	7.6 b	6.5 b	7.8 b	6.4	<b>0.021</b>
fresh green	32.4 a	23.7 b	14.8 c	20.2 bc	13.5 c	16.3 bc	8.4	<b>0.0005</b>
herbaceous	19.7 ab	20.2 a	17.3 abc	10.5 cd	12.3 bcd	8.8 d	7.6	<b>0.012</b>
cooked vegetables	10.3	13.0	9.0	11.0	13.8	9.6	6.9	0.67
rubbery	10.3	9.7	14.8	17.6	12.7	22.1	9.2	0.077
earthy	13.3	11.4	14.8	10.4	12.2	14.3	5.6	0.57
sweet spice	13.4	12.4	11.6	10.8	10.3	12.0	6.8	0.95
solvent	14.4 b	14.5 b	18.1 ab	20.0 ab	23.4 a	26.3 a	8.6	<b>0.045</b>

<sup>a</sup>Data for each treatment are presented as the mean with different letters across a row indicating significant differences ( $p \leq 0.05$ ) according to least significant difference (LSD) pairwise comparison with the interactions as error term. Bolded *p*-values indicate a significant difference for the attribute among the samples.



**Figure 2.** CVA plot generated from DA mean intensity data for different treated wines showing (A) scores with 95% confidence ellipses and (B) loadings designated by the significant ( $p \leq 0.05$ ) aroma attributes.

method<sup>31</sup> after thiols were derivatized and extracted from wine samples as previously described.<sup>32</sup>

**Olfactory Sensory Analysis.** Descriptive analysis (DA) was conducted by eight assessors (4 females and 4 males, aged 24 to 31 years) recruited from a pool of wine researchers from The University of Adelaide with some having previous DA experience. As the synthesized polymers were not food grade additives, only olfactory sensory analysis was undertaken. This sensory study was approved by Human Research Ethics Committee of The University of Adelaide (Ethics approval number: H-2017-093).

The assessors participated in four training sessions of 90 min each. Aroma descriptors were generated with definitions, and reference standards were discussed and finalized during the first two training sessions based on the wines produced for the study. Two subsequent training sessions were conducted in sensory booths to familiarize panelists with the tasting environment and sensory software. PanelCheck software (V1.4.2) was used to evaluate panel performance during the training sessions, and feedback was provided to panelists. On the basis of the panel consensus, 13 aroma attributes (Table S1) were determined and evaluated on an unstructured 15 cm line scale, anchored at 10%, 50%, and 90% corresponding to “low”, “medium”, and “high”, respectively.

Two formal sessions were held to evaluate wines in triplicate (18 in total). Wine samples (30 mL) were presented in standard black Institut National des Appellations d’Origine (INAO) approved tasting glasses (to eliminate biases caused by wine color differences) fitted with glass lids. Glasses were coded with random 3-digit codes and presented in a randomized and balanced order across panelists. RedJade software (RedJade, Redwood City, USA) was used to collect

the data. Assessors were required to wait 30 s between samples to avoid sensory fatigue.

**Data Analysis.** Data are presented as mean values with standard deviation from triplicate determinations using Microsoft Excel 2013. One-way analysis of variance (ANOVA) was conducted to determine significant differences between treatments with Tukey’s HSD multiple comparison posthoc test at  $p < 0.05$  using XLSTAT (version 2014.5.03, Addinsoft, Paris, France). Sensory data were analyzed using mixed model ANOVA to determine the effects of treatments, replicates, presentation order, judges (treating judges as a random factor), and their interactions with Fisher’s least significant difference (LSD) posthoc test for pairwise comparisons with the interactions as error term ( $p \leq 0.05$ ). Data were analyzed using SENPAQ (version 6.03, Qi Statistics, Reading, UK). Canonical variate analysis (CVA, SENPAQ) was used to analyze the sensory attributes with the number of dimensions chosen based on the corresponding eigenvalue. The means of significantly different chemical data and sensory attributes as supplementary variables were subjected to Pearson’s type principal component analysis (PCA) using XLSTAT.

## RESULTS AND DISCUSSION

**Olfactory Descriptive Analysis of Cabernet Sauvignon Wines with Different Treatments.** The aroma characters of the treated wines were described by panelists using the 13 descriptors listed in Table S1. Of those, four aroma attributes were significantly different: “capsicum”, “fresh green”, “herbaceous”, and “solvent” (Table 2). CVA was conducted to differentiate wines based on distinguishable attributes; Figure 2

**Table 3. Mean Concentrations ( $\mu\text{g/L}$ , Except Where Specified) and OAV<sup>a</sup> (in Parentheses) of Selected Volatile Compounds in Control and Treated Wines<sup>b</sup>**

	control	PLA	PIMP <sub>preferment</sub>	NIMP <sub>preferment</sub>	PIMP <sub>postferment</sub>	NIMP <sub>postferment</sub>	odor description <sup>c</sup>
IBMP (ng/L)	20.6 ± 1.7 a (2.05 <sup>d</sup> , 1.37 <sup>e</sup> )	16.8 ± 1.9 ab (1.68, 1.12)	11.6 ± 1.9 bc (1.16, 0.77)	11.3 ± 1.3 c (1.13, 0.75)	6.9 ± 1.2 cd (0.69, 0.46)	5.4 ± 0.8 d (0.53, 0.36)	green bell pepper, leafy, vegetative, herbaceous
$\beta$ -damascenone <sup>f</sup>	0.57 ± 0.06 a (11.3)	0.58 ± 0.04 a (11.5)	0.40 ± 0.04 b (8.1)	0.44 ± 0.02 b (8.8)	0.29 ± 0.02 c (5.7)	0.33 ± 0.04 bc (6.6)	apple, rose, honey
ethyl butanoate <sup>f</sup>	32 ± 3 abc (1.8)	31 ± 0 bc (1.8)	39 ± 1 a (2.2)	37 ± 1 ab (2.1)	27 ± 4 c (1.5)	28 ± 4 c (1.6)	strawberry, lactic
ethyl hexanoate <sup>g</sup>	73 ± 8 b (5.2)	73 ± 4 b (5.2)	87 ± 0 a (6.2)	81 ± 2 ab (5.8)	49 ± 1 c (3.5)	47 ± 3 c (3.4)	apple peel, fruit
ethyl octanoate <sup>h</sup>	52 ± 8 b (2.6)	50 ± 6 b (2.5)	73 ± 2 a (3.6)	59 ± 2 b (3.0)	22 ± 2 c (1.1)	17 ± 1 c (0.9)	melon, wood
ethyl 2-methylbutanoate <sup>f</sup>	2.9 ± 0.3 (2.9)	2.9 ± 0.5 (2.9)	2.8 a ± 0.4 (2.8)	2.4 ± 0.3 (2.4)	2.2 ± 0.1 (2.2)	2.6 ± 0.4 (2.6)	fruity, anise, strawberry
ethyl 3-methylbutanoate <sup>f</sup>	3.4 ± 0.4 (1.1)	3.3 ± 0.6 (1.1)	2.4 ± 2.0 (0.8)	3.0 ± 0.3 (1.0)	2.6 ± 0.1 (0.9)	3.1 ± 0.5 (1.0)	fruit
3-methylbutyl acetate <sup>f</sup>	56 ± 11 ab (1.9)	52 ± 2 bc (1.7)	70 ± 10 ab (2.4)	72 ± 3 a (2.4)	36 ± 2 c (1.2)	36 ± 1 c (1.2)	banana
1-octanol <sup>i</sup>	1.0 ± 0.1 a (1.4)	0.8 ± 0.1 b (1.1)	0.8 ± 0.0 b (1.1)	0.8 ± 0.0 b (1.1)	0.4 ± 0.0 c (0.6)	0.4 ± 0.0 c (0.6)	chemical, metal, burnt
(R)-3-SH (ng/L) <sup>j</sup>	199 ± 8 a (4.0)	206 ± 12 a (4.1)	164 ± 1 b (3.3)	177 ± 18 b (3.5)	180 ± 4 b (3.6)	176 ± 8 b (3.5)	grapefruit, citrus peel <sup>l</sup>
(S)-3-SH (ng/L) <sup>j</sup>	270 ± 4 ab (4.5)	282 ± 25 a (4.7)	224 ± 4 c (3.7)	234 ± 33 bc (4.1)	234 ± 5 c (3.9)	243 ± 4 bc (4.0)	passionfruit <sup>j</sup>

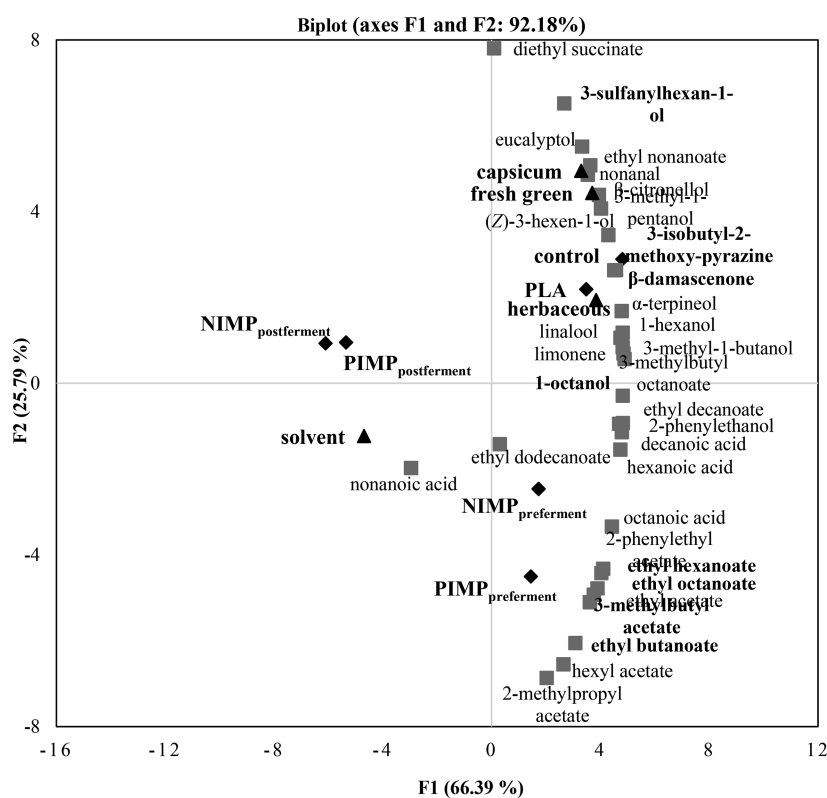
<sup>a</sup>OAV, odor activity value calculated from the ratio of concentration of the compound to its detection threshold. <sup>b</sup>Data for each treatment are presented as the mean ± SD ( $n = 3$ ) with different letters across a row indicating significantly different ( $p < 0.05$ ) means according to one-way ANOVA with Tukey (HSD) pairwise comparison. <sup>c</sup>Obtained from flavornet (<http://www.flavornet.org>). <sup>d</sup>Ratio between the concentration of IBMP and its sensory detection threshold in red wine of 10 ng/L. <sup>e</sup>Ratio between the concentration of IBMP and its recognition threshold in red wine of 15 ng/L. <sup>f</sup>Aroma detection threshold ( $\mu\text{g/L}$ ) refers to Guth. <sup>g</sup>Aroma detection threshold ( $\mu\text{g/L}$ ) refers to Ferreira et al. <sup>h</sup>Aroma detection threshold refers to Swiegers et al. <sup>i</sup>Aroma detection threshold refers to Bakker and Clarke. <sup>j</sup>Aroma detection threshold and odor description refer to Tominaga et al.<sup>41</sup>

shows scores for each wine with 95% confidence ellipses and loadings based on significant ( $p \leq 0.05$ ) sensory descriptors. The CVA plots showed that 97% of the variance was explained by the first two dimensions, with 76.4% of the variance explained by Dimension 1. The control wines were deemed richest in “capsicum” and “fresh green” aroma and were assessed to be higher in “herbaceous” aroma as well. The PLA treated wines, which were the only treatment that clustered with the control wines, were characterized by a “herbaceous” aroma attribute. Similar results were reported previously in terms of PLA treatment of Merlot wine showing no significant difference in “green” characters according to sensory analysis.<sup>17</sup> The prefermentation treatments involving PIMP (PIMP<sub>preferment</sub>) and NIMP (NIMP<sub>preferment</sub>) shared similar profiles and overlapped somewhat with the PLA treated wines. For postfermentation treatments with polymers, the PIMP<sub>postferment</sub> wines and NIMP<sub>postferment</sub> wines clustered with the prefermentation treated wines but were perceived to be higher in “solvent” notes. Notably, except for PLA treatments, all the other polymer treated wines were perceived to be much lower in “green” characters (“capsicum”, “fresh green”, and “herbaceous”) compared to the control wines and were generally perceived to be fruity.

**Basic Wine Composition.** The ethanol concentrations, pH, and TA were not significantly different among the treatments and in comparison to the control, with mean values of 12.1% alcohol by volume, pH 3.53, and 7.46 g/L TA (Table S2). In contrast, detectable differences in wine color properties were evident among the treatments (Table S3). Results of the color and phenolic parameters (total anthocyanins, color density, hue, SO<sub>2</sub>-resistant pigments, and total phenolics) showed the PLA treatment had little or no sorption of pigments and phenolics compared to that of the control wines, whereas postfermentation treatments with PIMP and NIMP had higher

sorption than those of the prefermentation treatments. The effect of PLA treatment on color parameters was in line with previous results,<sup>17</sup> and the decreases in phenols and color due to polymer addition were comparable to previously reported results for phenolic and color parameters after treatment of a red wine with polyvinylpyrrolidone (PVPP).<sup>33</sup> In the present study, polymer treatments were found to decrease SO<sub>2</sub>-resistant pigments, color density, and hue, which relate to decreased polymeric phenols, lightened color, and less brown color intensity relative to red, respectively.<sup>28</sup> PIMP was not differentiated from NIMP when comparing prefermentation or postfermentation treatments (Table S3).

**IBMP Analysis.** Data for IBMP concentrations in the wines are shown in Table 3, along with odor activity values (OAV) to help evaluate the contribution of IBMP to sensory perceptions. As threshold determinations are subjective measurements that depend greatly on the matrix and method used, the thresholds provided should be viewed as a guide when relating chemical constituents and sensory perceptions. Prefermentation treatments with synthetic polymers decreased the IBMP concentration in finished wines from 20.6 ng/L to around 11.5 ng/L, just above its detection threshold (10 ng/L)<sup>34</sup> but below the recognition threshold (15 ng/L).<sup>7</sup> In contrast, postfermentation treatments with the synthetic polymers yielded IBMP concentrations in finished wines that were well below the detection threshold (Table 3), whereas the PLA postfermentation treatment was much less effective, affording the removal of around 4 ng/L of IBMP (about 18% of the initial concentration) and leaving IBMP at levels in the finished wines that were still recognizable by olfaction. According to the study of Botzatu, Kemp, and Pickering,<sup>17</sup> PLA treatment with a surface area of 600 cm<sup>2</sup>/L led to a decrease in IBMP concentration of 77% (< 5 ng/L IBMP for a commercial Merlot spiked with 20 ng/L IBMP). The different results could



**Figure 3.** PCA biplot showing scores and loadings of the standardized means for significant ( $p < 0.05$ ) volatile compounds (gray square) and sensory attributes as supplementary variables (▲) for different treated wines (◆). Volatile compounds in bold indicate OAV > 1.

potentially be attributable to differences in film thickness or more likely to contact time; in the previous report, the 50  $\mu\text{m}$  PLA film was submerged in wine for 6 h, whereas the present study used a 75  $\mu\text{m}$  PLA film (due to availability) with 2 h contact time (to be consistent with the synthetic polymer treatments). Although increased film thickness has been reported to yield a higher sorption of aroma compounds,<sup>35</sup> it appears that contact time plays a more important role in the sorption effect,<sup>16,35</sup> and it is reported that the maximum sorption of IBMP on a PLA film was found after 8–24 h of contact time.<sup>16</sup> Under the conditions tested, it appeared that the PIMP had the same affinity toward IBMP as the NIMP; that is, greater selectivity for IBMP removal due to molecular imprinting was not observed in this study, even though an attempt was made to achieve specific binding by changing the functional monomer (to methacrylic acid from methyl methacrylate) and porogen solvent (to aqueous acetonitrile from toluene) compared to that in our previous study.<sup>36</sup> Therefore, sorption of IBMP from grape must and wine by PIMP and NIMP may be dominated by hydrophobic interactions that may have overshadowed any specific binding.

**Volatile Compounds Analysis.** A selection of wine volatiles was included to assess the impact on compounds other than IBMP. Of the 37 volatile compounds analyzed (Table S4), ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 2-methyl-1-propanol, 1-butanol, 2-ethyl-1-hexanol, ethyl furoate, and methionol were seemingly not affected by any treatments. For the other 30 compounds, significant differences were found between treatments and control. There were no significant differences between the magnetic polymer types (i.e., PIMP and NIMP), but the timing of treatments (pre- vs postfermentation) showed significantly different results.

Quantitative results for aroma compounds with OAV > 1 are shown in Table 3 to exemplify this outcome. The difference between prefermentation and postfermentation polymer treatments was associated with the prospective origin of the aroma compounds. Grape-derived compounds such as monoterpenoids (e.g., linalool, Table S4) and  $\text{C}_{13}$ -norisoprenoids (e.g.,  $\beta$ -damascenone, Table 3) were decreased by around 20–30% for prefermentation treatments, which was considerably less than the 40–60% seen for postfermentation polymer addition. In contrast, yeast-derived volatile metabolites including esters, volatile fatty acids, higher alcohols, and carbonyls were minimally decreased by prefermentation treatments (e.g., 3-methylbutyl acetate and ethyl butanoate; Table 3), and certain esters were apparently increased around 20% by prefermentation treatments compared to that in the control wines (e.g., ethyl hexanoate and ethyl octanoate; Table 3). Yeast-derived volatiles are produced through sugar and amino acid metabolism,<sup>38</sup> and equilibria are involved in ester-forming reactions. The prefermentation treatments seemed to shift some equilibria toward ester formation,<sup>42</sup> from ethanol and the corresponding acids, and from acetic acid and the corresponding alcohols. Among the treatments, the PLA film had the least effect on aroma compounds, and this treatment had the closest composition to the control wine. Most volatile compounds were either not affected by the PLA film or decreased by 20% at most, in contrast to the prefermentation treatment by polymers (Table 3 and S4). Quantitative data for 3-SH is presented as the respective enantiomers in Table 3. 3-SH levels were well above the reported sensory thresholds of 50 ng/L for (*R*)-3-SH and 60 ng/L for (*S*)-3-SH,<sup>40</sup> but 3-SHA was not detected. In accord with the other results, the PLA treatment did not differ in concentration for either enantiomer of 3-SH compared to

the control, and no difference was found for PIMP<sub>postferment</sub> or treatments involving NIMP. However, PIMP<sub>preferment</sub> wine was found to have significantly lower 3-SH levels in contrast to the postfermentation treated wines, as seen with the other volatile compounds. As 3-SH is released by yeast from nonvolatile precursors in juice during alcoholic fermentation,<sup>43</sup> treatments before fermentation might decrease the amount of precursors or modify the assimilation of the precursors by yeast and further affect the final concentration of 3-SH in wine. Similar results for thiols from must fining with bentonite were found by Vela et al.,<sup>44</sup> who reported that varietal thiols were significantly decreased by bentonite, especially when added to must compared to finished wine.

#### Sorption of Components by Different Treatments.

Besides the timing of treatments, sorption of wine components is influenced by the properties of the treatment materials, the nature of the compounds, and the external conditions.<sup>15</sup> The higher sorption of wine components by PIMP and NIMP treatments compared to that of the PLA film was hypothesized to be mainly caused by the larger surface area of the synthesized polymers, with a Brunauer–Emmett–Teller (BET) surface area of  $264 \pm 5 \text{ m}^2/\text{g}$  and  $242 \pm 4 \text{ m}^2/\text{g}$  for PIMP and NIMP, respectively. The putative imprinting may have led to the higher surface area of PIMP compared to that of NIMP, but a differential effect of putative imprinting on the sorption of wine components was not evident. On the other hand, other impactors such as altered sorption kinetics and polarity of the polymers may affect the sorption properties. Variances among compounds influenced by treatments could be explained by the concentration and polarity of prospective molecules. Generally, the more polar a compound, the less it was adsorbed by the treatments (e.g., ethyl 2-methylbutanoate and ethyl 3-methylbutanoate were not adsorbed by any treatment; Table S4), whereas volatile compounds with longer carbon chains have lower polarity and greater sorption ability (e.g., 1-octanol, which was adsorbed not only by magnetic polymers but also by the PLA film; Table 3). Additionally, a higher concentration of the adsorbed compound leads to greater sorption, e.g., ethyl butanoate, which despite being more polar than the esters stated above, had a higher sorption due to higher relative concentration.<sup>45</sup> The impactors described above could explain the sorption for most of the volatile compounds, as well for pigments, but more work is needed to further elucidate the underlying reasons.

**Relating Chemical and Sensory Data.** Principal component analysis (PCA) was performed on volatile compounds and sensory data that differed significantly ( $p < 0.05$ ) among the wines (Figure 3). The first two PCs explained 92% of the total variation among the samples, with 66% explained by PC1. Control and PLA treated wines were clearly differentiated from postfermentation treated wines along PC1 based on the higher content of most volatile compounds on the right of the plot. Wines treated prefermentation were separated from the control along PC2 based on their higher concentrations of fermentation-derived esters.

As the treatments led to sorption of a range of volatile compounds, the treated wines presented different aroma nuances due to the loss of different levels of aroma compounds. In a wine matrix, the aromas exhibited are a result of the effect of a mixture of volatile compounds; that is, synergistic or masking interactions among groups or individual compounds determine the final aroma perception.<sup>46</sup> As shown in Table 3, the two OAV values (based on detection and recognition

thresholds, respectively) of control and PLA treated wines were above one, which may indicate that the concentration of IBMP was high enough to be not only perceived in terms of vegetative and herbaceous nuances but also recognized as capsicum-like. The prefermentation treatments with PIMP and NIMP decreased the IBMP level to just above its detection threshold but below the recognition threshold, and the relative postfermentation treatments decreased the IBMP content to below detectable according to sensory analysis. As observed in the PCA plot (Figure 3), the content of IBMP was highly correlated with “capsicum” ( $r = 0.87$ ), “fresh green” ( $r = 0.90$ ), and “herbaceous” ( $r = 0.86$ ) aroma attributes according to Pearson’s correlation analysis. The “capsicum” and “fresh green” attributes were closely correlated with each other ( $r = 0.97$ ) more so than with the “herbaceous” attribute ( $r = 0.67$  and  $0.61$ , respectively), which was in line with the results shown in Figure 2. Apart from IBMP, several compounds were also found to be highly correlated (some expectedly so) with “capsicum” and “fresh green” notes, including 3-methyl-1-pentanol ( $r = 0.91, 0.91$ ), (*Z*)-3-hexen-1-ol ( $r = 0.88, 0.92$ ), ethyl nonanoate ( $r = 0.95, 0.97$ ),  $\beta$ -citronellol ( $r = 0.86, 0.87$ ), eucalyptol ( $r = 0.97, 0.94$ ), and 3-SH (sum of enantiomers,  $r = 0.87, 0.89$ ). Among these compounds, only 3-SH was considered odor-active based on the OAV values, whereas the others had an OAV below 0.1. Although the sensory character of 3-SH is reminiscent of grapefruit, passionfruit, and tropical fruit, it has been reported that volatile thiol combinations could contribute cooked vegetable aroma to wines as well.<sup>47</sup> As the “cooked vegetable” attribute was not differentiated (Table 2), the impact of volatile thiols may be reflected by the subtle enhancement of overall green aroma attributes, which in turn correlated with “fresh green” and “capsicum” attributes. As for the other correlated compounds, 3-methyl-1-pentanol (pungent, fusel, cognac and wine, and cocoa, with green fruity undernotes), (*Z*)-3-hexen-1-ol (green, grassy, and melon rind-like with a pungent freshness (aroma descriptors are from [www.thegoodscentcompany.com](http://www.thegoodscentcompany.com))), ethyl nonanoate (waxy, cognac, fruity, and tropical),  $\beta$ -citronellol (floral, rosy, sweet, and citrusy with green fatty terpene nuances), and eucalyptol (eucalyptus, herbal, camphor, and medicinal), were deemed to have more or less green aroma characters. Though they were all well below their respective sensory detection thresholds in the wines in this study, they may potentially be enhancers for green aroma characters (“fresh green” and “herbaceous”) when interacting with IBMP. The presence of IBMP, especially at high levels (e.g., 20 ng/L), has been shown to have a greater impact on sensory profile than the thiols,<sup>4,47</sup> that is, for wines containing both fruity and capsicum aroma bouquets, the overall aroma is driven by IBMP content. This may explain why the control and PLA wines were still perceived as “green” despite having higher concentrations of  $\beta$ -damascenone (Table 3).

As shown in Figure 3, PIMP<sub>preferment</sub> and NIMP<sub>preferment</sub> wines had higher levels of ethyl butanoate, hexanoate, and octanoate, and 3-methylbutyl acetate, which may likely contribute “fruity” and “tropical” nuances due to OAVs > 1 (Table 3). Thus, even though the prefermentation treated wines contained less 3-SH and overall esters compared to the control wines, they were not significantly different in overall aroma intensity and berry fruit characters, probably due to the removal of the strong masking effect of IBMP. Indeed, prefermentation treatment wines were perceived to be slightly more “tropical” and specifically as passionfruit and pineapple rather than grapefruit and citrus peel, which accords with the concentration of the 3-SH

enantiomers. As shown in Table 3, OAV values of (S)-3-SH (passionfruit nuance) were slightly higher than those of (R)-3-SH (grapefruit and citrus peel nuances). The large impact of IBMP on the fruity and green aroma balance was reported in terms of addition tests with IBMP or fruity esters (among other compounds).<sup>48</sup> Addition of certain ethyl and acetate esters had no significant effect, and addition of some berry fruit note ester combinations did not have a clear sensorial effect in red wine (dearomatized or neutral). However, addition of 5 ng/L IBMP was perceived as earthy in the dearomatized wine, and 15 ng/L IBMP was perceived as both green and earthy in the neutral wine.<sup>48</sup>

The PIMP<sub>postferment</sub> and NIMP<sub>postferment</sub> wines were perceived to be significantly higher in “solvent” aroma (Table 2); however, no aroma compounds were closely correlated with this attribute. Further decreasing of overall volatile compounds apparently made the pungent solvent aroma of ethanol and other higher alcohols stand out, as the “solvent” attribute was highly negatively correlated with most of the volatile compounds. Panelists commented that the PIMP<sub>postferment</sub> and NIMP<sub>postferment</sub> wines were perceived similarly to a Sauvignon Blanc wine. According to Sáenz-Navajas et al.,<sup>49</sup> the wine nonvolatile matrix has a great influence on the release of odorants, and the red wine nonvolatile matrix seems to retain esters and thiols. On the basis of the analysis of color parameters, treatments with PIMP and NIMP decreased the concentration of color pigments and could be considered as decreasing the retaining effect of the nonvolatile matrix on esters and thiols, thus enhancing their sensory impact.

In summary, this study evaluated the practical use of magnetic polymers as a postharvest remedial treatment for elevated IBMP concentrations in grape must and further investigated the balance between fruity and green aroma nuances in wines. PIMP has been shown to decrease relatively high initial levels of IBMP (20 ng/L) to around the sensory detection threshold or below in Cabernet Sauvignon wines, although the most effective postfermentation treatments also had a significant impact on most of the other wine volatile components. Despite this outcome, sensory results showed clear decreases in IBMP-related green aroma nuances without decrease of overall aroma intensity or fruity characters. In comparison, the PLA-based film was less effective as a remedial treatment, which may be a result of the limited surface area and slow sorption kinetics. In considering the entirety of the chemical analysis data, it seems advisable to adopt a prefermentation treatment approach for IBMP removal, but this may be less appropriate for other wines depending on varietal aromas such as 3-SH. Further research is still necessary to improve the imprinting process and enhance the selectivity of a magnetic polymer to arrive at an optimum dosage for a more specific remedial treatment under different wine matrix conditions. Furthermore, the format for magnetic separation of polymers would need to be investigated for an industry scale approach but would likely involve the use of magnetic filtering with an electromagnet, as used in the food industry.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b01397.

Sensory attributes, descriptions, and reference standards, wine basic chemical composition, wine color properties,

and mean concentrations of volatile compounds in wines (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

CVA, canonical variate analysis; DA, descriptive analysis; DAP, diammonium phosphate; GC–MS, gas chromatography–mass spectrometry; HPLC–MS/MS, high-performance liquid chromatography–tandem mass spectrometry; HS-SPME, head-space-solid phase microextraction; IBMP, 3-isobutyl-2-methoxy-pyrazine; IPMP, 3-isopropyl-2-methoxypyrazine; 3-SH, 3-sulfanylhexas-1-ol; 3-SHA, 3-sulfanylhexasyl acetate; MIP, molecularly imprinted polymer; MMIP, magnetic molecularly imprinted polymer; MPs, alkylmethoxypyrazines; NIMP, non-imprinted magnetic polymer; NIMP<sub>preferment</sub>, prefermentation treatment with nonimprinted magnetic polymer; NIMP<sub>postferment</sub>, postfermentation treatment with nonimprinted magnetic polymer; OAV, odor activity value; PCA, principal component analysis; PIMP, putative imprinted magnetic polymer; PIMP<sub>preferment</sub>, prefermentation treatment with putative imprinted magnetic polymer; PIMP<sub>postferment</sub>, postfermentation treatment with putative imprinted magnetic polymer; PLA, polylactic acid; PMS, potassium metabisulfite; PVPP, polyvinylpyrrolidone; SBMP, 3-sec-butyl-2-methoxy-pyrazine; TA, titratable acidity

## ■ REFERENCES

- (1) Ferreira, V.; Escudero, A.; Campo, E.; Cacho, J. In *The Chemical Foundations of Wine Aroma—A Role Game Aiming at Wine Quality, Personality and Varietal Expression, Thirteenth Australian Wine Industry Technical Conference, Adelaide, South Australia, 2007*; Blair, R. J., Williams, P. J., Pretorius, I. S., Eds.; Australian Wine Industry Technical Conference, Inc.: Adelaide, South Australia, 2007; pp 142–150.
- (2) Allen, M. S.; Lacey, M. J.; Harris, R. L. N.; Brown, W. V. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* **1991**, *42*, 109–112.
- (3) Allen, M.; Lacey, M. Methoxypyrazines of Grapes and Wines. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Publications: Washington, DC, 1998; Vol. 714, pp 31–38.
- (4) Hein, K.; Ebeler, S. E.; Heymann, H. Perception of fruity and vegetative aromas in red wine. *J. Sens. Stud.* **2009**, *24*, 441–455.

- (5) Pickering, G.; Lin, J.; Reynolds, A.; Soleas, G.; Riesen, R. The evaluation of remedial treatments for wine affected by *Harmonia axyridis*. *Int. J. Food Sci. Technol.* **2006**, *41*, 77–86.
- (6) Botezatu, A.; Kotseridis, Y.; Inglis, D.; Pickering, G. A survey of methoxypyrazines in wine. *J. Food Agric. Environ.* **2016**, *14* (1), 24–29.
- (7) Roujou de Boubée, D.; Van Leeuwen, C.; Dubourdiou, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830–4834.
- (8) Sidhu, D.; Lund, J.; Kotseridis, Y.; Saucier, C. Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 485–502.
- (9) Ryona, I.; Pan, B. S.; Sacks, G. L. Rapid measurement of 3-alkyl-2-methoxypyrazine content of winegrapes to predict levels in resultant wines. *J. Agric. Food Chem.* **2009**, *57*, 8250–7.
- (10) Hashizume, K.; Umeda, N. Methoxypyrazine content of Japanese red wines. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 802–805.
- (11) Ryona, I.; Pan, B. S.; Intrigliolo, D. S.; Lakso, A. N.; Sacks, G. L. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet Franc). *J. Agric. Food Chem.* **2008**, *56*, 10838–10846.
- (12) Kotseridis, Y. S.; Spink, M.; Brindle, I. D.; Blake, A. J.; Sears, M.; Chen, X.; Soleas, G.; Inglis, D.; Pickering, G. J. Quantitative analysis of 3-alkyl-2-methoxypyrazines in juice and wine using stable isotope labelled internal standard assay. *J. Chromatogr. A* **2008**, *1190*, 294–301.
- (13) Roujou de Boubée, D. Recherche sur la 2-méthoxy-3-isobutylpyrazine dans les raisins et les vins. Approches analytique, biologique et agronomique. Ph.D. Thesis, University of Bordeaux 2, 2000.
- (14) Ryona, I.; Reinhardt, J.; Sacks, G. L. Treatment of grape juice or must with silicone reduces 3-alkyl-2-methoxypyrazine concentrations in resulting wines without altering fermentation volatiles. *Food Res. Int.* **2012**, *47*, 70–79.
- (15) Sajilata, M.; Savitha, K.; Singhal, R.; Kanetkar, V. Scalping of flavors in packaged foods. *Compr. Rev. Food Sci. Food Saf.* **2007**, *6*, 17–35.
- (16) Botezatu, A.; Pickering, G. J. Application of plastic polymers in remediating wine with elevated alkyl-methoxypyrazine levels. *Food Addit. Contam., Part A* **2015**, *32*, 1199–1206.
- (17) Botezatu, A.; Kemp, B. S.; Pickering, G. J. Chemical and sensory evaluation of silicone and polylactic acid-based remedial treatments for elevated methoxypyrazine levels in wine. *Molecules* **2016**, *21*, 1238.
- (18) Haupt, K.; Linares, A. V.; Bompert, M.; Bui, B. T. Molecularly imprinted polymers. *Top. Curr. Chem.* **2011**, *325*, 1–28.
- (19) Ansari, S. Application of magnetic molecularly imprinted polymer as a versatile and highly selective tool in food and environmental analysis: Recent developments and trends. *TrAC, Trends Anal. Chem.* **2017**, *90*, 89–106.
- (20) Boss, P. K.; Pearce, A. D.; Zhao, Y.; Nicholson, E. L.; Dennis, E. G.; Jeffery, D. W. Potential grape-derived contributions to volatile ester concentrations in wine. *Molecules* **2015**, *20*, 7845–7873.
- (21) Chen, F. F.; Xie, X. Y.; Shi, Y. P. Preparation of magnetic molecularly imprinted polymer for selective recognition of resveratrol in wine. *J. Chromatogr. A* **2013**, *1300*, 112–8.
- (22) Zhang, H.-F.; Shi, Y.-P. Magnetic retrieval of chitosan: Extraction of bioactive constituents from green tea beverage samples. *Analyst* **2012**, *137*, 910–916.
- (23) Zeng, H.; Wang, Y.; Nie, C.; Kong, J.; Liu, X. Preparation of magnetic molecularly imprinted polymers for separating rutin from Chinese medicinal plants. *Analyst* **2012**, *137*, 2503–2512.
- (24) Lu, F.; Sun, M.; Fan, L.; Qiu, H.; Li, X.; Luo, C. Flow injection chemiluminescence sensor based on core-shell magnetic molecularly imprinted nanoparticles for determination of chrysoidine in food samples. *Sens. Actuators, B* **2012**, *173*, 591–598.
- (25) Belbruno, J. J.; Kelm, M. Molecularly Imprinted Polymers for Wine Extraction. U.S. Patent 2014/0242237 A1, 28 August 2014.
- (26) Euterpio, M. A.; Pagano, I.; Piccinelli, A. L.; Rastrelli, L.; Crescenzi, C. Development and validation of a method for the determination of (*E*)-resveratrol and related phenolic compounds in beverages using molecularly imprinted solid phase extraction. *J. Agric. Food Chem.* **2013**, *61*, 1640–5.
- (27) Li, S.; Bindon, K.; Bastian, S. E.; Jiranek, V.; Wilkinson, K. L. Use of winemaking supplements to modify the composition and sensory properties of Shiraz wine. *J. Agric. Food Chem.* **2017**, *65*, 1353–1364.
- (28) Mercurio, M. D.; Damberg, R. G.; Herderich, M. J.; Smith, P. A. High throughput analysis of red wine and grape phenolics adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. *J. Agric. Food Chem.* **2007**, *55*, 4651–4657.
- (29) Chapman, D. M.; Thorngate, J. H.; Matthews, M. A.; Guinard, J.-X.; Ebeler, S. E. Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid phase micro-extraction gas chromatography/mass spectrometry method. *J. Agric. Food Chem.* **2004**, *52*, 5431–5435.
- (30) Wang, J.; Gambetta, J. M.; Jeffery, D. W. Comprehensive study of volatile compounds in two Australian rosé wines: Aroma extract dilution analysis (AEDA) of extracts prepared using solvent-assisted flavor evaporation (SAFE) or headspace solid-phase extraction (HS-SPE). *J. Agric. Food Chem.* **2016**, *64*, 3838–3848.
- (31) Chen, L.; Capone, D. L.; Jeffery, D. W. Chiral analysis of 3-sulfanylhexan-1-ol and 3-sulfanylhexyl acetate in wine by high-performance liquid chromatography–tandem mass spectrometry. *Anal. Chim. Acta* **2018**, *998*, 83–92.
- (32) Capone, D. L.; Ristic, R.; Pardon, K. H.; Jeffery, D. W. Simple quantitative determination of potent thiols at ultratrace levels in wine by derivatization and high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis. *Anal. Chem.* **2015**, *87*, 1226–1231.
- (33) Sims, C. A.; Eastridge, J. S.; Bates, R. P. Changes in phenols, color, and sensory characteristics of muscadine wines by pre- and post-fermentation additions of PVPP, casein, and gelatin. *Am. J. Enol. Vitic.* **1995**, *46*, 155–158.
- (34) Kotseridis, Y.; Belouqui, A. A.; Bertrand, A.; Doazan, J. An analytical method for studying the volatile compounds of Merlot noir clone wines. *Am. J. Enol. Vitic.* **1998**, *49*, 44–48.
- (35) Licciardello, F.; Del Nobile, M. A.; Spagna, G.; Muratore, G. Scalping of ethylacetate and linalool from a model wine into plastic films. *LWT-Food Sci. Technol.* **2009**, *42*, 1065–1069.
- (36) Liang, C.; Jeffery, D. W.; Taylor, D. K. Preparation of magnetic polymers for the elimination of 3-isobutyl-2-methoxypyrazine from wine. *Molecules* **2018**, *23*, 1140.
- (37) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.
- (38) Ferreira, V.; López, R.; Cacho, J. F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667.
- (39) Swiegers, J.; Bartowsky, E.; Henschke, P.; Pretorius, I. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139–173.
- (40) Bakker, J.; Clarke, R. J. Volatile Components. In *Wine Flavour Chemistry*, 2nd ed.; Bakker, J., Clarke, R. J., Eds.; Wiley-Blackwell: Oxford, UK, 2012; pp 155–238.
- (41) Tominaga, T.; Niclass, Y.; Frerot, E.; Dubourdiou, D. Stereoisomeric distribution of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in dry and sweet white wines made from *Vitis vinifera* (Var. Sauvignon Blanc and Semillon). *J. Agric. Food Chem.* **2006**, *54*, 7251–7255.
- (42) Waterhouse, A. L.; Sacks, G. L.; Jeffery, D. W. Esters. In *Understanding Wine Chemistry*; John Wiley & Sons: Chichester, UK, 2016; pp 57–67.
- (43) Subileau, M.; Schneider, R. M.; Salmon, J.-M.; Degryse, E. New insights on 3-mercaptohexanol (3MH) biogenesis in Sauvignon Blanc

wines: Cys-3MH and (*E*)-hexen-2-al are not the major precursors. *J. Agric. Food Chem.* **2008**, *56*, 9230–9235.

(44) Vela, E.; Hernández-Orte, P.; Castro, E.; Ferreira, V.; Lopez, R. Effect of bentonite fining on polyfunctional mercaptans and other volatile compounds in Sauvignon blanc wines. *Am. J. Enol. Vitic.* **2017**, *68*, 30–38.

(45) Brody, A. L. Flavor scalping: Quality loss due to packaging. *Food Technol.* **2002**, *56*, 124–125.

(46) Styger, G.; Prior, B.; Bauer, F. Wine flavor and aroma. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 1145–1159.

(47) King, E. S.; Osidacz, P.; Curtin, C.; Bastian, S. E. P.; Francis, I. L. Assessing desirable levels of sensory properties in Sauvignon Blanc wines - consumer preferences and contribution of key aroma compounds. *Aust. J. Grape Wine Res.* **2011**, *17*, 169–180.

(48) Escudero, A.; Campo, E.; Fariña, L.; Cacho, J.; Ferreira, V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem.* **2007**, *55*, 4501–4510.

(49) Sáenz-Navajas, M.-P.; Campo, E.; Culleré, L.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V. Effects of the nonvolatile matrix on the aroma perception of wine. *J. Agric. Food Chem.* **2010**, *58*, 5574–5585.

## SUPPORTING INFORMATION FOR

### **Chemical and Sensory Evaluation of Magnetic Polymers as a Remedial Treatment for Elevated Concentrations of 3-Isobutyl-2-methoxypyrazine in Cabernet Sauvignon Grape Must and Wine**

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**Table S1. Attributes, Descriptions and Reference Standards Used in the Olfactory Sensory Descriptive Analysis**

attribute	description	reference standards <sup>a</sup>
overall aroma intensity	–	–
berry	raspberry, blackcurrant, blackberry, blueberry	2 fresh raspberries, 3 fresh blackberries, 4 fresh blueberries, all halved
jammy	cooked berry, apricot jam	1 tsp black cherry jam (St. Dalfour), 1 tsp apricot jam (Bonne Maman)
tropical	passionfruit, pineapple	3 pieces fresh pineapple (2 cm cube), ¼ fresh passionfruit
floral	violet, jasmine, muscat	3 drops <sup>b</sup> rose water, 0.17 g fresh jasmine flower
capsicum	fresh capsicum	1 slice of fresh cut green capsicum
fresh green	green apple, grass, green vegetable, leaf, stalk, mint, pine tree	2 g green grass, 1.5 g green apple peel, ¼ fresh cut green bean, tiny piece of fresh mint leaf
herbaceous	dried herbs, dried leaf, dried grass	2 g dried grass, ¼ tsp mixture dried herbs
cooked vegetables	cooked peas, beans, potato	2 boiled green beans
rubbery	rubber	2 rubber bands (3.0 mm, Belgrave)
earthy	wet cardboard, mushroom, dusty	¼ fresh cut mushroom, 1 tsp dried soil
sweet spice	cloves, liquorice	½ tsp mix baking spice
solvent	alcohols, medicine	3 drops ethanol, 1 drop acetone, 1 drop ethyl acetate

<sup>a</sup> All aroma standards were prepared in 30 mL of base wine (Cabernet Sauvignon 2016, Yalumba Wine Company). <sup>b</sup> Each drop was equal to approximately 0.05 g.

**Table S2. Basic Composition of Cabernet Sauvignon Wines from the Different Treatments<sup>a</sup>**

	control	PLA	PIMP <sub>preferment</sub>	NIMP <sub>preferment</sub>	PIMP <sub>postferment</sub>	NIMP <sub>postferment</sub>
ethanol (% v/v)	12.3 ± 0.2	12.2 ± 0.2	12.1 ± 0.0	12.1 ± 0.1	12.2 ± 0.1	12.1 ± 0.0
pH	3.50 ± 0.01	3.50 ± 0.01	3.56 ± 0.05	3.55 ± 0.03	3.56 ± 0.02	3.53 ± 0.03
TA (g/L)	7.6 ± 0.1	7.5 ± 0.0	7.5 ± 0.0	7.3 ± 0.1	7.5 ± 0.0	7.4 ± 0.1

<sup>a</sup>Data for each treatment are presented as the mean ± SD (standard deviation, n=3). There were no significant differences ( $p > 0.05$ ) among the treatments according to one-way ANOVA.

**Table S3. Color Parameters of Cabernet Sauvignon Wines from the Different Treatments<sup>a</sup>**

	control	PLA	PIMP <sub>preferment</sub>	NIMP <sub>preferment</sub>	PIMP <sub>postferment</sub>	NIMP <sub>postferment</sub>
total anthocyanins (mg/L)	323 ± 9 a	296 ± 24 abc	310 ± 6 ab	298 ± 9 abc	260 ± 16 c	270 ± 14 bc
color density ( $A_{520} + A_{420}$ )	7.6 ± 0.2 ab	8.0 ± 0.6 a	6.8 ± 0.2 bc	6.4 ± 0.3 c	5.2 ± 0.2 d	5.3 ± 0.1 d
hue ( $A_{420}/A_{520}$ )	0.60 ± 0.01 ab	0.60 ± 0.01 a	0.58 ± 0.01 abc	0.58 ± 0.001 abc	0.57 ± 0.01 bc	0.56 ± 0.01 c
SO <sub>2</sub> -resistant pigments (au)	2.0 ± 0.1 a	2.0 ± 0.1 a	1.6 ± 0.1 b	1.6 ± 0.1 b	1.2 ± 0.1 c	1.2 ± 0.0 c
total phenolics (au)	29.8 ± 1.0 a	27.6 ± 2.8 ab	25.5 ± 0.4 b	25.3 ± 0.5 b	20.0 ± 0.8 c	20.3 ± 0.5 c

<sup>a</sup>Data for each treatment are presented as the mean ± SD (n=3) with different letters across a row indicating significantly different ( $p < 0.05$ ) means according to one-way ANOVA with Tukey (HSD) pairwise comparison.

**Table S4. Mean Concentrations ( $\mu\text{g/L}$ ) of Volatile Compounds in Cabernet Sauvignon Wines from the Different Treatments<sup>a</sup>**

	control	PLA	PIMP <sub>preferment</sub>	NIMP <sub>preferment</sub>	PIMP <sub>postferment</sub>	NIMP <sub>postferment</sub>
<i>ethyl esters</i>						
ethyl butanoate	32 ± 3 abc	31 ± 0 bc	39 ± 1 a	37 ± 1 ab	27 ± 4 c	28 ± 4 c
ethyl 2-methylbutanoate	2.9 ± 0.3	2.9 ± 0.5	2.8 ± 0.4	2.4 ± 0.2	2.2 ± 0.1	2.6 ± 0.4
ethyl 3-methylbutanoate	3.4 ± 0.4	3.3 ± 0.6	2.4 ± 2.0	3.0 ± 0.3	2.6 ± 0.1	3.1 ± 0.5
ethyl hexanoate	73 ± 8 b	73 ± 4 b	87 ± 0 a	81 ± 2 ab	49 ± 1 c	47 ± 3 c
ethyl octanoate	52 ± 8 b	50 ± 6 b	73 ± 2 a	59 ± 2 b	22 ± 2 c	17 ± 1 c
ethyl nonanoate	0.05 ± 0.00 a	0.04 ± 0.00 a	0.02 ± 0.00 bc	0.02 ± 0.00 b	0.01 ± 0.00 c	0.02 ± 0.01 bc
ethyl decanoate	2.9 ± 0.3 a	2.7 ± 0.4 ab	2.6 ± 0.1 ab	2.1 ± 0.2 b	0.6 ± 0.1 c	0.4 ± 0.1 c
ethyl dodecanoate	0.5 ± 0.1 ab	0.5 ± 0.0 ab	0.6 ± 0.1 ab	0.4 ± 0.0 ab	0.7 ± 0.3 a	0.2 ± 0.0 b
ethyl furoate	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
3-methylbutyl octanoate	0.6 ± 0.0 a	0.5 ± 0.1 ab	0.4 ± 0.0 b	0.4 ± 0.0 b	0.2 ± 0.0 c	0.1 ± 0.0 c
diethyl succinate	134 ± 13 a	128 ± 17 a	81 ± 3 c	91 ± 10 bc	123 ± 1 ab	114 ± 13 ab
<i>acetate esters</i>						
ethyl acetate	9.7 ± 0.4 ab	9.4 ± 0.2 ab	10.2 ± 0.3 a	9.9 ± 0.1 a	8.7 ± 0.1 b	8.8 ± 0.5 b
2-methylpropyl acetate	0.27 ± 0.02 ab	0.26 ± 0.02 b	0.50 ± 0.19 a	0.35 ± 0.02 ab	0.20 ± 0.00 b	0.23 ± 0.02 b
3-methylbutyl acetate	56 ± 11 ab	52 ± 2 bc	70 ± 10 ab	72 ± 3 a	36 ± 2 c	36 ± 1 c
hexyl acetate	8 ± 1 b	8 ± 0 b	20 ± 2 a	19 ± 1 a	3 ± 0 c	3 ± 0 c

Table S4. cont.

	8 ± 1 b	8 ± 0 b	12 ± 0 a	11 ± 1 a	4 ± 0 c	3 ± 0 c
2-phenylethyl acetate						
<i>alcohols</i>						
2-methyl-1-propanol	949 ± 256	915 ± 143	1030 ± 223	838 ± 133	849 ± 41	942 ± 107
1-butanol	21 ± 2	22 ± 1	21 ± 2	18 ± 2	19 ± 1	18 ± 1
3-methyl-1-butanol	26327 ± 821 a	26140 ± 199 a	25559 ± 441 ab	25669 ± 361 ab	24650 ± 87 bc	24177 ± 228 c
3-methyl-1-pentanol	7.6 ± 0.1 a	7.3 ± 0.1 ab	6.8 ± 0.3 bc	6.7 ± 0.4 bc	6.5 ± 0.3 c	6.3 ± 0.2 c
1-hexanol	376 ± 26 a	377 ± 2 a	337 ± 5 bc	355 ± 3 ab	307 ± 2 cd	280 ± 10 d
(Z)-3-hexen-1-ol	2.8 ± 0.2 a	2.6 ± 0.0 ab	2.3 ± 0.0 bc	2.5 ± 0.1 abc	2.4 ± 0.0 bc	2.2 ± 0.1 c
2-ethyl-1-hexanol	26 ± 1	26 ± 2	26 ± 2	26 ± 1	24 ± 2	22 ± 2
1-octanol	1.0 ± 0.1 a	0.8 ± 0.1 b	0.8 ± 0.0 b	0.8 ± 0.0 b	0.4 ± 0.0 c	0.40 ± 0.0 c
methionol	48 ± 5	37 ± 9	42 ± 2	38 ± 9	34 ± 2	34 ± 9
2-phenylethanol	4759 ± 169 a	4846 ± 68 a	4772 ± 77 a	4631 ± 21 a	4344 ± 74 b	4246 ± 10 b
<i>isoprenoids</i>						
limonene	3.6 ± 0.3 a	3.4 ± 0.1 a	2.9 ± 0.1 b	3.1 ± 0.1 ab	1.9 ± 0.1 c	2.1 ± 0.1 c
linalool	0.4 ± 0.0 a	0.4 ± 0.0 a	0.3 ± 0.0 b	0.3 ± 0.0 b	0.2 ± 0.0 c	0.1 ± 0.0 c
α-terpineol	0.68 ± 0.05 a	0.64 ± 0.06 a	0.56 ± 0.04 abc	0.57 ± 0.05 ab	0.47 ± 0.03 bc	0.44 ± 0.04 c
β-citronellol	0.62 ± 0.04 a	0.62 ± 0.03 a	0.22 ± 0.00 b	0.20 ± 0.04 bc	0.14 ± 0.02 bc	0.11 ± 0.00 c
β-damascenone	0.57 ± 0.06 a	0.58 ± 0.04 a	0.40 ± 0.04 b	0.44 ± 0.02 b	0.29 ± 0.02 c	0.33 ± 0.04 bc
eucalyptol	0.200 ± 0.098 a	0.140 ± 0.034 ab	0.036 ± 0.004 c	0.039 ± 0.008 bc	0.043 ± 0.007 bc	0.040 ± 0.008 bc

Table S4. cont.

<i>carbonyls</i>	
nonanal	1.1 ± 0.2 a
<i>acids</i>	
hexanoic acid	203 ± 13 a
octanoic acid	187 ± 1 ab
nonanoic acid	4 ± 0 c
decanoic acid	54 ± 1 a

	1.0 ± 0.2 ab	0.6 ± 0.1 b	0.8 ± 0.1 ab	0.8 ± 0.1 ab	0.6 ± 0.1 b
hexanoic acid	202 ± 19 a	191 ± 1 a	210 ± 12 a	124 ± 7 b	114 ± 3 b
octanoic acid	185 ± 12 b	214 ± 3 a	216 ± 13 a	75 ± 11 c	69 ± 2 c
nonanoic acid	4 ± 0 c	6 ± 0 b	4 ± 0 c	7 ± 1 a	5 ± 0 bc
decanoic acid	52 ± 5 a	48 ± 2 a	49 ± 2 a	24 ± 12 b	12 ± 2 b

<sup>a</sup>Data for each treatment are presented as the mean ± SD (n=3) with different letters across a row indicating significantly different ( $p < 0.05$ ) means according to one-way ANOVA with Tukey (HSD) pairwise comparison.

## CHAPTER 4

### Preface

Given the similarities between magnetic and non-magnetic polymers in terms of sorptive properties, subsequent trials were focused on improving polymer selectivity for IBMP by preparing non-magnetic counterparts. Imprinted polymers were prepared with various combinations of templates, functional monomers, ratios with cross-linker and porogen solvents, along with non-imprinted counterparts as controls. However, higher affinity toward IBMP of imprinted polymers in respective porogen solvents was not observed compared to that of their non-imprinted controls. The attempts to prepare imprinted polymer specific for IBMP were not achieved.

Apart from preparing polymers to target a specific compound such as IBMP, various monomeric components could be chosen to improve the retention properties of polymers toward other compounds or groups of compounds in wine. As such, microwave and thermal syntheses of polymers were trialled with different functional monomers, ratios with cross-linker, and porogen solvents to investigate their sorptive properties in wine. This work also included assessment of commercial sorbents and discussion of sorbent properties in relation to their physicochemical characters.

## CHAPTER 4

### Extraction Properties of New Polymeric Sorbents Applied to Wine

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Contribution to the Paper	Conceived and designed the experiment. Synthesised and analysed the polymers. Carried out physical characterisation of polymers and sample preparation for GC-MS analysis of volatile compounds. Analysed and interpreted the data. Completed first draft of the manuscript and revised based on co-author input.			
Overall percentage (%)	80%			
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## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Paul K. Boss			
Contribution to the Paper	Helped with GC-MS analysis of volatile compounds. Critically evaluated and revised the manuscript.			
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Name of Co-Author	David W. Jeffery			
Contribution to the Paper	Contributed to the research idea. Supervised the project. Discussed the results and provided critical feedback. Critically evaluated and revised the manuscript. Acted as corresponding author.			
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# Extraction Properties of New Polymeric Sorbents Applied to Wine

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## S Supporting Information

**ABSTRACT:** Polymeric sorbents are frequently used in wine, either as solid phase extraction materials for isolation of analytes or as sorptive materials for removal of undesirable compounds (amelioration). Six new polymeric sorbents were produced thermally or in a microwave from various ratios of methacrylic acid, acrylic acid, and 4-vinylbenzoic acid as hydrophilic monomers, together with ethylene glycol dimethacrylate as cross-linker, using different porogen solvents. The relationship between physicochemical properties (pore size, surface area, and polarity) of the sorbents and their sorption properties for compounds in wine was investigated and compared to four commercially available sorbents. With some similarities to their commercial counterparts depending on hydrophobic and hydrophilic characteristics, the six new sorbents showed specificity toward different groups of compounds (e.g., volatiles and phenolics) and could be applied for targeted purposes. The results provide insight into the selection and utilization of new polymeric materials for extraction of components from wine.

**KEYWORDS:** gas adsorption isotherms, pore size distribution, hydrophobic, hydrophilic, GC–MS

## INTRODUCTION

In parallel with advances in analytical chemistry, the focus in understanding wine chemistry has shifted from determining major components such as ethanol and organic acids and quantifying spoilage compounds such as acetic acid to investigating trace compounds that may positively or negatively influence wine quality.<sup>1</sup> This has necessitated new approaches not only for the analysis of wine constituents but also for their prior isolation and enrichment. Volatile compounds that contribute aroma and flavor to wines tend to be small (low in molecular weight) and nonpolar and often require specialized analytical approaches.<sup>2</sup> For compounds of low concentrations or prone to interferences when being analyzed, solid-phase extraction (SPE) has often been adopted as an important sample preparation technique for either matrix simplification or trace enrichment.<sup>3</sup>

The most common material for SPE is silica-based sorbent, e.g., silica functionalized with octadecyl (C<sub>18</sub>) carbon chains, with an interaction mechanism that mainly relies on hydrophobic interactions (van der Waals forces) between the sorbent and analytes. However, reversed-phase silica sorbents have several disadvantages, such as low retention of polar compounds, instability at extreme pH, and limited ability for reuse.<sup>4</sup> In contrast, polymeric sorbents have the advantages of chemical stability and a broad range of physicochemical characteristics. The morphology (e.g., specific surface area, pore size distribution, particle size, etc.) and surface chemistry (promoting interactions between sorbents and the analytes), which directly affect the extraction and retention of analytes, could be designed and modified when developing a new polymeric sorbent.<sup>5</sup> The classic polymeric sorbent is polystyrene cross-linked with divinylbenzene (PS-DVB), which is also a hydrophobic phase that interacts through van der Waals forces but in addition exhibits  $\pi$ – $\pi$  interactions of

the aromatic rings in the sorbent structure. However, due to the low retention of polar compounds and poor wettability when applied to aqueous matrixes, introducing some polar functionality into sorbents has become another focus when developing new sorbents.<sup>4</sup>

Hydrophilic sorbents can be made by copolymerizing hydrophilic monomers or functionalizing polymers by chemical modification.<sup>5</sup> Several commercial or in-house synthesized hydrophilic polymers have been used to analyze minor and trace compounds from aqueous samples such as water, blood serum, urine, and alcoholic beverage, either as an SPE sorbent for sample preparation or as a stationary phase for liquid chromatography columns.<sup>2,6–10</sup> One commonly known hydrophilic SPE sorbent is Oasis HLB (Waters), a copolymer of *N*-vinylpyrrolidone and divinylbenzene (PVP-DVB), which is a hydrophilic–lipophilic balanced copolymer that has been applied to pesticides, metabolites, pharmaceuticals, and pollutants analysis.<sup>11</sup> As another example, poly(methacrylic acid-ethylene glycol dimethacrylate) (MAA-EGDMA) is one of the most widely used monolithic materials comprising MAA as hydrophilic monomer and EGDMA as bifunctional cross-linker. It can be used with aqueous or organic solvent matrixes within a wide range of pH values, and the carboxylic acid group offers the possibility of not only hydrophilic interactions but also weak electrostatic interactions with analytes carrying either positive or negative charges.<sup>7</sup> With proper mobile-phase conditioning, the sorbents are capable of being cycled. The poly(MAA-EGDMA) has been applied as a new coating material for solid-phase microextraction (SPME) fibers for the

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Table 1. Compositions of the Six Polymeric Sorbents

sorbent	monomer	structural unit	co-polymer	solvent	method
MAA <sub>(1:5/A/M)</sub>			MAA/EGDMA (1:5)	ACN	microwave
MAA <sub>(1:5/D/M)</sub>	MAA		MAA/EGDMA (1:5)	DCM	microwave
MAA <sub>(1:5/A/T)</sub>			MAA/EGDMA (1:5)	ACN	thermal
MAA <sub>(2:5/A/T)</sub>			MAA/EGDMA (2:5)	ACN	thermal
AA <sub>(1:5/D/M)</sub>	AA		AA/EGDMA (1:5)	DCM	microwave
4-VBA <sub>(1:5/D/M)</sub>	4-VBA		4-VBA/EGDMA (1:5)	DCM	microwave

Table 2. Properties of Commercial Sorbents

sorbent	monomer(s)	structural units	particle size (μm)	surface area (m <sup>2</sup> /g)	mean pore size (nm)
C <sub>18</sub>	Trifunctional octadecyl/silica based		40	500	6
Oasis HLB	PVP-DVB		30	800	8
Strata SDB-L	Styrene-DVB		100	500	26
PVPP	PVP		N/A	N/A	N/A

determination of chlorophenols in water coupled with gas chromatography (GC).<sup>12</sup> To achieve selective performance and better extraction, molecularly imprinted polymers have also been developed as tailor-made SPE sorbents and applied to analytes in wine.<sup>13,14</sup>

Apart from determination of volatile compounds, analysis of phenolics is another focus in understanding wine chemistry, as polyphenols are important wine components that contribute to color, texture, stability, and matrix effects on both aroma and flavor. Hydrophilic polymeric sorbents such as polyamide have been used as a fractionating medium for phenolic analysis.<sup>15</sup> In addition, due to the sorptive characters of polymeric sorbents, they have been adopted and developed to remove undesirable compounds from wines, albeit relatively nonselectively. Polyvinylpyrrolidone (PVPP) has been routinely used as a winemaking aid, known as a fining agent, to adjust color and decrease polyphenol content.<sup>16–18</sup> Other polymers have been investigated to remove off-flavor compounds from wines, such as application of plastic polymers in remediating wine with elevated methoxypyrazines.<sup>19</sup>

There are almost unlimited combinations of monomers, cross-linkers, and porogen solvents to improve the retention properties of a sorbent toward a certain compound or groups of compounds.<sup>8</sup> Wine as a hydroalcoholic beverage is a challenging matrix for sorptive materials due to its low pH and the retaining effect of ethanol on nonpolar compounds in the aqueous environment.<sup>20</sup> Carboxylic acids and EGDMA are primary interests because they are among the most commonly used components for selective imprinted polymer development, and their hydrophilic properties have also been adopted to prepare monolithic stationary phases. Thus, with the aim of

introducing some specificity and provide better wettability, six polymeric sorbents were synthesized, either thermally or microwave-assisted, by copolymerizing different carboxylic acid monomers with EGDMA as cross-linker at different ratios in various solvents. Microwave-assisted polymerization was adopted for closed reaction vessels that broaden the choice of porogen solvents with low boiling point, and the fast and homogeneous heating from microwave irradiation leads to less side reactions, cleaner products, and higher yields.<sup>21</sup> The extraction properties of the six sorbents toward wine components (volatile and phenolic compounds) was investigated, and the relationship between the physicochemical properties of sorbents and their sorption properties in a wine matrix was examined, along with that of four commercial sorbents (C<sub>18</sub>, Oasis HLB, Strata SDB-L, and PVPP) to evaluate their performance. This preliminary investigation provides insight into the selection and utilization of new polymeric materials for extraction of components from wine.

## ■ MATERIALS AND METHODS

**Chemicals.** MAA, 99%, acrylic acid (AA, 99%), EGDMA, 98%, 4-vinylbenzoic acid (4-VBA, 97%), and 2,2'-azobis(isobutyronitrile) (AIBN) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). MAA, AA, and EGDMA were purified by distillation under vacuum. AIBN was recrystallized from ethanol. HPLC-grade acetonitrile (ACN) and dichloromethane (DCM) were purchased from Merck (Bayswater, VIC, Australia) and distilled prior to use. Analytical reagent (AR) grade methanol and acetic acid were purchased from Chem-Supply (Adelaide, SA, Australia). Water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). The following SPE cartridges were obtained: Bond Elut C<sub>18</sub> (500 mg/6 mL, Agilent Technologies, Mulgrave, VIC, Australia); Oasis HLB (60 mg/3 mL, Waters, Rydalmere, NSW,

Table 3. BET Surface Areas of the Six Polymeric Sorbents (Refer to Table 1 for Sorbent Details)

	MAA <sub>(1:5/A/M)</sub>	MAA <sub>(1:5/D/M)</sub>	MAA <sub>(1:5/A/T)</sub>	MAA <sub>(2:5/A/T)</sub>	AA <sub>(1:5/D/M)</sub>	4-VBA <sub>(1:5/D/M)</sub>
mean surface area (m <sup>2</sup> /g)	112 ± 1	143 ± 4	234 ± 6	126 ± 4	139 ± 4	30 ± 0.4

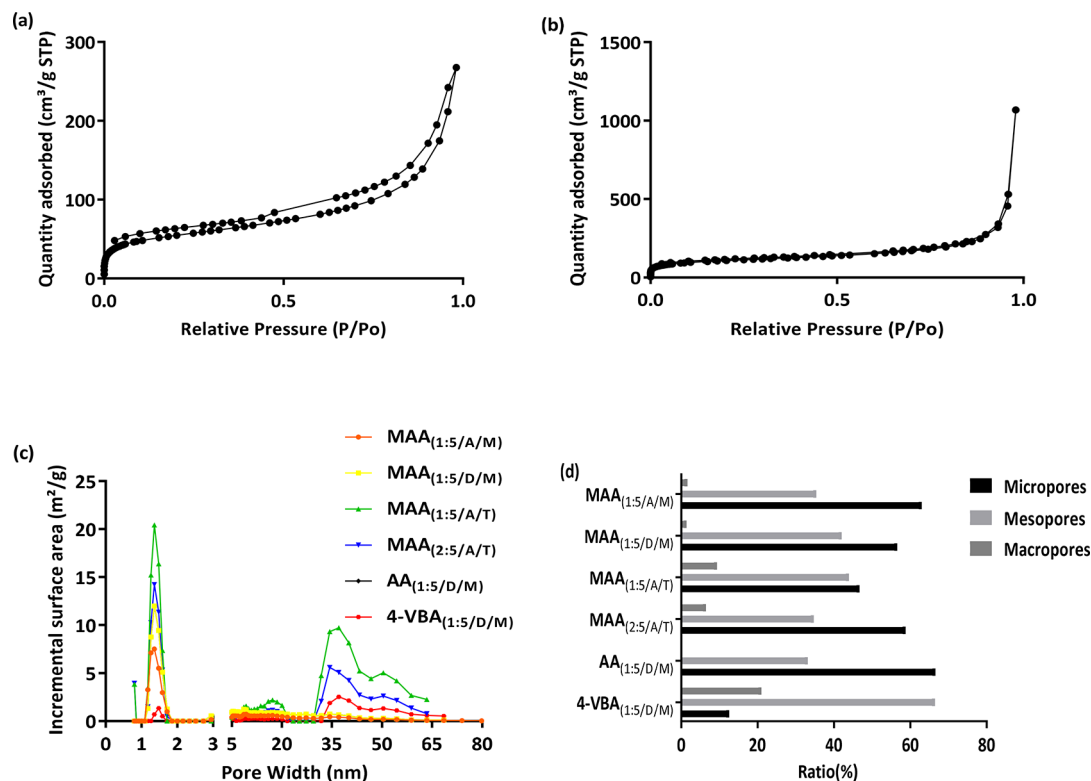


Figure 1. Representative gas adsorption isotherms of (a) MAA<sub>(1:5/A/M)</sub> and (b) MAA<sub>(1:5/A/T)</sub>, (c) pore size distributions, and (d) relative pore size proportions of the six sorbents (refer to Table 1 for sorbent details).

Australia); Strata SDB-L (200 mg/3 mL, Phenomenex, Torrance, CA, United States). PVPP was obtained from WINEQUIP (Newton, SA, Australia).

**Preparation of Polymeric Sorbents.** As listed in Table 1, polymeric sorbents with various monomers and porogen solvents were synthesized thermally or in a microwave synthesizer (CEM microwave synthesizer, Discover S, DKSH, Melbourne, VIC, Australia). Monomers (0.4 or 0.8 mmol) and EGDMA (2 mmol) as copolymer were added to the respective porogen solvent (1.2 mL). The mixture was stirred at ambient temperature for 30 min before addition of initiator AIBN (10 mg). After purging with nitrogen for 15 min, the mixture was sealed and submerged in a 60 °C oil bath for 24 h for conventional thermal synthesis or transferred to a microwave synthesizer for microwave synthesis. Thermal synthesis was performed in a round-bottom flask, whereas microwave synthesis was performed in a 10 mL sealed reaction vessel (CEM, DKSH). The microwave reaction was performed at 60 °C for 1 h with the synthesizer power in dynamic mode and pressure limit of 150 psi. The obtained bulk polymers were separately crushed using a mortar and pestle, and ground polymer was passed through a 150 μm sieve (Retsch test sieve, 200 mm DIA × 50 mm, 150 μm, VWR, Tingalpa, QLD, Australia) and collected. The sieved particles were rinsed with methanol:acetic acid (9:1 v/v) and then methanol three times each by immersing in the respective solvent and shaking at 120 rpm (Ratek orbital mixer incubator, Adelab, Adelaide, SA, Australia) at ambient temperature for 1 h. The washed polymers were dried under high vacuum before analysis. All polymers were prepared in duplicate.

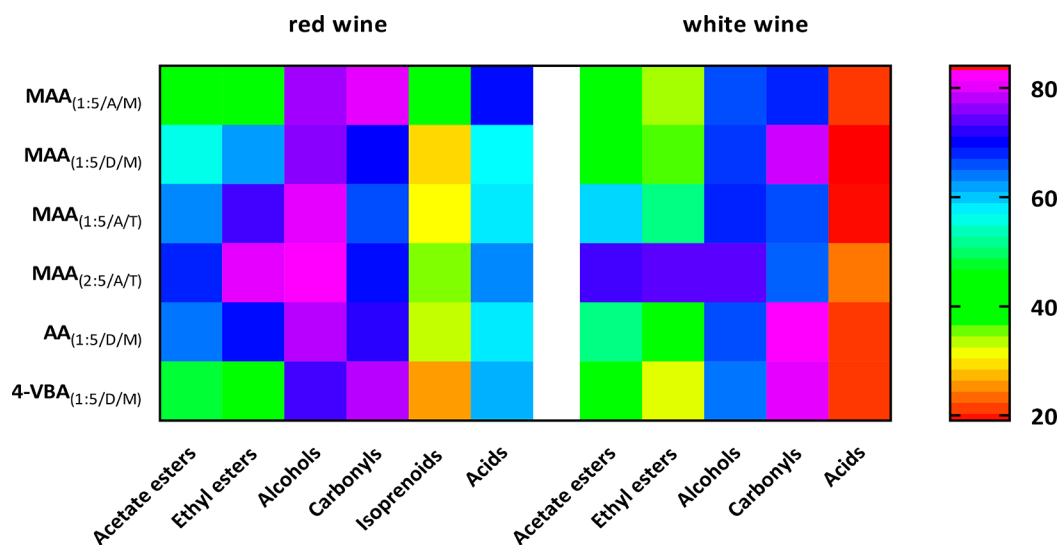
Commercial C<sub>18</sub> and copolymeric (Oasis HLB, Strata SDB-L) sorbents were obtained from SPE cartridges and PVPP was adopted directly. Properties of commercial sorbents as provided by the supplier are listed in Table 2.

**Gas Adsorption Measurements.** Gas adsorption measurements were performed on an ASAP 2020 surface area and pore size analyzer. The sorbent samples were degassed overnight in ASAP 2020 analysis tubes at ambient temperature before analysis. Nitrogen (77 K) adsorption analysis was performed in the relative pressure (P/P<sub>0</sub>) range of 10<sup>-6</sup> to 1. UHP-grade (99.999%) N<sub>2</sub> was used for all measurements.

**Sorption Properties in Wine Matrix.** Each polymeric sorbent (10 mg) was separately added to 1 mL each of white wine (Chardonnay 2015, alcohol 10.9% v/v, pH 3.26) and red wine (Cabernet Sauvignon 2014, alcohol 12.5% v/v, pH 3.36). After shaking at ambient temperature for 2 h at 120 rpm, the sorbents were separated by centrifugation (8200g, 20 °C, 10 min) (Eppendorf, 5415D, Adelab), and the supernatants were transferred for color measures and volatile compound analysis. Control wines were prepared the same as the treatments but without sorbent addition. All measurements were conducted in triplicate.

**Wine Color and Phenolic Measures.** Color parameters for red wine treatments were measured using a modified Somers assay<sup>22</sup> and for white wine treatments by determining absorbance values (300 μL in Greiner UV star 96-well plates) at 280 and 420 nm.<sup>23,24</sup>

**Analysis of Volatile Compounds.** Headspace solid-phase microextraction (HS-SPME)-gas chromatography-mass spectrometry (GC-MS) was used to analyze the volatile components of the wines treated with different sorbents. Aliquots of the wines (0.5 mL) were analyzed at 1 in 10 dilution with water to a final volume of 5 mL in 20 mL headspace vials containing 2 g NaCl added prior to sample addition. Samples were spiked with four internal standards prior to GC-MS analysis: d<sub>13</sub>-hexanol (920 μg/L, C/D/N Isotopes, Pointe-Claire, Canada), d<sub>11</sub>-hexanoic acid (930 μg/L, C/D/N Isotopes), d<sub>16</sub>-



**Figure 2.** Relative amounts of grouped volatile compounds remaining in wines compared to the control (%) after treatment with the six sorbents (refer to Table 1 for sorbent details).

octanal (82.1  $\mu\text{g/L}$ , C/D/N Isotopes), and  $d_5$ -ethyl nonanoate (6.12  $\mu\text{g/L}$ , synthesized as previous reported<sup>25</sup>).

Samples were analyzed with an Agilent 7890A GC coupled to a 5975C MSD and fitted with a Gerstel MPS2 autosampler. A DVB/CAR/PDMS fiber (2 cm, 23-gauge, 50/30  $\mu\text{m}$ , Supelco, Bellefonte, PA) was used for extraction. Sampling and instrumental analysis followed the procedure described by Dennis et al.<sup>26</sup> Data were collected with Agilent ChemStation software (E.02.02.1431) in scan mode (range,  $m/z$  35–350; scan rate, 4.45 scans/s). The identity of compounds was determined by spectral library matches (NIST-05a) and by comparing spectra and linear retention indices (LRI) with those of authentic compounds. The LRI were calculated relative to the retention of a series of *n*-alkanes ( $\text{C}_8$ – $\text{C}_{26}$ , Sigma-Aldrich). Compounds were quantified based on equivalence to internal standards used for calibration of each analyte.<sup>27</sup>

**Data Analysis.** Data are presented as mean values with standard deviation from triplicate determinations using Microsoft Excel 2013. One-way analysis of variance (ANOVA) was conducted to determine significant differences between samples with Fisher's LSD multiple comparison posthoc test at  $p \leq 0.05$  using XLSTAT (version 2018.2, Addinsoft, Paris, France). Pearson correlation analysis was conducted to examine the strength and direction of the linear relationship between sorption and lipophilicity with 95% confidence intervals using XLSTAT. The means of significantly different grouped volatile compounds and color parameters relative to control were subjected to Pearson's type principal component analysis (PCA) using XLSTAT. Graphs were prepared using GraphPad Prism 7.02 (La Jolla, CA, United States).

## RESULTS AND DISCUSSION

**Gas Adsorption Properties of the Sorbents.** Nitrogen adsorption isotherms were performed on the six polymeric sorbents in the dry state to assess their permanent porosity. Although porous properties in the dry state may vary from polymer hydrodynamic status in solution, the parameters obtained could still serve as a good reference.<sup>28</sup> The calculated Brunauer–Emmett–Teller (BET) surface areas are shown in Table 3. According to IUPAC recommendations for classification of adsorption isotherms,<sup>29</sup> the data were best described as type II in shape for microwave synthesized and thermal synthesized MAA sorbents, respectively (Figures 1a and b). The type II isotherm represents monolayer-multilayer adsorption and is normally obtained with a nonporous or macroporous (pore width  $>50$  nm) sorbent, with macro-

porosity applying in this case, as evident from existence of pores that were wider than 50 nm (Figures 1c and d). Multilayer adsorption has also been reported for PVPP as determined by computational methods, such that PVPP particles have micropockets on the surface that could interact and capture large molecules and interior cavities capable of retaining smaller molecules.<sup>17</sup> The hysteresis loop (Figure 1a) is usually associated with capillary condensation in mesopore structures.<sup>29</sup> Indeed, as shown in the pore size distribution graph (Figure 1c) and relative pore size proportion graph (Figure 1d), except for the sorbent prepared with 4-VBA monomer, sorbents prepared by microwave synthesis had narrow pore size distributions, with most pores having a width below 2 nm (micropores) and a few pores being between 2 and 50 nm (mesopores).

Twofold higher surface area was observed for the sorbent obtained by thermal synthesis compared to its microwave synthesis counterpart ( $\text{MAA}_{(1:5/A/T)}$  vs  $\text{MAA}_{(1:5/A/M)}$ , Table 3). A similar result was found by Turner et al.<sup>30</sup> whereby thermally synthesized molecularly imprinted polymer had a surface area higher than that of its microwave equivalent. The use of different porogens (DCM vs ACN) had less of an effect, leading to slightly higher surface area for  $\text{MAA}_{(1:5/D/M)}$  in contrast to  $\text{MAA}_{(1:5/A/M)}$ . As for choice of different monomers,  $\text{MAA}_{(1:5/D/M)}$  and  $\text{AA}_{(1:5/D/M)}$  had similar surface area (Table 3) due to the structural similarity of MAA and AA, but 4-VBA<sub>(1:5/D/M)</sub> had much lower surface area and divergent pore size distribution. The aromatic ring of 4-VBA may lead to a more compact backbone of the sorbent, thus affording a lower surface area, and the extent of mesoporosity may also have a relationship with the structure of the monomer.<sup>31</sup> With the same amount of EGDMA copolymer, doubling of the monomer  $\text{MAA}_{(2:5/A/T)}$  also increased the compactness of the sorbent and resulted in surface area lower than that of  $\text{MAA}_{(1:5/A/T)}$ .

Pore formation could be affected by reaction time and temperature; for conventional thermal synthesis, higher temperature and longer reaction time would result in less macropores with narrower pore size distribution of the final product.<sup>8,31,32</sup> Despite reaction temperatures nominally being the same, sorbents from microwave synthesis were obtained in

a much shorter reaction time of 1 h compared to 24 h for thermal synthesis and had narrower pore size distributions and more micropores and mesopores rather than macropores. This may be explained by the fact that the more energy-efficient “in-core” heating of the microwave compared to classic oil-bath heating could lead to a higher reaction temperature and dramatically enhanced reaction rate.<sup>33</sup> Additionally, it has been reported that products from microwave synthesis have many evenly distributed pores whereas thermal synthesis produces irregular pores, as determined by scanning electron microscopy analysis,<sup>21</sup> which was in line with the findings in the present study. Finally, the sealed-vessel system used for microwave synthesis provides more reproducible reaction conditions than conventional thermal heating, especially for solvents with low boiling point (e.g., DCM).<sup>33</sup>

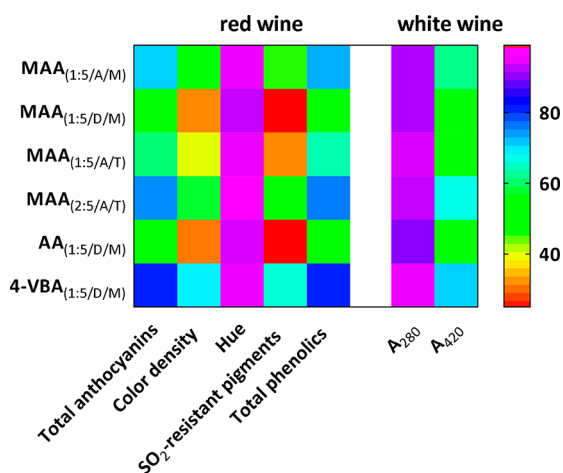
**Sorption of Wine Volatile Compounds by the Sorbents.** The extent of sorption of volatile compounds of different origins from wine by the six sorbents is shown in Tables S1 and S2 of the Supporting Information. The amounts of significant ( $p \leq 0.05$ ) compounds relative to control in the same group were calculated as grouped volatile compounds and presented in a heatmap for each sorbent (Figure 2). For volatile compounds in general, 4-VBA<sub>(1:5/D/M)</sub> had the highest sorption, and MAA<sub>(2:5/A/T)</sub> had the lowest sorption. In contrast, sorbents differing only in porogen solvent (i.e., MAA<sub>(1:5/A/M)</sub> and MAA<sub>(1:5/D/M)</sub>) had similar sorption properties, and the sorption of volatiles by AA<sub>(1:5/D/M)</sub> was close to that of MAA<sub>(1:5/A/T)</sub>, although the sorption of MAA<sub>(1:5/A/M)</sub> and MAA<sub>(1:5/D/M)</sub> was slightly higher in both red and white wines than AA<sub>(1:5/D/M)</sub> and MAA<sub>(1:5/A/T)</sub>.

Sorption rates of the sorbents in the white wine tended to be higher than those of sorbents in the red wine (Figure 2 and Tables S1 and S2 of the Supporting Information). Among the volatile compounds, all the sorbents had the highest sorption toward isoprenoids, including  $\alpha$ -terpineol,  $\beta$ -damascenone, and eucalyptol in the red wine. On the other hand, the highest sorption was of acids in the white wine, in which no isoprenoids were detected. Alcohols (10 significant compounds in the red wine and 9 in the white wine) and carbonyls (nonanal and furfural in the red wine and furfural in the white wine) had the least sorption on all the sorbents. The sorption of acetate and ethyl esters on each sorbent was similar in both red and white wines. 4-VBA<sub>(1:5/D/M)</sub> had higher affinity toward esters, isoprenoids, and alcohols than the other sorbents, MAA<sub>(1:5/D/M)</sub> had higher sorption of acids, and MAA<sub>(2:5/A/T)</sub> had lower affinity toward most of the volatile compounds but relatively higher retention of carbonyls (furfural in particular, Tables S1 and S2 of the Supporting Information), together with another thermally made sorbent MAA<sub>(1:5/A/T)</sub>. For microwave synthesized sorbents in DCM, the more branched-chain the monomer, the higher sorption toward volatile compounds. For sorbents with the same structural components prepared in different solvents, the general retention of volatile compounds was similar, but sorbent made in ACN had higher affinity toward acetate and ethyl esters, whereas its counterpart made in DCM had higher affinity toward acids in the red wine (i.e., MAA<sub>(1:5/A/M)</sub> and MAA<sub>(1:5/D/M)</sub>, Figure 2).

#### Sorption of Color and Phenolics by the Sorbents.

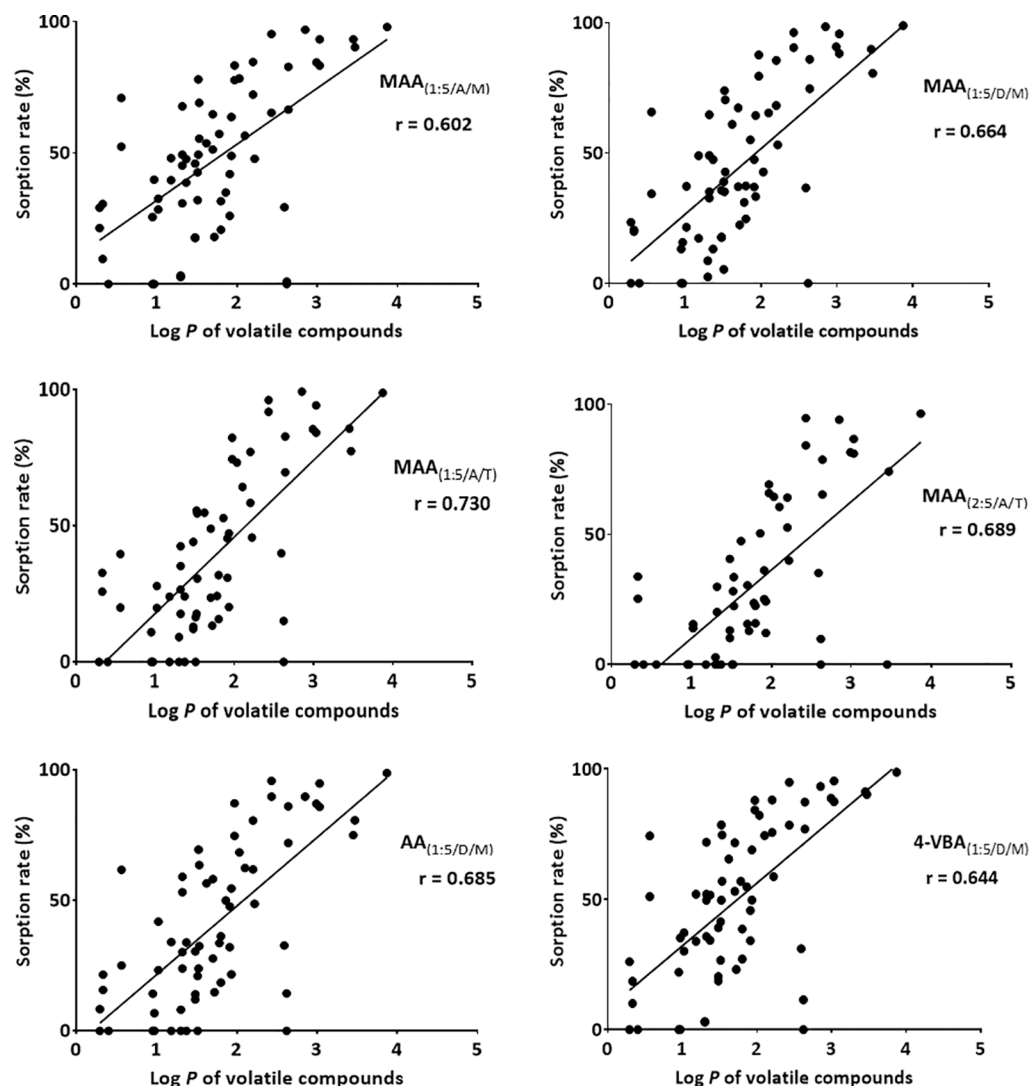
Sorption of colored matter and phenolics from wines by the different sorbents is shown in Tables S3 and S4 of the Supporting Information. The values of significant ( $p \leq 0.05$ ) color/phenolic parameters (Somers measures for red wine,

A<sub>280</sub> and A<sub>420</sub> for white wine) relative to the control are presented in a heatmap for each sorbent (Figure 3). In contrast



**Figure 3.** Values of color/phenolic parameters relative to the control (%) for wines treated with the six sorbents (refer to Table 1 for sorbent details).

to the outcomes for volatile compounds, the sorption of pigments on 4-VBA<sub>(1:5/D/M)</sub> was the lowest compared to other sorbents in both red and white wines. MAA<sub>(1:5/D/M)</sub> and AA<sub>(1:5/D/M)</sub> had the same sorption of color, with sorptions that were the highest among all the sorbents. MAA<sub>(2:5/A/T)</sub> had relatively lower sorption of phenolic compounds, which was also the case for volatiles. Thermally produced sorbent MAA<sub>(1:5/A/T)</sub> had higher retention of phenolic compounds than its microwave synthesized counterpart MAA<sub>(1:5/A/M)</sub> in red and white wines, which was opposite to the results for volatile compounds. Sorbent made using DCM as porogen had higher sorption of pigments than its counterpart made in ACN (e.g., MAA<sub>(1:5/D/M)</sub> and MAA<sub>(1:5/A/M)</sub> in red and white wines). Among the parameters in the red wine, SO<sub>2</sub>-resistant pigments revealed the greatest decrease with all the sorbents, followed by color density, whereas hue was barely affected (Figure 3). The decreases in SO<sub>2</sub>-resistant pigments and color density in the red wine indicated higher retention of polymeric polyphenols by the sorbents,<sup>34</sup> as more stable polymeric pigments will replace monomer phenolic compounds during aging and account for up to 50% of color density within the first year.<sup>35</sup> On the other hand, the influence of the same sorbent on total anthocyanins and total phenolics was similar. In the white wine, absorbance at 420 nm (indicator of browning) was greatly decreased by all the sorbents compared to slight decreases of absorbance at 280 nm (indicator of total phenolics). The decrease of absorbance at 420 nm showed the lightening effect of sorbents on the yellow-brown hue, which relates to flavan-3-ol derivatives, oxidized phenols, and their polymerized macromolecules.<sup>36</sup> The higher affinity toward polymeric phenols was also found with PVPP,<sup>23</sup> for which it was reported that flavan-3-ols were the most affected polyphenols in rosé wine, and anthocyanins were not strongly absorbed on average.<sup>17</sup> The lightening effect and the ability to improve resistance to browning of PVPP addition was reported for white wine as well.<sup>23</sup> Those previous findings are in good agreement with the results for the polymeric sorbents presented in this study.



**Figure 4.** Correlations between the lipophilicity ( $\text{Log } P$ ) of volatile compounds and sorption rates for the six sorbents (from Tables S1 and S2 of the Supporting Information).

**Relationship Between Physical Structures and Sorption Properties.** The degree of sorption is related to surface area in contact with adsorbates;<sup>37</sup> however, sorption in the present study was not directly in accordance with surface area. Among the six sorbents, 4-VBA<sub>(1:5/D/M)</sub> had the smallest surface area (Table 3), whereas it typically had the highest sorption of volatile compounds (Figure 2). The multi-component sorption could be affected by sorbent pore size distribution: sorption strength could increase with decreasing pore size due to the increasing contact points between adsorbent and adsorbate, and small pores could exclude certain compound sizes and shapes due to size exclusion limits.<sup>38</sup> As most volatile compounds are small, the presence of micropores and mesopores is important for retention of such molecules. Indeed, adsorbates of similar size and structure have been observed to have direct competition toward binding sites in the accessible pore region of micropores, and broadening the pore size distribution to include mesopores could lead to decreased competition.<sup>39</sup> As shown in Figure 1(d), pore size distribution of 4-VBA<sub>(1:5/D/M)</sub> was dominated by mesopores rather than micropores, as seen for the other sorbents. Thus, the sorption rate for volatile compounds could be explained by

the presence of abundant mesopores together with micropores, leading to higher sorption with lower competition.

The most substantial contributions to overall surface area are from micropores followed by mesopores, whereas large macropores make an insignificant contribution to the surface area.<sup>28</sup> Such a result was found for 4-VBA<sub>(1:5/D/M)</sub>, in which the overall surface area was not significantly increased despite the higher macropore constitution (Table 3 and Figure 1d). Due to the insignificant contribution of macropores and competition effect and size exclusion limits of micropores (molecular weight up to 10 kDa),<sup>28</sup> it is ultimately the mesopores (molecular weight below 500 kDa)<sup>40,41</sup> that play an important role in sorption of large molecules such as condensed polyphenols in wine, of which the majority can be found in the molecular weight range 2–5 kDa (with some up to 50 kDa).<sup>42</sup> For thermally synthesized sorbents MAA<sub>(1:5/A/T)</sub> in both red and white wine, higher sorption of phenolics was observed compared to its microwave synthesized counterpart MAA<sub>(1:5/A/M)</sub> due to the higher mesopore ratio. The same observation was found with sorbents differing in solvents (i.e., MAA<sub>(1:5/A/M)</sub> and MAA<sub>(1:5/D/M)</sub>), whereby the higher mesopore constitution of MAA<sub>(1:5/D/M)</sub> had higher retention of

phenolics in the red and white wine. The smallest overall surface area and the strong competition from volatiles may account for the lowest sorption of pigments on 4-VBA<sub>(1.5/D/M)</sub>, in spite of its proportionately more abundant mesopores. On the other hand, the relatively higher sorption of phenolics on AA<sub>(1.5/D/M)</sub> was found in spite of its proportionately less abundant mesopores as for MAA<sub>(2.5/A/T)</sub> (Figures 1d and 3).

In aqueous solutions, oxygen-containing functional groups of sorbents could adsorb water through hydrogen bonding and result in water clustering at these sites, which could further prevent compounds accessing hydrophobic regions and/or micropores in the sorbents.<sup>38</sup> The relatively lower sorption of MAA<sub>(2.5/A/T)</sub> toward volatile compounds and color pigments in the red and white wine may be explained by water clustering of its binding sites, as the sorbent contained double the amount of carboxylic acid functional groups that could hydrogen bond with water compared with the other sorbents. As implied by this role of water, besides pore structure, pore surface chemistry also plays an important role in wine component sorption.

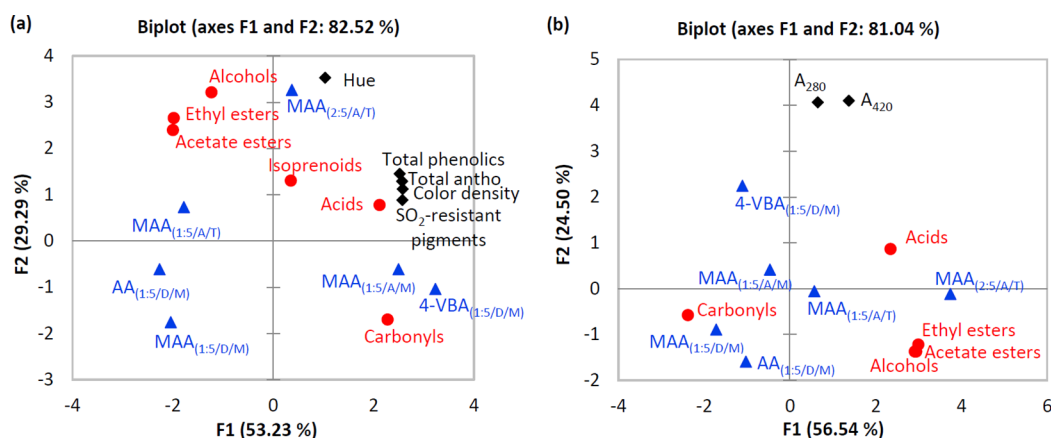
**Relationship Between Surface Chemistry and Sorption Properties.** Chemical functionality is responsible for the surface chemistry of polymeric sorbents and further influences the interactions between the sorbents and extracted compounds. Based on the hydrophobic nature of polymeric sorbents<sup>5</sup> and the sorption pattern of the wine components described earlier, the sorption for a hydroalcoholic matrix like wine was driven by hydrophobic interactions. Indeed, although copolymerization was conducted with hydrophilic monomers, the retention of polar compounds requires a much higher organic solvent matrix (e.g., ACN% > 60%) to ensure significant hydrophilic interaction with a methacrylate-based solid phase.<sup>7</sup> Thus, the sorption of volatile compounds on the sorbents in the red and white wine had a strong correlation ( $r = 0.6-0.7$ ) with lipophilicity (Log  $P$ , Tables S1 and S2 of the Supporting Information) of the absorbed volatile compounds (Figure 4). Another observation was that the sorbents had higher affinity toward alcohols containing aromatic rings than some of the alcohols with even higher lipophilicities (e.g., compare benzyl alcohol, phenylethyl alcohol, and 3-hexen-1-ol, especially in the red wine, Table S1 of the Supporting Information). The  $\pi-\pi$  stacking between the aromatic rings of 4-VBA<sub>(1.5/D/M)</sub> and benzyl alcohol and phenylethyl alcohol may account for the higher sorption. Hydrophobic interactions could account for the retention of phenolic compounds as well, as the sorption of derived pigments (SO<sub>2</sub>-resistant pigments) was higher than that of the highly water-soluble monomeric anthocyanins, which was in line with the finding that sorption of phenolic compounds by PVPP increased with the degree of polymerization (trimers > dimers > monomers).<sup>17</sup> The  $\pi-\pi$  stacking between the aromatic rings of 4-VBA<sub>(1.5/D/M)</sub> and phenolic compounds may lead to a higher sorption; however, it was apparently limited by overall surface area and porosity.

The polarity of polymeric sorbents could be affected by the percentage of incorporated hydrophilic monomers.<sup>9</sup> Among the sorbents, MAA<sub>(2.5/A/T)</sub> had double the amount of hydrophilic monomer compared to the other sorbents and tended to be more polar and hydrophilic. As a result, it had the least sorption toward volatile compounds and second least sorption toward pigments in the red and white wine (Figures 2 and 3). The higher sorption of volatiles by 4-VBA<sub>(1.5/D/M)</sub> (Figure 2) may be due to higher hydrophobicity of its monomer 4-VBA (Log  $P$  2.23) compared to MAA (Log  $P$

0.73) and AA (Log  $P$  0.38). The highest retention of isoprenoids among the other volatiles in the red wine correlated with their high lipophilicities, with Log  $P$  values of 1.86, 2.10, and 2.99 for eucalyptol,  $\alpha$ -terpineol, and  $\beta$ -damascenone, respectively, falling at the upper end of the range of  $-0.47$  to  $3.03$  for volatile compounds in the red wine (Table S1 of the Supporting Information). The highest extraction of acids compared to other volatiles in the white wine could also be related to lipophilicity, with Log  $P$  values of 1.91, 2.43, and 2.85 for hexanoic acid, octanoic acid, and nonanoic acid, respectively, being on the higher end of the range of  $0.29-3.87$  for volatile compounds in the white wine (Table S2 of the Supporting Information). The sorption of acids may involve not only hydrophobic interactions but also hydrogen bonds between the carboxylic acid groups.

Hydrophilic interactions may also be found between phenolics and the sorbents. The hydrogen bonds between polyphenolics and PVPP have been confirmed by computational methods.<sup>16,17</sup> A lower concentration of phenolic compounds in the white wine has likely led to less competition of both hydrophobic and hydrophilic interactions with the sorbents and to higher sorption of volatile compounds on a grouped basis. The hydrophilic monomers used in this work are weak acids, with one acidic carboxyl group and pK<sub>a</sub> values of 4.25, 4.65, and 4.29 for AA,<sup>41</sup> MAA,<sup>42</sup> and 4-VBA,<sup>43</sup> respectively. The pK<sub>a</sub> values of the monomers may vary according to the degree of polymerization and nature of the cross-linkers but may not be dramatically different after polymerization, with pK<sub>a</sub> values of 4.5, 4.8, and 7.1 for poly(acrylic acid) (PAA),<sup>43</sup> poly(methacrylic acid) (PMAA),<sup>44</sup> and poly(4-vinylbenzoic acid) (P4-VBA).<sup>45</sup> At wine pH, it is possible to find the monomers in their deprotonated form (COO<sup>-</sup>) and in much less proportion (<10%) than their acid form (COOH).<sup>46</sup> This may be relevant to mixed-mode interactions as the cationic form of anthocyanins and derived pigments (A<sup>+</sup>) exists at wine pH, although in smaller proportions compared to their hydrated form (AOH).<sup>47</sup> Thus, there may be weak cation exchange between anthocyanins and the acidic residues of the sorbents except for 4-VBA<sub>(1.5/D/M)</sub>, whose deprotonated form would barely exist at wine pH. However, as the sorbents and sorbates are most in their hydrated forms, hydrogen bonds may be more dominant than ionic interactions. A similar assumption was evaluated by computational modeling, in which higher interaction energies were found with hydrated forms of anthocyanins when interacting with PVPP, and a higher correlation was found between interaction energy and sorption of hydrated anthocyanins than for their cationic forms.<sup>17</sup>

The higher sorption of furfural on MAA<sub>(1.5/A/T)</sub> and MAA<sub>(2.5/A/T)</sub> may mainly rely on H-bonding between the proton donor of the carboxylic acid monomers and the carbonyl group of furfural, as the lipophilicity of furfural is only 0.33 and the sorption on 4-VBA<sub>(1.5/D/M)</sub> was not high, indicating the hydrophobic interaction and  $\pi-\pi$  stacking are not very evident. Polarity of the sorbents and the hydrophilic interactions between the sorbents and sorbates could be affected by porogen solvent. As observed in Figures 2 and 3, MAA<sub>(1.5/D/M)</sub> prepared with DCM had affinity toward acids and phenolic compounds higher than that of its counterpart MAA<sub>(1.5/A/M)</sub> made with ACN. The higher hydrophilic interactions between MAA<sub>(1.5/A/T)</sub> and furfural compared to that of MAA<sub>(1.5/A/M)</sub> may indicate that thermal heating leads to fewer hydrophilic interactions between the monomer and



**Figure 5.** PCA biplots showing scores and loadings of the means for significant ( $p \leq 0.05$ ) grouped volatile compounds (red circles) and color parameters (black diamonds) relative to the control for (a) a red and (b) a white wine treated with the six sorbents (blue triangles).

porogen solvent than microwave heating, as “nonthermal” effect of microwave heating has been proposed. It is thought that polar compounds of a reaction may be more reactive under microwave irradiation than thermal heating.<sup>21</sup>

PCA was performed on grouped volatile compounds and phenolics data that differed significantly ( $p \leq 0.05$ ) among the sorbent treated wines (Figure 5). The first two PCs explained 83 and 81% of the total variation among the samples in the red and white wine, respectively. 4-VBA<sub>(1:5/D/M)</sub> and MAA<sub>(1:5/A/M)</sub> were differentiated from the other sorbents based on the higher sorption of volatile compounds and lower retention of phenolics in both wines types. Conversely, MAA<sub>(1:5/D/M)</sub>, AA<sub>(1:5/D/M)</sub>, and MAA<sub>(1:5/A/T)</sub> were differentiated based on the higher amount of volatile compounds and phenolics removed from the treated wines, especially for the wines treated with MAA<sub>(1:5/D/M)</sub> and AA<sub>(1:5/D/M)</sub>. Separation of MAA<sub>(2:5/A/T)</sub> was based on the higher concentration of most wine components that remained in the wine, except for carbonyls.

**Relationship Between Physicochemical Properties and Sorption Properties of Four Commercial Sorbents.** Surface areas of the four commercial sorbents (Table 4) were

**Table 4. BET Surface Areas of Four Commercial Sorbent (Refer to Table 2 for Sorbents Details)**

	C <sub>18</sub>	Oasis HLB	Strata SDB-L	PVPP
mean surface area (m <sup>2</sup> /g)	110 ± 7	522 ± 8	384 ± 7	N/A
pore size proportions (%)				
micropores		39	56	
mesopores	100	61	42	N/A
macropores			2	

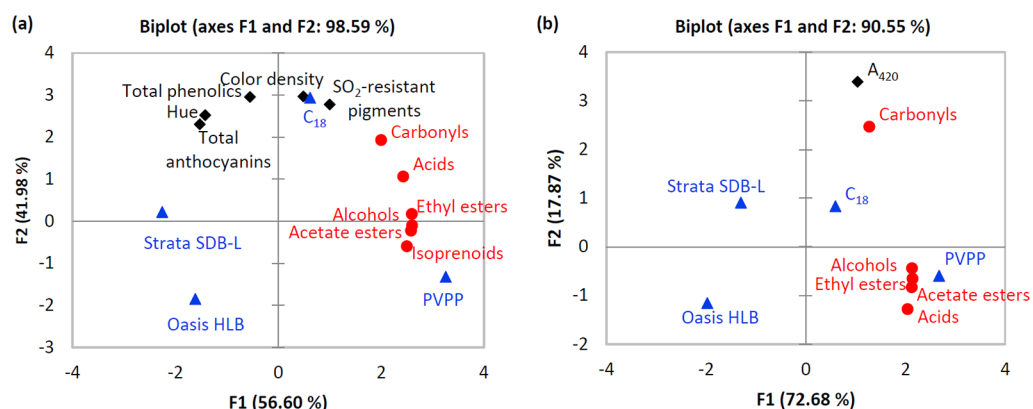
found to be different from those provided by producers (Table 2), and different measurement methods may account for the variance. No uptake of nitrogen was observed with PVPP at relative low pressure ( $P/P_0$ ), and the obtained gas adsorption isotherm was best described as type III in shape (Figure S1a of the Supporting Information). In this case, the BET method is unlikely to yield the actual surface area, and an alternative method (e.g., mercury porosimetry) would best be adopted.<sup>29</sup> Moreover, the BET method is more suitable for the measurement of materials with small pores (microporous or mesoporous),<sup>48</sup> whereas the PVPP sorbent used in this experiment may be macroporous. Gas adsorption isotherms

of the other three sorbents (Figure S1 of the Supporting Information) indicated monolayer-multilayer adsorption, similar to our synthesized sorbents. However, abundant mesopores and a lack of macropores (Table 4) contributed to the plateau of gas adsorption isotherms of C<sub>18</sub> and Oasis HLB (Figures S1b and S1c of the Supporting Information), which led to isotherms described as type IV. The isotherm for Strata SDB-L (Figure S1d of the Supporting Information) could be described as type II in shape, similar to our synthesized sorbents.

Sorption properties of the four commercial sorbents in a red and a white wine were examined by PCA and presented in biplots (Figure 6). The first two PCs explained 99 and 91% of the total variation among the samples in the red and white wine, respectively. The four sorbents had similar performance in the two wine matrixes. The C<sub>18</sub> sorbent was separated for low retention of phenolics and high retention of volatile compounds. Conversely, the PVPP was differentiated due to its high sorption of phenolic compounds and low sorption of volatiles. Stata SDB-L and Oasis HLB had higher retention of wine components compared to the C<sub>18</sub> and PVPP in general, but Stata SDB-L was differentiated from Oasis HLB based on low retention of phenolic compounds. Detailed sorption of grouped volatile compounds and phenolics have been presented in heatmaps in the Supporting Information (Figure S2), and relevant data can be found in Tables S5–S8 of the Supporting Information.

Surface area of the four commercial sorbents may relate to sorption properties in wines, given the higher surface areas of Oasis HLB and Strata SDB-L coinciding with higher retention ability of the polymers. However, the sorption properties were not determined by surface area alone, as was the case for our synthesized sorbents. Higher sorption of moderately polar volatile and phenolic compounds by Oasis HLB compared to Stata SDB-L may largely due to the hydrophilic monomer component of the former. The divinylbenzene monomer common to both sorbents contributes to hydrophobic and  $\pi$ - $\pi$  interactions between sorbent and analytes, whereas the vinylpyrrolidone monomer in Oasis HLB may form H-bonds with polar analytes such as alcohols and phenolic compounds. As for the C<sub>18</sub> sorbent, interactions are mainly hydrophobic, which explains the preferential sorption of nonpolar volatiles (Figure S2 of the Supporting Information), but overall, polymeric sorbents and especially Stata SDB-L likely had stronger hydrophobic capabilities, as indicated by





**Figure 6.** PCA biplots showing scores and loadings of the means for significant ( $p \leq 0.05$ ) grouped volatile compounds (red circles) and color parameters (black diamonds) relative to the control for (a) a red and (b) a white wine treated with the four commercial sorbents (blue triangles).

their higher sorption of nonpolar volatiles (Figure S2 of the Supporting Information). The hydrophilic components of PVPP may account for the preferential sorption of phenolics; however, the contribution of physical characteristics was unknown in this case. Correlations between the Log  $P$  of volatile compounds and the degree of retention by the four sorbents were assessed. All correlations were positive and ranged from strong ( $r = 0.619$ ) for C<sub>18</sub> to moderate ( $r = 0.477$ ,  $0.470$ , and  $0.366$ , respectively) for Stata SDB-L, Oasis HLB, and PVPP. The strong sorption ability of Stata SDB-L led to sorption of even moderately polar volatile compounds, which resulted in a smaller correlation coefficient compared to that of the C<sub>18</sub> sorbent despite the greater apparent hydrophobic nature of Stata SDB-L.

The hydrophilic sorbents synthesized in-house or commercially available (Oasis HLB and PVPP) still have high capacities for retaining less polar compounds; however, in contrast to sorbents such as C<sub>18</sub>, the hydrophilic character supposedly introduces much better wettability and consequently could aid the transfer of compounds from aqueous solution to the sorbent.<sup>49</sup> Improved sorption ability and wettability would also require less sorbent.<sup>50</sup> Interestingly, the synthesized 4-VBA<sub>(1.5/D/M)</sub> showed similar sorption properties to the C<sub>18</sub> sorbent, but better wettability could be expected. The stronger sorption ability of Stata SDB-L and Oasis HLB compared to the synthesized polymers may be due to the large particle sizes and small surface areas in the latter case, which could be further improved.

In summary, six new polymeric sorbents were prepared and analyzed to investigate their ability to separate specific analytes or potentially undesirable compounds from wine. The sorption of wine components depended on physicochemical properties (surface area, pore size, and polarity) of sorbents and was connected with the constituents and production methods of the polymers. The observed relationship was considered in the context of four commercial sorbents, and together, the information could be used to guide sorbent selection depending on the task. Good specificity potential of the six synthesized sorbents was found: 4-VBA<sub>(1.5/D/M)</sub> may favor extraction of small, nonpolar compounds (e.g., volatiles); the moderate hydrophobicity and hydrophilicity of MAA<sub>(1.5/D/M)</sub> and AA<sub>(1.5/D/M)</sub> may make these suitable for extraction of small to large (molecular weight below 500 kDa), moderately polar compounds (e.g., phenolics), and the greater polarity of MAA<sub>(2.5/A/T)</sub> may be suited to carbonyl compound extraction.

Overall, sorption testing of the six sorbents has shown some specificity toward certain groups of compounds and, based on the current results, further optimization could be achieved by modifying aspects such as monomer:cross-linker ratio, porogen solvent, polymerization method, and particle size.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b04641.

Tables containing lipophilicity (Log  $P$ ) and quantified results of volatile compounds in the red wine treated with the six sorbents (Table S1), lipophilicity (Log  $P$ ) and quantified results of volatile compounds in the white wine treated with the six sorbents (Table S2), color parameters of the red wine treated with the six sorbents (Table S3), absorbance at 280 and 420 nm of the white wine treated with the six sorbents (Table S4), lipophilicity (Log  $P$ ) and quantified results of volatile compounds in the red wine treated with the four commercial sorbents (Table S5), lipophilicity (Log  $P$ ) and quantified results of volatile compounds in the white wine treated with the four commercial sorbents (Table S6), color parameters of the red wine treated with the four commercial sorbents (Table S7), and absorbance at 280 and 420 nm of the white wine treated with the four commercial sorbents (Table S8); figures showing gas adsorption isotherms of (a) PVPP, (b) C<sub>18</sub>, (c) Oasis HLB, and (d) Strata SDB-L and (e) pore size distributions of four commercial sorbents (Figure S1), and relative amounts of (a) grouped volatile compounds remaining in wines compared to the control (%) and (b) values of color/phenolic parameters relative to the control (%) after treatment with the four commercial sorbents (Figure S2) (PDF)

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### ABBREVIATIONS USED

AA, acrylic acid; ACN, acetonitrile; AIBN, 2,2'-azobis(isobutyronitrile); AR, analytical reagent; BET, Brunauer–Emmett–Teller; DCM, dichloromethane; EDGMA, ethylene glycol dimethacrylate; HS-SPME-GC–MS, headspace solid-phase microextraction-gas chromatography/mass spectrometry; MAA, methacrylic acid; P4-VBA, poly(4-vinylbenzoic acid); PAA, poly(acrylic acid); PCA, principal component analysis; PMAA, poly(methacrylic acid); PS-DVB, polystyrene-divinylbenzene; PVPP, polyvinylpyrrolidone; SPE, solid phase extraction; TCA, 2,4,6-trichloroanisole; 4-VBA, 4-vinylbenzoic acid

### REFERENCES

(1) Ebeler, S. E.; Thorngate, J. H. Wine chemistry and flavor: looking into the crystal glass. *J. Agric. Food Chem.* **2009**, *57*, 8098–8108.

(2) Lopez, R.; Aznar, M.; Cacho, J.; Ferreira, V. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A* **2002**, *966*, 167–177.

(3) Ortega, L.; Lopez, R.; Cacho, J.; Ferreira, V. Use of solid–liquid distribution coefficients to determine retention properties of Porapak-Q resins: Determination of optimal conditions to isolate alkyl-methoxy-pyrazines and beta-damascenone from wine. *Journal of Chromatography A* **2001**, *931*, 31–39.

(4) Fontanals, N.; Marcé, R.; Borrull, F. New hydrophilic materials for solid-phase extraction. *TrAC, Trends Anal. Chem.* **2005**, *24*, 394–406.

(5) Fontanals, N.; Marcé, R.; Borrull, F. New materials in sorptive extraction techniques for polar compounds. *Journal of Chromatography A* **2007**, *1152*, 14–31.

(6) Fan, Y.; Feng, Y.-Q.; Da, S.-L.; Shi, Z.-G. Poly (methacrylic acid–ethylene glycol dimethacrylate) monolithic capillary for in-tube solid phase microextraction coupled to high performance liquid chromatography and its application to determination of basic drugs in human serum. *Anal. Chim. Acta* **2004**, *523*, 251–258.

(7) Jiang, Z.; Smith, N. W.; Ferguson, P. D.; Taylor, M. R. Hydrophilic interaction chromatography using methacrylate-based monolithic capillary column for the separation of polar analytes. *Anal. Chem.* **2007**, *79*, 1243–1250.

(8) Jandera, P. Advances in the development of organic polymer monolithic columns and their applications in food analysis—a review. *Journal of Chromatography A* **2013**, *1313*, 37–53.

(9) Bielicka-Daszkiwicz, K.; Voelkel, A.; Szejner, M.; Osypiuk, J. Extraction properties of new polymeric sorbents in SPE/GC analysis of phenol and hydroquinone from water samples. *Chemosphere* **2006**, *62*, 890–898.

(10) Li, A.; Zhang, Q.; Chen, J.; Fei, Z.; Long, C.; Li, W. Adsorption of phenolic compounds on Amberlite XAD-4 and its acetylated derivative MX-4. *React. Funct. Polym.* **2001**, *49*, 225–233.

(11) Pérez-Magariño, S.; Ortega-Heras, M.; Cano-Mozo, E. Optimization of a solid-phase extraction method using copolymer sorbents for isolation of phenolic compounds in red wines and quantification by HPLC. *J. Agric. Food Chem.* **2008**, *56*, 11560–11570.

(12) Li, Y.; Li, W.; Wang, Y.; Zhou, H.; Hu, G.; Zhang, N.; Sun, C. Development of a solid-phase microextraction fiber coated with poly(methacrylic acid-ethylene glycol dimethacrylate) and its application for the determination of chlorophenols in water coupled with GC. *J. Sep. Sci.* **2013**, *36*, 2121–2127.

(13) Wang, L.; Zhang, Z. Molecular imprinted polymer-based chemiluminescence imaging sensor for the detection of trans-resveratrol. *Anal. Chim. Acta* **2007**, *592*, 115–120.

(14) Molinelli, A.; Weiss, R.; Mizaikoff, B. Advanced solid phase extraction using molecularly imprinted polymers for the determination of quercetin in red wine. *J. Agric. Food Chem.* **2002**, *50*, 1804–1808.

(15) Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B.; Laureano, O.; Ricardo da Silva, J. M. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003**, *51*, 6475–6481.

(16) Durán-Lara, E. F.; López-Cortés, X. A.; Castro, R. I.; Avila-Salas, F.; González-Nilo, F. D.; Laurie, V. F.; Santos, L. S. Experimental and theoretical binding affinity between polyvinylpyrrolidone and selected phenolic compounds from food matrices. *Food Chem.* **2015**, *168*, 464–470.

(17) Gil, M. I.; Avila-Salas, F.; Santos, L. S.; Iturmendi, N.; Moine, V.; Cheynier, V. r.; Saucier, C. d. Rosé wine fining using polyvinylpyrrolidone: colorimetry, targeted polyphenomics, and molecular dynamics simulations. *J. Agric. Food Chem.* **2017**, *65*, 10591–10597.

(18) Laborde, B.; Moine-Ledoux, V.; Richard, T.; Saucier, C.; Dubourdieu, D.; Monti, J.-P. PVPP– polyphenol complexes: a molecular approach. *J. Agric. Food Chem.* **2006**, *54*, 4383–4389.

(19) Botezatu, A.; Pickering, G. J. Application of plastic polymers in remediating wine with elevated alkyl-methoxy-pyrazine levels. *Food Addit. Contam., Part A* **2015**, *32*, 1199–1206.

(20) Ferreira, V.; Ortega, L.; Escudero, A.; Cacho, J. F. A comparative study of the ability of different solvents and adsorbents to extract aroma compounds from alcoholic beverages. *J. Chromatogr. Sci.* **2000**, *38*, 469–476.

(21) Hoogenboom, R.; Schubert, U. S. Microwave-assisted polymer synthesis: recent developments in a rapidly expanding field of research. *Macromol. Rapid Commun.* **2007**, *28*, 368–386.

(22) Mercurio, M. D.; Damberg, R. G.; Herderich, M. J.; Smith, P. A. High throughput analysis of red wine and grape phenolics adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. *J. Agric. Food Chem.* **2007**, *55*, 4651–4657.

(23) Sims, C. A.; Eastridge, J. S.; Bates, R. P. Changes in phenols, color, and sensory characteristics of muscadine wines by pre- and post-fermentation additions of PVPP, casein, and gelatin. *Am. J. Enol. Vitic.* **1995**, *46*, 155–158.

(24) Ortega, A.; Lopez-Toledano, A.; Mayen, M.; Merida, J.; Medina, M. Changes in color and phenolic compounds during oxidative aging of sherry white wine. *J. Food Sci.* **2003**, *68*, 2461–2468.

- (25) Boss, P. K.; Pearce, A. D.; Zhao, Y.; Nicholson, E. L.; Dennis, E. G.; Jeffery, D. W. Potential grape-derived contributions to volatile ester concentrations in wine. *Molecules* **2015**, *20*, 7845–7873.
- (26) Dennis, E. G.; Keyzers, R. A.; Kalua, C. M.; Maffei, S. M.; Nicholson, E. L.; Boss, P. K. Grape contribution to wine aroma: production of hexyl acetate, octyl acetate, and benzyl acetate during yeast fermentation is dependent upon precursors in the must. *J. Agric. Food Chem.* **2012**, *60*, 2638–2646.
- (27) Boss, P. K.; Böttcher, C.; Davies, C. Various influences of harvest date and fruit sugar content on different wine flavor and aroma compounds. *Am. J. Enol. Vitic.* **2014**, *65*, 13137.
- (28) Viklund, C.; Svec, F.; Fréchet, J. M.; Irgum, K. Monolithic, “molded”, porous materials with high flow characteristics for separations, catalysis, or solid-phase chemistry: control of porous properties during polymerization. *Chem. Mater.* **1996**, *8*, 744–750.
- (29) Sing, K.; Everett, D.; Haul, R.; Moscou, L.; Pierotti, R.; Rouquerol, J.; Siemieniowska, T. Physical and biophysical chemistry division commission on colloid and surface chemistry including catalysis. *Pure Appl. Chem.* **1985**, *57*, 603–619.
- (30) Turner, N. W.; Holdsworth, C. I.; Donne, S. W.; McCluskey, A.; Bowyer, M. C. Microwave induced MIP synthesis: comparative analysis of thermal and microwave induced polymerisation of caffeine imprinted polymers. *New J. Chem.* **2010**, *34*, 686–692.
- (31) Svec, F.; Frechet, J. M. Kinetic control of pore formation in macroporous polymers. Formation of “molded” porous materials with high flow characteristics for separations or catalysis. *Chem. Mater.* **1995**, *7*, 707–715.
- (32) Greiderer, A.; Trojer, L.; Huck, C. W.; Bonn, G. K. Influence of the polymerisation time on the porous and chromatographic properties of monolithic poly(1,2-bis(*p*-vinylphenyl))ethane capillary columns. *Journal of Chromatography A* **2009**, *1216*, 7747–7754.
- (33) Kappe, C. O. Controlled microwave heating in modern organic synthesis. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250–6284.
- (34) Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *Am. J. Enol. Vitic.* **2001**, *52*, 67–87.
- (35) Somers, T. The polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175–2186.
- (36) Recamales, A. F.; Sayago, A.; González-Miret, M. L.; Hernanz, D. The effect of time and storage conditions on the phenolic composition and colour of white wine. *Food Res. Int.* **2006**, *39*, 220–229.
- (37) Sajilata, M.; Savitha, K.; Singhal, R.; Kanetkar, V. Scalping of flavors in packaged foods. *Compr. Rev. Food Sci. Food Saf.* **2007**, *6*, 17–35.
- (38) Li, L.; Quinlivan, P. A.; Knappe, D. R. Effects of activated carbon surface chemistry and pore structure on the adsorption of organic contaminants from aqueous solution. *Carbon* **2002**, *40*, 2085–2100.
- (39) Pelekani, C.; Snoeyink, V. L. Competitive adsorption between atrazine and methylene blue on activated carbon: the importance of pore size distribution. *Carbon* **2000**, *38*, 1423–1436.
- (40) Wang, Q. C.; Svec, F.; Frechet, J. M. Macroporous polymeric stationary-phase rod as continuous separation medium for reversed-phase chromatography. *Anal. Chem.* **1993**, *65*, 2243–2248.
- (41) Wang, Q. C.; Svec, F.; Fréchet, J. M. Hydrophilization of porous polystyrene-based continuous rod column. *Anal. Chem.* **1995**, *67*, 670–674.
- (42) Somers, T. Wine tannins— isolation of condensed flavonoid pigments by gel-filtration. *Nature* **1966**, *209*, 368.
- (43) Tjipangandjara, K.; Somasundaran, P. Effects of the conformation of polyacrylic acid on the dispersion-flocculation of alumina and kaolin fines. *Adv. Powder Technol.* **1992**, *3*, 119–127.
- (44) Sonia, T. A.; Sharma, C. P. *Oral Delivery of Insulin*; Elsevier: 2014.
- (45) De las Heras Alarcón, C.; Pennadam, S.; Alexander, C. Stimuli responsive polymers for biomedical applications. *Chem. Soc. Rev.* **2005**, *34*, 276–285.
- (46) Waterhouse, A. L.; Sacks, G. L.; Jeffery, D. W. Acids. In *Understanding Wine Chemistry*; John Wiley & Sons, Ltd: 2016; pp 19–33.
- (47) Waterhouse, A. L.; Sacks, G. L.; Jeffery, D. W. Anthocyanins. In *Understanding Wine Chemistry*; John Wiley & Sons, Ltd: 2016; pp 131–139.
- (48) Rouquerol, J.; Avnir, D.; Fairbridge, C.; Everett, D.; Haynes, J.; Pernicone, N.; Ramsay, J.; Sing, K.; Unger, K. Recommendations for the characterization of porous solids (Technical Report). *Pure Appl. Chem.* **1994**, *66*, 1739–1758.
- (49) Rodriguez, I.; Llompart, M.; Cela, R. Solid-phase extraction of phenols. *Journal of Chromatography A* **2000**, *885*, 291–304.
- (50) Huck, C.; Bonn, G. Recent developments in polymer-based sorbents for solid-phase extraction. *Journal of Chromatography A* **2000**, *885*, 51–72.

## **SUPPORTING INFORMATION FOR**

### **Extraction Properties of New Polymeric Sorbents Applied to Wine**

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**Table S1.** Lipophilicity (Log *P*) and quantified results of volatile compounds in the red wine treated with the six sorbents.

	<b>Log <i>P</i><sup>a</sup></b>	<b>MAA<sub>(1:5/A/M)</sub></b>	<b>MAA<sub>(1:5/D/M)</sub></b>	<b>MAA<sub>(1:5/A/T)</sub></b>	<b>MAA<sub>(2:5/A/T)</sub></b>	<b>AA<sub>(1:5/D/M)</sub></b>	<b>4-VBA<sub>(1:5/D/M)</sub></b>	<b>Control</b>
<i>Acetate esters<sup>b</sup></i>								
Ethyl acetate	0.29	26 ± 1c (79%) <sup>c</sup>	38 ± 3a (100%)	42 ± 2a (100%)	42 ± 3a (100%)	41 ± 2a (100%)	30 ± 2b (100%)	33 ± 1b
Isobutyl acetate	1.18	0.22 ± 0.02c (60%)	0.30 ± 0.03b (83%)	0.36 ± 0.03a (100%)	0.39 ± 0.03a (100%)	0.36 ± 0.02a (100%)	0.24 ± 0.02c (66%)	0.36 ± 0.01a
Isoamyl acetate	1.53	8.8 ± 1.1e (45%)	11.3 ± 0.8d (57%)	13.7 ± 0.7c (69%)	15.3 ± 1.2b (78%)	13.3 ± 0.5c (67%)	8.5 ± 0.6e (43%)	19.8 ± 0.5a
Hexyl acetate	2.03	0.014 ± 0.003de (22%)	0.016 ± 0.004cde (25%)	0.017 ± 0.001cd (27%)	0.022 ± 0.003b (35%)	0.020 ± 0.003bc (32%)	0.011 ± 0.002e (18%)	0.063 ± 0.004a
2-Phenylethyl acetate	1.97	0.37 ± 0.06de (17%)	0.45 ± 0.05d (20%)	0.57 ± 0.02c (26%)	0.76 ± 0.05b (34%)	0.56 ± 0.04c (25%)	0.35 ± 0.01e (16%)	2.22 ± 0.12a
<b>Mean<sup>c</sup></b>		<b>44%</b>	<b>57%</b>	<b>64%</b>	<b>69%</b>	<b>65%</b>	<b>49%</b>	-
<i>Ethyl esters<sup>b</sup></i>								
Ethyl isobutyrate	1.51	1.6 ± 0.1d (68%)	2.2 ± 0.2c (95%)	2.5 ± 0.1ab (100%)	2.6 ± 0.2a (100%)	2.6 ± 0.1a (100%)	1.7 ± 0.1d (73%)	2.4 ± 0.1bc
Ethyl butanoate	1.37	2.3 ± 0.2c (61%)	3.2 ± 0.2b (85%)	3.6 ± 0.1a (100%)	3.9 ± 0.3a (100%)	3.6 ± 0.2a (100%)	2.4 ± 0.2c (66%)	3.7 ± 0.0a
Ethyl 2-methylbutanoate	1.93	0.96 ± 0.11e (51%)	1.25 ± 0.10d (67%)	1.50 ± 0.08bc (80%)	1.65 ± 0.14b (88%)	1.47 ± 0.04c (78%)	0.94 ± 0.08e (50%)	1.88 ± 0.04a
Ethyl 3-methylbutanoate	1.70	1.3 ± 0.1e (49%)	1.7 ± 0.2d (63%)	2.1 ± 0.1bc (76%)	2.3 ± 0.2b (84%)	2.0 ± 0.2c (72%)	1.3 ± 0.1e (47%)	2.7 ± 0.1a
Ethyl pentanoate	1.78	0.031 ± 0.006c (43%)	0.049 ± 0.011b (69%)	0.054 ± 0.005b (76%)	0.055 ± 0.004b (76%)	0.048 ± 0.003b (66%)	0.031 ± 0.002c (43%)	0.072 ± 0.003a
Ethyl hexanoate	2.20	5.6 ± 1.0de (28%)	6.4 ± 0.6d (32%)	8.3 ± 0.4bc (42%)	9.5 ± 0.6b (47%)	7.6 ± 0.3c (38%)	4.9 ± 0.3e (24%)	20.0 ± 1.2a
Ethyl octanoate	3.03	0.63 ± 0.06bc (17%)	0.45 ± 0.04d (12%)	0.60 ± 0.05c (16%)	0.72 ± 0.08b (19%)	0.54 ± 0.02cd (14%)	0.47 ± 0.05d (12%)	3.79 ± 0.12a
Isobutyl lactate	0.97	0.33 ± 0.01d (60%)	0.46 ± 0.04c (84%)	0.55 ± 0.02b (100%)	0.61 ± 0.04a (100%)	0.51 ± 0.05bc (93%)	0.36 ± 0.02d (65%)	0.55 ± 0.02b
Ethyl 2-furoate	1.52	0.062 ± 0.005e (51%)	0.084 ± 0.006d (65%)	0.10 ± 0.003c (82%)	0.12 ± 0.004b (100%)	0.10 ± 0.008c (76%)	0.064 ± 0.003e (50%)	0.17 ± 0.007a
Isoamyl lactate	1.32	4.1 ± 0.1d (51%)	5.2 ± 0.5c (65%)	6.6 ± 0.2b (82%)	8.2 ± 0.5a (100%)	6.2 ± 0.6b (76%)	4.1 ± 0.3d (50%)	8.1 ± 0.4a
Diethyl succinate	0.56	62 ± 3d (48%)	86 ± 9c (66%)	104 ± 4b (80%)	125 ± 6a (100%)	98 ± 9.6b (75%)	64 ± 3d (49%)	130 ± 6a
<b>Mean<sup>c</sup></b>		<b>47%</b>	<b>63%</b>	<b>74%</b>	<b>81%</b>	<b>70%</b>	<b>47%</b>	-



Table S1. cont.

Eucalyptol	1.86	3 ± 0b (65%)	2 ± 0c (45%)	2 ± 0c (50%)	2 ± 0c (50%)	2 ± 0c (45%)	4 ± 0a
<b>Mean<sup>c</sup></b>		<b>41%</b>	<b>30%</b>	<b>36%</b>	<b>34%</b>	<b>27%</b>	-
<i>Acids<sup>f</sup></i>							
Acetic acid	-0.31	74 ± 5a (100%)	72 ± 3a (100%)	60 ± 3bc (100%)	71 ± 2ab (100%)	70 ± 4ab (100%)	58 ± 2c
Hexanoic acid	1.91	73 ± 1b (74%)	62 ± 3d (63%)	74 ± 1b (75%)	67 ± 2c (68%)	65 ± 2cd (66%)	99 ± a
Octanoic acid	2.43	41 ± 15b (35%)	11 ± 3cd (10%)	18 ± 4cd (16%)	12 ± 5cd (10%)	25 ± 2c (21%)	117 ± 15a
<b>Mean<sup>c</sup></b>		<b>70%</b>	<b>58%</b>	<b>64%</b>	<b>59%</b>	<b>62%</b>	-
<i>Lactones<sup>e</sup></i>							
Butyrolactone	-0.47	8 ± 0a (100%)	8 ± 2ab (88%)	8 ± 0ab (83%)	8 ± 1ab (84%)	9 ± 1a (100%)	9 ± 1a

Data ( $\mu\text{g/L}$  of internal standard equivalents) for each sorbent treatment are presented as mean  $\pm$  SD ( $n = 3$ ) with different letters across a row indicating significantly different ( $p \leq 0.05$ ) means according to one-way ANOVA with Fisher's (LSD) pairwise comparison. <sup>a</sup> Lipophilicity value was obtained by Chemdraw prediction. <sup>b</sup> Compounds were quantified as *d*<sub>5</sub>-ethyl nonanoate equivalents. <sup>c</sup> Amount relative to the control. <sup>d</sup> Compounds were quantified as *d*<sub>13</sub>-hexanol equivalents. <sup>e</sup> Compounds were quantified as *d*<sub>16</sub>-octanal equivalents. <sup>f</sup> Compounds were quantified as *d*<sub>11</sub>-hexanoic acid equivalents.



**Table S2.** Lipophilicity (Log *P*) and quantified results of volatile compounds in the white wine treated with the six sorbents.

	<b>Log <i>P</i><sup>a</sup></b>	<b>MAA<sub>(1:5/A/M)</sub></b>	<b>MAA<sub>(1:5/D/M)</sub></b>	<b>MAA<sub>(1:5/A/T)</sub></b>	<b>MAA<sub>(2:5/A/T)</sub></b>	<b>AA<sub>(1:5/D/M)</sub></b>	<b>4-VBA<sub>(1:5/D/M)</sub></b>	<b>Control</b>
<i>Acetate esters<sup>b</sup></i>								
Ethyl acetate	0.29	21 ± 3d (71%) <sup>c</sup>	23 ± 1cd (77%)	31 ± 8b (100%)	41 ± 7a (100%)	27 ± 2bcd (92%)	22 ± 2cd (74%)	30 ± 1bc
Isobutyl acetate	1.18	0.12 ± 0.02c (52%)	0.12 ± 0.00c (51%)	0.18 ± 0.06b (76%)	0.25 ± 0.05a (100%)	0.16 ± 0.01bc (66%)	0.11 ± 0.01c (48%)	0.24 ± 0.01a
Isoamyl acetate	1.53	0.4 ± 0.1d (31%)	0.4 ± 0.0d (30%)	0.6 ± 0.2c (45%)	0.9 ± 0.2b (66%)	0.5 ± 0.1cd (36%)	0.4 ± 0.0d (25%)	1.4 ± 0.1a
2-Phenylethyl acetate	1.97	0.06 ± 0.02bc (22%)	0.03 ± 0.00c (12%)	0.05 ± 0.01c (18%)	0.08 ± 0.02b (31%)	0.03 ± 0.00c (13%)	0.03 ± 0.00c (12%)	0.27 ± 0.04a
<b>Mean<sup>c</sup></b>		<b>44%</b>	<b>42%</b>	<b>60%</b>	<b>74%</b>	<b>52%</b>	<b>40%</b>	-
<i>Ethyl esters<sup>b</sup></i>								
Ethyl propanoate	0.95	0.2 ± 0.0c (63%)	0.2 ± 0.0c (70%)	0.3 ± 0.1b (100%)	0.4 ± 0.0a (100%)	0.2 ± 0.0bc (100%)	0.2 ± 0.0c (63%)	0.3 ± 0.0b
Ethyl isobutyrate	1.51	0.8 ± 0.1c (57%)	0.8 ± 0.0c (61%)	1.1 ± 0.3b (84%)	1.5 ± 0.3a (100%)	1.0 ± 0.1bc (79%)	0.8 ± 0.1c (59%)	1.3 ± 0.0ab
Ethyl butanoate	1.37	1.4 ± 0.2c (52%)	1.4 ± 0.0c (52%)	2.0 ± 0.5b (76%)	2.8 ± 0.5a (100%)	1.8 ± 0.1bc (66%)	1.3 ± 0.1c (48%)	2.7 ± 0.1a
Ethyl 2-methylbutanoate	1.93	0.15 ± 0.03d (36%)	0.14 ± 0.00d (36%)	0.21 ± 0.06c (53%)	0.30 ± 0.07b (76%)	0.18 ± 0.01cd (45%)	0.12 ± 0.01d (31%)	0.40 ± 0.02a
Ethyl 3-methylbutanoate	1.70	0.16 ± 0.03d (35%)	0.15 ± 0.00d (33%)	0.23 ± 0.06c (51%)	0.34 ± 0.07b (70%)	0.19 ± 0.01cd (42%)	0.13 ± 0.01d (28%)	0.45 ± 0.03a
Ethyl hexanoate	2.20	4 ± 1de (15%)	4 ± 0de (14%)	6 ± 1c (23%)	9 ± 2b (36%)	5 ± 0cd (19%)	3 ± 0e (12%)	26 ± 1a
Ethyl octanoate	3.03	0.7 ± 0.2bc (7%)	0.5 ± 0.0c (4%)	0.6 ± 0.1bc (6%)	1.4 ± 0.3b (13%)	0.6 ± 0.0c (5%)	0.5 ± 0.1c (5%)	10.5 ± 1.1a
Ethyl nonanoate	3.45	0.04 ± 0.01c (7%)	0.06 ± 0.01bc (10%)	0.08 ± 0.01bc (14%)	0.51 ± 0.10a (100%)	0.15 ± 0.03b (25%)	0.05 ± 0.02c (9%)	0.59 ± 0.08a
Isoamyl lactate	1.32	0.7 ± 0.2c (32%)	0.7 ± 0.1c (35%)	1.4 ± 0.3b (65%)	2.3 ± 0.7a (100%)	0.9 ± 0.1bc (41%)	0.6 ± 0.1c (28%)	2.1 ± 0.3a
Methyl decanoate	3.53	0.6 ± 0.2ab (100%)	0.7 ± 0.1ab (100%)	1.3 ± 0.3a (100%)	1.6 ± 1.4a (100%)	0.6 ± 0.5ab (100%)	0.2 ± 0.3b (100%)	1.2 ± 0.2ab
Ethyl 2-furoate	1.52	0.07 ± 0.02d (22%)	0.08 ± 0.01d (26%)	0.14 ± 0.04c (44%)	0.23 ± 0.06b (72%)	0.10 ± 0.01cd (31%)	0.07 ± 0.01d (21%)	0.32 ± 0.03a
Ethyl decanoate	3.87	0.1 ± 0.0b (2%)	0.1 ± 0.0b (1%)	0.1 ± 0.0b (1%)	0.2 ± 0.0b (4%)	0.1 ± 0.0b (1%)	0.1 ± 0.0b (1%)	6.1 ± 1.0a
Diethyl succinate	0.56	7 ± 2c (29%)	8 ± 1c (34%)	14 ± 3b (60%)	24 ± 7a (100%)	9 ± 1bc (38%)	6 ± 1c (26%)	24 ± 3a
<b>Mean<sup>c</sup></b>		<b>35%</b>	<b>37%</b>	<b>52%</b>	<b>75%</b>	<b>44%</b>	<b>33%</b>	-



**Table S3.** Color parameters of the red wine treated with the six sorbents.

	MAA <sub>(1:5/A/M)</sub>	MAA <sub>(1:5/D/M)</sub>	MAA <sub>(1:5/A/T)</sub>	MAA <sub>(2:5/A/T)</sub>	AA <sub>(1:5/D/M)</sub>	4-VBA <sub>(1:5/D/M)</sub>	Control
Total anthocyanins (mg/L)	138 ± 4c (72%) <sup>a</sup>	107 ± 2d (56%)	118 ± 4d (62%)	145 ± 6bc (76%)	109 ± 3d (57%)	157 ± 18b (82%)	191 ± 4a
Color density (au)	5.9 ± 0.1d (54%)	3.6 ± 0.0f (33%)	4.5 ± 0.3e (41%)	6.4 ± 0.2c (59%)	3.5 ± 0.2f (32%)	7.6 ± 0.1b (70%)	10.8 ± 0.2a
Hue	0.89 ± 0.002c (96%)	0.87 ± 0.004f (94%)	0.89 ± 0.003cd (96%)	0.91 ± 0.004b (99%)	0.88 ± 0.003e (95%)	0.89 ± 0.001d (96%)	0.93 ± 0.003a
SO <sub>2</sub> -resistant pigments (au)	1.8 ± 0.0d (46%)	1.0 ± 0.00f (25%)	1.2 ± 0.1e (33%)	1.9 ± 0.1c (51%)	0.9 ± 0.1f (25%)	2.5 ± 0.0b (67%)	3.8 ± 0.0a
Total phenolics (au)	40 ± 1c (74%)	30 ± 0e (55%)	35 ± 2d (65%)	42 ± 1c (77%)	29 ± 1e (53%)	44 ± 0b (82%)	54 ± 1a

Data for each sorbent treatment are presented as mean ± SD (n = 3) with different letters across a row indicating significantly different ( $p \leq 0.05$ ) means according to one-way ANOVA with Fisher's (LSD) pairwise comparison. <sup>a</sup> Value relative to the control.

**Table S4.** Absorbance at 280 and 420 nm of the white wine treated with the six sorbents.

	MAA <sub>(1:5/A/M)</sub>	MAA <sub>(1:5/D/M)</sub>	MAA <sub>(1:5/A/T)</sub>	MAA <sub>(2:5/A/T)</sub>	AA <sub>(1:5/D/M)</sub>	4-VBA <sub>(1:5/D/M)</sub>	Control
A <sub>280</sub>	4.98 ± 0.02cd (93%) <sup>a</sup>	4.98 ± 0.05cd (93%)	5.13 ± 0.01b (95%)	5.05 ± 0.08bc (94%)	4.90 ± 0.06d (91%)	5.13 ± 0.10b (96%)	5.37 ± 0.05a
A <sub>420</sub>	0.11 ± 0.00cd (63%)	0.09 ± 0.00e (50%)	0.10 ± 0.00de (56%)	0.12 ± 0.01bc (68%)	0.09 ± 0.00e (51%)	0.12 ± 0.01b (72%)	0.17 ± 0.02a

Data for each sorbent treatment are presented as mean ± SD (n = 3) with different letters across a row indicating significantly different ( $p \leq 0.05$ ) means according to one-way ANOVA with Fisher's (LSD) pairwise comparison. <sup>a</sup> Value relative to the control.

**Table S5.** Lipophilicity (Log *P*) and quantified results of volatile compounds in the red wine<sup>a</sup> treated with the four commercial sorbents.

	Log <i>P</i> <sup>b</sup>	C <sub>18</sub>	Oasis HLB	Strata SDB-L	PVPP	Control
<i>Acetate esters</i> <sup>c</sup>						
Ethyl acetate	0.29	404 ± 25a (100%) <sup>d</sup>	360 ± 7b (84%)	364 ± 24b (85%)	421 ± 13a (100%)	428 ± 3a
Isobutyl acetate	1.18	4.5 ± 0.4c (65%)	2.0 ± 0.1d (29%)	1.1 ± 0.1e (16%)	6.3 ± 0.3b (91%)	6.9 ± 0.1a
Isoamyl acetate	1.53	193 ± 33c (39%)	45 ± 2d (9%)	21 ± 2d (4%)	466 ± 13b (94%)	495 ± 4a
Hexyl acetate	2.03	0.33 ± 0.10c (15%)	0.06 ± 0.01d (3%)	0.06 ± 0.01d (3%)	2.00 ± 0.08b (91%)	2.19 ± 0.07a
2-Phenylethyl acetate	1.97	33 ± 5b (38%)	2 ± 0c (2%)	1 ± 0c (1%)	79 ± 5a (100%)	86 ± 7a
<b>Mean<sup>d</sup></b>		<b>51%</b>	<b>25%</b>	<b>22%</b>	<b>95%</b>	-
<i>Ethyl esters</i> <sup>c</sup>						
Ethyl isobutyrate	1.51	19 ± 2c (59%)	8 ± 1d (25%)	5 ± 1e (16%)	29 ± 2b (91%)	32 ± 1a
Ethyl butanoate	1.37	38 ± 4c (64%)	14 ± 1d (24%)	8 ± 1e (14%)	54 ± 2b (92%)	59 ± 1a
Ethyl 2-methylbutanoate	1.93	7 ± 1c (39%)	2 ± 0d (11%)	1 ± 0d (6%)	16 ± 1b (89%)	18 ± 0a
Ethyl 3-methylbutanoate	1.70	13 ± 2c (38%)	4 ± 0d (12%)	2 ± 0d (6%)	31 ± 1b (91%)	34 ± 0a
Ethyl pentanoate	1.78	0.5 ± 0.1b (29%)	0.1 ± 0.0c (6%)	0.1 ± 0.0c (6%)	1.6 ± 0.0a (100%)	1.7 ± 0.1a
Ethyl hexanoate	2.20	80 ± 17c (19%)	11 ± 0d (3%)	5 ± 0d (1%)	404 ± 8b (95%)	425 ± 1a
Ethyl octanoate	3.03	8 ± 2c (9%)	1 ± 0d (1%)	1 ± 0d (1%)	80 ± 3b (94%)	86 ± 2a
Ethyl propanoate	0.95	30 ± 3b (81%)	19 ± 1c (51%)	15 ± 1d (41%)	34 ± 2a (100%)	37 ± 1a
Ethyl 2-furoate	1.52	2.7 ± 0.2c (73%)	0.6 ± 0d (16%)	0.5 ± 0.0d (14%)	3.4 ± 0.1b (92%)	3.7 ± 0.2a
Isoamyl lactate	1.32	119 ± 14b (75%)	54 ± 5c (34%)	31 ± 2d (19%)	163 ± 10a (100%)	159 ± 9a
Diethyl succinate	0.56	1713 ± 132b (82%)	926 ± 67c (44%)	509 ± 37d (24%)	2028 ± 74a (100%)	2086 ± 151a
<b>Mean<sup>d</sup></b>		<b>52%</b>	<b>21%</b>	<b>13%</b>	<b>95%</b>	-
<i>Alcohols</i> <sup>c</sup>						
Isobutanol	0.95	146 ± 14	146 ± 5	156 ± 2	156 ± 10	155 ± 9
1-Butanol	0.97	795 ± 39	747 ± 29	751 ± 24	723 ± 109	770 ± 20
3-Methyl-1-butanol	1.30	2783 ± 149b (94%)	2598 ± 51c (88%)	2555 ± 107c (86%)	2906 ± 27ab (98%)	2967 ± 61a
4-Methyl-1-pentanol	1.72	15 ± 1b (71%)	11 ± 0c (52%)	8 ± 0d (38%)	20 ± 1a (100%)	21 ± 1a
1-Hexanol	1.80	183 ± 17b (78%)	116 ± 4c (50%)	90 ± 4d (38%)	232 ± 7a (100%)	234 ± 7a

Table S5. cont.

(E)-3-Hexen-1-ol	1.48	4.0 ± 0.3b (87%)	2.9 ± 0.1c (63%)	2.3 ± 0.1d (50%)	4.5 ± 0.1a (100%)	4.6 ± 0.2a
(Z)-3-Hexen-1-ol	1.48	4.0 ± 0.2b (87%)	3.0 ± 0.1c (65%)	2.6 ± 0.1d (57%)	4.5 ± 0.1a (100%)	4.6 ± 0.1a
3-Methyl-1-pentanol	1.72	33 ± 3a (100%)	15 ± 0c (54%)	12 ± 0d (43%)	27 ± 1b (100%)	28 ± 1b
1-Heptanol	2.22	23 ± 4b (43%)	9 ± 1c (17%)	6 ± 0c (11%)	52 ± 2a (100%)	54 ± 2a
2-Ethyl-1-hexanol	2.62	91 ± 55a (100%)	33 ± 3b (100%)	27 ± 3b (100%)	29 ± 2b (100%)	30 ± 2b
1-Octanol	2.64	3 ± 1b (20%)	1 ± 0c (7%)	1 ± 0c (7%)	14 ± 1a (100%)	15 ± 1a
Methionol	0.40	7 ± 1b (78%)	4 ± 1c (44%)	6 ± 1b (67%)	8 ± 1ab (89%)	9 ± 2a
Benzyl alcohol	1.02	8 ± 1b (80%)	5 ± 0c (50%)	6 ± 1bc (60%)	10 ± 1a (100%)	10 ± 2a
Phenylethyl alcohol	1.32	622 ± 49b (78%)	367 ± 32c (46%)	385 ± 52c (49%)	776 ± 41a (100%)	793 ± 107a
<b>Mean<sup>d</sup></b>		<b>80%</b>	<b>60%</b>	<b>58%</b>	<b>99%</b>	-
<i>Carbonyls<sup>e</sup></i>						
Nonanal	2.59	6 ± 2a (100%)	3 ± 0b (100%)	3 ± 1b (100%)	3 ± 1 b (100%)	3 ± 0b
Furfural	0.33	20.8 ± 2.4a (100%)	15.4 ± 1.4c (83%)	15.7 ± 1.9bc (85%)	17.6 ± 0.5bc (95%)	18.5 ± 0.6ab
<b>Mean<sup>d</sup></b>		<b>100%</b>	<b>92%</b>	<b>93%</b>	<b>98%</b>	-
<i>Isoprenoids<sup>e</sup></i>						
α-Terpineol	2.10	4 ± 1c (67%)	2 ± 0d (33%)	2 ± 0d (33%)	5 ± 0b (83%)	6 ± 0.4a
β-Damascenone	2.99	0.2 ± 0.1c (6%)	0.1 ± 0c (3%)	0.1 ± 0c (3%)	2.9 ± 0.1b (83%)	3.5 ± 0.3a
Eucalyptol	1.86	0.9 ± 0.3b (36%)	0.5 ± 0bc (20%)	0.5 ± 0c (20%)	2.4 ± 0.2a (100%)	2.5 ± 0.2a
<b>Mean<sup>d</sup></b>		<b>36%</b>	<b>19%</b>	<b>19%</b>	<b>89%</b>	-
<i>Acids<sup>f</sup></i>						
Hexanoic acid	1.91	111 ± 11b (82%)	47 ± 5c (35%)	41 ± 2c (30%)	127 ± 8a (100%)	136 ± 6a
Octanoic acid	2.43	66 ± 53a (100%)	9 ± 3b (10%)	3 ± 0b (8%)	102 ± 30a (100%)	123 ± 36a
<b>Mean<sup>d</sup></b>		<b>91%</b>	<b>23%</b>	<b>19%</b>	<b>100%</b>	-

Data ( $\mu\text{g/L}$  of internal standard equivalents) for each sorbent treatment are presented as mean  $\pm$  SD ( $n = 3$ ) with different letters across a row indicating significantly different ( $p \leq 0.05$ ) means according to one-way ANOVA with Fisher's (LSD) pairwise comparison.<sup>a</sup> Cabernet Sauvignon 2015, alcohol 14% v/v. <sup>b</sup> Lipophilicity value was obtained by Chemdraw prediction. <sup>c</sup> Compounds were quantified as  $d_{13}$ -hexanol equivalents. <sup>d</sup> Amount relative to the control. <sup>e</sup> Compounds were quantified as  $d_{16}$ -octanal equivalents. <sup>f</sup> Compounds were quantified as  $d_{11}$ -hexanoic acid equivalents.

**Table S6.** Lipophilicity (Log *P*) and quantified results of volatile compounds in the white wine treated with the four commercial sorbents.

	Log <i>P</i> <sup>a</sup>	C <sub>18</sub>	Oasis HLB	Strata SDB-L	PVPP	Control
<i>Acetate esters</i> <sup>b</sup>						
Ethyl acetate	0.29	509 ± 24a (100%) <sup>c</sup>	407 ± 38cd (96%)	376 ± 15d (89%)	453 ± 6b (100%)	422 ± 8bc
Isobutyl acetate	1.18	4.0 ± 0.3b (80%)	1.2 ± 0.1c (24%)	0.6 ± 0.1d (12%)	5.2 ± 0.1a (100%)	5.0 ± 0.3a
Isoamyl acetate	1.53	8 ± 1c (35%)	1 ± 0d (4%)	1 ± 0d (4%)	25 ± 1a (100%)	23 ± 1b
2-Phenylethyl acetate	1.97	2.0 ± 0.1c (27%)	0.2 ± 0.1d (3%)	0.1 ± 0.0d (1%)	6.6 ± 0.5b (88%)	7.5 ± 0.2a
<b>Mean<sup>c</sup></b>		<b>61%</b>	<b>32%</b>	<b>27%</b>	<b>97%</b>	-
<i>Ethyl esters</i> <sup>b</sup>						
Ethyl propanoate	0.95	4.5 ± 0.3a (100%)	2.1 ± 0.3b (48%)	1.5 ± 0.1c (34%)	4.6 ± 0.1a (100%)	4.4 ± 0.1a
Ethyl isobutyrate	1.51	22 ± 2b (79%)	5 ± 1c (18%)	3 ± 0d (11%)	30 ± 0a (100%)	28 ± 1a
Ethyl butanoate	1.37	33 ± 4b (72%)	8 ± 1c (17%)	5 ± 0c (11%)	48 ± 1a (100%)	46 ± 2a
Ethyl 2-methylbutanoate	1.93	4.0 ± 0.4c (42%)	0.7 ± 0.0d (7%)	0.2 ± 0.1d (2%)	10.2 ± 0.3a (100%)	9.5 ± 0.5b
Ethyl 3-methylbutanoate	1.70	3.8 ± 0.3b (38%)	0.5 ± 0.0c (5%)	0.2 ± 0.0c (2%)	10.0 ± 0.3a (100%)	10.0 ± 0.7a
Ethyl hexanoate	2.20	102 ± 9c (18%)	9 ± 1d (2%)	4 ± 0d (1%)	617 ± 20a (100%)	572 ± 43b
Ethyl octanoate	3.03	53 ± 13c (16%)	3 ± 0c (1%)	3 ± 1c (1%)	337 ± 10b (99%)	340 ± 63a
Ethyl nonanoate	3.45	5 ± 1c (14%)	8 ± 2c (22%)	10 ± 3c (28%)	20 ± 2b (56%)	36 ± 5a
Isoamyl lactate	1.32	39 ± 4b (64%)	10 ± 1c (16%)	6 ± 1c (10%)	56 ± 2a (100%)	61 ± 4a
Methyl decanoate	3.53	25 ± 2b (81%)	1 ± 0d (3%)	5 ± 1c (16%)	32 ± 1a (100%)	31 ± 1a
Ethyl 2-furoate	1.52	6 ± 1c (67%)	1 ± 0d (11%)	1 ± 0d (11%)	8 ± 0b (89%)	9 ± 0a
Ethyl decanoate	3.87	5 ± 1c (2%)	1 ± 0c (0.4%)	1 ± 0c (0.4%)	160 ± 12b (66%)	242 ± 14a
Diethyl succinate	0.56	442 ± 39c (66%)	93 ± 7d (14%)	46 ± 6d (7%)	596 ± 20b (89%)	673 ± 43a
<b>Mean<sup>c</sup></b>		<b>51%</b>	<b>13%</b>	<b>10%</b>	<b>92%</b>	-
<i>Alcohols</i> <sup>b</sup>						
Isobutanol	0.95	141 ± 7a (100%)	112 ± 14b (100%)	110 ± 3b (100%)	109 ± 2b (100%)	102 ± 3b
1-Butanol	0.97	704 ± 65a (100%)	574 ± 58bc (100%)	552 ± 15c (88%)	644 ± 4ab (100%)	624 ± 18bc
3-Methyl-1-butanol	1.30	1698 ± 107a (100%)	1328 ± 125c (89%)	1287 ± 33c (86%)	1550 ± 19b (100%)	1494 ± 43b
1-Hexanol	1.80	151 ± 11b (88%)	56 ± 6c (33%)	47 ± 3c (27%)	176 ± 3a (100%)	172 ± 7a
( <i>Z</i> )-3-Hexen-1-ol	1.48	38 ± 11b (76%)	16 ± 4c (32%)	34 ± 5b (68%)	43 ± 1ab (86%)	50 ± 6a

Table S6. cont.

2-Ethyl-1-hexanol	2.62	114 ± 34bc (72%)	153 ± 18ab (96%)	96 ± 17c (60%)	150 ± 15ab (94%)	159 ± 31a
1-Octanol	2.64	2.3 ± 0.4b (14%)	1.4 ± 0.1bc (9%)	0.7 ± 0.2c (4%)	16.2 ± 0.8a (100%)	16.1 ± 0.6a
( <i>E</i> )-3-Hexen-1-ol	1.48	2 ± 0a (100%)	1 ± 0b (50%)	1 ± 0b (50%)	2 ± 0a (100%)	2 ± 0a
Benzyl alcohol	1.02	3 ± 0b (75%)	2 ± 0c (50%)	2 ± 0c (50%)	3 ± 0b (75%)	4 ± 0a
Phenylethyl alcohol	1.32	103 ± 11c (68%)	38 ± 4d (25%)	45 ± 2d (30%)	127 ± 5b (84%)	151 ± 15a
<b>Mean<sup>f</sup></b>		<b>79%</b>	<b>58%</b>	<b>56%</b>	<b>94%</b>	-
<i>Carbonyls<sup>d</sup></i>						
Furfural	0.33	76 ± 24bc (81%)	48 ± 10c (51%)	106 ± 22a (100%)	82 ± 2ab (100%)	94 ± 12ab
<i>Acids<sup>e</sup></i>						
Hexanoic acid	1.91	150 ± 14b (79%)	41 ± 9c (22%)	40 ± 1c (21%)	179 ± 15a (100%)	189 ± 11a
Octanoic acid	2.43	9 ± 5b (5%)	4 ± 2b (2%)	1 ± 0b (0.5%)	174 ± 29a (100%)	197 ± 32a
Nonanoic acid	2.85	0.4 ± 0.6c (1%)	2.6 ± 0.8c (5%)	1.7 ± 0.3c (3%)	41.5 ± 7.1b (78%)	52.9 ± 10.6a
<b>Mean<sup>f</sup></b>		<b>28%</b>	<b>10%</b>	<b>8%</b>	<b>93%</b>	-

Data ( $\mu\text{g/L}$  of internal standard equivalents) for each sorbent treatment are presented as mean  $\pm$  SD ( $n = 3$ ) with different letters across a row indicating significantly different ( $p \leq 0.05$ ) means according to one-way ANOVA with Fisher's (LSD) pairwise comparison. <sup>a</sup> Lipophilicity value was obtained by Chemdraw prediction. <sup>b</sup> Compounds were quantified as  $d_{13}$ -hexanoic acid equivalents. <sup>c</sup> Amount relative to the control. <sup>d</sup> Compounds were quantified as  $d_{16}$ -octanoic acid equivalents. <sup>e</sup> Compounds were quantified as  $d_{11}$ -hexanoic acid equivalents.

**Table S7.** Color parameters of the red wine treated with the four commercial sorbents.

	<b>C<sub>18</sub></b>	<b>Oasis HLB</b>	<b>Strata SDB-L</b>	<b>PVPP</b>	<b>Control</b>
Total anthocyanins (mg/L)	159 ± 1a (100%) <sup>a</sup>	110 ± 4d (72%)	143 ± 2c (94%)	87 ± 1e (57%)	152 ± 3b
Color density (au)	12 ± 0a (100%)	3 ± 0c (25%)	6 ± 0b (50%)	5 ± 0b (42%)	12 ± 0a
Hue	0.86 ± 0.00c (99%)	0.80 ± 0.00d (92%)	0.87 ± 0.00a (100%)	0.77 ± 0.00e (89%)	0.87 ± 0.00b
SO <sub>2</sub> -resistant pigments (au)	4.0 ± 0.0b (98%)	0.7 ± 0.0e (17%)	1.5 ± 0.0d (37%)	1.9 ± 0.1c (46%)	4.1 ± 0.1a
Total phenolics (au)	63 ± 1a (100%)	29 ± 1c (47%)	45 ± 1b (73%)	26 ± 1d (42%)	62 ± 1a

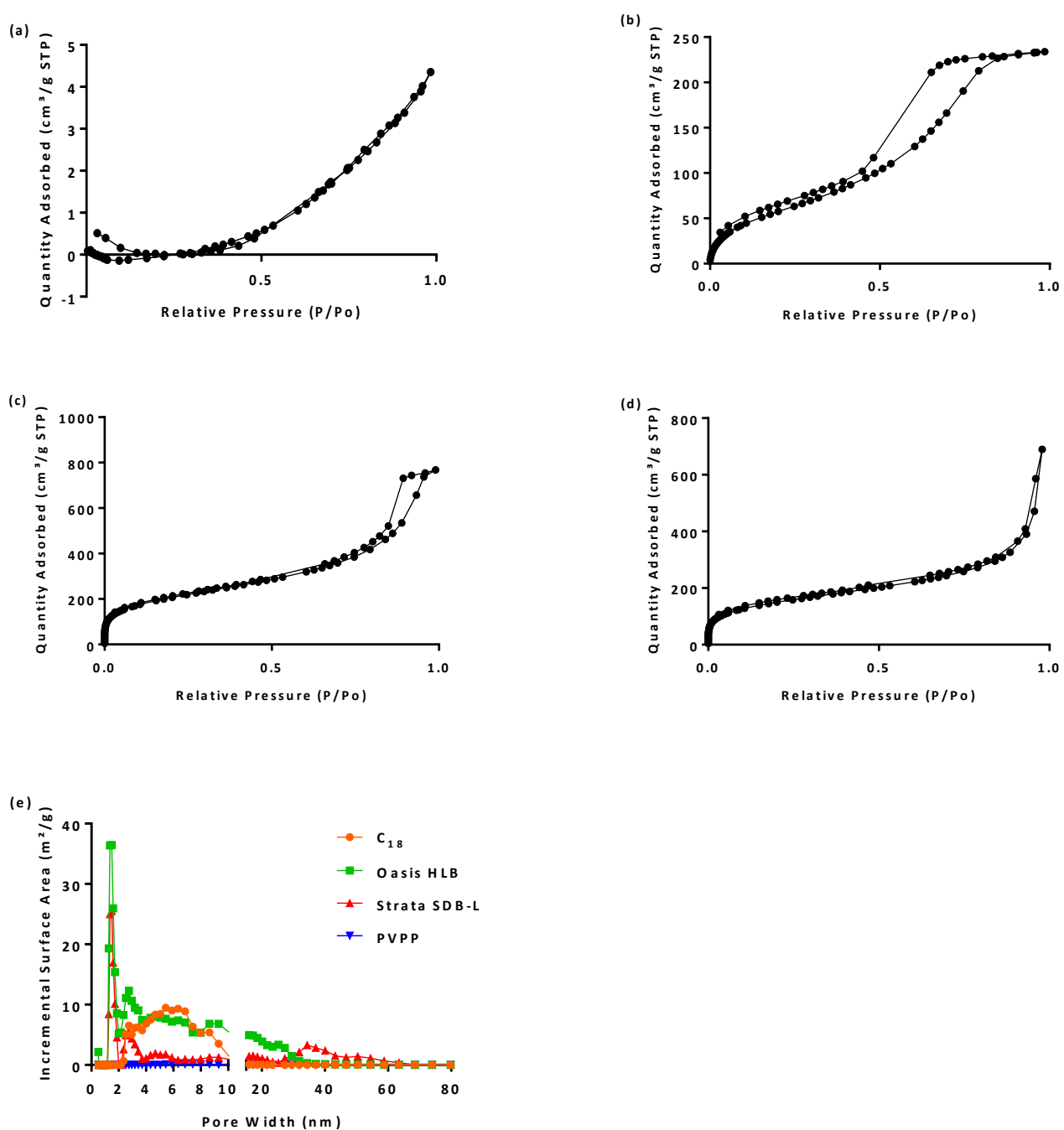
Data for each sorbent treatment are presented as mean ± SD (n = 3) with different letters across a row indicating significantly different ( $p \leq 0.05$ ) means according to one-way ANOVA with Fisher's (LSD) pairwise comparison. <sup>a</sup> Value relative to the control.

**Table S8.** Absorbance at 280 and 420 nm of the white wine treated with the four commercial sorbents.

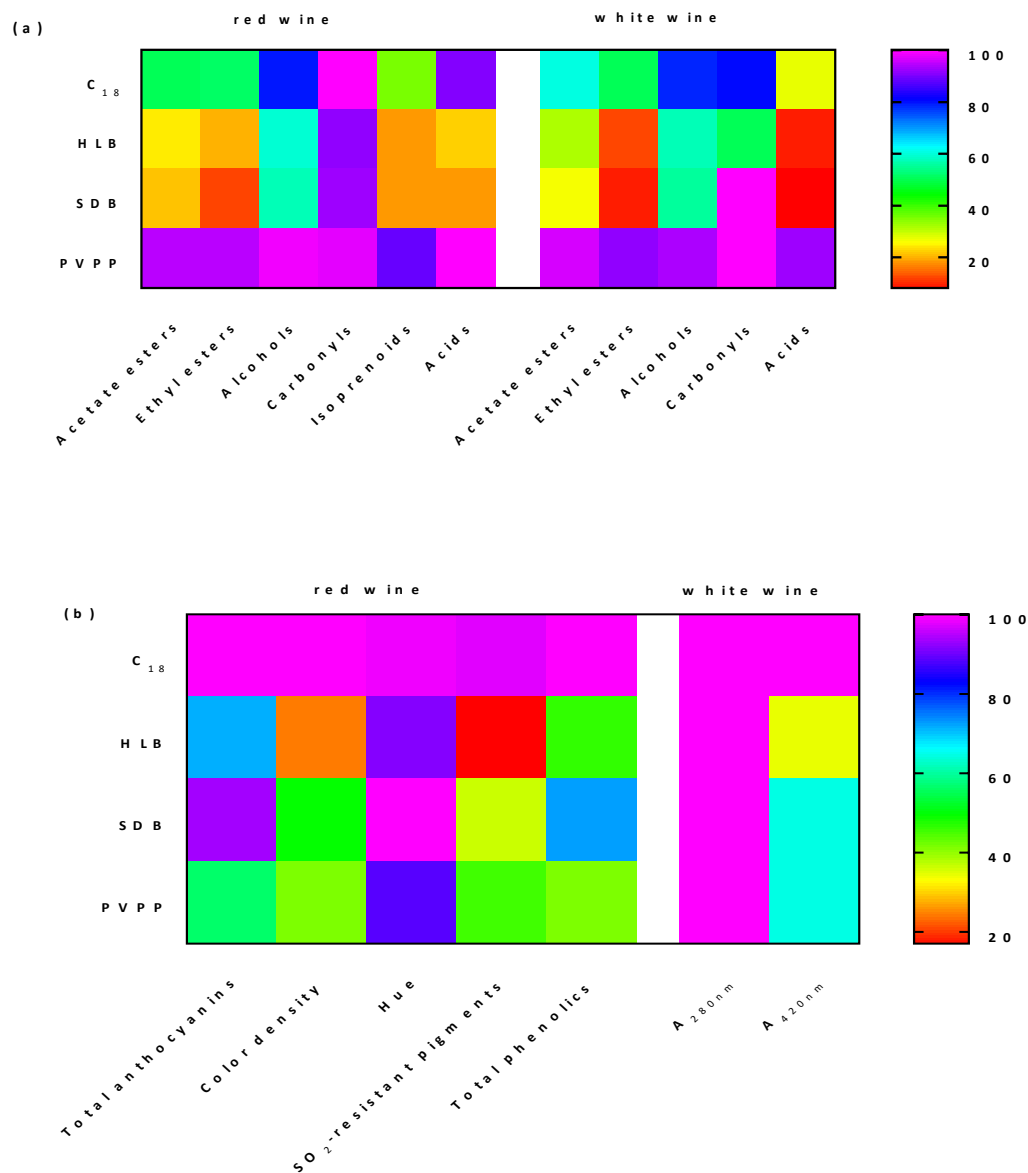
	<b>C<sub>18</sub></b>	<b>Oasis HLB</b>	<b>Strata SDB-L</b>	<b>PVPP</b>	<b>Control</b>
A <sub>280</sub>	5.6 ± 0.3a (100%) <sup>a</sup>	5.3 ± 0.1b (100%)	5.4 ± 0.1ab (100%)	5.4 ± 0ab (100%)	5.2 ± 0.1b
A <sub>420</sub>	0.18 ± 0.00a (100%)	0.06 ± 0.00d (35%)	0.11 ± 0.00c (65%)	0.11 ± 0.00c (65%)	0.17 ± 0.00b

Data for each sorbent treatment are presented as mean ± SD (n = 3) with different letters across a row indicating significantly different ( $p \leq 0.05$ ) means according to one-way ANOVA with Fisher's (LSD) pairwise comparison. <sup>a</sup> Value relative to the control.





**Figure S1.** Gas adsorption isotherms of (a) PVPP; (b)  $\text{C}_{18}$ ; (c) Oasis HLB; (d) Strata SDB-L, and (e) pore size distributions of the four commercial sorbents.



**Figure S2.** Relative amounts of (a) grouped volatile compounds remaining in wines compared to the control (%) and (b) values of color/phenolic parameters relative to the control (%) after treatment for the four commercial sorbents.

## **CHAPTER 5**

### **Concluding Remarks and Future Perspectives**

## 5. Concluding remarks and future perspectives

### 5.1 Conclusions

A series of polymers (magnetic, non-magnetic, putative imprinted, non-imprinted, thermally synthesised, microwave synthesised, different components) were prepared to remove excessive potent odorant-IBMP as post-harvest remedial treatments. The polymers were analysed by physical characterisations (SEM, FTIR, gas adsorption analysis) and adsorption tests towards IBMP and other wine components. Moderate removal of IBMP, specificities towards groups of compounds and magnetic separation were observed among the polymers in different wine matrices, however, specific binding of IBMP was not achieved.

#### 5.1.1 Preparation of magnetic polymers for the elimination of 3-isobutyl-2-methoxypyrazine from wine

Magnetic polymers have been successfully prepared via multi-step polymerisation, along with their non-magnetic counterparts, and an attempt to produce (magnetic) imprinted polymer was made, using 2-methoxypyrazine as a template molecule, fulfilling Objective 1. Thermal and microwave-assisted synthesis were adopted and compared and magnetic separation was achieved by externally applying a permanent magnet. Physical characterisation results (SEM and FTIR) confirmed the incorporation of magnetic nanoparticles and showed no evident difference between the putative imprinted and non-imprinted polymers, as well for the thermally and microwave synthesised polymers. Adsorption isotherms were determined in model wine solution spiked with IBMP to evaluate the sorption properties of the polymers. No significant difference was found among the putative imprinted and non-imprinted, and magnetic and non-magnetic polymers. Isotherm models (linear and non-linear) were applied to the thermally synthesised magnetic polymers (putative imprinted and non-imprinted) to further investigate the sorption pattern and linear models (Langmuir Type 2 and Freundlich) were found to have better fit with the polymers. Adsorption kinetics and reusability analysis were carried out on the thermally synthesised magnetic polymers (putative imprinted and non-imprinted) and results showed a prompt equilibrium within ten minutes and stable sorption ability for at least five cycles of the polymers. The thermally synthesised magnetic polymers (putative imprinted and non-imprinted) could remove up to 40% or more of IBMP from two spiked white wines as was the case for model wine.

Higher sorption of IBMP on putative imprinted polymer compared to non-imprinted polymer was not observed in either white wine or model wine, and this may indicate the specific binding, if there was any, was overwhelmed by hydrophobic interactions. Several factors could be considered to improve polymer selectivity; functional monomers with greater H-bonding capability could be adopted; and solvents of different polarities (non-polar, apolar, polar) may be trialled to reinforce the interactions between template and monomers.

#### 5.1.2 Chemical and sensory evaluation of magnetic polymers as a remedial treatment for elevated concentrations of 3-isobutyl-2-methoxypyrazine in Cabernet Sauvignon grape must and wine

Preliminary investigation of IBMP sorption on putative imprinted magnetic polymer and non-imprinted magnetic polymer in model wine matrix was performed. The practical usage of magnetic polymer as a pre- or post-fermentation treatment for IBMP removal in winemaking, and effects on other wine volatiles and colour parameters as outlined in Objective 2, were further investigated along with a polylactic acid (PLA) based film used as post-fermentation treatment for comparison.

A different functional monomer, methacrylic acid (MAA) instead of methyl methacrylate (MMA), and porogen solvent (acetonitrile: H<sub>2</sub>O 9:1 v/v as a substitute for toluene) were trialled to improve the specificity of the putative imprinted polymers, however, no evident differences were observed between the putative imprinted and non-imprinted polymers on either wine volatiles including IBMP, or colour according to chemical and sensory analysis. Despite this, the magnetic polymers could remove up to 74% of IBMP (20 ng/L) through post-fermentation treatment, compared to 18% for that of PLA treatment. Sensory analysis was in line with the chemical analysis, that wines treated post-fermentation with magnetic polymers were detected without any IBMP related 'green' attributes, whereas the PLA treated wines were still perceived to be herbaceous. The lower remedial treatment effect of PLA may be due to the limited surface area and slower adsorption kinetics. Although the most effective treatment (post-fermentation addition of polymer) had evident impact on most of the wines volatiles and colour, there was no detectable difference in overall aroma intensity and fruity attributes among all the treatments. In terms of pre-fermentation addition, the magnetic polymers removed 20-30% less IBMP than the post-fermentation counterparts but their impact on other wine components was also lower. The role of

IBMP as a character impact compound in wine aroma profiles was observed and synergistic or masking interactions among other wine aroma compounds was apparently also perceived.

### 5.1.3 Extraction properties of new polymeric sorbents applied to wine

Given the lack of difference in sorption properties between magnetic and non-magnetic polymers, subsequent trials were carried out with non-magnetic polymers in an attempt to improve the polymer imprinting process for more selective removal of IBMP. Prepared polymers were also potentially suited to acting as solid-phases for extraction of other wine components such as phenolic and volatile compounds. Several combinations of different functional monomers with various ratios to cross-linker, and multiple porogen solvents were trialled by both thermal and microwave synthesis. The newly prepared polymers were found to have different physicochemical properties (surface area, pore size distribution, polarity) and sorption specificities towards different groups of compounds in wine, which met Objective 3. The polymers could potentially be used as fining agents, applied as polymeric sorbents for solid-phase extraction or stationary phase for liquid chromatography.

Thermal synthesis was found to produce polymers with higher surface area, and on the other hand, narrower pore size distribution with a dominance of micro- and mesopore was observed with microwave synthesis. Polarities of the sorbents were influenced by the copolymerised functional monomers as a function of the hydrophilic components. Sorbent made by microwave synthesis in dichloromethane with 4-vinylbenzoic acid as co-monomer was found to have higher affinity towards non-polar, small molecular weight compounds (e.g., various volatiles) in a white and a red wine. Sorbents made by microwave in dichloromethane with methacrylic acid and acrylic acid as hydrophilic monomers, respectively, were observed to have higher retention of moderately polar, small to large molecular weight compounds (e.g., pigments and other phenolics) in wines. Sorbent made thermally in acetonitrile with double the amount of methacrylic acid as co-monomer may be more polar and has the potential for preferential extraction of carbonyl compounds. Four commercial sorbents ( $C_{18}$ , Oasis HLB, Strata SDB-L, and PVPP) were investigated alongside as comparison. Oasis HLB and Strata SDB-L had higher retention of both volatile and phenolic compounds among the commercial sorbents, with Oasis HLB having higher sorption toward moderately polar compounds than that of Strata SDB-L.  $C_{18}$  had preferential sorption toward volatile compounds, and on the other hand, PVPP had higher affinity

toward phenolics. Our synthesised polymers had some similarities with the analysed commercial sorbents regarding hydrophobicity and hydrophilicity, moreover, with supposedly better wettability and selectivity.

## 5.2 Future perspectives

Computational modelling, UV-vis spectrophotometry and nuclear magnetic resonance titration could be adopted to evaluate the interactions between template and functional monomer molecules prior to polymer synthesis, and thus be used as pre-selection tools for optimal polymer design. Interactions between the templates and functional monomers involved in this study were mainly non-covalent. On the other hand, covalent and cleavable semi-covalent binding may also be considered for stronger interactions and greater imprinting effect. The selection of porogen solvent is crucial to successful imprinting, as the solvent may interfere with the interactions between template and monomers, either reinforcing or diminishing the interactions. The more similar the porogen solvent to the composition of the matrix in which the polymers are to be used, the higher chance there is to observe the same specificity in porogen solvent and treated samples.

An aqueous environment is challenging for imprinting of polymers due to its H-bonding polar attributes, which may largely interfere with the interactions between template/target molecule and functional monomer. Furthermore, most monomers, cross-linkers and initiators are insoluble/immiscible in water. One of the solutions to recover specific binding in aqueous solutions is to modify an imprinted polymer with hydrophilic shell/brushes to make it water-compatible (Pan, et al. 2011). However, wine as a hydroalcoholic beverage, has another significant interfering factor, being an abundant amount of tartaric acid that influences pH and ionic strength. These factors and challenges could all be taken into account in future studies to achieve imprinted polymers for specific removal of IBMP or other fault compounds in wine.

The incorporation of magnetic nanoparticles provided an innovative and promising separation method, especially for water compatible/soluble polymeric sorbents. However, to scale up from laboratory trials to industry application, electromagnet separation should be considered for faster and more efficient polymer removal after wine treatment, as the adsorption efficiency of magnetic particles on a permanent magnet is quite limited and therefore impractical.

Based on the results from the present study with respect to use polymers as sorbents for analytical purposes, more efficient polymeric sorbents could be synthesised by adjusting the type and amount of hydrophilic monomers, solvents and production methods. The challenges for polymeric sorbents applied in wines may be the same as those stated above for imprinted polymers for specific IBMP removal. In summary, a range of new polymeric sorbents (magnetic, imprinted, selective and specific) could be applied in wine for both analytical and remedial purposes.

### Reference

Pan, G., Zhang, Y., Ma, Y., Li, C., & Zhang, H. (2011). Efficient one-pot synthesis of water-compatible molecularly imprinted polymer microspheres by facile RAFT precipitation polymerization. *Angew. Chem. Int. Ed.* 50(49), 11731-11734.



## List of abbreviations

3-SH, 3-sulfanylhexasan-1-ol

3-SHA, 3-sulfanylhexasyl acetate

4-VBA, 4-vinylbenzoic acid

AA, acrylic acid

ACN, acetonitrile

AIBN, 2,2'-azobisisobutyronitrile

AR, analytical reagent

ATR-FTIR, attenuated total reflectance Fourier transform-infrared spectroscopy BET,

Brunauer–Emmett–Teller

CRP, controlled radical polymerisation

CVA, canonical variate analysis

DA, descriptive analysis

DAP, diammonium phosphate

DCM, dichloromethane

DMMP, 2,5-dimethyl-3-methoxypyrazine

DVB, divinylbenzene

EDGMA, ethylene glycol dimethacrylate

GC–MS, gas chromatography–mass spectrometry

HPLC–MS/MS, high-performance liquid chromatography–tandem mass spectrometry

HS-SPME, headspace-solid phase microextraction

IBHP, 3-isobutyl-2-hydroxypyrazine

IBMP, 3-isobutyl-2-methoxypyrazine

IPMP, 3-isopropyl-2-methoxypyrazine

LBT, ladybug taint

MAA, methacrylic acid

MALB, multicoloured asian lady beetles

MIP, molecularly imprinted polymer

MMA, methyl methacrylate

MMIP, magnetic molecularly imprinted polymer

MPs, alkylmethoxypyrazines  
MPS, 3-(trimethoxysilyl)propyl methacrylate  
MW, microwave  
NIMP, non-imprinted magnetic polymer,  
NIMP<sub>preferment</sub>, pre-fermentation treatment with non-imprinted magnetic polymer  
NIMP<sub>postferment</sub>, post-fermentation treatment with non-imprinted magnetic polymer  
NIP, non-imprinted polymer  
OAV, odor activity value  
PAA, poly(acrylic acid)  
PCA, principal component analysis  
PIMP, putative imprinted magnetic polymer  
PIMP<sub>preferment</sub>, pre-fermentation treatment with putative imprinted magnetic polymer  
PIMP<sub>postferment</sub>, post-fermentation treatment with putative imprinted magnetic polymer  
PLA, polylactic acid  
PMAA, poly(methacrylic acid)  
PMMA, poly(methylmethacrylate)  
PMS, potassium metabisulfite  
PS-DVB, polystyrene-divinylbenzene  
P4-VBA, poly(4-vinylbenzoic acid)  
PVPP, polyvinylpyrrolidone  
RAFT, reversible addition/fragmentation chain-transfer  
SBMP, 3-*sec*-butyl-2-methoxypyrazine  
SEM, scanning electron microscopy  
SPE, solid phase extraction  
TA, titratable acidity  
TCA, 2,4,6-trichloroanisole  
TEOS, tetraethoxysilane  
VSM, vibrating sample magnetometer  
XRD, X-ray diffraction