

Methods for reducing methoxypyrazines in grape & grape products



Introduction to the Innovation

3-Alkyl-2-methoxypyrazines (MPs) represent an important & potent class of grape- & insect-derived odor-active compounds associated with wine quality¹⁻⁴. Specifically, 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) & 3-sec-butyl-2-methoxypyrazine (SBMP) are 3 grape-derived volatile compounds that elicit green & vegetative aroma & flavour descriptions in wine. Although these MPs can positively influence wine quality in varieties like Sauvignon Blanc, at higher concentrations they are dominant & unpleasant, can mask fruity/floral aromas, & are associated with cooler climates & underripe, low quality fruit⁵. Recently, a further source of MPs has been identified in wine, of insect origin. Specifically, MPs from the Multicoloured Asian ladybeetle (MALB), Harmonia axyridis & the seven-spotted ladybeetle (C7), Coccinella septempunctata, have been found in wine⁶⁻⁹. When these ladybeetles are inadvertently incorporated with grapes at harvest, elevated MP concentrations are found in the wine, concurrent with development of an off-flavor coined 'ladybug taint' (LBT)^{10,11}. IPMP is the primary compound responsible for LBT^{2,3,9} although IBMP^{2,12} & SBMP¹² have also been identified at lower concentrations in some affected wine. Subsequently, MALB is one of the greatest threats to the industry this decade¹³. The industry has instigated a zero tolerance policy for ladybeetles in grapes intended for juice & wine production, with subsequent substantial economic losses to grape growers. Wine grapes are a high value crop. worth \$1,416/tonne or >\$14,000/ha¹⁴. MPs, regardless of source, are very resistant to removal by traditional grape & wine processing measures¹⁵. Thus, novel approaches to MP remediation, are both timely & necessary to protect & grow Ontario's \$1.5 billion¹⁶ wine industry.

Results

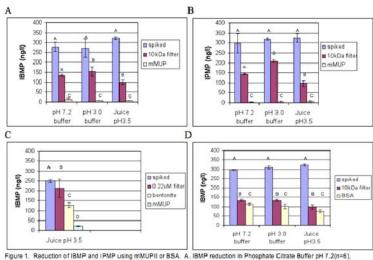


Figure 1. Reduction of IBMP and IPMP using mMUPII or BSA. A. IBMP reduction in Phosphate Citrate Buffer pH 7.2(n=8). Phosphate Citrate Buffer pH 3.0 (n=6) and Chardonnay Juice pH 3.5 (n=3) using mMUPII and the 10 MWCO PES filtration system; B. IPMP reduction in Phosphate Citrate Buffer pH 7.2 (n=3), Phosphate Citrate Buffer pH 3.0 (n=3) and Chardonnay Juice pH 3.5 (n=3) using mMUPII and the 10 MWCO PES filtration system; C. IBMP reduction in Chardonnay Juice pH 3.5 (n=3) using mMUPII and the bentonite system; D. IBMP reduction in Phosphate Citrate Buffer pH 7.2 (n=3), Phosphate Citrate Buffer pH 3.0 (n=3) and Chardonnay Juice pH 3.5 (n=3) using BSA and the 10 MWCO PES filtration system. Treatments which resignificantly different are represented by different letters. Data analysis was performed using ANOVA and mean separation by Fisher LSD (P<0.05)

Two technologies are currently in development to reduce MP levels in juice &/or wine; firstly, the use of proteins with naturally high affinity for MPs, & secondly, a plastic polymer-based system for direct sorption of MPs in juice & wine. The lipocalin protein family contain such proteins¹⁷. We have cloned two proteins^{18,19} (piglet Odorant Binding Protein pIOBP & murine Major Urinary Protein mMUPII) & expressed them in Pichia pastoris, purified these secreted proteins from the growth media & shown they are able to bind to & remove MPs from both buffer & grape juice²⁰. Two systems removed the soluble protein-MP complex from the buffer/juice media after protein addition: a filtration system using a polyethersulfone (PES) membrane resulting in

MP levels in the filtrate below the LOQ of the measurement technique (Fig 1A, B); & the second using bentonite fining to bind the soluble protein-MP complex, reducing MPs when the bentonite is separated from the media (Fig 1C) 20 . Similar results for mMUPII & pIOBP were found for both IBMP & IPMP using both protein removal systems 20 . The reduction of MPs down to the LOQ requires the high affinity MP-binding proteins as demonstrated through lack of MP reduction using bovine serum albumin 20 . The PES membrane itself removed up to 1/3 of the starting MPs from the reaction prior to protein addition (Fig 1), even when MP levels were only at 40 ng/L 20 .

We recently also demonstrated the capacity of synthetic wine closures to reduce MP concentrations in wine²¹. Synthetic closures (5 units/L & 10 units/L) were soaked in a Chardonnay wine enriched with 40 ng/L each of IBMP, SBMP & IPMP, resulting in a 70% - 89% reduction in MPs. These findings show unequivocally for the first time, that synthetic cork has significant binding affinity for this important class of wine impact odorants. The closures used consist primarily of various polyethylene- (PE) based polymers, which we hypothesize are the active constituents in this result through direct adsorption of MPs. While volatile sorption by these polymeric materials has been studied extensively in food products, it is poorly described in wine²², thus presenting an exciting opportunity for novel technology development. In other foodstuffs, flavour compound sorption is heavily dependent on polymer polarity, density & surface area as well as volatile polarity & concentration; the interactions are complex & no comprehensive models exist to predict polymer-volatile interactions, therefore studies must be designed to test a range of polymeric materials to preserve/create optimal aroma profile²².

Application of Technology

We are continuing to examine the capacity of various polymers & immobilized MP-binding protein(s) in removing MPs to levels acceptable by wine consumers, & investigate potential delivery systems on a lab & then 'micro-vinification' scale. Criteria for optimal performance of specific polymers, immobilized proteins & delivery systems will also consider other constituents of wine quality, particularly odor-active compounds²³. By removing MPs that cause ladybug taint & greenness-eliciting MPs from juice & wine, the sensory quality of Canadian wine & profitability of the industry will be improved in (i) wine affected by LBT, (ii) wine from climatically marginal appellations within Canada, & (iii) wine from throughout Canada produced in poor vintages (wet &/or reduced growing days or when fruit breakdown occurs prior to reaching physiological ripeness).

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¹Allen et al 1991 AJEV 42(2):109-112. ²Pickering et al 2005 J Food Sci 70 (2):S128-S135. ³Pickering et al 2006 IN Dris, R(ed) Crops: Growth, Quality & Biotechnology!II. Quality Management of Food Crops for Processing Technology.WFL Publisher, Meri-Rastilan tie 3 C, Helsinki, Finl&,785-794. ISBN 952-91-8601-0. ⁴Pickering et al 2007 Vitis 46(2):85-90.⁵Allen & Lacey1998 In:Waterhouse & Ebeler eds Chemistry of Wine Flavor American Chemical Society New York 31-38.⁵Nalepa et al 2004 Am Entomol 50:165-166. ⁵Nalepa et al 2005 Environ Entomol 34:425-431. ³Glemser 2010 MSc Thesis School of Environmental Sciences, University of Guelph. ¹Botezatu & Pickering 2010 IN:Reynolds, A. G. Underst&ing & Managing Wine Quality & Safety. Woodhead Publishing Limited, Cambridge, U.K. (in press). ¹¹Pickering et al 2004 AJEV 55 (2):153-159. ¹¹Pickering & Lin 2006 IN Dris, R(ed.) Crops:Growth, Quality & Biotechnology. III. Quality Management of Food Crops for Processing Technology. WFL Publisher, Meri-Rastilan tie 3 C, Helsinki, Finl&, 778-784. ISBN 952-91-8601-0. ¹²Unpublished data from Pickering Lab.¹³OGWRSC Annual Report 2002-2008 Printer of the Gov't of Ont.¹⁴ OMAFRA 2009.http://www.omafra.gov.on.ca/English/stats/hort/index.html.¹⁵Pickering et al 2006 Inter J Food Sci Tech 41:77-86. ¹⁴Bramble et al 2007 IN:The Golden Grape -Wine, Society & Globalization. Multidisciplinary Perspectives on the Wine Industry. Palgrave Macmillan, NY, 63-86. ¹¹Flower, D.1996 Biochem J 318:1-14. ¹³Tegoni et al 2000 Biochem, Biophys Acta 1482:229-240. ¹³Cavaggioni et al 1990 Comp Biochem Physiol 968:513-520. ²⁰Inglis et al. 2010. Method For Reducing Methoxypyrazines in Grapes & Grape Products. PCT application based on US Provisional Patent No. 61/169,121 filed April 14, 2010. ²²Pickering et al 2010 J Food Agric & Envir 8: in press. ²²Sajilata et al 2007 Comp Rev Food Sci Food Safety 6:17-35. ²³Blake et al 2009 J Agric Food Chem 57:4680-4690.