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The Australian Wine Research Institute

Flavour precursors: contribution to wine aroma, in-mouth sensory properties and flavour release

FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AWRI 3.1.3**

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Project 3.1.3 – Flavour precursors: contribution to wine aroma, in-mouth sensory properties and flavour release

Abstract

Glycosides were previously thought to be flavourless, needing the action of enzymes during fermentation or slow chemical reactions during wine ageing to release and express their flavour. This project has established that the presence of glycosides in wine and their concomitant breakdown during tasting can boost desirable 'fruity' and 'floral' lingering flavour attributes. As a persistent aftertaste is a hallmark of quality wines, flavour release from glycosides may be a key factor differentiating between good and excellent wines.

In this project, experiments were conducted with sensory panels tasting glycosides from white wines and parallel measurement of flavour compounds in saliva or in the mouth. The results confirmed that enzymes in the saliva act like a key to a locked door, releasing a wave of additional flavour that can be perceived over the time after swallowing, creating a positive long-lasting fruity flavour sensation.

Adding purified glycosides to a juice or wine resulted in increased flavour with no negative characteristics; it was also established that white grape skins are a readily available source for extraction of glycosides which can be used as a natural flavour boost that has potential for an easily controlled new way of enhancing a wine's sensory properties. Finally, knowledge about the profile and/or concentrations of glycosides in grapes or a wine could be used by winemakers as a quality or style indicator.

Executive summary

This project demonstrated the potential of non-volatile glycosides as flavour precursors during wine consumption. The studies on floral varieties such as Riesling and Gewurztraminer showed that there is a surprising ability of in-mouth enzymes, most likely from salivary bacteria, to quickly liberate volatile aroma compounds from their bound form during wine drinking, enhancing flavour and contributing to a lingering aftertaste. The quantity of flavour release and/or retronasal perception of flavour released by this mechanism seems to be fairly variable across individuals, suggesting one reason for variation in people's sensory perception of wine and, potentially, their preferences.

To further corroborate this work, a winemaking experiment was completed to explore various methods winemakers might use to intensify the contribution of these precursors in their wines. Several sets of grape juices were treated to increase their level of glycosides, and following fermentation, sensory and chemical analyses were completed on the finished wines, as well as wines with glycosides added prior to bottling. The addition of glycosides had a major effect on wine aroma and flavour, enhancing 'fruity'/'floral' attributes with no effect on bitterness or astringency. Chemical data showed an increase in key aroma compounds as a result of glycoside addition, as well as higher levels of intact glycosides, acting as flavour precursors and contributing to an enhanced persistence of 'fruity'/'floral' flavour.

The demonstrated benefit of increasing the amount of glycosides present in wines to boost desirable flavour and flavour persistence opens up the option for wine producers to make additions of glycosides isolated from grape skins, or indeed changing vineyard management practices to increase glycoside concentrations. Further studies of the practical application of glycoside preparations from grapes in a production setting should be completed, as well as work on other commercially important grape varieties and investigations of other precursor classes, notably amino acid conjugates of sulfur aroma compounds.

Background

Potent aroma compounds can be present in grapes and wines in a bound form (e.g. glycoconjugates or amino acid conjugates) and may be released during winemaking and storage (Parker et al. 2017a). The pool of precursors can break down through chemical hydrolysis reactions, or through the action of yeast or bacterial enzymes, significantly changing the aroma profile of a wine, a fact that can be exploited by winemakers to influence flavour profiles and persistence in wine. Figure 1 provides a schematic representation of flavour release.

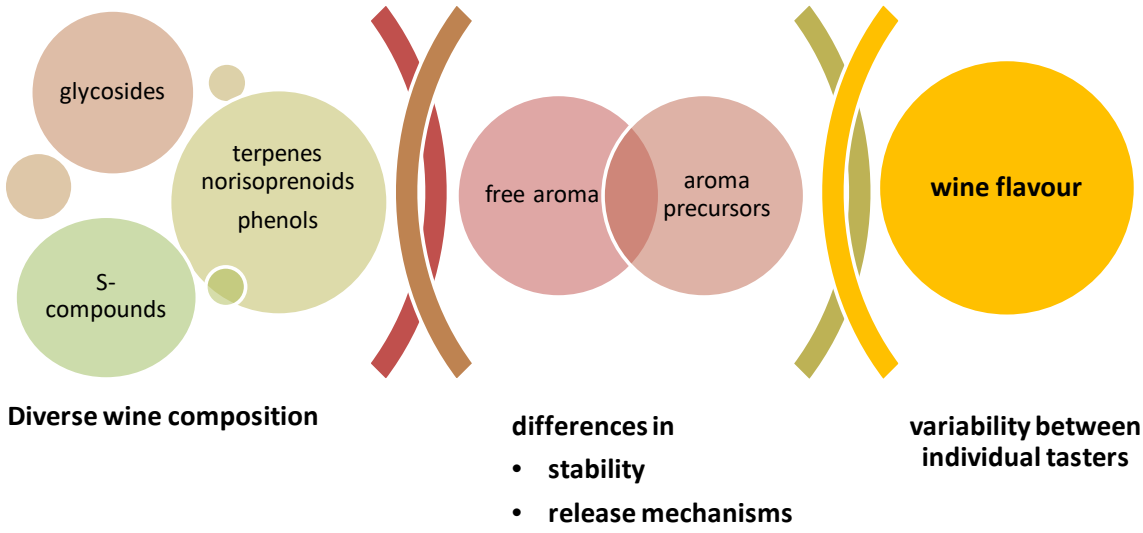

Figure 1. Illustration of free volatiles and precursor compounds involved in wine flavour release

Previous studies conducted at the AWRI from the late 1980s to the late 1990s found that there are numerous glycoconjugates of aroma compounds in grapes and wine and a relatively simple, although broad, analytical quality measure (the 'GG assay') was developed from this work. The important variety Shiraz was not studied to any extent at that time. Since then, analytical tools have progressed significantly, with options now available to characterise key precursors, glycosides and sulfur-conjugates by LC-MS and the ability to measure volatiles in expired air.

An important outcome from recent AWRI smoke taint research was that glycosidically bound aroma compounds can be released in-mouth and add directly to the flavour and aftertaste of a wine (Parker et al. 2012). As some sulfur compounds have very low odour thresholds, they are of particular interest, and an interaction of saliva with precursors to give free 'tropical fruit' thiol aroma compounds has previously been demonstrated in other food areas. The release of volatiles in-mouth after swallowing was described as a delayed perception of retronasal odour, and is of particular interest. A more comprehensive understanding of the role of aroma precursors and of their sensory impact is indispensible to fully understanding wine flavour, flavour complexity and perception.

Model studies so far have shown that some glycosides - including those of non-volatile phenolics - are susceptible to hydrolysis in the mouth, through the activity of human enzymes and/or enzymes derived from oral microflora, although their direct sensory significance was not demonstrated. An early indication of hydrolysis of esters in-mouth was reported. Studies in vegetable flavour showed that a cysteine conjugate precursor to the potent thiol 3-mercaptohexanol was broken down in-mouth by salivary bacteria, with sensory impact of aftertaste for up to three minutes. In recent studies at the AWRI, smoke taint-related volatile phenols were released from their bound

glycoconjugate form to produce important retronasal flavour (Parker et al. 2012), and it was found that this effect occurred in the presence of ethanol and acid. The specificity of release in-mouth is not known, that is, whether all glycoside types will break down as a result of certain salivary bacteria and enzymes or only a small proportion. From preliminary work carried out at the AWRI, non-volatile fractions of glycosides from wines not affected by smoke taint have also been shown to be broken down in-mouth, releasing 'fruity' flavour. The degree of importance of fruit flavour released from glycosides relative to other sources is not clear.

Australian winemakers aim to produce wine styles to suit specific markets, which is reliant on sourcing a suitable supply of grapes and selecting production methods to achieve the target style. Markers for measuring style have long been sought to inform production. However, relatively few objective measures of volatile grape and wine compounds which can be directly related to style are currently available. Precursor compounds have previously been considered a pool of potential flavour rather than as flavorants themselves, and this research will open up the identity of classes of compounds that are highly important to wine flavour intensity and which can act as flavour markers. The slow breakdown of precursors during wine ageing in bottle may be a cause of loss of some flavour in-mouth over time, but degradation of key free volatiles such as thiols is typically much faster and an understanding of these phenomena could open ways of increasing shelf-life or cellar-life of wines.

Despite their relevance in the consumer liking of wines, important phenomena such as the role of retronasal aroma and in-mouth aroma release are scarcely characterised in food analysis, and the wine industry could greatly benefit from the development of this knowledge, as such information will result in helpful decision tools and analytical methods for producing wines with certain desired in-mouth sensory characteristics.

Highlights

- Glycosides in wine, previously considered to be flavourless, were shown to release flavour during consumption, enhancing fruit characters and aftertaste without giving any bitterness.
- The flavour release was highly variable across individuals, providing further insight into possible reasons for individual differences in perception and wine style preferences.
- Increasing glycoside concentration in wines, by addition of glycosides isolated from grape skins from a floral variety high in monoterpene glycosides using a simple procedure, gave increased 'fruit'/ 'floral' aroma and flavour with no bitterness and has potential as a practical means of enhancing flavour in some wine types

Objectives

The project has the overall goal of understanding the flavour of wine in-mouth that is due to precursor compounds as well as retronasal (aroma by mouth) perception of volatiles. The project has the following specific objectives:

- To characterise glycoconjugates and amino acid conjugates of volatile compounds from Shiraz and other varieties, including volatile hydrolysis products.
- To assess the extent and sensory significance of in-mouth breakdown of glycosides, amino acid conjugates and free volatiles such as esters.
- To determine the relationship between levels of key precursors in grapes and the amounts remaining in wines.
- To determine the effect of other wine constituents such as alcohol and non-volatiles on in-mouth release and sensory properties of precursor-derived volatiles.

The project will provide insight into the consumer experience when drinking a wine, including the intensity of flavour and the persistence of aftertaste, rather than on perceptions by orthonasal

evaluation by simple sniffing as often practiced by winemakers and other experts. The project will lead to analytical targets for quality markers for grapes and wines.

Method

Glycoconjugates were isolated from grapes and wines using polymeric adsorbents and analysed using LC-MS methods (Parker et al. 2017b). Importantly, the phenolic glycosides that contribute to bitterness were removed using a high pH wash. The hydrolysis products of the glycoconjugates were determined through enzyme and elevated temperature acid hydrolysis, followed by GC-MS (Parker et al 2017b). Synthetic glycosides were also produced for study.

In assessing in-mouth release, conditions were developed for capturing volatiles in the oral cavity following from preliminary work conducted at the AWRI, using in-mouth solid phase microextraction (Mayr et al. 2014). Volatiles released from isolated and synthetic precursors were determined with in-mouth volatile release *in vivo* studied as well as incubation with saliva *in vitro* (Parker et al 2017b). Matrix conditions were evaluated, notably alcohol and pH. Sensory studies were conducted, using time intensity methods (Parker el al 2017b), expert assessments and a sensory descriptive analysis study. A range of subjects were used, with pre-screening to assess variation in salivary breakdown ability, as previously observed. Application of chlorhexidine gluconate mouthwash was used to investigate whether in-mouth biota elimination is a cause of this variation.

A winemaking study was completed with precursors derived from grapes added to juices and wines, followed by sensory and compositional analysis, using procedures detailed in Mayr et al. (2014).

Results and discussion

In initial studies, extracts isolated from Chardonnay and Shiraz juice gave negligible flavour in contrast to Gewürztraminer or Riesling samples, which showed major flavour effects. Further investigations concentrated on glycosides from Gewürztraminer and Riesling (Parker et al 2017b).

Experiments showed that detectable monoterpenes were released both *in vivo* and *in vitro* through interaction of glycosides with saliva. Up to 80% release of monoterpenes could be achieved during 30-minute incubation of glycosides isolated from Gewürztraminer with saliva. From expired air trapping using a stir bar sorptive extraction technique, it was surprising to be able to detect monoterpenes using GC/MS after subjects tasted glycoside material, as it was expected that any release would be only apparent by GC-olfactometry, as previously demonstrated with smoke glycosides. The ability of subject's saliva to breakdown the glycosides varied in extent across individuals, from 26% to 76% release, in line with previous work on volatile phenol glycosides. Interestingly, even the saliva from those individuals who could not perceive any flavour when tasting precursors could break down glycosides and release monoterpenes, which indicates perceptual or in-mouth retronasal air flow differences among individuals may be more important than differences in saliva microflora. This experiment showed that enzymes or microflora in saliva are capable of liberating free monoterpenes from their glycosides during 30 minutes of incubation, but to be relevant to flavour, this must happen in a shorter time frame in the range of seconds to minutes.

The glycoside profile of 15 Gewürztraminer and 16 Riesling wines was determined. There is little quantitative information in the literature on the concentration of specific glycosides in wine. Using a LC-MS method developed for the purpose, with synthesised internal standards, it was found that geranyl glucoside was the major glucoside, with multiple disaccharide monoterpene glycosides also observed. The Gewürztraminer wines had much higher total monoterpene glycoside concentration than the Riesling wines, from 300-1800 µg/L compared to 20-140 µg/L.

Volatiles released from the Gewürztraminer glycosides upon enzyme hydrolysis included the monoterpenes α-terpineol, nerol and geraniol, as well as the less important octanol, decanol, benzyl alcohol and 2-phenylethanol. The Riesling glycosides gave similar volatiles, with a lower relative abundance of geraniol, and higher octanol and decanol abundance. These results confirmed the presence of enzyme-labile precursors, and provided further supporting evidence that glycosides in wines from these varieties have the potential to give rise to aroma-active compounds during consumption.

The sensory importance of monoterpene glycosides during tasting was assessed, to determine whether odorous aglycones were released in-mouth. Monoterpene glycosides were isolated from Gewürztraminer and Riesling wines and juices, characterised using LC-MS, and studied using a time-intensity technique, together with a synthesised monoterpene glucoside. When assessed in model wine at five times the concentration expected in wine, Gewürztraminer glycosides isolated from either juice or wine, together with geranyl glucoside, gave significant flavour and enhanced aftertaste (Figure 2).

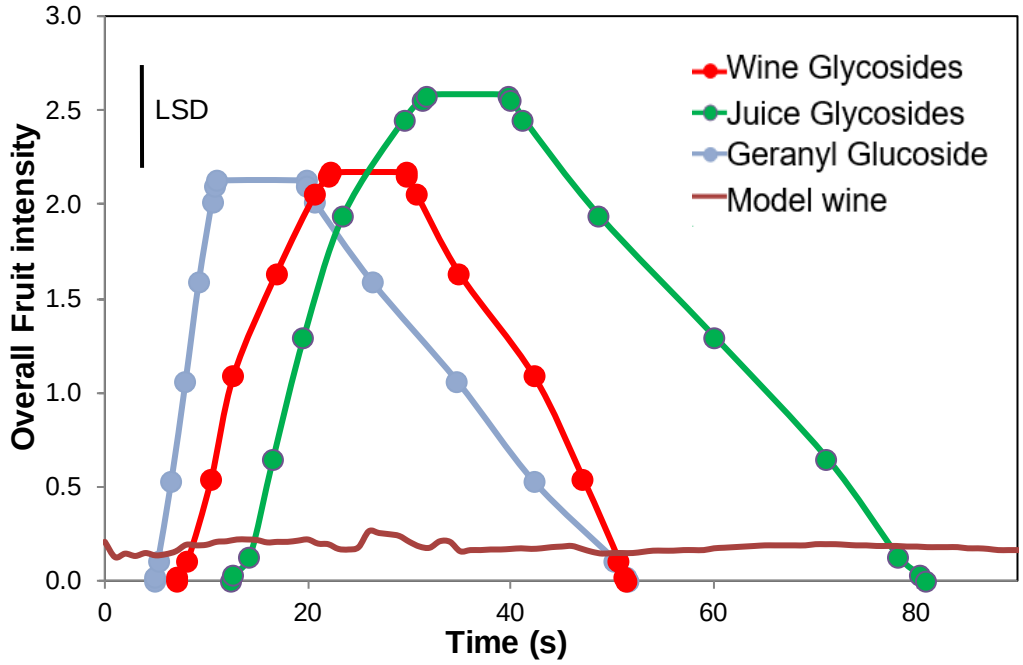

Figure 2. Mean time-intensity sensory rating curves for precursor samples assessed in a model wine. Also shown is data for a model wine control. LSD: least significant difference (P=0.05) for the maximum intensity parameter.

To assess the relative effect of glycoconjugates of Riesling and Gewürztraminer compared to free volatiles, they were assessed in a more challenging, but more realistic, model wine system at single strength using a time intensity methodology. Combinations of the glycoconjugates and volatiles were tested at levels closely comparable to those found in Riesling and Gewürztraminer wines. There was an indication that the monoterpene glycosides enhanced the duration and intensity of the perceived flavour. A third of the judges were most responsive to the flavour from the glycosides, and for this sub-group, the glycosides isolated from Riesling contributed significant flavour, with the combination of Riesling volatile aroma compounds and the precursors giving the longest duration of aftertaste compared to the volatiles alone. An example of one judge's responses is shown in Figure 3. This result means that at wine-like concentrations, with the influence of ethanol and wine pH, release of monoterpenes from glycoside precursors for most people is relatively subtle, but likely to

be an important source of flavour for varieties such as Riesling. It may be that the effect is larger for wines of lower alcohol or higher pH. The results indicate that important flavour may be contributed by the glycosides at wine-like concentrations for a proportion of the population.

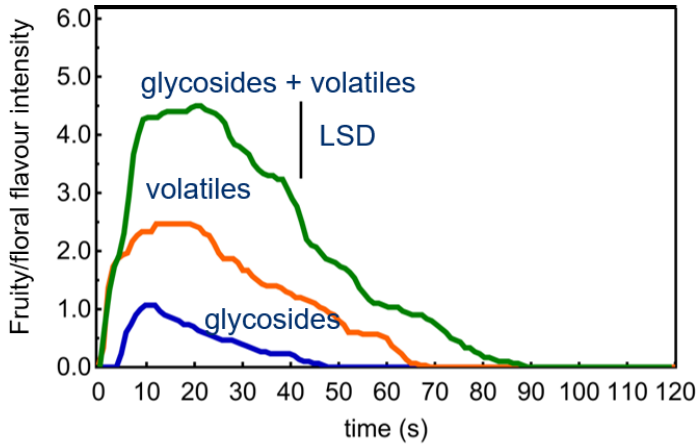

Figure 3. Average time-intensity curves (mean of three presentation replicates) for one judge who responded to the glycosides: for glycosides (blue), free volatiles (orange) and glycosides plus free volatiles (green) isolated from a Riesling, and assessed at wine-like concentration. The solid vertical bar is the least significant difference at the 5% level of significance for the maximum intensity parameter.

Gewürztraminer glycosides, geranyl glucoside and guaiacyl glucoside were investigated using a larger sensory panel (*n*=39). Results confirmed that there was large inter-individual variability, with 77% of panellists responding to at least one glycoside, and 28% responded to all three (Figure 4). The variation in responses is likely due to differences in ability of saliva enzymes to break down the glycosides, due to differing populations of microflora in the mouth cavity, and may also be related to differing ability to perceive the volatile compounds released.

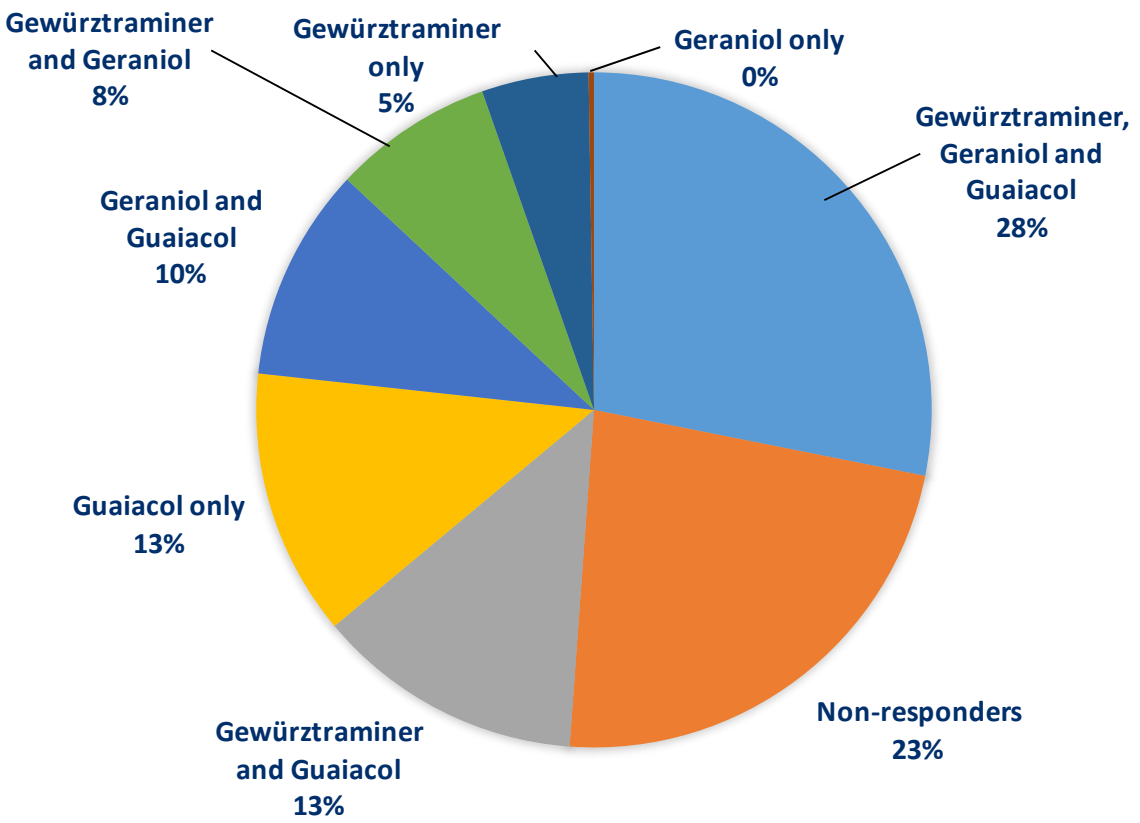

Figure 4. Variability in response to three glycosides: Gewürztraminer glycosides assessed at double strength; and two synthesised glycosides, geranyl glucoside and guaiacol glucoside.

Overall, these studies have demonstrated for the first time that non-volatile glycosides of monoterpenes can contribute to retronasal olfaction and after-odour through hydrolysis in-mouth. The effect can be eliminated through use of antibacterial mouthwash, and the extent of hydrolysis is almost certain to be related to microbiota populations in the oral cavity.

For wine producers, enhancing glycosides remaining in wines could be a feasible way of improving intensity and persistence of desirable flavour. As glycosides are present in other fruits and foods, breakdown in-mouth could be of importance to the consumption experience for other products, and glycosides could be valuable ingredients to enhance flavour. The study showed that grape-derived glycosides can give 'fruity' flavour, providing a means of enhancing flavour in wines, and confirms that the effect is very different across people, adding to evidence that sensory experiences are highly variable within a population.

In a follow-up experiment, glycosides were extracted from a large quantity of Gewürztraminer marc and purified using a polymeric resin column to remove phenolic compounds that contribute bitterness. The glycosides were added to Riesling and Chardonnay juices at two concentrations, and fermentation was conducted using standardised conditions. A treatment involving addition of glycosides when the wines were bottled was also included in the study. The sensory properties of the wines were quantified using sensory descriptive analysis, and chemical determination of monoterpenes and residual glycosides was also completed. The addition of glycosides significantly increased the concentrations of free monoterpenes and monoterpene glycosides in all treatments, resulting in significant increases in 'fruity'/'floral' aroma, flavour and aftertaste, while not significantly altering the bitterness or astringency. The timing of the addition was not as important as the overall amount of glycosides added. Interestingly, the sensory panellists who were confirmed in separate testing to be able to perceive flavour from glycosides (46% of the panel), rated 'floral aftertaste' in the wines with enhanced glycoside concentration substantially higher than those who could not, providing evidence that the residual monoterpene glycosides were a significant factor contributing to the flavour enhancement for these assessors. The concentration of geranyl glucoside, together with free beta-damascenone and linalool, was found to be strongly associated with the intensity rating of 'fruity'/'floral' attributes.

Consumer preference testing was conducted using a subset of the wines from the winemaking study, and showed that the Riesling wine made with an addition of glycosides to the juice was well liked for a sizeable cluster of consumers, while the larger additions were not appreciated. Moscato and Riesling drinkers showed a trend for increased liking of the Riesling addition wines compared to drinkers of other wine types.

The project was completed as planned, with an agreed shift early in the project from the varieties Chardonnay and Shiraz to Riesling and Gewürztraminer. A scientific paper was published describing the main outcomes of the project (Parker et al 2017b), with a further manuscript submitted. The work was communicated as an oral presentation at the 16th Australian Wine Industry Technical Conference in July 2016, and was awarded a best poster prize (Parker et al. 2017c), as well as presented at a flavour workshop at the conference. An *AWRI Technical Review* article was published (Parker et al. 2015), and outcomes have been presented at AWRI roadshows.

Outcome and conclusion

These studies have shown that non-volatile glycosides, previously considered a reserve of flavour that may be slowly broken down over time in-bottle, releasing flavour, can in fact contribute directly to wine flavour and aftertaste through in-mouth breakdown, most likely by microbiota in the saliva. Wines made from juices with added glycosides had more intense aroma and flavour, including a more intense and prolongated aftertaste. As aftertaste is one of the hallmarks of a high quality wine, the recognition of this hitherto unrecognised source of flavour is an important step in better understanding the factors required for an attractive, enjoyable and complex wine.

The project established a technique of isolating non-phenolic glycosides, eliminating any potential for bitterness. As glycosides can be quite easily isolated from white grape skins, there is potential to make use of a resource previously considered mainly as a waste material in the industry. The extraction procedure used in the project to generate a glycoside fraction suitable for winemaking was relatively simple and straightforward, and could be applied either by wineries using equipment available through winery engineering firms, or potentially by a company interested in supplying such material. There could be major benefits economically from improving flavour of lower value wines through use of a waste product.

Recommendations

Further studies are warranted into the cause of individual differences in perception and release of glycosides in-mouth. Studies involving other varieties, as well as investigations into the role of other precursor classes, notably cysteine and glutathione conjugates of sulfur compounds, would likely prove highly informative.

Regarding the effect of increasing glycoside concentration through isolation of non-phenolic glycosides from grape skins, several approaches could be taken. There is a need to consult with wine producers regarding their potential use of this technology, notably the relevance and potential for enhancing products, suitability for particular styles and also whether cost or certain processing constraints might be serious obstacles for uptake.

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