Flavour release from wine glycosides during tasting

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Abstract

Grape-derived glycosides contribute some of the most important aroma characteristics to wine, with volatiles released from glycosides during vinification. Wine can retain high concentrations of these non-volatile flavour precursors. Juice and wine made from aromatic varieties such as Gewürztraminer and Riesling are particularly rich in glycosides of the monoterpenes geraniol, linalool, nerol and α-terpineol. Glycosides from these varieties were extracted and purified to remove phenolics and free volatiles, and extensively characterised. GC/MS analysis following enzyme hydrolysis, hydrolysis by human saliva, and analysis of breath after tasting glycosides showed that monoterpene glycosides can release monoterpenes upon hydrolysis *in vitro* and *in vivo*. The possibility that hydrolysis could contribute to flavour via retronasal odour perception was investigated in a series of sensory experiments. Time-intensity sensory studies showed that *fruity* flavour resulted from assessors tasting glycosides at elevated concentrations. The effect was not significant at wine-like concentrations. There was substantial variability in response to glycosides, and a study of 39 people and several glycosides showed that 77% could detect flavour from at least one glycoside. This study provided evidence that non-volatile glycoconjugates can contribute previously unrecognised flavour during tasting, as well as contributing to aftertaste, a sought-after aspect of wine quality. Following on from these experiments, wines were made with additional glycosides extracted from grape skins. The addition of glycosides increased *floral*, *fruity* and *confectionary* aromas and flavours. Floral aftertaste was especially increased for those panellists who were tested as perceiving flavour from geranyl glucoside.

Introduction

Austria and Australia are home to some of the world's best regarded Riesling wines [1], notable for their floral, citrus, perfumed and fruity aroma. Wine grapes generally contain low concentrations of free volatiles, and it is accepted that varietal flavour predominantly arises from non-volatile precursors in the grapes, with the main classes being glycosides of volatiles possessing an alcohol functional group, and amino acid conjugates of volatile thiols [2-4]. Free volatiles are present in some grapes, such as monoterpenes in Muscat grapes, rotundone in Shiraz, and methoxypyrazines in Cabernet Sauvignon and Sauvignon Blanc. However, these are exceptions rather than the rule.

Glycosides and other precursors are readily transferred from the grapes into wine, where hydrolysis can occur through the enzyme or acid hydrolysis [5].

Studies have shown that precursors can hydrolyse in the human mouth, including thiol precursors [6], and hexyl glucoside [7]. Glycosides hydrolysed in-mouth have also been shown to be important to the smoky flavour of wines made from grapes exposed to bushfire smoke, although in this case the concentration of smoke-related phenol glycosides is unusually high [8].

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The human oral cavity is a complex system, with many factors influencing sensory perception, especially retronasal perception of odorants from food. Large inter-individual variability has been observed in factors such as oral microbiota, salivary flow rate, saliva composition, breathing and swallowing behaviour [9-12].

For wine glycosides to impart retronasal odour, they must be present in sufficient concentrations, even after swallowing or expectorating the sample, and then be hydrolysed in the mouth cavity. The released odorant must then travel via the retronasal route to the olfactory cleft where it can be perceived by olfaction if the concentration is high enough. And these steps must happen quite quickly to be noticed as a part of the flavour of the wine, in the first 30 seconds to 2 minutes after consuming the wine.

The hypothesis for this research is that grape-derived odourless aroma precursors in wine can be hydrolysed to release odorants in the mouth, leading to perceivable retronasal odour.

Experimental

Wines and chemicals

A Riesling wine from Eden Valley, a Gewürztraminer wine from Goulburn Valley and a Gewürztraminer juice from Adelaide Hills were chosen for the study. Geranyl glucoside, guaiacol glucoside and d_2-geranyl glucoside were synthesised in-house. Gewürztraminer marc from Eden Valley and Riesling juice from Adelaide were used for the winemaking study.

Glycoside extraction, purification and characterisation

Glycosides were extracted using polymeric resin Amberlite FPX66, and purified to remove phenolic glycosides and volatile impurities. Glycosides were incubated with a commercial enzyme preparation with a wide glycosidase activity, Lallzyme beta, and glycosides were also incubated with whole fresh saliva used within one hour of sampling. The volatiles released were measured using HS-SPME-GC-MS. Glycosides, including monoterpene glucosides, pentosylglucosides and rutinosides were also quantified directly using LC-MS-MS. Experimental details have been previously reported [13].

Sensory time-intensity studies

Glycosides from Gewürztraminer juice and wine were assessed by sensory analysis at five times the original concentration in the juice or wine, in model wine with 10.7% v/v aqueous ethanol and pH 3.50. Preliminary sensory assessment of the aroma of the glycosides confirmed the absence of *fruity* or *floral* aroma. Geranyl glucoside was also included in the study, at 3,080 µg/L. A panel (n=11, eight females) was recruited from AWRI staff with at least two years' wine descriptive analysis experience. Details of the panel training and sensory methods have been previously reported [13]. A second study assessed Riesling and Gewürztraminer volatiles and glycosides at wine-like concentrations, using a different sensory panel (n=11, five females), all of whom had previous sensory analysis experience, and none had participated in the first time-intensity experiment

For both studies, *overall fruit* flavour (defined as citrus, floral, stone fruit and confectionary-like) was then rated continuously using FIZZ data acquisition software, over a period of 120 s. Samples were presented monadically, with a forced rest of at least ten minutes between each sample in the formal sessions. All sensory data were obtained in compliance with institutional ethical procedures for sensory evaluation, involving risk

assessment and informed consent, and all samples were expectorated. Fisher's least significant difference (P=0.05) was calculated using analysis of variance of the maximum intensity.

Inter-individual variability in response to a range of glycosides

Thirty-nine people (18 males) experienced in wine sensory evaluation were assessed for their ability to perceive flavour from three different glycosides assessed individually in water: geranyl glucoside (3,080 µg/L), glycosides isolated from Gewürztraminer wine (2,930 µg/L), and guaiacyl glucoside (500 µg/L). A water control sample was also evaluated. The participants were instructed to hold the entire sample in the mouth for five seconds, then expectorate and rate *floral/fruity flavour, smoke/medicinal flavour* and, if needed, *other flavour*, rinse with water, and then rest for two minutes before the next sample. Individual judge responses for flavour attributes *fruity/floral* and *smoky* were examined using analysis of variance with P<0.15, compared to the water blank. Those with a significant response to a glycoside were classed as tasters of that glycoside.

Winemaking with added glycosides from Gewürztraminer marc

Glycosides were extracted from Gewürztraminer marc (the skin, stem and seed by-product of grape juice production) and purified using a polymeric resin column to remove phenolic compounds. The glycosides were added to Riesling juices at 0.4 g/L ('juice add') and to the wine at 0.4 g/L at bottling ('wine add'). The wines were fermented in 20 L stainless steel containers, in duplicate.

Sensory descriptive analysis of wines made with added glycosides

A panel of AWRI staff members with previous wine sensory experience (n=11, five females, six tasters of geranyl glucoside) was convened and a consensus-based descriptive methodology was used as described previously [14]. Nine aroma, twelve flavour and five aftertaste attributes were rated using an unstructured 15 cm line scale. The wines were assessed in duplicate over four days of formal sessions. All samples were expectorated, and there were forced one-minute rests between samples and a ten-minute rest after the fifth sample.

Analysis of variance assessed the effects of wine, judge (random effect), replicate, variety, and the corresponding two-way interactions. The least significant difference (Fisher's, 95% confidence) was calculated using Minitab 18.

Results and discussion

Chemical characterisation of extracted glycosides

Glycosides from Gewürztraminer juice and wine liberated geraniol and other monoterpene alcohols when hydrolysed with enzyme and whole fresh saliva. Direct analysis of the glycosides using LC-MS-MS confirmed the presence of geraniol glucoside as the major glycoside present, with minor components of monoterpene glycosides of diverse structures, including glucosides, pentosylglucosides and rutinosides.

Sensory time-intensity studies

Gewürztraminer wine glycosides tasted at five times original concentration gave a fruity flavour with onset approximately 7 s after the sample was taken into the mouth, reaching maximum intensity at 22 s, and lasting until 52 s. Gewürztraminer juice glycosides had a slightly longer delay with flavour onset at 12 s, reaching maximum intensity at 31 s and lasting until 80 s. Pure geranyl glucoside had a similar profile with a slightly

earlier flavour onset. Close examination of the individual panellist data revealed that only six of the eleven panellists were consistently rating the flavour effect.

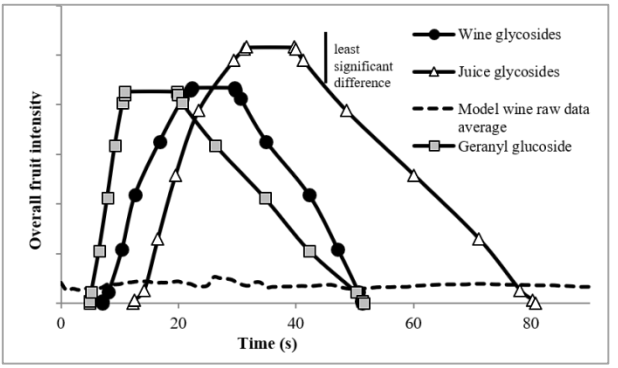

Figure 1. Mean time intensity curves for 'overall fruit' flavour intensity, generated by extracting parameters from individual raw data curves from 11 judges x 3 replicates for the three samples with added glycosides assessed in model wine (10% v/v ethanol, pH 3.50). Gewürztraminer wine and juice glycosides were tasted at five times original concentration, geranyl glucoside at 3,080 µg/L. The Fisher's least significant difference value (P=0.05), calculated from the maximum intensity data for the effect of sample, is also shown. (Originally published in AWRI Technical Review issue 214, 2015, with minor modifications.)

There was no significant flavour effect from the Riesling or Gewürztraminer glycosides when tasted at wine-like concentrations in model wine, in the presence or absence of wine volatiles for the sensory panel mean. However, close examination of the individual panellist responses revealed that five panellists out of the twelve responded to some of the glycosides in the study. In the first sensory study six out of eleven panellists perceived flavour. Perhaps only half of the population can perceive flavour from glycosides?

Inter-individual variability in response to a range of glycosides

There was large inter-individual variation in response to glycosides (Figure 2), with 77% responding to one or more of the glycosides. Some people responded to all three glycosides, some people responded to two of the glycosides, some responded to one of the glycosides, and some responded to none. Overall, 54% of the panellists rated a significant response to the Gewürztraminer glycosides, 46% rated a significant response to geranyl glucoside, and 64% rated a significant response to guaiacyl glucoside.

Winemaking with added glycosides from Gewürztraminer marc

Glycoside additions increased the concentration of geranyl glucoside and free monoterpenes in the resulting wines, regardless of whether the glycosides were added to the juice or to the wine. The concentration of geranyl glycoside increased by more than 2,000 µg/L, and linalool increased by approximately 50 µg/L, presumably due to hydrolysis during the winemaking process, followed by rearrangement of the monoterpene alcohols in the acidic wine matrix.

Fruity, floral or *confectionary* aroma and flavour attributes were boosted by the glycosides. Six of the eleven panellists in this study were separately assessed as able to detect flavour from geranyl glucoside, and the tasters rated much higher floral aftertaste in the wines with glycoside additions than the non-tasters. Bitterness was not significantly higher in the glycoside addition wines.

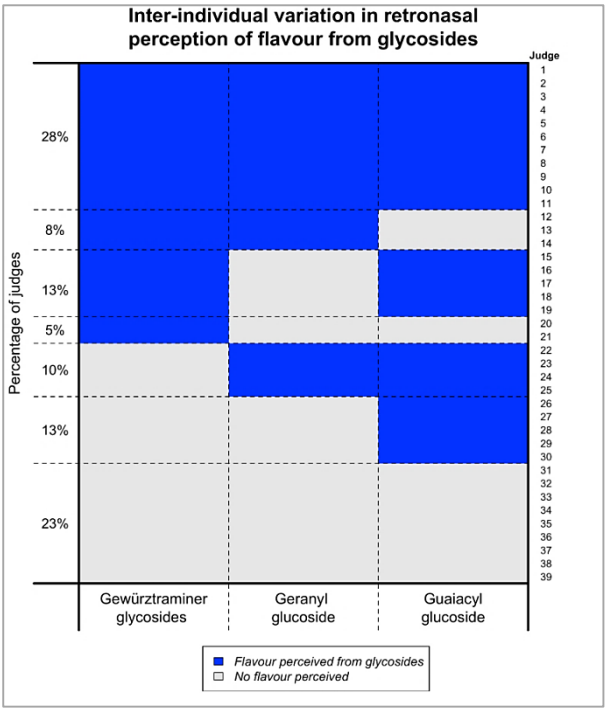

Figure 2: Response to various types of glycosides tasted individually in water. Gewürztraminer wine glycosides tasted at five times original concentration, geranyl glucoside at 3080 µg/L, guaiacyl glucoside tasted at 500 µg/L. n=39 people, triplicate presentations, ANOVA 0.15 significance.

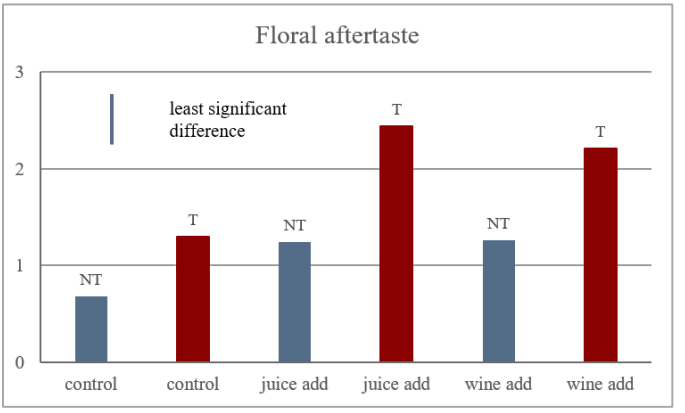

Figure 3: Floral aftertaste intensity mean score of Riesling wines made with single additions of glycosides from Gewürztraminer marc, added to the juice before fermentation (juice add), or to the wine at bottling (wine add). The panel was divided into two groups, those who had a significant ($p<0.15$) flavour response to geranyl glucoside in water at 3080 µg/L, who were labelled as tasters (T) (n=6), and nontasters (NT) (n=5) who did not have a significant flavour response to the geranyl glucoside. The Fisher's least significant difference value (P=0.05), calculated for the effect of wine, is also shown.

Overall, monoterpene glycosides were shown to break down in the mouth and contribute to flavour by retronasal perception of the released volatile odorants when tasted

at elevated concentrations. The effect was not significant when tested at wine-like concentrations, and large inter-individual variability was observed. A small survey of 39 individuals and three types of glycosides showed most people (77%) were capable of detecting flavour from some glycosides. Additions of glycosides from Gewürztraminer marc increased *fruity* and *floral* aromas and aftertaste in Riesling wines. The *floral* aftertaste was enhanced for panellists able to taste geranyl glucoside, even in the control wine, providing evidence that release of glycosides in-mouth is an important part of the sensory experience for many people.

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