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Glucose, fructose and sucrose increase the solubility of protein–tannin complexes and at high concentration, glucose and sucrose interfere with bisulphite bleaching of wine pigments

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ABSTRACT

Wines were modified with increasing sugar concentrations and decreasing tannin concentrations and analysed by a combination of protein precipitation and bisulphite bleaching. Increasing sugar concentration decreased the precipitation of tannin and protein-precipitable polymeric pigments (PPP). The use of a hydrogen bond disruptor (urea) to reduce protein–tannin and protein–pigment complex formation showed that the effect of sugar concentration occurred by increasing the solubility of the tannin–protein complex, not by interfering with protein–tannin complex formation. By increasing the solubility of pigment–protein complexes, non-protein-precipitable polymeric pigments (nPPP) appeared to increase. There was also an increase in total polymeric pigments at each tannin concentration with increasing glucose and sucrose concentration, indicating that sugar concentration might also affect bisulphite bleaching of wine pigments. While a significant effect of sugar concentration on tannin–protein complex solubility was observed, these effects were greatest at sugar concentrations far in excess of normal wine making conditions. Under normal wine making conditions, sugar concentration will have a negligible effect on protein-precipitable tannin, PPP and nPPP concentrations.

1. Introduction

Increasingly, tannins and polymeric pigments are routinely measured during commercial wine fermentation to evaluate important wine quality parameters, such as wine astringency and colour stability (Harbertson & Spayd, 2006). Because tannins and polymeric pigments are such important components of the sensory perception of wine, it is important to evaluate how various grape juice and wine components impact on the efficacy and therefore the usefulness or validity of an assay employed to measure tannins and polymeric pigments. The tannin and polymeric pigment assay based on protein precipitation is a robust and accessible method gaining increasing acceptance in the wine industry as well as in grape and wine research (Harbertson & Spayd, 2006). With increasing reliance of the methodology and its incorporation into the business model of winery production systems, it is important that such an assay has been thoroughly and critically evaluated under a range of conditions that occur in grape and wine analysis.

Protein precipitation (Hagerman & Butler, 1978; Harbertson, Picciotto, & Adams, 2003) has been adopted by wineries to monitor tannin extraction and pigmented polymer formation during wine making. In tannin protein precipitation assays, an insoluble tannin–protein complex (precipitate) is formed (Haslam, 1998; Swain, 1965). The commonly used protein for grape and wine tannin analysis is bovine serum albumin (BSA) (Harbertson & Spayd, 2006), which behaves similarly to human saliva in precipitating condensed tannin. Protein precipitable tannins have been demonstrated to correlate strongly with sensory evaluations of astringency (Kennedy, Ferrier, Harbertson, & Peyrot des Gachons, 2006; Mercurio & Smith, 2008). The long term stability of colour in red wines is also attributed to the presence of condensed tannin in the form of pigmented polymers (Cheynier et al., 2006; Somers, 1968).

Protein precipitation has been adapted to measure polymeric pigments in combination with bisulphite bleaching (Harbertson et al., 2003). Polymeric pigments are defined as those pigments that are resistant to bisulphite bleaching. These can be differentiated into two classes, protein-precipitable polymeric pigments (PPP) and non-protein-precipitable polymeric pigments (nPPP). Previously these have been called long and short pigmented polymers (Harbertson et al., 2003); however the size distinction between these has not been defined. Therefore, in a protein precipitation study the revised terminology of precipitable and non-precipitable pigments is more appropriate.

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It is uncertain to what extent the interaction of tannin with protein and subsequent precipitation might be affected by changes in the wine matrix, such as decreasing sugar and increasing ethanol concentration. One of the major changes that occur during wine making is the alcoholic fermentation of sugar (Boulton, Singleton, Bisson, & Kunkee, 1998). During wine making, the concentration of soluble sugars changes considerably from a high concentration at grape harvest and declines during alcoholic fermentation. Typically harvest concentrations of soluble solids can range from 20 to 30 °Brix. Commensurately, the concentration of alcohol increases and can range from 10 to 12% up to 18% alc/vol or more in some wines, but typically for red wines falls in the range of around 14–16% alc/vol.

The effect of ethanol on tannin–protein interactions has previously been examined. Increasing ethanol concentration reduces protein precipitation of tannin in wine compared to an alcohol-free wine (Serafini, Maiani, & Ferro-Luzzi, 1997). However, this was only observed to occur above 11% ethanol, not at lower concentrations, e.g., 5% ethanol, and the effect has been attributed to disruption of hydrogen bonding (Serafini et al., 1997). Increasing ethanol concentration has also been reported to decrease co-pigmentation effects in wine (or at least wine colour) (Hermosín Gutiérrez, 2003), although no direct effect on nPPP or PPP has been reported. While hydrogen bond disruptors such as ethanol, urea and caffeine are known to interfere with protein precipitation assays for condensed tannins (Rowe et al., 2010; Serafini et al., 1997), it is unclear whether these hydrogen bond disruptors interfere with the tannin's initial interaction with the protein, or alter the solubility of the protein–tannin complex.

Grape sugars are primarily sucrose and fructose, but also include a number of other open-chain sugars (Boulton et al., 1998). Some grape sugars (glucose, raffinose) can form stable adducts with bisulphite due to the presence of a free carbonyl functional group and when at high concentrations, such as in juice, they have been estimated to bind approximately 50% of the sulphur dioxide added (Boulton et al., 1998). Fructose binds bisulphite less strongly. However it is not clear whether the glucose and raffinose present during fermentation interfere with bisulphite bleaching assays for polymeric pigments.

The kosmotropic effect of sugar (and salt) concentration on the solubility and stability of proteins (including BSA) has previously been explored (Arakawa, Kita, & Carpenter, 1991). This work concluded that sugars decreased the solubility of proteins and stabilised them against denaturation (e.g., unfolding) by increasing the surface tension of water, and while interaction with the protein may occur through hydrogen bonding, hydrophobic interactions or electrostatic interactions, these were outweighed by the impact on surface tension. Where the binding forces were stronger, protein solubility increased or the structure was destabilised (denatured). The implication of this for an assay that relies on precipitation of a protein–tannin complex is that the complex may be less soluble in a solution where sugar is present and decreases the effectiveness of the assay. To the best of our knowledge the effect of sugar concentration on the precipitation by protein of condensed tannin has not been investigated. Based on previous work, a reasonable hypothesis might be that sugar would decrease solubility of the protein–tannin complex, potentially increasing precipitation of tannin–protein complexes that might otherwise remain in solution and thereby increase the measured concentration of precipitated tannin.

This work aimed to determine what effect sugar concentration had on the formation and precipitation of protein–tannin complexes necessary for measuring protein precipitable tannin and whether sugars would interfere with the effectiveness of bisulphite bleaching used in determining precipitable and non-precipitable polymeric pigments in wines and fermenting musts.

2. Materials and methods

2.1. Chemicals

Sucrose, D-glucose, D-fructose, urea, potassium metabisulphite, bovine serum albumin (BSA, fraction V, lyophilised powder), sodium dodecyl sulphate (SDS, lauryl sulphate, sodium salt, 95%), triethanolamine (TEA, 98%), ferric chloride hexahydrate (98%), and (+)-catechin hydrate (98%, powder) were purchased from Sigma (St. Louis, MO), as were materials for preparing buffers used in analyses. Reagents were prepared (Harbertson et al., 2003) and stored (Heredia, Adams, Fields, Held, & Harbertson, 2006) as described elsewhere.

2.2. Tannin analysis

Tannin analysis was performed as described previously (Hagerman & Butler, 1978) with minor modifications. The method is a variation on an existing method modified to incorporate measures of pigmented polymers (Harbertson, Kennedy, & Adams, 2002). Briefly, 500-μL aliquots of red wine diluted into a model wine buffer containing 5 g/L potassium bitartrate adjusted to pH 3.3 with HCl were added to 1 mL of pH 4.9, 200 mM acetic acid, 170 mM NaCl containing 1.5 mg/mL BSA and incubated at room temperature for 15 min. Samples were then centrifuged at 13,500×*g* for 5 min to form a pellet with a clear supernatant. The supernatant was discarded, and the remaining pellet was incubated for 10 min after adding 875 μL TEA buffer containing 5% TEA (v/v) and 5% SDS (w/v) adjusted to pH 9.4 with HCl. After the incubation period the sample was mixed mechanically to dissolve the tannin–protein pellet. To each sample, a 125-μL aliquot of ferric chloride reagent containing 10 mM $FeCl_3$ in 0.01 N HCl was added to the tube and allowed to stand at room temperature for 10 min. After the incubation period the absorbance at 510 nm was determined in a Beckman DU 640 spectrophotometer (Fullerton, CA) using the TEA buffer as a blank. Tannin values are reported in catechin equivalents (C.E.) as described by Harbertson et al. (2002).

2.3. Polymeric pigment analysis

Protein-precipitable polymeric pigments (PPP) and non-protein-precipitable pigments (nPPP) were measured in the wine as previously described (Harbertson et al., 2003). Briefly, following addition of the sample containing tannin (and polymeric pigments) and centrifugation of the tannin–protein complex the supernatant is transferred to a fresh microfuge tube and potassium metabisulphite is added, and absorbance at 520 nm is recorded. This value represents non-protein-precipitable tannins (nPPP). Total polymeric pigments (TPP) are determined by recording the absorbance (520 nm) of the sample containing tannin and the assay buffer without protein. Protein-precipitable polymeric pigments (PPP) are calculated as the difference between TPP and nPPP. The analysis for each sugar and each dilution was conducted separately including 0 g/L with four analytical replicates ($n = 4$). Data for each analysis were analysed and presented separately. Thus, in Fig. 3, differences were reported for 0 g/L sugar where theoretically there should be none. These are exaggerated for PPP where this number is derived by subtraction and by the *y*-axis scale.

2.4. Sugar effect on the polymeric pigment and tannin of wine

Various amounts of urea and sugars (glucose, fructose, sucrose and raffinose) were added at different concentrations during the tannin analysis in the place of the normal model wine buffer to determine the impact of the concentration of sugars or urea on