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Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine

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ABSTRACT

The effects of ethanol (8, 10, 12, 14, and 16% v/v), tannin (500, 1000, and 1500 mg/L) and fructose (200 and 2000 mg/L) concentrations on the headspace of eight selected odorants were investigated using headspace solid phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS). Analysis of variance results (ANOVA) showed significant interaction effects for the majority of odorants ($P<0.05$). In general, higher tannin concentration enhanced the release of odorants while fructose induced a retention effect, both of which were largely dependent upon ethanol concentration. The net magnitude effect was a substantial reduction in the headspace concentration of odorants with the dominant contribution from ethanol concentration. The percent reduction in extracted odorant was more pronounced on larger molecular weight compounds. Further multivariate analysis discriminated model wines with different ethanol concentrations and, to a lesser extent, separated model wines with different fructose and tannin concentrations. Subsequent gas chromatography–olfactometry (GC–O) analysis revealed differences in the estimated odor thresholds of odorants in the model wines. Threshold values increased between 2 and 10,000-fold for 2-methoxyphenol and eugenol, respectively, at higher ethanol, tannin and fructose concentrations. Consequently, odor unit values (OUV) of odorants decreased indicating a reduction in the potential contribution of the odorants to the aroma of model wine. These results highlighted the significant impact that wine matrix interactions can have on wine aroma quality.

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1. Introduction

Wine aroma is one of the major determining factors influencing consumer acceptance (Charters & Pettigrew, 2007; Jover, Llorens Montes, & Fuentes Fuentes, 2004). As with other food products, the perception of wine aroma is related predominantly to the nature and concentration of aroma compounds in the gaseous phase above the wine. More than 800 volatile compounds have been estimated to be present in wine (Maarse & Vischer, 1989) while only a few compounds exist at concentrations above the sensory perception threshold (Cullerè, Escudero, Cacho, & Ferreira, 2004; Guth, 1997; Li, Tao, Wang, & Zhang, 2008; Zhang, Xu, Duan, Qu, & Wu, 2007).

Various factors influence the release of volatile compounds from the wine matrix. Wine is a complex alcoholic beverage, which contains both the volatile (including ethanol) and non-volatile components (polyphenolic compounds, proteins and carbohydrates) that may interact with aroma compounds affecting their volatility and/or concentration in the headspace, and ultimately modify aroma perception. The impact of these interactions may be concentration-dependent; wine components exist in wide range of concentrations in wine as direct or indirect consequences of winemaking practices employed. Ethanol, the major component of wines, contributes to aroma and overall flavor at concentration above its threshold (0.1 to 100 ppm), enhances sourness, sweetness (between 2 and 4% v/v ethanol) (as cited by Pozo-Bayon & Reineccius, 2009), bitterness (50% increase in bitterness with 3% v/v ethanol increase) (Fischer & Noble, 1994), and influences viscosity and density (between 3 and 15% v/v ethanol) (Nurgel & Pickering, 2005). Wine polyphenols, particularly tannins, are positively correlated to perceived astringency and bitterness (Landon, Weller, Harbertson, & Ross, 2008). While wine sugar content indicates completeness of fermentation and sweetness (Amerine & Joslyn, 1970), owing to its very low content in wine (close to 0.2%), its contribution to wine aroma profile has not been reported. Understanding the combined effects of wine matrix interactions at varied concentrations on the release behavior of aroma compounds from the wine matrix is important to predict the wine aroma characteristics for informed decisions during winemaking. Several studies conducted to understand wine aroma perception have focused on the contribution of single components within a wine matrix using static headspace solid phase microextraction (HS-SPME) and dynamic headspace analytical techniques. Ethanol has been shown to decrease the partition coefficient of various classes of volatile compounds by increasing the solubility of

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volatile compounds in model wine systems (Camara, Arminda Alves, & Marques, 2006; Conner, Birkmyre, Paterson, & Piggott, 1998; Hartmann, Mc Nair, & Zoecklein, 2002; Voilley, Beghin, Charpentier, Charpentier, & Peyrond, 1991; Whiton & Zoecklein, 2000). Aznar, Tsachaki, Linforth, Ferreira, and Taylor (2004) measured the effect of ethanol on the headspace partitioning of 11 compounds and reported that the hydrophobicity of the compound was also a determining factor influencing the partition of volatile compounds in the headspace of ethanolic solutions. The suppression effect of ethanol on aroma corresponded to the changes in odor detection threshold in the presence or absence of ethanol. As reviewed by Grosch (2001), gas chromatography–olfactometry (GC–O) results showed a dramatic increase in threshold values (a result of the suppression effect) in the presence of ethanol (55.6 mg/L). The increase in odor threshold ranged between factors of 10 for ethylhexanoate to a factor of 312 for methylpropanol (Grosch, 2001).

The effect of polyphenols on the release of odorants and their impact on aroma perception has also been reported. Few studies using headspace-solid phase microextraction/gas chromatography–mass spectrometry (HS-SPME/GC–MS) demonstrated different effects of monomeric phenols on the partitioning of odorants (Aronson & Ebeler, 2004; Jung & Ebeler, 2003). Dufour and Bayonove (1999) investigated the influence of condensed wine tannins on the volatility of some volatile compounds using a dynamic headspace technique. The authors found that addition of tannin (0–5 g/L) resulted in increased volatility of limonene and a slight increase in benzaldehyde volatility but had no effect on isoamyl acetate and ethylhexanoate. However, in a recent study, the addition of increasing concentrations of natural tannin extract (1–10 g/L) from grape skin in model wine (with 0.3 g tartaric acid in 10% v/v ethanol/water mixture) increased the volatility of the ester isoamyl acetate and other hydrophilic compounds, including 2-methyl-1-butanol, diethyl succinate, and phenylethyl alcohol (Mitropoulou, Hatzidimitriou, & Paraskevopoulou, 2011). Lund, Nicolau, Gardner, and Kilmartin (2009) conducted sensory difference tests (*R*-index methodology) to evaluate the effect of polyphenols on the perception of key odorants found in New Zealand Sauvignon Blanc wines. The results showed an increase in the threshold of isobutyl methoxypyrazine, 3-mercaptohexanol and ethyldecanoate after the addition of catechin (12 mg/L), caffeic acid (102 mg/L) and quercetin (10 mg/L) in a dilute base wine. In another study, panelist found lower perception of fruity, citrus, strawberry, cooked fruit and floral aromas in Malbec wines containing high polyphenols (5.4–7.2 g/L) compared to the low polyphenol content (1.4–3.2 g/L), indicating a matrix effect (Goldner, Lira, van Baren, & Bandoni, 2011).

However, few studies on the simultaneous effects of different wine components have been described in the literature. Robinson et al. (2009) reported significant reduction of peak areas for most volatile compounds due to ethanol, and this effect was slightly increased in the presence of glucose in model solutions. In another study using model white wine, Jones, Gawel, Francis, and Waters (2008) demonstrated the influence of interactions among major wine components on perceived aroma intensity. Their results showed more evident interaction effects of wine proteins, alcohol and glycerol concentration at the lower volatile compound concentration. Ethanol and glycerol were also shown to be involved in either polysaccharides or protein interactions, with the lowest overall aroma observed when polysaccharides and glycerol were present at lower ethanol concentration (11%, v/v). Both of these investigations demonstrated the importance of multi-factorial approach in studying wine matrix interactions, which provides more information on the behavior of odorants in various combinations of conditions.

Thus, the objective of the present study was to investigate the combined influence of ethanol, tannin and fructose concentration, factors which may impact the headspace concentration of selected odorants and their relative potential significance to the aroma of model wine.

2. Materials and methods

2.1. Chemicals and reagents

All odorants including the internal standards (IS) were purchased from Sigma-Aldrich (St. Louis, MO): 1-octen-3-one (50 wt.% in 1-octen-3-ol), β-damascenone (1.1–1.3 wt.% in 190 proof ethanol), 2-phenylethanol (≥99%), 3-methyl-1-butanol, dimethyl disulfide, 1-hexanol, 2-methoxyphenol, eugenol, and 1-pentanol (IS) and 1-dodecanol (IS) (≥98%). Pure ethanol (100%) was obtained from Decon Labs, Inc. (King of Prussia, PA) while grape tannin, Biotan, was provided by Laffort Company (Sonoma, CA). D-(−)-Fructose, L-(+)-tartaric acid, NaCl and NaOH were procured from Sigma-Aldrich (St. Louis, Mo). Water used was purified by Milli-Q (Millipore, Bedford, MA).

2.2. Model wine preparation

Thirty different model wine solutions replicated five times were prepared using a full-factorial design used to assess the effect of ethanol (8, 10, 12, 14, and 16%, v/v), grape tannin, Biotan (500, 1000 and 1500 mg/L) and fructose (200 and 2000 mg/L). Ethanol levels used reflected the concentration range found in commercial red wines. The low (500 mg/L), medium (1000 mg/L), and high (1500 mg/L) grape tannin concentrations were selected based in part on the groupings established previously for the low, medium and high tannin commercially available red wines (Landon et al., 2008). Fructose concentrations used were within the range present in wines considered as "dry" (Zoecklein, Fugelsang, Gump, & Nury, 1995).

All model wines contained tartaric acid (5000 mg/L) and 0.65 g NaCl, and were spiked with eight selected odorants of different physicochemical and aroma properties commonly found in red wines: 50 mg/L 3-methyl-1-butanol (caramel/cooked), 4 mg/L dimethyl disulfide (chemical/sulfury), 2 mg/L 1-hexanol (herbaceous/green), 1 mg/L 1-octen-3-one (earthy/mushroom), 4 mg/L methoxyphenol (woody/medicinal), 14 mg/L 2-phenylethanol (floral/rose), 0.5 mg/L eugenol (spicy/clove), and 2 mg/L β-damascenone (fruity) (Table 1). The above concentrations of odorants were within the range present in wines and could be analyzed using the developed HS-SPME/GC–MS method in this study. For each odorant, a stock solution was prepared every 2 weeks in 50% ethanol/Milli Q (v/v) water and stored at 5 °C in a 5 mL amber vial sealed with a polytetrafluoroethylene (PTFE)/silicone septum until the solution was used. Prior gas chromatography–mass spectrometry (GC–MS) analysis results showed no change in odorant concentration within two weeks of storage. The pH of the model wines was adjusted to 3.4 with 1 M NaOH.

2.3. HS-SPME/GC–MS analysis

The volatiles were isolated and concentrated using the headspace solid-phase microextraction (HS-SPME) technique. A sample consisting of two (2) milliliter of each model wine was spiked with the odorants and then introduced into a 10-mL amber vial sealed with a magnetic stainless steel screw cap (PTFE/silicone septum). Volatiles were extracted using a CTC Combi PAL autosampler (Zwingen, Switzerland). The optimized parameters wherein optimum odorant concentration was obtained for a reasonable extraction time were: pre-incubation time for 5 min, incubation temperature at 30 °C, agitator speed at 250 rpm, agitator-on time for 5 s, agitator-off time for 2 s, extraction time at 30 min and desorption time for 5 min. Prior to use, a 65 μm polydimethysiloxane/divinylbenzene (PDMS/DVB) coated fiber (Supelco, Bellefonte, PA) was conditioned at 250 °C for 30 min.

Samples were then analyzed using a GC 6890N chromatograph coupled with a mass spectrometer (MS 5975) (Agilent technologies, Avondale, PA) functioning in the EI mode. The following GC column was used: HP-5MS (5%-phenyl-methylpolysiloxane, 30.0 m × 250 μm ×