Influence of Wine Structurally Different Polysaccharides on the Volatility of Aroma Substances in a Model System

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The influence of native polysaccharides on wine organoleptic quality has not been yet clarified. Hence, the effect of purified fractions of arabinogalactan−proteins (AGPs), monomeric and dimeric rhamnogalacturonans II (mRG-II and dRG-II), and mannoproteins (MPs) on the volatility of various aroma substances was examined using the exponential dilution technique. The volatility of isoamyl acetate and ethyl hexanoate in a model wine system was not affected upon addition of wine polysaccharides in the range 5−20 g/L. At higher levels, the two esters were significantly retained in solution in the presence of the protein-rich polysaccharides MP0 and AGP0 and weakly salted out in the presence of the uronic acid-rich fractions AGP4, mRG-II, and dRG-II. In addition, the volatility increase caused by dRG-II on the esters was reversed when water was used as the solvent. 1-Hexanol exhibited retention in water with four fractions in the strength order AGP0 > dRG-II > mRG-II > AGP4 but was strongly salted out in the presence of fraction MP0. Furthermore, the diacetyl activity in water was overall not modified increasing only at high concentrations of AGP4. Reference polysaccharides dextrin and dextran were used for binding strength comparison.

Keywords: *Activity coefficient; polysaccharide; wine; aroma compound; exponential dilution*

INTRODUCTION

The aromatic quality of a wine is assessed first by sensory analysis of the headspace containing the aroma substances. The aroma equilibrium between vapor and aqueous phases is expected to be influenced by nonvolatile molecules present in wine. Macromolecules found in wine, polysaccharides, tannins, and proteins appear as potential partners for aroma−macromolecule interactions. Wine polysaccharides, ranging from 500 to 1500 mg/L (Will et al., 1991), originate from grape primary cell wall and from autolysis of microorganisms such as yeasts used in winemaking or *Botrytis cinerea*, a parasitic mould of the vine. Hence, this origin diversity leads to polysaccharides which are different in composition and structure. Two criteria widely used for polysaccharide discrimination are acidity and protein contents. Neutral pectic substances mainly comprise type II arabinogalactans (AGs) and arabinogalactan−proteins (AGPs) which represent ca. 40% of all wine polysaccharides (Pellerin et al., 1993, 1995). Their common structural feature is a (1→3)-β-D-galactan backbone with (1→6) linked β-D-galactan side chains highly substituted by arabinofuranose residues. AGPs typically contain less than 10% protein. Other neutral polysaccharides are weakly branched (1→5)-α-L-arabinans (Belleville et al., 1993) and type I arabinogalactans which are (1→4)-β-D-galactans substituted in the position 6 by arabinofuranose residues. Acidic pectic polysaccharides, characterized by a high proportion of galacturonic acid, involve homogalacturonans and rhamnogalacturonans. Rhamnogalacturonans II (RG-IIs) account for 20% of all the ethanol-precipitable polysaccharides in wine; they are (1→4)-α-D-galacturonans branched with four different side chains containing primarily rhamnopyranose, arabinofuranose, and galactopyranose residues (Pellerin et al., 1996). O'Neill et al. (1996) reported the existence of a dimeric form (dRG-II) resulting from the cross-linking of two monomeric polymers (mRG-II) through a borate diester. Microorganisms are known to release exocellular and cell wall polysaccharides during fermentation. *Saccharomyces cerevisiae* exocellular mannoproteins have structural features in common with cell wall mannoproteins but present lower protein contents (Saulnier et al., 1991). The interaction between polysaccharides and other wine constituents has recently started to trigger interest. An AGP fraction (Waters et al., 1994a) and a *Saccharomyces* cell wall mannoprotein (Waters et al., 1994b) were reported having a protein haze protective effect. The rate of crystallization of potassium hydrogen tartrate was found to be influenced by wine polysaccharides (Gerbaud et al., 1997; Moine-Ledoux et al., 1997).

However, little is known on the influence of these structurally different polysaccharides on the aroma of wine except for yeast-released mannoproteins. The influence of wine total colloids and purified yeast mannoproteins from *Saccharomyces* on the sensory properties of a Riesling wine was investigated by Will et al. (1991). No significant sensory differences were found for ultrafiltrated wines enriched with the abovementioned colloids in the range 600−1500 mg/L. Lubbers et al. (1994a) reported that the retention of ethyl hexanoate and β-ionone by exocellular mannoproteins was dependent on the fraction protein content. Besides, studies with yeast wall mannoproteins suggested that increasing aroma hydrophobicity and mannoprotein higher content in lipids enhanced the binding ability (Lubbers et al., 1994b).

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The aim of the present work is to study the interactions between aroma substances and polysaccharides isolated from a red wine. The determination of the aroma vapor−liquid equilibrium will be addressed using the dynamic exponential dilution technique (Leroi et al., 1977; Sadafian and Crouzet, 1987). Up to now, the use of this technique has been reserved to the sole study of binary systems. We, here, report the extension of this technique to the ternary system isoamyl acetate−ethyl hexanoate−water. Influence of dextrin on the ternary system was assessed by statistical analysis.

MATERIALS AND METHODS

Materials. Purity (>98%) of ethyl hexanoate, isoamyl acetate, 1-hexanol, and diacetyl was checked by GC-MS and ^{1}H NMR analyses. Aroma compounds and potassium hydrogen tartrate were from Aldrich Chemical Co. (Milwaukee, WI); dextrin (average dp 20; type I from corn) and dextran (MW 60 000−90 000 Da; from *Leuconostoc mesenteroides* strain no. B-512), from Sigma (St Louis, MI). Polysaccharides isolated from red wine were kindly donated by Dr. P. Pellerin from the Laboratoire des Polymères et des Techniques Physicochimiques (IPV-INRA, Montpellier, France). Rhamnogalacturonans RG-IIs were purified as described in Pellerin et al. (1996). Fractions mRG-II and dRG-II were predominantly monomeric (90% pure) and dimeric (87% pure). Type II arabinogalactan−protein fractions, AGP0 and AGP4, were isolated by anion exchange chromatography and discriminated on the basis of their uronic acid contents (respectively 3 and 20%) according to Pellerin et al. (1995). Mannoproteins MP0 were purified by affinity chromatography according to Vernhet et al. (1996). The sugar composition of the various wine polysaccharides was checked at the end of the study confirming the stability of the five fractions. Model wine was a 11.76% EtOH/aqueous tartrate solution (v/v) made with 2 g/L potassium hydrogen tartrate adjusted at pH 3.5 with concentrated NaOH.

Exponential Dilution. *Influence of Dextrin and Mixing on Ester Activity Coefficients.* The experimental setup was similar to the devices described by Leroi et al. (1977) and Sadafian and Crouzet (1987). In a double-jacketed glass cell were placed water (10 mL), dextrin when required, and the ester(s) via syringe (0.3 μL of pure aroma compound or a 1:1 (v/v) mixture). The solution was incubated for 30 min under magnetic stirring. Stripping of the solution was achieved by flowing nitrogen (15−20 mL/min) into the stirred solution through a glass frit disk (no. 4). Warming of the N_2 flow was obtained by a heat exchanger constituted of steel tubing (8 m × $^1/_{16}$ in. i.d.) placed in the thermostated water bath already used for cell temperature regulation (25 ± 0.1 °C). The headspace concentration decrease was followed by repeated injections of 250 μL of the leaving gas through a six-port electropneumatic valve (Valco series W) onto a HP 5890A chromatograph (Hewlett-Packard) equipped with a FID detector and a fused silica capillary column (DB-5, J&W Scientific, 60 m × 0.32 mm i.d., 1 μm film thickness). The carrier gas was N_2, and the flow rate, 0.7 mL/min. Column and sampling valve were set at 150 °C, and the detector temperature was 250 °C. Data acquisition and treatment were carried out under APEX software (Autochrom Inc., version 2.15).

Aroma−Polysaccharide Interactions. The experiments were conducted as above. Ethyl hexanoate and isoamyl acetate (0.3 μL in 10 mL of water) were studied individually with dextrin and dextran. The esters were studied as a 1:1 (v/v) mixture (0.2 μL in 5 mL of model wine) in the presence of wine polysaccharides. 1-Hexanol and diacetyl (0.5 μL in 5 mL of water) were studied individually with all polysaccharides. The stripping N_2 flow rate was then 30−40 mL/min. Column and sample valve were set at 130 °C.

Determination of Activity Coefficients at Infinite Dilution. Dynamic headspace analysis proceeds by gas stripping of an aqueous solution containing the volatile compound.

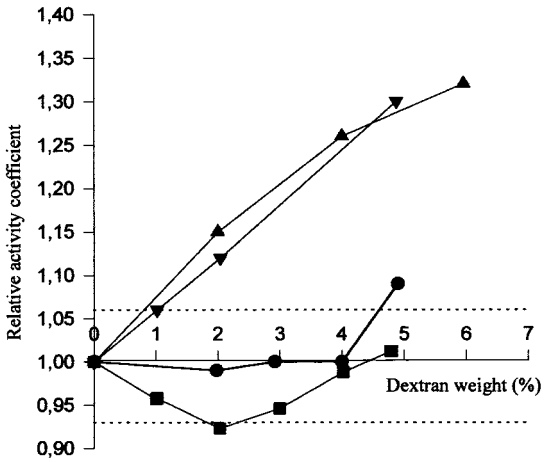

Figure 1. Variation of activity coefficients in water of individual aroma compounds in function of dextran weight fraction at 298 K: (●) hexanol; (■) diacetyl; (▲) isoamyl acetate; (▼) ethyl hexanoate.

The aroma concentration has been shown to decrease exponentially against time according to eq 1 (Leroi et al., 1977),

$$\ln S = -\frac{D_{corr}}{RT}\frac{P^s_{solute}}{N}\gamma^{\infty}t + \ln S_0 \qquad (1)$$

where S is the GC peak area, D_{corr} is the cell flow rate (mL/min, measured with a soap bubble flowmeter at the gas exit and corrected as in Sadafian and Crouzet (1987)), P^s_{solute} is the vapor pressure of the solute (atm) at T, R is the gas constant (mL·atm·mol^{-1}·K^{-1}), T is the cell temperature (K), N is the solvent number of moles (mol), γ^{∞} is the activity coefficient at infinite dilution, t is the time (min), and S_0 is the extrapolated area peak at zero time. The γ^{∞} values were obtained by linear regression of ln S vs time. The variation of the activity coefficient accounts for a retention (activity decrease) of the volatile molecule or a salting-out effect (activity increase).

Statistical Data Treatments. *Influence of Dextrin and Mixing on Ester Activity Coefficients by the Student t-Test.* In this part, activity coefficients were determined in triplicate. The standard deviation (s) was derived from the mean activity coefficient (γ^{∞}_{mean}) using the following relationship:

$$s = \sqrt{\frac{\sum(\gamma^{\infty} - \gamma^{\infty}_{mean})^2}{(N-1)}} \qquad (2)$$

Series of triplicates with and without dextrin and series in binary and ternary systems were compared two by two using the Student t-statistical test. An F-test was initially conducted to check variance homogeneity in the two groups ($p = 0.05$).

Aroma−Polysaccharide Interactions by the Confidence Limit Method. Replicates (3 or 4) were only carried out for solutions of aroma compounds in the pure solvent. The standard deviation (s) was calculated as above, and the 95% confidence limits (95% CL) were derived using relationship (3) with t extracted from the Student t-table with a degree of freedom of $N - 1$ and a 0.05 significant level.

$$95\%\ \mathrm{CL} = \gamma^{\infty}_{mean} \pm st/\sqrt{N} \qquad (3)$$

Due to minute quantities of isolated wine polysaccharides, single or duplicate experiments (average data in figures) were performed for each substrate concentration. γ^{∞} values lying outside the confidence limits pointed out a substrate influence on the aroma volatility. The broken lines in Figures 1−7 (representing the 95% confidence limits for reported data) have been arbitrarily chosen as the closest values to a relative

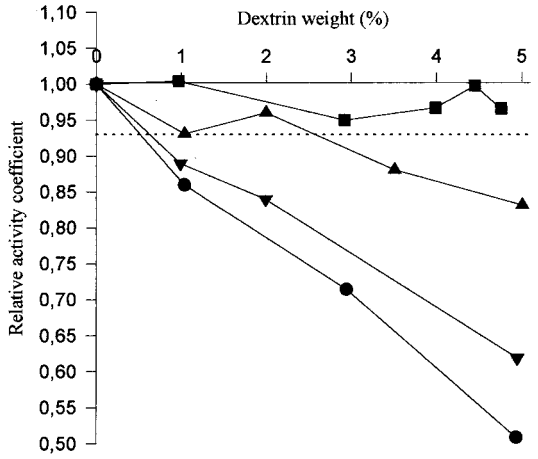

Figure 2. Variation of activity coefficients in water of individual aroma compounds in function of dextrin weight fraction at 298 K: (●) hexanol; (■) diacetyl; (▲) isoamyl acetate; (▼) ethyl hexanoate.

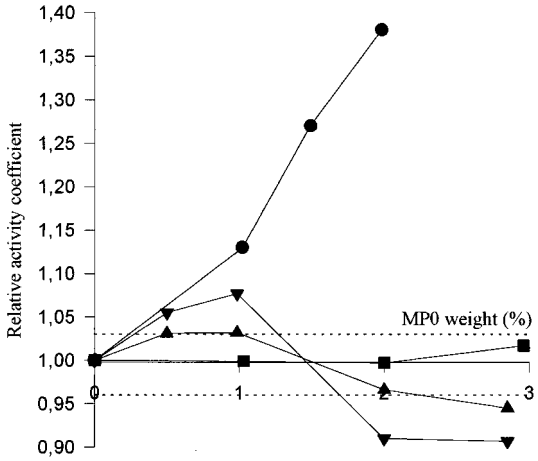

Figure 3. Variation of activity coefficients of aroma compounds in function of mannoprotein MP0 weight fraction at 298 K. In water: (●) hexanol; (■) diacetyl. In model wine: (▲) isoamyl acetate; (▼) ethyl hexanoate.

activity coefficient of 1 separating significant from nonsignificant experimental values in a particular figure.

RESULTS AND DISCUSSION

Influence of Dextrin and Mixing on Isoamyl Acetate and Ethyl Hexanoate Activity Coefficients. Activity coefficients at infinite dilution were measured for isoamyl acetate alone in water (IA_{water}), alone in 2% dextrin ($IA_{dextrin}$), and in the presence of ethyl hexanoate (ternary system in water and in 2% dextrin) (Table 2). Similar data are reported for ethyl hexanoate (Table 3). When comparing activity coefficients with literature data, one should take into account the value of P^s_{solute} (Table 1). Hence, our values for isoamyl acetate (4614) and ethyl hexanoate (15 481) alone are respectively 11 and 6% higher than Langourieux' data (Langourieux and Crouzet, 1994) and 21% higher than the ethyl hexanoate activity coefficient reported by Lubbers et al. (1994b). Differences in values obtained for individual and combined aroma substances were assessed by the Student *t*-test (Tables 2 and 3). For $t_{calcd} > t_{table}$, the mean difference is significant at the selected probability level. Activity coefficients γ^∞ for isoamyl acetate and ethyl hexanoate in water are similar whether they are measured individually or in

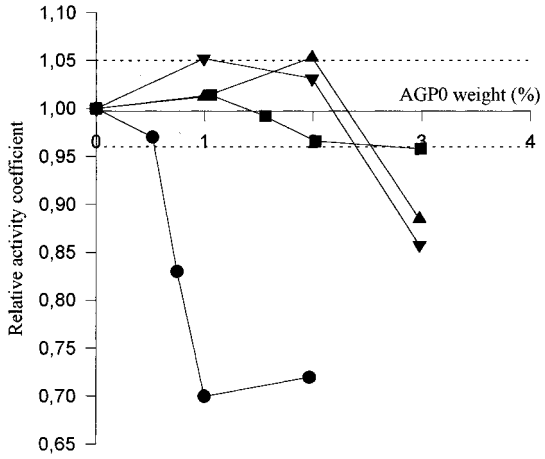

Figure 4. Variation of activity coefficients of aroma compounds in function of arabinogalactan−protein AGP0 weight fraction at 298 K. In water: (●) hexanol; (■) diacetyl. In model wine: (▲) isoamyl acetate; (▼) ethyl hexanoate.

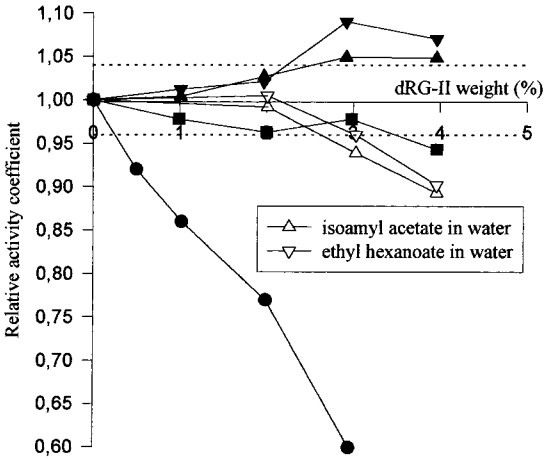

Figure 5. Variation of activity coefficients of aroma compounds in function of arabinogalactan−protein AGP4 weight fraction at 298 K. In water: (●) hexanol; (■) diacetyl. In model wine: (▲) isoamyl acetate; (▼) ethyl hexanoate.

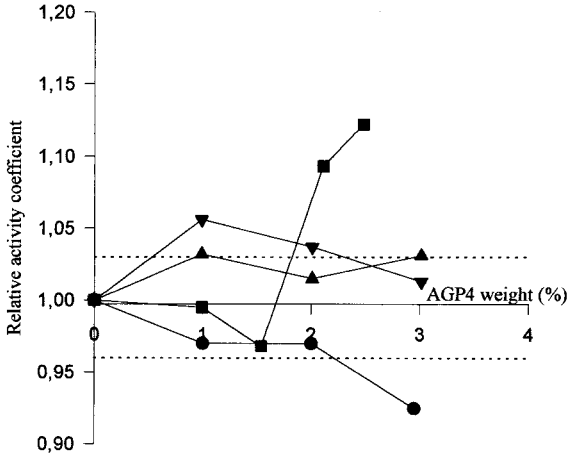

Figure 6. Variation of activity coefficients of aroma compounds in function of rhamnogalacturonan dimer dRG-II weight fraction at 298 K. In water: (●) hexanol; (■) diacetyl. In model wine: (▲) isoamyl acetate; (▼) ethyl hexanoate.

mixture (column comparison). This experimental result is in agreement with the theory developed by Leroi et al. (1977). Indeed, one can write differential equations for both solutes relating the variations of the amounts

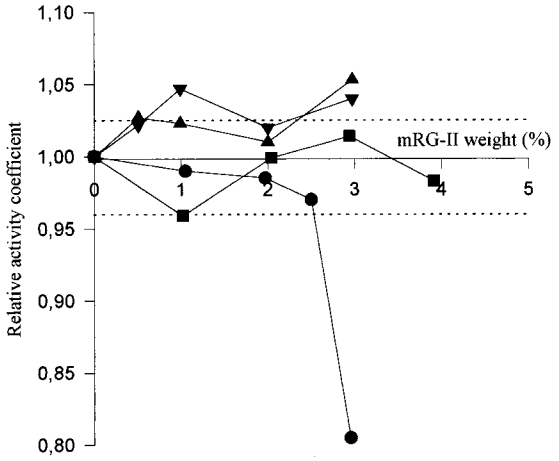

Figure 7. Variation of activity coefficients of aroma compounds in function of rhamnogalacturonan monomer mRG-II weight fraction at 298 K. In water: (●) hexanol; (■) diacetyl. In model wine: (▲) isoamyl acetate; (▼) ethyl hexanoate.

of solutes with time (for a nonvolatile solvent). In the case of infinite dilution for both volatiles, the resulting equations are independent of each other. Therefore, activity coefficients of several aroma compounds at infinite dilution can be determined in a single experiment using the dynamic exponential dilution technique. The activity coefficients for individual and combined esters were also determined in the presence of 2% dextrin (line comparison). In the case of binary systems, no binding was detected for isoamyl acetate although a highly significant retention (18%) was exhibited by ethyl hexanoate in the presence of 2% dextrin. A dextrin with an average dp of 20 can present at least a helical turn where part of the alkyl chain of ethyl hexanoate could fit as a result of hydrophobic interactions. Indeed, maltoheptaose, a left-handed helix with 6.5 residues per turn, was shown to form a "pseudo" inclusion complex with the hydrophobic ligand naproxen (Bettinetti et al., 1996). This hypothesis can be further supported by X-ray diffraction studies between starch and various organic ligands (Rutschmann and Solms, 1990). These authors found that the number of D-glucose residues per helical turn depended on the ligand structure. For decanal and monostearate, the helix had six D-glucosyl residues per turn and an internal diameter of 4.5−6 Å while menthone and (−)-limonene induced a seven D-glucose containing helix with an internal diameter closer to 6 Å. Steric hindrance may prevent the insertion of the isoamyl chain in a small cavity while weak binding may result in a larger one. Langourieux and Crouzet (1994) observed a stronger interaction of ethyl hexanoate as compared to isoamyl acetate with corn starch, respectively 60 and 10% retentions for a 10% starch weight fraction. Our results are consistent with these data as the respective retention levels for individual ethyl hexanoate and isoamyl acetate were determined to be 38 and 17% in a 5% dextrin solution (Figure 2).

Activity coefficient evaluation for the combined esters in the presence of dextrin led to a weak significant retention (6%) of isoamyl acetate concomitant with a strong retention (14%) of ethyl hexanoate. Moreover, the γ^{∞} values for $EH_{dextrin}$ in binary (12 675) and ternary (13 717) systems were discriminated at the 0.05 significant level by a *t*-test (Table 3). These results may indicate a competitive binding of the two esters. Isoamyl acetate seems to have a moderate influence on the retention level of ethyl hexanoate which in turn could display a positive effect on the binding of isoamyl acetate. The simultaneous occurrence of a synergistic effect of EH and an antagonistic effect of IA could be the result of a conformational change of dextrin. Similar trends were observed by Rutschmann and Solms (1991) for the ternary system (−)-limonene−starch−menthone. Menthone, which had a higher affinity for starch than limonene, helped in the binding of the latter while a small decrease of the menthone affinity for starch was measured. According to the small γ^{∞} variations registered at 2% dextrin, the hypothesis of a competitive binding mechanism should be verified by varying the concentration of one ligand.

Comparison of Statistical Treatments. Data from Tables 2 and 3 can be used further to compare the confidence limit method (used in the next part) with the more reliable Student *t*-test. The 3 experimental values giving the means presented in the second column (line) were determined to lie within or outside the 95% confidence limits calculated for the first column (line) data. The two data treatments were in total agreement when no difference was detected between the two series. Discrepancies were highlighted when differences were detected leading to lower significant levels with the confidence limit method. As expected, the *t*-test was more discriminating although both tests were in good agreement for most conclusions.

Aroma−Wine Polysaccharide Interactions. For comparison purpose, two well-studied polysaccharides, dextran and dextrin, were used for their opposite effect on volatile esters (Langourieux and Crouzet, 1994). Diacetyl and 1-hexanol activities were too low in model wine to be measured correctly under standard exponential dilution conditions. Hence, they were evaluated in water.

Dextran. The effects of dextran on the activity in water of four wine aroma compounds selected for their physicochemical properties are presented in Figure 1. The relative infinite dilution activity coefficient is defined as the ratio $\gamma^{\infty}_{solvent\ +\ macromolecule}/\gamma^{\infty}_{solvent}$. Both esters, isoamyl acetate and ethyl hexanoate, were salted out in the vapor phase as the result of a solubility lowering in the presence of dextran. Interestingly, the activity of 1-hexanol, a more hydrophilic compound than ethyl hexanoate, was not affected at dextran concentrations below 4%. Furthermore, diacetyl, a highly polar and water soluble molecule (log $P = -2.0$, Landy et al., 1997), was overall not affected upon increasing dextran additions.

Dextrin. An increasing decrease of the volatilities of ethyl hexanoate, isoamyl acetate, and 1-hexanol was correlated to increasing dextrin weight fractions (Figure 2). Ethyl hexanoate and 1-hexanol sharing the same structural hexyl group were the most retained resulting from stronger inclusion complexes as compared to isoamyl acetate. Similarly, Kieckbusch and King (1979) showed that partition coefficients of various *n*-alkyl acetates were differently influenced by maltodextrins: release of ethyl and propyl acetates, no effect on butyl acetate, and retention of pentyl acetate. In their studies dealing with *n*-alcohol−maltodextrin interactions, Lebert and Richon (1984) reported that the relative infinite dilution activity coefficient of 1-hexanol decreased linearly reaching 0.70 for a maltodextrin concentration of 23%. In addition, these authors established that the

Table 1. Saturated Vapor Pressure and Mean Activity Coefficient for Various Aroma Compounds in Water or in Model Wine at 298 K

aroma compd	P^s_{solute} (10^{-3} atm)	γ^{∞}_{mean}	s/γ^{∞}_{mean} (%)	γ^{∞} other authors (P^s_{solute}, 10^{-3} atm)
isoamyl acetate	7.092[a]	4614,[c] 2695[d]	3.4, 2.4	4647 (6.29),[e] 2035,[f] 1514[g]
ethyl hexanoate	3.422[a]	15481,[c] 7177[d]	4.0, 1.2	18954 (2.211),[h] 14634 (3.416),[e] 9424[i]
1-hexanol	1.389[a]	1226[c]	1.4	636 (1.316),[j] 645,[k] 417[g]
diacetyl	76.316[b]	13.26[c]	2.4	13.1 (76.316),[b] 14.0 (75.79)[l]

[a] Calculated from vapor pressure/temperature couples (*Handbook of Chemistry and Physics*, 64th ed.; West, R. C., Ed.; CRC Press: Boca Raton, FL, 1983−1984); ethyl isocaproate vapor pressure for ethyl hexanoate. [b] P^s calculated from Antoine's formula $\log Pi = A - B(C + T)^{-1}$; γ^{∞} in water (Voilley and Bosset, 1986). [c] In water. [d] In model wine IA/EH combined. [e] In water (Langourieux and Crouzet, 1994). [f] In water or [g] in model wine (Voilley et al., 1991). [h] In water or [i] in model wine (Lubbers et al., 1994b). [j] In water (Voilley, 1986). [k] In water (Lebert and Richon, 1984). [l] In water (Landy et al., 1997).

Table 2. Influence of 2% Dextrin or Mixing with Ethyl Hexanoate on the Activity Coefficient of Isoamyl Acetate (IA) in Water at 298 K

	γ^{∞}_{mean} (std dev)			
	binary system	ternary system	t_{calcd}(binary/ternary)	associated probability[a]
IA_{water}	4614 (164)	4741 (132)	1.04	−
$IA_{dextrin}$	4446 (177)	4456 (53)	0.10	−
t_{calcd}(water/dextrin)	1.21	3.46		
associated probability[a]	−	0.05		

[a] Values of t_{table} at $p = 0.05$ (2.776), $p = 0.01$ (4.604), and $p = 0.001$ (8.61).

Table 3. Influence of 2% Dextrin or Mixing with Isoamyl Acetate on the Activity Coefficient of Ethyl Hexanoate (EH) in Water at 298 K

	γ^{∞}_{mean} (std dev)			
	binary system	ternary system	t_{calcd}(binary/ternary)	associated probability[a]
EH_{water}	15481 (621)	15607 (243)	0.32	−
$EH_{dextrin}$	12675 (398)	13717 (477)	2.90	0.05
t_{calcd}(water/dextrin)	6.59	6.12		
associated probability[a]	0.01	0.01		

[a] Values of t_{table} at $p = 0.05$ (2.776), $p = 0.01$ (4.604), and $p = 0.001$ (8.61).

retention level was independent of the alcohol nature for C1−C6 *n*-alcohols with maltodextrins and β-cyclodextrin. As to diacetyl, its volatility was not found to vary significantly in the presence of 1−5% dextrin although Voilley and Bosset (1986) reported a large salting-out effect ($\gamma^{\infty}_{relative} = 2.3$) for diacetyl in a 50% maltodextrin solution.

MP0. Mannoproteins MP0 in water and model wine provided highly foaming solutions under exponential dilution conditions. A larger foaming ability was exhibited at higher nitrogen sweeping rates. The foaming extent appeared to be correlated to the protein content of the studied polysaccharides: MP0 (6.2%) > AGP0 (3.6%) > AGP4 (0.8%). Only concentrations below 3% could then be experimented. No effect was registered for diacetyl while 1-hexanol displayed a striking salting-out effect in water (Figure 3). The isoamyl acetate volatility in model wine was not significantly influenced up to 30 g/L MP0 where a slight retention appeared. The effect of mannoproteins on jointly studied ethyl hexanoate was more pronounced leading to a 10% retention level for concentrations above 20 g/L. Furthermore, mannoproteins (protein content 10−15%) released by yeasts during alcoholic fermentation of a synthetic must were shown to reduce ethyl hexanoate volatility by 12% at a level of 1 g/L in model wine (Lubbers et al., 1994a). When the mannoproteins were further purified, the retention disappeared even at 10 g/L. Lubbers et al. (1994b) also tested the effect of yeast walls used in particular winemaking procedures and containing 18% of both protein and lipid matters. At 1 g/L yeast walls, the ethyl hexanoate partition coefficient was 14% lower than in model wine. As to Langourieux and Crouzet (1997), they pointed out a 20% retention for the same ester at a yeast wall concentration of 25 g/L. These differences appear to be linked to lipid and protein contents of mannoproteins, although extraction and purification effects cannot be totally ruled out.

AGP0. Diacetyl volatility remained unaffected in water upon addition of arabinogalactan−proteins AGP0 in a 0−30 g/L range (Figure 4). High levels of AGP0 were required to decrease the activity of isoamyl acetate and ethyl hexanoate in model wine. However, a strong depressive effect was registered for 1-hexanol indicative of either an AGP0−hexanol bimolecular interaction or a texture modification. The dynamic flavor release is known to be affected by texture rather than by phase partitioning (Godshall, 1997). Here, factors influencing mass transfer are viscosity and foaming as well as nitrogen bubble size. Viscosity only increased visibly for concentrated MP0 and dextrin solutions. Foaming was observed for all protein-containing macromolecules, and nitrogen bubble size was reduced upon ethanol addition.

AGP4. No influence was evidenced for isoamyl acetate in model wine although AGP4 seemed to exert a weak salting-out effect on ethyl hexanoate (Figure 5). Interestingly, AGP4 had a depressive effect on 1-hexanol activity although less strongly than AGP0. The main differences between the two AGPs lie in the protein and uronic acid contents. AGP0 is richer in protein (3.6%) and is a less negatively charged polymer with an uronic acid level of 2.7%. The protein and uronic acid contents of AGP4 are respectively 0.8 and 20.4% (Pellerin et al., 1995). Electrostatic interactions should be ruled out especially as a salting-out effect was detected for diacetyl. The protein part of the macromolecule may play a role in the interaction of AGPs with lipophilic volatile substances.

dRG-II. The activities of all four aroma compounds were evaluated in water (Figure 6). A prominent result was obtained with 1-hexanol where the binding ability of dRG-II was found superior to the one observed with dextrin. The retention level was 40% with 30 g/L dRG-II as compared to 30% in the presence of 30 g/L dextrin. Besides, dRG-II in water bound significantly the mixture of esters at concentrations above 30 g/L. The comparison of model wine vs water gave an insight into the roles of ethanol and pH in the medium. The addition of 11.76% (v/v) ethanol led to a 2-fold decrease of the ester activity coefficients as the result of a better solubility (Table 1). In model wine, the ester activities

were slightly increased as observed with the other uronic acid-rich polysaccharides AGP4 and mRG-II. Rhamnogalacturonans present a significant charge density difference between pH 3.5 and pH 6 linked to a 40% level in uronic acid residues (Vernhet et al., 1996). In model wine, RG-IIs are less negatively charged polymers and should be less repellent for neutral substances. As a matter of fact, the opposite influence of dRG-II on the two ester volatilities must be linked to ethanol as cosolvent. This latter is known for disrupting hydrophobic interactions through perturbation of the three-dimensional network of hydrogen-bonded water molecules (Brouillard et al., 1989). Finally, dRG-II did not show any affinity for diacetyl in the studied concentration range.

mRG-II. Similar trends were observed for esters with the monomeric polymer mRG-II. The activity of 1-hexanol was sharply reduced at mRG-II concentrations above 20 g/L. This could be due to the small fraction of dRG-II (10%) contained in the monomer. Besides, polymer aggregation resulting from increasing mRG-II concentrations may give rise to an interaction site or a texture modification slowing then flavor release. Diacetyl behavior remained unaffected.

CONCLUSION

Although the studied polysaccharide concentrations were superior to those usually found in wine, this work provides grounds for the area of interactions between native wine polysaccharides and aroma substances. Interaction studies showed a tendency of the uronic acid-rich polysaccharide fractions AGP4, mRG-II, and dRG-II to salt out the mixture of esters in a model wine. However, high levels of the protein-rich fractions MP0 and AGP0 led to a slight retention of the two esters. In water, the addition of dRG-II (at concentration above 30 g/L) induced a decrease in the volatility of the esters. Ethanol rather than pH played a role in disrupting hydrophobic interaction through modification of the solvation state of the molecules. None of the model or wine polysaccharides affected the diacetyl volatility, except AGP4. Valuable results for enology were obtained with 1-hexanol. Increasing polysaccharide addition led to an activity reduction in the decreasing strength order AGP0, dRG-II, dextrin, mRG-II, and AGP4 as well as to a dramatic release of 1-hexanol in the presence of MP0.

Because of difficulties in purifying wine polysaccharides, only works dealing with *Saccharomyces* exocellular and wall mannoproteins had been reported so far. Wine proteins and glycosylated proteins (10−100 mg/L) should be considered as candidates for interaction with volatiles. Proteins are known to interact strongly with lipophilic ligands (Druaux *et al.*, 1995, and references therein). In addition to charge densities, a conformational study of these highly branched polymers is required to shed light on the type of binding involved in intermolecular interactions.

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