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Interactions between aroma compounds and whole mannoprotein isolated from *Saccharomyces cerevisiae* strains

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Abstract

The interactions of four wine aroma compounds (isoamyl acetate, hexanol, ethyl hexanoate, β-ionone) either with a whole mannoprotein extract or with mannoprotein fractions at a level encountered in wine (150 mg/L) were studied by dynamic and static headspace techniques. The mannoproteins were isolated from a synthetic medium subjected to an alcoholic fermentation with two enological yeast strains. They were fractionated by exclusion chromatography and characterized through glycosyl composition analysis.

While the volatility of isoamyl acetate was affected neither by whole extracts nor by mannoprotein fractions, a significant decrease of the volatility (up to 80%) was observed for the other aroma compounds. If the behaviour of mannoproteins towards ethyl hexanoate differed between the two strains, a similar retention was found for the other compounds. The retention level was not the same when whole mannoprotein extract and isolated mannoprotein fractions were used, suggesting the possible interactions between mannoprotein fractions.

Keywords: Flavour; Mannoproteins; Interactions; *Saccharomyces cerevisiae*

1. Introduction

Mannoproteins produced by yeasts are second most abundant family of polysaccharides in wine representing ca. 35% of the total polysaccharides (Vidal, Williams, Doco, Moutounet, & Pellerin, 2003). Two groups of mannoproteins are found in wine. The first one is made up of those secreted into wine by yeast during alcoholic fermentation at the levels close to 100–150 mg/L. They possess very varied sizes extending from 5000 at more than 800,000 Da (Doco, Brillouet, & Moutounet, 1996). These macromolecules have structural analogy with those from yeast cell walls (Ballou, 1982; Doco, Vuchot, Cheynier, & Moutounet, 2003; Saulnier, Mercereau, & Vezinhet, 1991; Vidal et al., 2003; Waters, Pellerin, & Brillouet, 1994). The second group of mannoproteins deals with those released to wine due to the autolysis of yeasts during ageing on lees, probably through the cleavage of linkages between mannoproteins, glucans and chitin (Doco, Quellec, & Moutounet, 1999).

The presence of these mannoproteins in wines has many consequences: (i) reduction of the visible protein haze in white wine (Dupin et al., 2000; Waters et al., 1994), (ii) decrease in astringency of red wines (Vidal et al., 2004), (iii) increase in colour stability, (iv) inhibition of tannin aggregation (Riou, Vernhet, Doco, & Moutonnet, 2002), (v) stimulation of malolactic fermentation (Guilloux-Benatier, Guerreau, & Feuillat, 1995), (vi) protection of wine from tartric acid precipitation (Gerbaud, Gabas, Bloiun, Pellerin,

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& Moutonnet, 1997; Moine-Ledoux & Dubourdieu, 1999) and (vii) interaction with wine volatile compounds (Dufour & Bayonove, 1999; Lubbers, Leger, Charpentier, & Feuillat, 1993; Lubbers, Voilley, Feuillat, & Charpentier, 1994a; Lubbers, Charpentier, Feuillat, & Voilley, 1994b; Voilley, Lamer, Dubois, & Feuillat, 1990; Voilley, Beghin, Charpentier, & Peyron, 1991).

It is known that aroma compounds are able to interact with polysaccharides and proteins. Aroma retention has been observed with starches, galactomannans while with dextrans or sucrose a salting-out phenomenon occurs (Langourieux & Crouzet, 1994). For starches, the binding of volatiles has been classified into two types: inclusion complexes in the amylose helix and polar interactions with hydroxyl groups of starch (Arvisenet, Voilley, & Cayot, 2002; Heinemamn, Escher, & Conde-Petit, 2003; Rutschmann & Solms, 1990). Strong retentions have been shown between soy proteins, milk proteins and pea legumin with different aroma compounds as aldehydes, alcohols, esters, terpenes, methyl ketones, or ionones through hydrophobic interactions or hydrogen bonds (Damodaran & Kinsella, 1981; Guichard & Langourieux, 2000; Heng et al., 2004; Landy, Druaux, & Voilley, 1995; van Ruth & Villeneuve, 2002). The strength of interactions was found to depend on the nature of the aroma compound and on the nature of macromolecule.

The few studies regarding interactions between aroma compounds and yeast cell walls or wine mannoproteins have shown the importance of the physico-chemical nature of the volatile compounds: a greater degree of interactions was often observed with hydrophobic compounds (Lubbers et al., 1994a). However, high levels of mannoprotein extracts, from 1 to 30 g/L, had been used to demonstrate the binding capacity of these macromolecules against esters (ethyl hexanoate and isoamyl acetate) hexanol and β-ionone (Dufour & Bayonove, 1999; Lubbers et al., 1994a, 1994b).

The aim of this work was to determine the interactions between volatile compounds of wine not only with whole mannoprotein extract but also with isolated mannoprotein fractions at a concentration that these macromolecules may occur in wine (150 mg/L). Mannoproteins were produced by two strains of *Saccharomyces cerevisiae* and characterized with regard to glycosyl-residue composition. Volatile compounds were those described in previous studies related to the interactions of wine volatiles with mannoproteins: isoamyl acetate, hexanol, ethyl hexanoate, β-ionone. Dynamic (exponential dilution) and static headspace techniques were used to study the interactions in a model wine solution.

2. Materials and methods

2.1. Aroma compounds

The volatile compounds, hexanol, isoamyl acetate, ethyl hexanoate and β-ionone were purchased from Sigma–Aldrich (St. Louis, MO) and ethanol from Carlo Erba Reactive (Rodano, Italy). Their purities were >98%. The four aroma compounds selected are among wine aroma contributors and differ in vapour pressure and hydrophobicity estimated by the logarithm of the partition coefficient between water and *n*-octanol (log P) (Table 1).

Table 1
Physico-chemical characteristics of aroma compounds

Aroma compound	Molecular weight (g/mol)	$P_{s,i}$ (10^{-3} atm) at 25 °C[a]	Log P[b]
Ethyl hexanoate	144.2	1.559	2.78
Isoamyl acetate	130.1	7.046	2.11
β-Ionone	192.3	0.0331	3.92
Hexanol	116.2	0.1448	1.82

[a] Saturated vapour pressure of aroma compound calculated from vapour pressure/temperature data of Handbook of Chemistry and Physics, 85th, for β-ionone saturated vapour pressure of α-ionone was used.
[b] Estimated with modelling molecular Pro software.

2.2. Yeast strains

S. cerevisiae strains ICV D21 and ICV D80 were commercial yeasts from Institut Coopératif du Vin (ICV), (La Jasse de Maurin, 34970 Lattes, France).

2.3. Mannoprotein preparations

2.3.1. Culture media and growth conditions

The synthetic medium described by Salmon, Doco, Vuchot, and Moutounet (2003) was inoculated either with ICV D21 or ICV D80 strains using starter cultures (5 mL of inoculum/litre issued from a overnight culture at 24 °C). Fermentations (168 h), monitored by measuring CO_2 production, were carried out in fermentors (1.1 L, 24 °C) with agitation (magnetic stirring) and aerated on a regular basis (1 oxygen saturation per day) throughout the fermentation oxygenation (Bely, Sablayrolles, & Barre, 1990).

2.3.2. Separation of mannoproteins from fermented media

Yeast cells and medium were separated by centrifugation (6000*g*, 20 min) at the end of alcoholic fermentation corresponding to the concentration of sugars less than 2 g/L; the concentration was determined by dinitrosalicylic acid reagent (Miller, 1959). Supernatants containing secreted mannoproteins were dialyzed against distilled water (MWCO 6–8 kDa), concentrated and freeze-dried. The samples constituted the whole mannoprotein extracts.

2.3.3. Fractionation of the whole mannoprotein extract

Twenty milligrams of mannoprotein extract from ICV D21 and ICV D80 strains were dissolved in 2 mL of water, centrifuged as above to eliminate residual insoluble material, and the supernatant was loaded on Superdex-30 HR column (1.6 × 60 cm, Pharmacia, Sweden) equipped with a pre-column (0.6 × 4 cm). The column was equilibrated at 1 mL/min with ammonium formiate (30 mM; pH 5.6). The elution of mannoproteins was monitored with an

Erma-ERC 7512 (Erma®, Japan) refractive index detector combined with a Waters Baseline 810-software. Three peaks were observed and pooled. First pool (37–48 min) constituted the first mannoprotein fraction, second pool (49–91 min) the second mannoprotein fraction and the last pool (92–99 min) the third mannoprotein fraction. The fractions F1, F2, and F3 were freeze-dried, re-dissolved in water and freeze-dried again. This procedure was repeated four times to eliminate the ammonium salt.

2.3.4. Analytical methods

Neutral monosaccharides were released from polysaccharide fractions by treatment with 2 M trifluoroacetic acid (75 min at 120 °C) according to Albersheim, Nevins, English, and Karr (1967). They were then converted to the corresponding alditol acetate derivatives (Harris, Henri, Blakeney, & Stone, 1984) and quantified by GC analysis using a fused silica DB-225 (210 °C) capillary column (30 m × 0.32 mm i.d., 0.25 μm film) with H_2 as carrier gas on a Hewlett–Packard Model 5890 gas chromatograph. Protein amount was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951) with bovine serum albumin as standard.

2.3.5. Glycosyl-linkage compositions

The Glycosyl-linkage composition of polysaccharides was determined by GC of the partially methylated alditol acetates. Polysaccharides (1 mg) in 0.3 mL dimethylsulfoxide were methylated using methyl sulfinyl carbanion and methyl iodide (Hakomori, 1964). Methylated samples were then submitted to acidic hydrolysis, $NaBH_4$ reduction and acetylation. Partially methylated alditol acetates were analysed by GC–EI-Mass Spectrometry with a 30 m × 0.25 mm OV-1 column (temperature programming: 135 °C for 10 min, then 1.2 °C/min to 180 °C). The carrier gas was hydrogen (2mL/min). GC–MS was performed with a Hewlett–Packard HP-6890 GC coupled to a HP 5973 mass selective detector operated in the EI mode (70 eV, 34.6 μA and *m/z* 50–650 uma) and under the control of a HP Productivity ChemStation.

2.4. Determination of interactions

2.4.1. Exponential dilution and determination of activity coefficients

The device used for exponential dilution study was the same as previously described (Langourieux & Crouzet, 1994). Aroma compound (1 μL) was spiked with 20 g of 12% (v/v) EtOH/aqueous tartrate solution adjusted to pH 3.5 (model wine solution:blank) or with the same solution containing whole mannoprotein extract or mannoprotein fractions (0.015% w/w). The solutions were placed in a diffusion cell at 25 °C and stripped by an inert gas (N_2) at a flow rate varying from 30 to 100 mL/min according to the volatile compound analysed. The stripping gas was dispersed into small diameter bubbles through a glass frit disk (no. 4, cell size 10–16 μm). Under the effect of gas bubbling and magnetic stirring, an exponential decrease of the concentration of the volatile compound in the effluent gas was observed. A six ways pneumatic valve was used to sample 200 μL of the vapour phase from the diffusion cell, which was injected onto a Varian 3300 GC-FID equipped with a DB-1 column (30 m × 0.25 mm i.d, 0.25 μm film, J&W Scientific) and isothermally operated at 50 or 60 °C with a nitrogen flow rate of 2mL/min.

The aroma concentration decrease obeyed the equation below (Leroi, Masson, Renon, Fabries, & Sannier, 1977):

$$\ln S = \frac{-FP_{s,i}}{RTN}\dot{\gamma}_i t + \ln S_0,$$

where S is the GC peak area, t is the time (min), S_0 is the GC peak area extrapolated to zero time, F is the stripping gas flow rate (mL/min), N is the number of moles of solvent (water and ethanol), R is the gas constant (mL atm/mol K), $P_{s,i}$ is the vapour pressure of the pure solute (atm) at 25 °C and T is the temperature in Kelvin. $\dot{\gamma}_i$ was calculated from the slope of the straight line obtained by plotting $\ln S$ versus time.

2.4.2. Static headspace analysis

Model wine solutions (5 mL) spiked with target aroma compounds with or without mannoproteins (whole extract or fractions at 0.015% w/v) were placed in glass flasks (20 mL) closed with Teflon septa in plastic screw caps and equilibrated at 25 °C during 24 h. In order to detect each aroma compound, the concentration was 20 ppm for ethyl hexanoate, 24 ppm for isoamyl acetate, 189 ppm for β-ionone and 173 ppm for hexanol.

Injections of vapour phase (500 μL) were performed automatically by a comBipal Varian. A Varian 3300 GC-FID equipped with a DBwax column (30m × 0.32 mm i.d., 0.25 μm film, J&W Scientific) was used and isothermally operated (40 °C for hexanol, 50 °C for isoamyl acetate and ethyl hexanoate and 140 °C for β-ionone). Gas carrier (hydrogen) flow rate was 2 mL/min. Injector and detector temperatures were set at 250 °C. The GC peak area obtained from the mannoprotein solution divided by the peak area from blank solution was used to estimate the level of retention. A value below 1 indicated a retention phenomenon while a value above 1 indicated a salting out effect.

2.5. Sensory evaluation

Preliminary sensory evaluation has been carried out by a limited panel (only four assessors). In training sessions, the panel was familiarized with selected aroma compounds added to the model wine solution to determine one or two descriptors for each aroma compound by consensus. A linear scale (10 cm verbally anchored at both ends, e.g., weak–strong) was used for intensity evaluation of the descriptors as practised in descriptive analysis. The samples coded with three-digit numbers and with or without the mannoproteins (150 mg/L) were presented to the judges in a randomised order at controlled temperature.

2.6. Statistical analysis

All the experiments were performed at least in triplicate. A one-way analysis of variance was performed using Statview software. Means comparison was made by Newman–Keuls test at $p < 0.05$.

3. Results and discussion

3.1. Characterisation of polysaccharides from whole mannoprotein extracts and mannoprotein fractions

The levels of mannoproteins secreted by the yeast strains ICV D21 and ICV D80 at the end of fermentation were quite similar, 605 and 583 mg/L, respectively. The sugar composition of the macromolecules secreted by the two yeasts was very close, with however more mannose for yeast ICV D80 than for ICV D21. The relative percentages of mannose were 73% and 79% for macromolecules from yeast strains ICV D21 and ICV D80, respectively, and those of glucose were 28% and 22%. Lubbers et al. (1994a) for the strain Levuline BRG, or Llauberes, Dubourdieu, and Villetaz (1987) for the strain Uvaferm CM, reported similar values. The major monosaccharide was mannose in agreement with previous studies (Vuchot et al., 2000; Llauberes et al., 1987; Lubbers et al., 1994a; Saulnier et al., 1991; Waters et al., 1994). Quite high levels of protein and in particular for the mannoproteins from the strain ICV D80 (236 mg/L against 178 mg/L) were detected.

Whole mannoprotein extracts from strains ICV D21 and ICV D80 were fractionated through a preparative chromatography on Superdex-30 HR column (data not shown). For both strains three fractions were obtained. The first fraction presented a high MW over 100 kDa, the second one showed mass ranging from 5 to 50 kDa and the third one was a complex mixture of oligosaccharides and small peptides. The latter was partially analysed in this study due to the minute amounts (Table 2). The fractions F1 and F2 were composed mainly of mannose, glucose, and proteins. Fractions F1 secreted by both strains were similar in composition containing from 11% to 13% of proteins and resembling to the mannoproteins described by Ballou (1976). On the contrary, fractions F2 were much richer in proteins than fraction F1, in particular, that from the strain ICV D80 (72%). Contrary to the fractions F1 and F2, the fractions F3 were richer in glucose than in mannose residues.

Analysis of the glycosidic linkages in the fractions F1 and F2 from both strains showed that terminal mannose and 2- and 3-linked mannose were the main structural features, followed by 2,6-linked mannose. These structural features are in good agreement with the model proposed by Ballou (1982), and match with those typically found in yeast mannoproteins (Saulnier et al., 1991) or with those from mannoproteins secreted into the wine (Vidal et al., 2003; Waters et al., 1994). Mannose from fraction F1 was linked in −2, in −3, in −2.6 and in non reducing terminal positions in molar ratio of 1.2, 0.8, 1.0, 1.3 and 1.2, 0.6, 1.0, 1.3 for strain ICV D21 and strain ICV D80, respectively. The structure of mannoproteins in these fractions consisted of a long 6-linked backbone, highly substituted on position two with 2- and 3-linked mannose side chains, and attached to protein at asparagin residues. ICV D80 strain secreted high molecular mannoproteins with less mannose in position 3 and thus leading to the shorter side chains than those of ICV D21. Presence of glucans in these fractions was indicated by identification of 6-linked glucose in the methyl ethers detected. It has been reported that the mannoproteins bound at a yeast cell wall were made up of α-(1 → 6) glucans by a glycophosphate molecule possessing chains of five mannose residues (Kollar et al., 1997). Mannoproteins possessing small chains of glucans are issued from glucanase catalysed hydrolysis of α-(1 → 6) glucans during the growth of yeasts.

Fractions F2 contained mannose linked in −2, in −3, and in no reducing terminal positions in molar ratio of 2.0, 1.0, 1.3 and 1.9, 1.0, 2.2 for strains ICV D21 and ICV D80, respectively. In contrast to F1 fraction, no mannose was linked in 2,6- or 6- positions. These particular glycosidic compositions observed in fractions F2 clearly indicated that majority of mannoproteins were composed of short 2- and 3-linked mannose chains attached to serine and threonine residues. It was noted also that the F2 fraction of the strain ICV D80 had short mannose chains in α-(1 → 3) and that the F2 fraction of ICV D80 was particularly rich in proteins. The presence of these O-linked mannoproteins with low molecular weights (5–50 kDa) during growth of yeasts is the consequence of the enzymatic hydrolysis of the cell walls (Babayan & Bezrukov, 1985).

Table 2
Glycosyl residue, glycosyl-linkage compositions and protein contents of mannoprotein fractions from yeasts ICV-D21and ICV-D80

Superdex 30 fractions	ICV-D21			ICV-D80		
	F1	F2	F3	F1	F2	F3
MW (kDa)	225	41	10	225	41	10
% of macromolecules	36.8	38.6	24.6	41.1	42.2	16.7
Proteins (%) [a]	13	30	nd	11	72	nd
Glycosyl residue [b]						
Mannose	88	65	13	91	68	13
Glucose	12	35	87	9	32	87
Glycosyl-linkage[c]						
Glc-(1→	1	5	nd	1	4	nd
→6)-Glc-(1→	3	10	nd	3	7	nd
Man-(1→	28	24	nd	30	33	nd
→2)-Man-(1→	26	36	nd	28	38	nd
→3)-Man-(1→	18	18	nd	14	17	nd
→6)-Man-(1→	2	–	nd	1	–	nd
→2,3)-Man-(1→	–	7	nd	–	–	nd
→2,6)-Man-(1→	21	–	nd	23	–	nd

nd: not determined.
[a] Percent of dry matter.
[b] Molar ratio, neutral sugars determined by GC of alditols acetates, and expressed as anhydrosugars.
[c] Molar ratio.

3.2. Interactions between aroma compounds and whole mannoprotein extract

Measurements of interactions between mannoproteins and flavour compounds were performed using the exponential dilution technique (or dynamic method) and headspace static method. The dynamic method could only be used for the two esters: ethyl hexanoate and isoamyl acetate due to their high vapour pressure in comparison to that from β-ionone and hexanol (Table 1). First, it could be notified that the activity coefficients of these compounds were significantly lower in the water–alcoholic solution than in water (Table 3). This dramatic decrease in the volatility of compounds has already been observed and explained by an increase of solubility in ethanol (Dufour & Bayonove, 1999; Lubbers et al., 1994a, 1994b; Ragazzo-Sanchez et al., 2005; Ramirez Ramirez et al., 2001). Furthermore, the presence of mannoproteins from both yeast strains in the wine model solution lowered clearly the volatility of ethyl hexanoate but not affected that of isoamyl acetate. The retention of ethyl hexanoate was more pronounced by mannoproteins obtained from ICV D80 strain (44%) than those from ICV D21 strain (32%).

Similar tendency was observed for the retention of both esters by static headspace method (Table 4). For isoamyl acetate no effect was observed with mannoproteins from ICV D21 strain but a slight and significantly salting-out effect appeared in the presence of mannoproteins from ICV D80 strain. For ethyl hexanoate, an important retention and particularly with mannoproteins from ICV D80 strain (64%) was again shown. However, the effect was higher (ca. 20%) than that estimated by activity coefficients measurement. These differences observed by the two methods could probably be due to a displacement of the equilibrium in dynamic headspace, which reduced the retention effect of protein (Fabre, Aubry, & Guichard, 2002).

Dufour and Bayonove (1999) had shown, using the exponential dilution technique, that isoamyl acetate and ethyl hexanoate volatility in a model wine was not significantly influenced by mannoproteins isolated from a red wine, and used at concentrations between 5 and 20g/L. Above these concentrations, which were several fold higher than that used in our study (0.15 g/L), these authors observed a slight retention for isoamyl acetate (5%) and ethyl hexanoate (10%). However, they did not report the conditions of mannoprotein separation and purification. It has been reported that the procedure used for the recovery and purification of mannoproteins and consequently their conformation state could influence their ability to interact with aroma compounds (Langourieux & Crouzet, 1997). Ethyl hexanoate retention by mannoproteins was clearly more important in our study than that found in several studies (12–20%) using yeast cell walls rich in mannoproteins or high concentrations of mannoproteins ($\geqslant 1$ g/L) (Langourieux & Crouzet, 1994; Lubbers et al., 1994a, 1994b). Mannoprotein aggregates could be formed when they are used at high concentrations, thereby decreasing the accessibility of volatiles compounds to binding sites of these macromolecules. In a study with sodium caseinate, it has been suggested that increasing concentrations of the macromolecule favoured protein–protein interactions and consequently the accessibility of the hydrophobic sites of protein for aroma compounds decreased (Landy et al., 1995).

As for ethyl hexanoate, the volatility of β-ionone decreased in the presence of mannoproteins: a retention of 40% with those from ICV D21 strain and 54% with those

Table 3
Effect of whole mannoproteins extract (MPE) from ICV D21 and ICV D80 strain on activity coefficient at infinite dilution of ethyl hexanoate and isoamyl acetate in model wine solution at 25 °C

Aroma compound	Activity coefficient at infinite dilution at 25 °C			
	In water	In model wine solution	In model wine solution added with (MPE) ICVD21	In model wine solution added with (MPE) ICVD80
Ethyl hexanoate	32,700[a] (920)	15,600[b] (2030)	10,900[c] (695)	9150[d] (264)
Isoamyl acetate	4870[a] (160)	1800[b] (200)	1600[b] (413)	1600[b] (386)

(a–d) Different labels indicate that means significantly differ at $p < 0.05$ (based on Neuwman–Keuls test).
Values between parentheses are standard deviation.

Table 4
Effect of whole mannoprotein extract (MPE) from ICVD21 and ICVD80 on aroma retention measured by static headspace analysis at 25 °C

	Peak area in reference solution	Peak area in solution of ICV D21 MPE	Peak area in solution of ICV D80 MPE	Relative area (%) ICV D21	Relative area (%) ICV D80
Ethyl hexanoate	51,800[a] (7480)	28,470[b] (290)	17,680[c] (1700)	55	34
Isoamyl acetate	217,100[a] (10,090)	214,100[a] (11,896)	229,500[b] (11,655)	99	106
Hexanol	167,000[a] (19,721)	121,580[b] (3156)	123,500[b] (1347)	83	84
β-Ionone	21,500[a] (2041)	12,800[b] (1324)	100,00[b] (1772)	60	46

(a)–(c) Different labels indicate that means significantly differ at $p < 0.05$ (based on Neuwman–Keuls test).
Values between parentheses are standard deviation.

from ICV D80 strain occurred (Table 4). However, no significant differences were observed between mannoproteins from both strains. Retention of β-ionone was found to be 23% and 70% in the presence of yeast cell walls at 1 and 10 g/L, respectively (Lubbers et al., 1994b). This phenomenon was explained by the interactions not only with proteins from the cell walls but also with lipids.

Hexanol was retained to a lesser extent compared to ethyl hexanoate and β-ionone. Hydrophobic constant log *P* values show that this compound is less hydrophobic than ethyl hexanoate or β-ionone (Table 1). However, the hydrophobicity of hexanol is quite close to isoamyl acetate while their retention by mannoproteins was found to be different. Indeed, the nature of aroma compounds could intervene in the strength of interactions. Pea legumin, soy proteins and milk proteins as β-lactoglobulin are known to interact strongly with hydrophobic compounds (Guichard & Langourieux, 2000; Heng et al., 2004; Landy et al., 1995; van Ruth & Villeneuve, 2002). However, it has been demonstrated with β-lactoglobulin, a protein with a binding hydrophobic core, that the protein effect was often selective and aroma compound dependent.

Contrary to our results, a salting out effect (13%) was reported for hexanol in the presence of mannoproteins at a concentration of 10 g/L (Dufour & Bayonove, 1999). This difference may be attributed to the high concentration of the mannoproteins used by these authors and to the absence of ethanol in their conditions of analysis. Indeed, we have observed that hexanol volatility was decreased by 35% when water–alcoholic solution (12% v/v) was used instead of aqueous solution (data not shown).

Sensory evaluations were performed to verify the effect of mannoprotein on the aroma perception. The presence of the mannoproteins in wine model solutions added with aroma compounds resulted in the decrease of aroma intensity. In addition, differences between the two types of mannoprotein were detected in terms of descriptor intensity. These results are in agreement with those obtained by headspace gas chromatography technique except for isoamyl acetate. Indeed, no change in volatility of this compound in the presence of mannoproteins was observed by headspace gas chromatography technique. Sensory analysis is often considered more relevant to real life situation than GC method and is a good complement of analytical method.

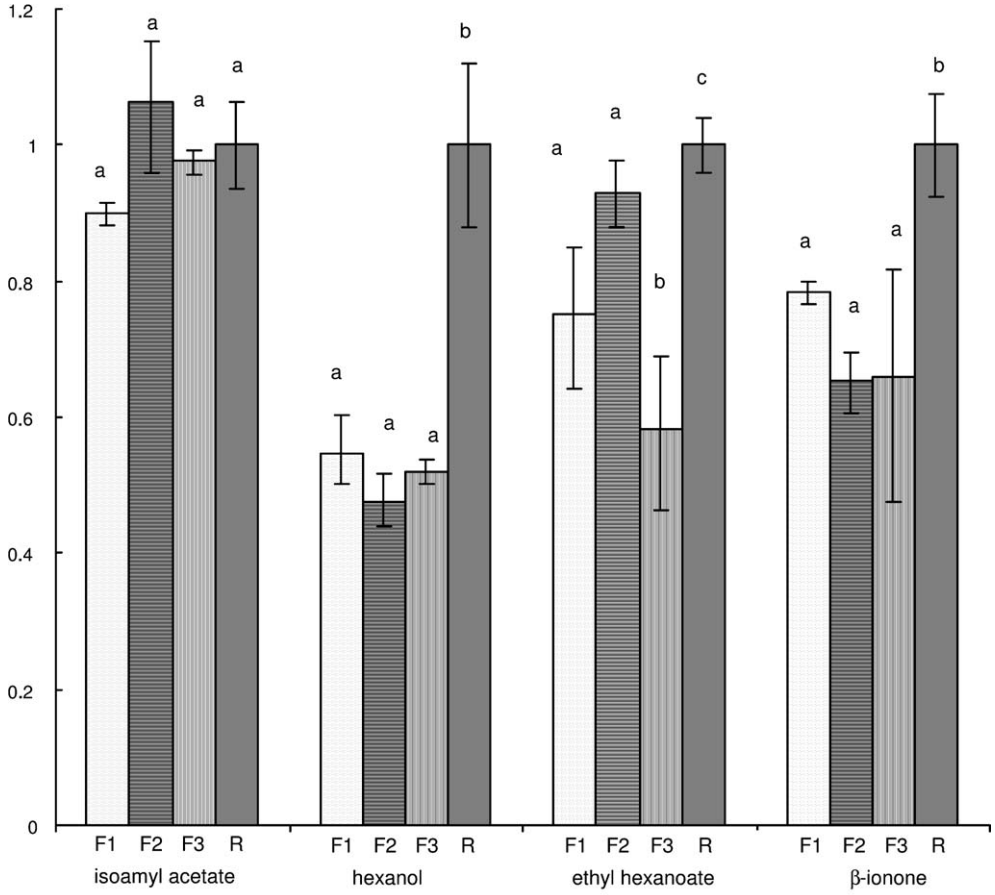

Fig. 1. Effect of mannoproteins fractions (F1, F2 and F3) isolated from ICVD80 strain on aroma peak area in the headspace at 25 °C. Relative peak area is obtained by dividing the peak area of a volatile compound in the model solution added with mannoprotein fraction by the peak area in the model solution without mannoprotein. *R* represents relative peak area in the absence of mannoprotein. Errors bars represent standard deviation. Different labels (a–c) indicate that means significantly differ at $p < 0.05$ (based on Neuwman–Keuls test).

3.3. Interactions between aroma compounds and mannoprotein fractions

Measurements of interactions between aroma compounds and three mannoprotein fractions were reported in Fig. 1, for ICV D80 strain and Fig. 2, for ICV D21 strain. The interactions with the four aroma compounds were studied with ICV D80 fractions, while only interactions with ethyl hexanoate and β-ionone were measured with ICV D21 fractions.

In agreement with the results using whole mannoprotein extract, the volatility of isoamyl acetate was not significantly affected by mannoprotein fractions from ICV D80 strain. In contrast, the volatility of the other aroma compounds differed when compared to the results obtained with whole mannoprotein extract. The effect of the different fractions of ICV D80 mannoproteins on hexanol volatility was similar but surprisingly the volatility was almost threefold lower than that in the presence of whole extract (50% against 16%). This could be attributed to the amount of mannoprotein fractions used (0.15 g/L) without considering their proportion in whole extract and also to the existence of interactions between the different fractions in whole extract. In this case, the purification steps could modify the accessibility of interaction site.

Regardless of the fractions and the origin of mannoproteins, the retention of ethyl hexanoate was found weaker than with whole mannoprotein extract. However, while no significant differences in behaviour of the two fractions of high molecular weight towards this compound were observed, the fraction of low molecular weight (F3) from both yeast strains bound it more strongly. The fractions F3 were poorer in macromolecules than the two other fractions and mannose was not the major sugar, but glucose. The richness in protein of the fraction F2 of ICV D80 (72%) had no significant effect on ethyl hexanoate retention compared to fraction F1, which was different by its low protein content (11%). Therefore, this volatile compound seemed to have a higher affinity for glycosidic parts of mannoproteins than for protein parts. It could be assumed that as for hexanol, the retention depended on the accessibility of the binding site. However, the nature of binding sites for the two compounds seems to be different.

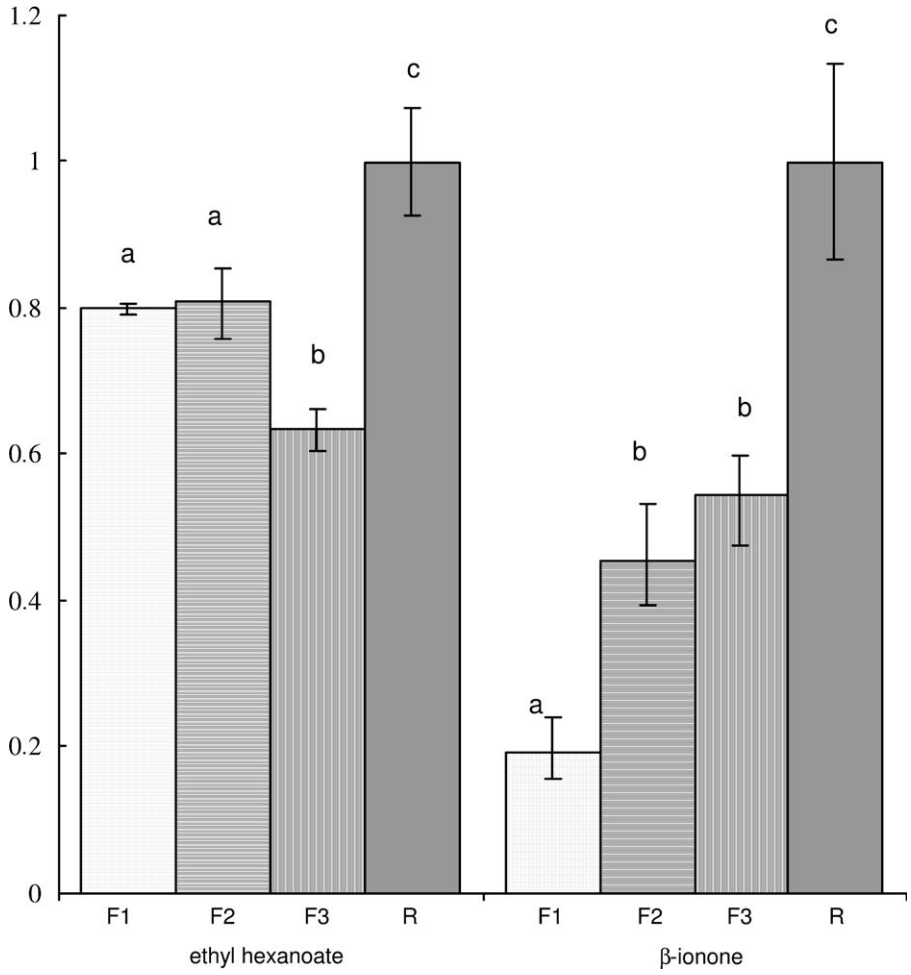

Fig. 2. Effect of mannoprotein fractions (F1, F2 and F3) isolated from ICVD21 strains on aroma peak area in the headspace at 25 °C. Relative peak area is obtained by dividing the peak area of a volatile compound in the model solution added with mannoprotein fraction by the peak area in the model solution without mannoprotein. *R* represents relative peak area in the absence of mannoprotein. Errors bars represent standard deviation. Different labels (a–c) indicate that means significantly differ at $p < 0.05$ (based on Neuwman–Keuls test).

In contrast to whole extract, the fractions obtained from ICV D80 had a little effect on the volatility of β-ionone. No specific retention by one of the fractions could be observed. On the contrary, the retention of this compound was more pronounced with the mannoprotein fractions from yeast ICV D21 than with the corresponding whole extract. In particular, the higher molecular weight fraction (100 kDa F1), which contained 11% of proteins and composed mainly of *N*-glycane mannoproteins, bound strongly (80%) β-ionone. It has been shown that a whole mannoprotein extract obtained from an alcoholic fermentation bound more β-ionone (17%) than the fraction rich in mannose obtained by chromatography on concavaline (Lubbers et al., 1994a). Yeast mannans with a molecular mass of 23.5 kDa and poor in protein (ca. 5%) retained slightly β-ionone (15%) when added into a model wine solution (10% v/v of ethanol) (Voilley et al., 1991). On the basis of these results, the conformational structure of the mannoproteins seems to be an important factor on the strength of interactions.

Differences in binding capacity for β-ionone of the fractions F1 from both strains could be explained by the differences in protein levels (8.5 mg/L for ICV D21 against 10.6 mg/L for ICV D80) and/or in lateral chain length of polysaccharide (that from ICV D80 is shorter than that from ICV D21 strain). Due to the cyclic structure and high hydrophobicity of β-ionone, strong interactions have been observed with β-lactoglobulin by fixation on hydrophobic cavity of the protein (Dufour & Haertlé, 1990). Furthermore, this compound was also retained through the formation of inclusion complexes with amylose from starches (Rutschmann & Solms, 1990). The fraction F1 of ICV D21 may possess a conformational structure allowing the establishment of strong interactions with β-ionone.

In conclusion, evidence for interactions between mannoproteins secreted by yeasts and aroma compounds was obtained at the concentration that these macromolecules occur in wine. Mannoproteins from two commercial yeast strains differed with regard to the strength of interactions with aroma compounds. Such difference between yeast strains may have a consequence in aroma quality of wines and should be confirmed by sensory evaluation. The retention of aroma compounds by whole mannoprotein extract from both strains and by fractions of mannoprotein isolated was not to the same extent, suggesting the role of conformational and compositional structure of these macromolecules in the interactions with aroma compounds. Finally, the difficulty to understand the interactions between aroma compounds and mannoproteins is due to the fact that both glycosidic and peptidic parts of these glycoproteins may have interactions with aroma compounds.

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