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Fate of yeast and grape pectic polysaccharides of a young red wine in the cross-flow microfiltration process

by

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Devenir des polysaccharides pectiques de raisin et de levure d'un vin rouge jeune dans le procédé de microfiltration tangentielle

R é s u m é : La microfiltration tangentielle d'un vin rouge jeune sur membrane minérale d'oxyde de zirconium de taille moyenne de pore 0,2 µm (support carbone aggloméré microporeux) réduit de 44 % la concentration du vin brut en polysaccharides solubles. Ces polymères glucidiques sont essentiellement constitués de mannose, arabinose, galactose et acide galacturonique et à un degré moindre de rhamnose, glucose, xylose et fucose. Les polysaccharides du vin brut et du perméat final ont été séparés par tamisage moléculaire sur Ultrogel AcA 34 (limite d'exclusion des protéines globulaires 750.000) en au moins quatre fractions (I—IV) de Kav respectifs 0,22, 0,50, 0,75 et 0,90. Chaque fraction polysaccharidique contenait des proportions variables de mannanes de levure, alors que les polysaccharides issus du raisin étaient inégalement répartis, la fraction I contenant des arabinogalactanes de type II neutres et les fractions II à IV étant constituées de mélanges complexes d'arabinogalactanes de type II, d'arabinanes et de formes dégradées de rhamnogalacturonanes acides (pectines). Les pertes dues à la microfiltration étaient corrélées positivement au volume hydrodynamique (masse moléculaire) des molécules: (I) ≈ 79 %, (II) ≈ 58 %, (III) ≈ 38 %, et (IV) pas de pertes. Les polysaccharides issus de la levure et du raisin coexistant dans une fraction donnée (ayant le même Kav) n'ont pas été affectés de façon identique par le procédé de microfiltration, les mannanes de levures passant préférentiellement la membrane alors que les polymères du raisin étaient plus retenus. Cette rétention différentielle n'a été observée que pour les fractions de haute masse moléculaire (I et II) et est discutée au regard des modifications potentielles au niveau moléculaire (taille et forme des polysaccharides) pouvant survenir dans la couche de polarisation. L'application d'une contre-pression destinée à décolmater la membrane a eu pour effet de réintroduire dans le perméat les polysaccharides présents dans le vin brut à hauteur de 82 %.

K e y w o r d s : red wine, filtration, yeast, berry, pectin, polysaccharide, carbohydrate, analysis, technique.

Introduction

Modern filtration processes, namely ultra- or microfiltration, have been increasingly considered since the recent years as an alternative to traditional filtration techniques (deep-bed filtration) for clarification, sterilisation and stabilisation of musts and wines (CASTINO and DELFINI 1984; GAILLARD and BERGER 1984; BARILLÈRE *et al.* 1985; FEUILLAT and BERNARD 1985; MARIGNETTI *et al.* 1986; CATTARUZZA *et al.* 1987; FEUILLAT *et al.* 1987; WUCHERPFENNIG *et al.* 1987; SERRANO *et al.* 1988). Potential use and limitations of these techniques in enology have been recently reviewed (PERI 1987). Theoretical basis for the fouling of mineral membranes in tangential filtration has also been given by BENNASAR and TARODO DE LA FUENTE (1987).

The behaviour of must and wine polysaccharides in the ultra- and microfiltration processes — though extensively studied (CASTINO and DELFINI 1984; CATTARUZZA *et al.* 1987; FEUILLAT *et al.* 1987; SERRANO *et al.* 1988) — is not fully understood, partly due to

lack of fundamental structural data on grape polysaccharides. Since it is well known that colloids of carbohydrate nature are the components of must and wine mainly affected by these processes (retention), they are suspected to play a detrimental role by participation in forming the concentration polarisation layer and by progressively plugging membranes, thus reducing permeation rates.

The aim of the present work was to study the distribution, composition and structure of soluble polysaccharides occurring in a young red wine and their differential behaviour upon the cross-flow microfiltration process using a prototype mineral membrane.

Material and methods

1. Experimental wine

Mature sound grapes of the cultivar Carignan were harvested from the experimental vineyards of the INRA, Pech Rouge-Narbonne Experimental Station (Gruissan), destemmed, then rapidly heated at 70 °C in a Rotatermic concentric pipe heat exchanger (Gasquet, France) and macerated for 20 min before cooling down to 25 °C (thermotreatment). Crushed berries were then pressed in a horizontal wine press, and after adding of yeast the must was allowed to ferment at 30—32 °C for 6 d.

2. Cross-flow microfiltration

The cross-flow microfiltration experiment was performed on a S 37 type filtering device from Tech-Sep (Mirebel, France; surface for filtration 0.8 m^2) equipped with a set of 37 prototype tubular mineral membranes (inserted in a stainless steel cartridge) of the following characteristics: length 120 cm; external Ø 1 cm; internal Ø 0.6 cm; flow rate with deionized water 2,700 $lh^{-1}m^{-2}$ (400 kPa; 25 °C). The filtrating element of the membrane was a layer of zirconium oxide (average pore Ø 0.2 μm; thickness 10 μm; exclusion level for a $2 \cdot 10^6$ MW dextran 8 %) on a microporous amorphous carbon support. The wine (40 hl) was continuously filtrated for 18 h (400 kPa; linear tangential flow 5 m s^{-1}; 18 °C), then back-flush pulse was repeatedly applied (4 ×) up to 20 h 30 min. The cross-flow experiment ended after 22 h 40 min (see Fig. 1). An aliquot of the starting wine (A) was kept for further analyses as well as permeate samples which were taken at the end of the experiment (final permeate, B) and 2 min after the first back-flush pulse (permeate after back-flush, C).

3. Isolation of polysaccharides from wine

Wine aliquots (750 ml) were centrifuged (5,000 r min^{-1}; 10 min), extensively dialyzed against distilled water for 2 d at ambient temperature, then for additional 4 d in the cold (4 °C). After 7 × vacuum concentration (< 40 °C), 4 M sodium chloride (16.6 ml) was added to aliquots (50 ml) to give a final 1 M concentration. Then samples were centrifuged (14,000 r $min.^{-1}$; 5 min) and decolourized on a column (10 × 1.5 cm) of Polyamide CC6 previously equilibrated with 1 M sodium chloride (flow rate 100 ml h^{-1}). After recovery of 3 total column volumes, the eluate was dialyzed against distilled water, concentrated, then freeze-dried.

4. Chemicals

Dimethyl sulfoxide, sodium hydride (80 % in white oil), methyl iodide and standard monosaccharides were of the highest purity (Fluka, Buchs, Switzerland). *m*-phenyl phenol was obtained from Eastman Kodak (Rochester, N.Y., USA). Polyamide CC6 (81561) was from Macherey-Nagel (Düren, Germany).

5. Carbohydrate analysis

Neutral polysaccharides (≈ 2 mg) were hydrolysed with 2 M trifluoroacetic acid (1 ml) containing 0.25 mg of inositol for 1 h 15 min at 120 °C (ALBERSHEIM *et al.* 1967). Hydrolysates were dried at 40 °C under an air stream and the liberated monosaccharides were reduced to alditols by 2 % sodium borohydride in 1 M ammonia (0.5 ml) for 1 h at 60 °C (HARRIS *et al.* 1984). Excess borohydride was eliminated by 3 successive additions of glacial acetic acid (3 × 50 µl); borate ions were removed by methanol containing 10 % acetic acid (3 × 0.5 ml), followed by methanol (3 × 0.5 ml) with intermittent evaporations. Alditols were then acetylated by acetic anhydride (0.25 ml) for 3 h at 120 °C (ALBERSHEIM *et al.* 1967). After addition of water (0.15 ml), samples were dried, then twice extracted with chloroform (0.25 ml) in presence of water (0.25 ml). After phase separation by centrifugation, both organic phases were pooled and evaporated. Alditol acetates were dissolved in dichloromethane (0.5 ml) and analysed by gas-liquid chromatography.

6. Methylation analysis

Freeze-dried decolourized colloids (≈ 2 mg) were placed in a 5 ml vial with a small magnetic bar and heated in an oven for 1 h at 100 °C. The vial provided with a Mininert valve (Pierce Eurochemie B.Y., Netherlands), was filled with dry nitrogen, closed and cooled to ambient temperature. All further operations were conducted under nitrogen. Dimethyl sulfoxide (0.5 ml) was added and the polysaccharides were dissolved under stirring with the help of intermittent heating (100 °C) and/or sonication. Sodium methyl sulfinyl carbanion (1.9 M; 0.5 ml) (HAKOMORI 1964; JANSSON *et al.* 1976) prepared by mixing dry pentane-extracted sodium hydride (1.28 g) with dimethyl sulfoxide (25 ml) at 60 °C for 2 h under N_2 was injected into the vial and the mixture was stirred for 1 h at ambient temperature. The vial was cooled in an ice bath and cold methyl iodide (0.5 ml) was added to the frozen medium (HARRIS *et al.* 1984). The vial was then progressively warmed to normal temperature, with intermittent thorough mixing of the thawing phase and the alkylating agent. The opalescent medium turned clear after 1 h and excess methyl iodide was eliminated after removal of the valve under an air stream. A chloroform/methanol (2 : 1) mixture was then added (3 ml) followed by distilled water (2 ml) (HARRIS *et al.* 1984). After thorough mixing, the phases were separated by centrifugation (9,000 *g*; 5 min) and the upper aqueous phase was discarded. The organic phase was washed twice with water (2 ml), then transferred into a 2 ml reacti-vial and dried. Methylated polysaccharides were treated with 90 % formic acid (0.5 ml; 100 °C; 1 h) and after evaporation at 40 °C hydrolysed with 2 M trifluoroacetic acid (120 °C; 1 h 15 min). Finally, partially methylated sugars were transformed into alditol acetates as described above.

7. Gas-liquid chromatography (GLC)

Alditol acetates of monosaccharides (compositional analysis) and partially methylated alditol acetates (structural analysis) were separated by GLC with two Delsi 30C chromatographs (Suresnes, France) equipped with two fused silica capillary columns: (A) bonded OV-1 (50 m × 0.32 mm internal Ø; film thickness 0.20 µm) (Delsi Instruments, Suresnes, France) and (B) bonded OV-225 (30 m × 0.32 mm internal Ø; film thickness 0.25 µm) (DB-225, J and W Scientific, USA). Operating parameters for separation of partially methylated sugars were as follows: column (A): hydrogen carrier gas 120 kPa; column temperature: on-column injection at 35 °C, then 15 °C/min to 165 °C, then after 30 min rise to 210 °C at 15 °C/min; FID temperature 250 °C; column (B): hydrogen carrier gas 65 kPa; column temperature: 170 °C for 15 min, then rise to 210 °C

at 5 °C/min; split ratio 1/10; injector and FID temperature 250 °C. For separation of alditol acetates, the column (B) operated under the following conditions: hydrogen carrier gas 65 kPa; column temperature: 210 °C isothermal; split ratio 1/10; injector and FID temperature 250 °C. Identification of partially methylated alditol acetates was achieved on a retention time basis using methyl ethers from standard polysaccharides (BRILLOUET 1987). Peak areas were corrected by response factors as described by SWEET *et al.* (1975).

8. Gel permeation chromatography

Decolourized colloids (≈ 20 mg) were dissolved in water (3 ml) and submitted to gel permeation chromatography on an Ultrogel AcA 34 column (90 × 2.2 cm) equilibrated with 0.05 M acetate buffer (pH 4.8) containing 0.3 M sodium chloride at a flow rate of 38 ml h^{-1}. Fractions (6 ml) were collected and analysed automatically for their galacturonic acid and neutral sugar contents by the *m*-phenyl phenol (BLUMENKRANTZ and ASBOE-HANSEN 1973; THIBAULT 1979) and orcinol (TOLLIER and ROBIN 1979) methods, respectively, with correction for mutual interferences. Galacturonic acid and arabinose were used for calibration. Appropriate fractions (having a constant acidic/neutral sugar ratio; hatched areas in Fig. 2) were pooled, extensively dialyzed against distilled water and analysed for their monosaccharide content and structure as described above.

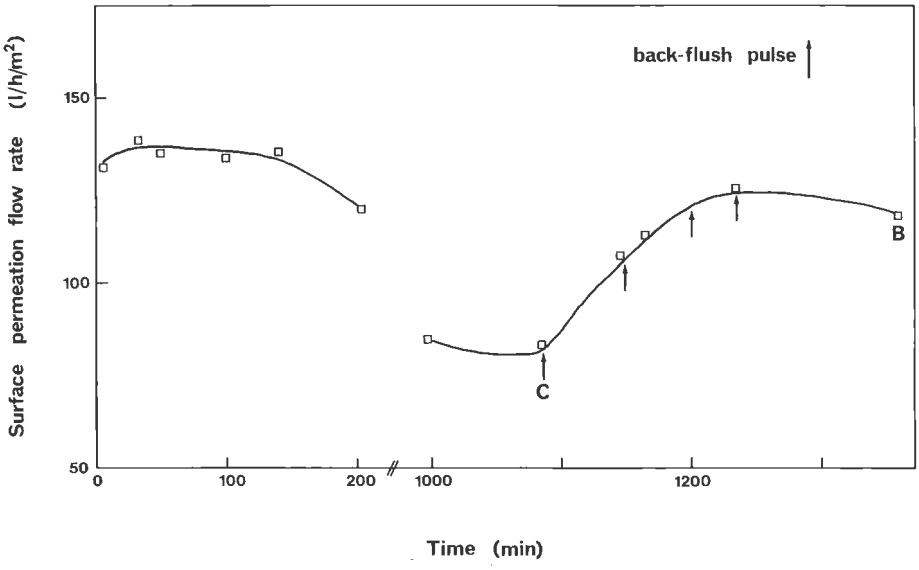


Fig. 1: Surface permeation flow rate (l $h^{-1}m^{-2}$) as a function of time of cross-flow microfiltration of a young red wine on a prototype mineral membrane (average pore size 0.2 µm). Samples of the wine permeate were withdrawn at times 18 h (2 min after the first back-flush pulse, sample C) and 22 h 40 min (sample B, final permeate).

Débit de perméation surfacique (l $h^{-1}m^{-2}$) en fonction du temps de microfiltration tangentielle d'un vin rouge primeur sur membrane minérale prototype (porosité moyenne 0,2 µm). Des échantillons de perméat sont prélevés aux temps 18 h (2 min après la première contre-pression, échantillon C) et 22 h 40 min (échantillon B, perméat final).

Results and discussion

1. Cross-flow microfiltration experiment

The surface permeation flow rate ($lh^{-1}m^{-2}$) measured along the filtration period is shown in Fig. 1. A classical curve shape was observed with a stable flow rate ($\approx$ 140 $lh^{-1}m^{-2}$) up to 2 h, followed by a progressive and slow decrease down to 80 $lh^{-1}m^{-2}$ at 18 h. After application of 4 back-flush pulses, the initial flow rate was almost recovered at 20 h 30 min. No changes were observed in the final permeate (B) as compared to the crude starting wine (A) with regard to gross characteristics (alcohol content, pH, acidity, sulfur dioxide content, colour, etc.), except for the turbidity (NTU) which was 94 for the crude wine (A) and 0.51 for the final permeate (B).

2. Characterization of colloids

The colloids from the three wine samples (A,B,C) were analysed for their polysaccharide constituents by colorimetric analysis or uronic acids and GLC of the alditol acetate derivatives of neutral sugars obtained after acid hydrolysis. Data are shown in Table 1. The starting wine contained 800 mg l^{-1} of decolourized colloids, $\approx$ 65 % being of polysaccharidic nature. The remaining portion was composed of proteinic and residual polyphenolic material (data not shown). The final permeate was strongly impoverished since it contained only about half of the starting colloids ($\approx$ 65 % carbohydrate). Similar losses in colloids were observed after cross-flow microfiltration of a Burgundy red wine on tubular alumina membranes of close average pore size (FEUILLAT *et al.* 1987). After application of a back-flush pulse, the permeate was reenriched in colloids (85 % as compared to the starting wine; $\approx$ 62 % carbohydrate content).

Mannose, arabinose and galactose were the major neutral sugars followed by minor quantities of rhamnose, glucose, xylose and fucose. Uronic acids, which were presumably mainly of the galacturonic type, represented $\approx$ 24 % of total polysaccha-

Table 1

Carbohydrate constituents of wine colloids

Composition polysaccharidique des colloïdes des vins

	Crude wine (A)	Final permeate (B)	Permeate after back-flush (C)
Decolourized colloids	800	448	682
Uronic acids	122	104	123
Neutral sugars			
Rhamnose	27	16	21
Fucose	3	1	2
Arabinose	112	59	80
Xylose	6	4	4
Mannose	129	60	110
Galactose	99	42	76
Glucose	19	9	10
Total	395	191	303

All values given as mg l^{-1} of wine.

rides of the starting wine, ≈ 35 % in the final permeate and ≈ 29 % in the permeate after back-flush. It must be noted that the uronic acid concentration was only lowered by ≈ 15 % after cross-flow microfiltration (final permeate), while the neutral polysaccharides decreased by ≈ 52 %. After the back-flush treatment, the uronic acid level reached back to the starting concentration (123 mg l^{-1}) while the recovery of neutral polysaccharides was only ≈ 77 %.

3. Fractionation of polysaccharides by gel permeation

The wine polysaccharides were further fractionated on a column of Ultrogel AcA 34 (exclusion limit for globular proteins 750,000) and analysed for their galacturonic acid and neutral sugar contents by the *m*-phenyl phenol and orcinol methods respectively. Chromatograms are shown in Fig. 2. The starting wine (A) exhibited a complex distribution, at least four populations (I—IV) being detected by both techniques. Their respective Kav [(Ve-Vo)/(Vt-Vo)] were: (I) 0.22, (II) 0.50, (III) 0.75 and (IV) 0.90. Part of the polysaccharidic material eluted at the void volume of the column was not further investigated. Populations I and III constituted the major part of total polysaccharides. The acidic/neutral sugar ratio increased from I to IV: (I) 0.06, (II) 0.20 and (III) 0.42. The ratio for IV was difficult to estimate due to overlapping with III; it was however in the range 1.40—1.60. USSEGLIO-TOMASSET and CASTINO (1975) already observed by gel permeation on Sepharose 6B of colloids from a Barbera wine a fraction of low molecular weight (Kav ≈ 1) having an acidic/neutral sugar ratio of 0.83 and being composed of galacturonic acid, rhamnose and arabinose in the ratio 1 : 0.8 : 0.4. So the uronic acid content of each polysaccharide class increased with Kav, population I being almost neutral while the major parts of acids was located in the populations of lower molecular weight — III and IV. It must be noted that the maxima for acidic and neutral polysaccharides coincided for the four populations, the neutral fraction of IV being detected only as a shoulder of III.

After microfiltration (B), the four populations were still visible but with a quite different relative distribution. Populations I and II were strongly diminished, III was reduced to a lower extent. It has been possible to estimate the losses due to microfiltration by integrating for each fraction the concentrations of neutral and acidic sugars. Losses were similar for a given population in neutral and acidic carbohydrates. Values were as follows: (I) ≈ 79 %, (II) ≈ 55—62 %, (III) ≈ 36—40 % and (IV) no loss. These data were confirmed by considering the neutral and acidic sugar contents of the colloids and their respective concentration in wines (Table 1). Due to weakening of III, the neutral polysaccharides of IV became visible as an individual peak. Similar effects were observed by gel permeation chromatography of polysaccharides from two wines obtained from a native or the corresponding ultrafiltrated must (FEUILLAT and BERNARD 1985) as well as on an ultrafiltrated (25,000 cut-off) red wine (CATTARUZZA *et al.* 1987). So the losses in each polysaccharide population were obviously related to its hydrodynamic volume (molecular weight), the larger molecules being retained to a greater extent than the smaller ones. No attempt was made to determine their molecular weight by calibration of the column with standard polysaccharides since their molecular shape was not known.

The back-flush process (C) clearly reintroduced in the permeate the populations of high molecular weights (I and II) but not at the same level as in the starting wine.

4. Structural analysis of fractionated polysaccharides

Neutral polysaccharides (fractions I—IV) were methylated by methyl iodide according to HAKOMORI (1964) and their derived partially methylated alditol acetates

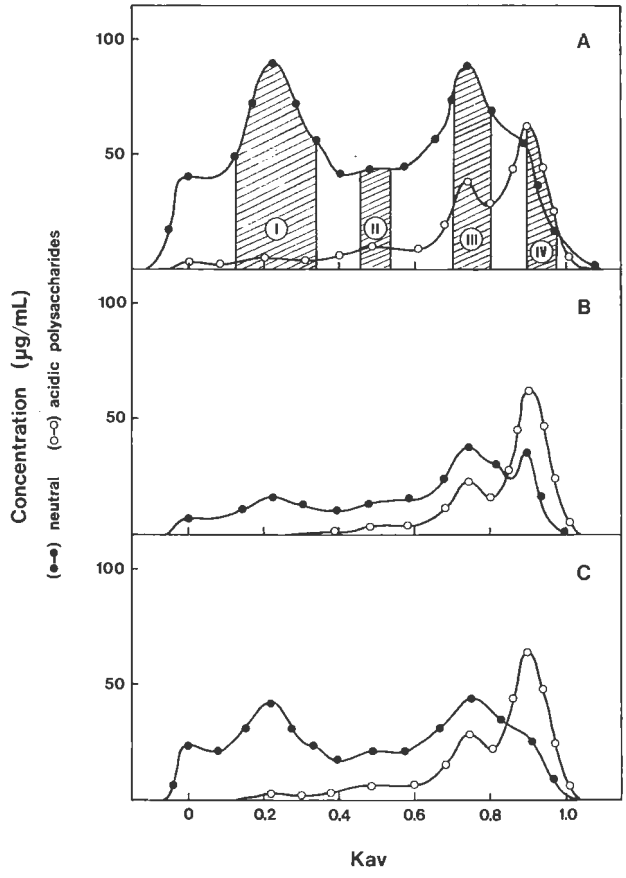
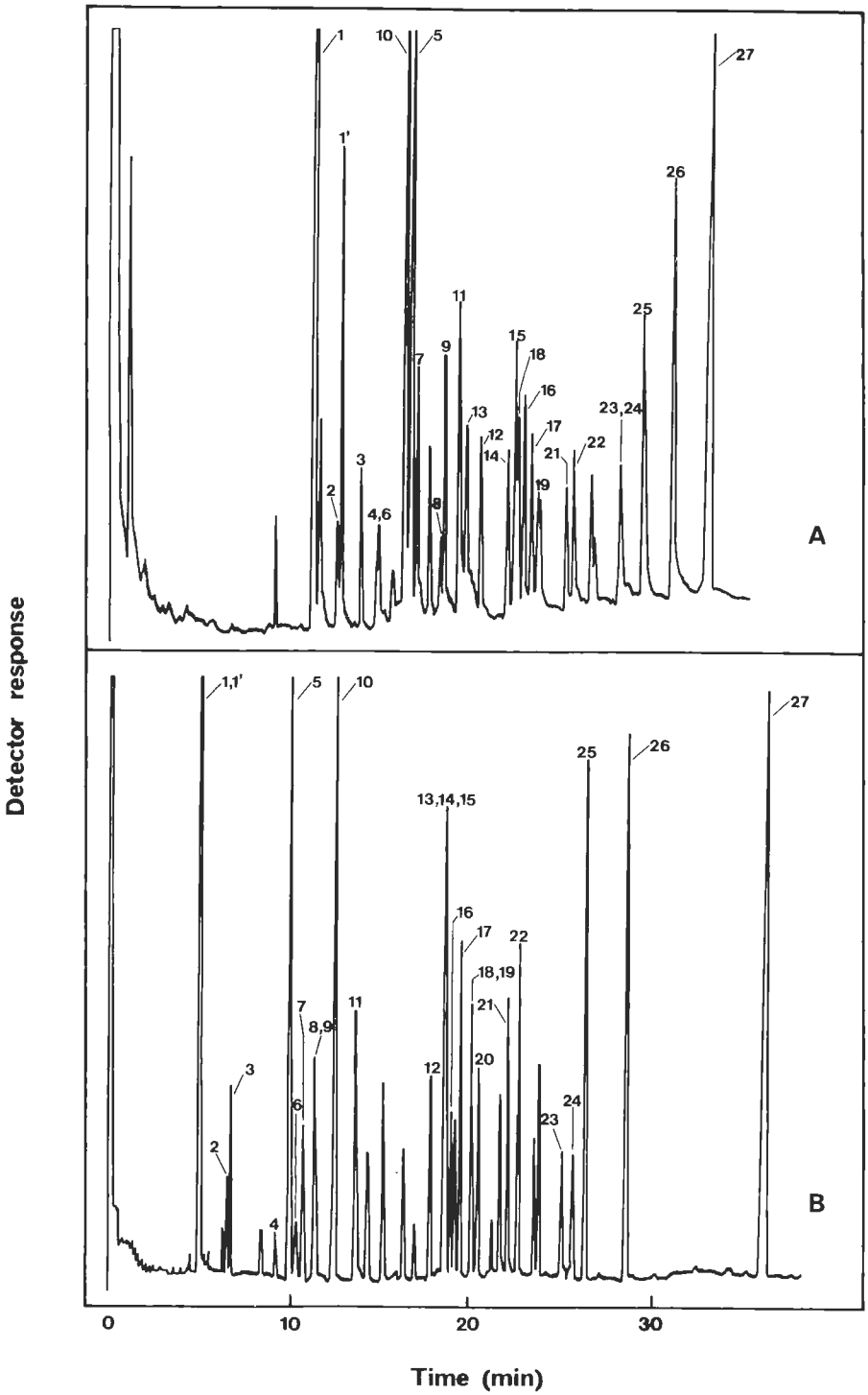


Fig. 2: Gel permeation chromatography of soluble polysaccharides from starting wine (A), final permeate (B) and permeate after a back-flush pulse (C) on a column (90 × 2.2 cm) of Ultrogel AcA 34 (flow rate 38 ml h^{-1}). Uronic acids and neutral polysaccharides were automatically analysed by the *m*-phenyl phenol and orcinol methods respectively. Chromatograms were recalculated to show identical uronic acid content in fraction IV for the three wine samples (A, B, C). Hatched areas were selected for methylation structural analysis of fractions I, II, III and IV.

Chromatographie de tamisage moléculaire des polysaccharides solubles du vin de départ (A), du perméat final (B) et du perméat après contre-pression (C) sur une colonne (90 × 2,2 cm) d'Ultrogel AcA 34 (débit 38 ml h^{-1}). Les acides uroniques et les polysaccharides neutres ont été analysés automatiquement par les méthodes au *m*-phenylephenol et à l'orcinol. Les chromatogrammes ont été recalculés afin de présenter un profil identique en acides uroniques pour la fraction IV dans les trois échantillons de vin (A, B, C). Les zones hachurées ont été sélectionnées pour l'analyse structurale par méthylation des fractions I, II, III et IV.

were separated by GLC on two capillary columns. Chromatograms are shown in Fig. 3. 26 derivatives were consistently observed in the four fractions in various proportions. Co-elutions observed on the OV-225 column (peaks 1, 1'; 8, 9; 13, 14, 15; 18, 19) were resolved on the OV-1 column. Quantitative data are presented in Table 2 under two forms: relative percent of methyl ethers within the parent sugar family and mole percent of each parent sugar (calculated by summing corrected surfaces of all ethers from one sugar type) relative to total sugars. These last figures agreed well with values


Detector response
A
1
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30
Time (min)

obtained by direct analysis of neutral sugars. It must be noted that minor glucose methyl ethers were not included in Table 2 due to difficulties in proper identification.

All populations (I—IV) were constituted of two families of polysaccharides originating from yeast — mannans — and from grape — arabinogalactans, arabinans and rhamnogalacturonans. They were present in each fraction from the crude wine (A) in various proportions: mannans represented respectively ≈ 29 % of neutral polymers (I), ≈ 34 % (II), ≈ 12 % (III) and ≈ 25 % (IV), the complement being the grape polysaccharides. All structural features were encountered in various proportions in each population. Considering the relative distribution of methyl ethers in fractions I A, II A, III A and IV A (starting wine), it becomes clear that strong structural differences existed between these fractions: arabinofuranose was present in fraction I A essentially (82 %) as terminal non-reducing units (2,3,5-Me_3-ara) associated with minor proportions of 5-linked (2,3-Me_2-ara) (9 %) and 3,5-linked (2-Me-ara) (7 %) arabinose. Conversely the relative proportions of 5-linked arabinose was much more important in fractions II A, III A and IV A (≈ 36 %), while terminal arabinose represented only 46—59 % of total arabinose as compared to I A. Variations are also seen at the galactose level: the major galactose methyl ether in I A was 2,4-Me_2-gal (3,6-linked galactose), followed by 2,4,6-Me_3-gal (3-linked) and 2-Me-gal (3,4,6-linked), while in II A, III A and IV A the distribution was more complex, the relative percent of terminal (2,3,4,6-Me_4-gal), 6-linked (2,3,4-Me_3-gal) and 3,4-linked (2,6-Me_2-gal) galactose being increased, 2,4-Me_2-gal being lowered. Rhamnose methyl ethers also showed strong variations: terminal rhamnose (2,3,4-Me_3-rha) was the major feature in I A, while 2-linked rhamnose (3,4-Me_2-rha) became more prominent in II A, III A and IV A. In addition, 3-linked (2,4-Me_2-rha) and 2,3,4-linked (unmethylated) rhamnose were detected in fractions III A and IV A. It must be noted that the content in rhamnose increased from fraction I A (≈ 3 %) to III A and IV A (≈ 16—18 %) in parallel with the relative proportion of uronic acids.

Some structural variations were also seen at the mannan level: 2,6-linked (3,4-Me_2-man) and 3-linked (2,4,6-Me_3-man) mannose tended to decrease from I A to IV A, while terminal mannose (2,3,4,6-Me_4-man) remained more or less constant. 2-linked mannose (3,4,6-Me_3-man) increased with elution volume.

Although each selected population was a complex mixture of several polysaccharides, some structural assignments could be made on the basis of already described structures. According to the relative distribution of major structural features in fraction I A (terminal arabinose, 3,6-linked galactose, 3-linked galactose and 3,4,6-linked galactose), it could be concluded that typical neutral type II arabinogalactans (or 3,6-linked arabinogalactans) (ara/gal 0.77) (ASPINALL 1980) were present showing close structural similarities with polysaccharides from grape must (BRILLOUET 1987) and neutral sugar side-chains of grape water-soluble pectic substances (SAULNIER *et al.* 1988 a). Presence of terminal rhamnose, a known component of such polysaccharides (ASPINALL

Fig. 3: Chromatograms of partially methylated alditol acetates obtained by methylation of soluble neutral polysaccharides from fraction III B on two fused silica capillary columns (A) bonded OV-1 and (B) OV-225. Explanation of peak numbers see below.

Chromatogrammes d'acétates d'alditol partiellement méthylés obtenus par perméthylation des polysaccharides solubles neutres de la fraction III B sur deux colonnes capillaires en silice fondue (A) OV-1 greffée et (B) OV-225. Identification des pics ci-dessous:

1: 2,3,5-Me_3-ara; 1': 2,3,4-Me_3-rha; 2: 2,3,4-Me_3-xyl; 3: 2,3,4-Me_3-fuc; 4: 3,5-Me_2-ara; 5: 3,4-Me_2-rha; 6: 2,5-Me_2-ara; 7: 2,4-Me_2-rha; 8: 2,3,4,6-Me_4-glc; 9: 2,3,4,6-Me_4-man; 10: 2,3-Me_2-ara; 11: 2,3,4,6-Me_4-gal; 12: 3-Me-rha; 13: 2-Me-ara; 14: rha; 15: 3,4,6-Me_3-man; 16: 2,4,6-Me_3-man; 17: 2,4,6-Me_3-gal; 18: 2,3,6-Me_3-gal; 19: 2,3,4-Me_3-glc; 20: 2,3,6-Me_3-glc; 21: 2,3,4-Me_3-gal; 22: 2,6-Me_2-gal; 23: 3,4-Me_2-man; 24: 2,3-Me_2-gal; 25: 2,4-Me_2-gal; 26: 2-Me-gal; 27: inositol hexaacetate.

Table 2

Methylation analysis of wine polysaccharides

Analyse structurale par méthylation des polysaccharides des vins

Methyl ether	Linkage	Fraction I			Fraction II			Fraction III			Fraction IV		
		A	B	C	A	B	C	A	B	C	A	B	C
2,3,4-Me_3-rha[1]	L-Rha*p*-(1→	66[2]	57	72	15	15	11	30	22	23	20	23	20
2,4-Me_2-rha	→ 3)-L-Rha*p*-(1→							10	13	14	8	10	8
3,4-Me_2-rha	→ 2)-L-Rha*p*-(1→	13[3]	16	11	60	55	58	43	47	43	60	55	60
3-Me-rha	→ 2,4)-L-Rha*p*-(1→	21[2]	27	17	25	30	31	9	11	10	9	9	9
rha	→ 2,3,4)-L-Rha*p*-(1→							8	7	10	3	3	3
Total		2.8[3]	2.1	3.2	7.2	5.7	7.8	17.6	19.1	17.3	16.1	21.8	13.6
2,3,4-Me_3-fuc	l-Fuc*p*-(1→	100	100	100	100	100	100	100	100	100	100	100	100
Total		0.3	0.1	0.3	0.9	0.5	0.2	1.6	2.1	1.5	1.2	1.6	1.9
2,3,5-Me_3-ara	L-Ara*f*-(1→	82	78	83	49	48	43	59	56	53	46	52	48
2,3-Me_2-ara	→ 5)-L-Ara*f*-(1→	9	14	10	37	36	41	35	33	35	37	35	28
2,5-Me_2-ara	→ 3)-L-Ara*f*-(1→	1	1	1	1	2	2	2	3	2	4	4	2
3,5-Me_2-ara	→ 2)-L-Ara*f*-(1→	1	1	1	2	1	1	1	1	1	4	3	5
2-Me-ara	→ 3,5)-L-Ara*f*-(1→	7	6	6	11	13	13	3	7	9	9	6	17
Total		29.3	23.9	26.5	34.2	29.4	32.6	38.2	41.3	39.0	19.9	20.6	23.4
2,3,4-Me_3-xyl	D-Xyl*p*-(1→	71	30	42	58	71	58	64	73	68	20	32	21
2,3-Me_2-xyl	→ 4)-D-Xyl*p*-(1→	29	70	58	42	29	42	36	27	32	80	68	79
Total		0.7	0.5	0.4	1.7	1.1	1.3	2.5	1.8	2.2	7.9	4.6	8.9

Methyl ether	Linkage	Fraction I			Fraction II			Fraction III			Fraction IV		
		A	B	C	A	B	C	A	B	C	A	B	C
2,3,4,6-Me_4-gal	D-Gal*p*-(1→	7	7	7	12	12	11	13	16	14	13	16	15
2,4,6-Me_3-gal	→ 3)-D-Gal*p*-(1→	18	18	17	24	18	18	15	11	12	28	21	27
2,3,4-Me_3-gal	→ 6)-D-Gal*p*-(1→	6	6	5	12	9	10	12	8	10	10	11	10
2,3,6-Me_3-gal	→ 4)-D-Gal*p*-(1→	1	2	1	1	7	5	4	5	3	9	4	10
2,6-Me_2-gal	→ 3,4)-D-Gal*p*-(1→	7	6	6	10	9	10	13	8	12	10	10	12
2,3-Me_2-gal	→ 4,6)-D-Gal*p*-(1→	2	2	2	4	3	3	7	5	4	4	5	3
2,4-Me_2-gal	→ 3,6)-D-Gal*p*-(1→	44	41	43	25	30	28	16	19	17	13	15	9
2-Me-gal	→ 3,4,6)-D-Gal*p*-(1→	15	18	19	12	12	15	20	28	28	13	18	14
Total		38.0	35.4	38.0	21.9	21.2	23.7	28.1	25.2	27.6	30.2	23.9	28.0
2,3,4,6-Me_4-man	D-Man*p*-(1→	34	34	33	34	31	32	26	29	27	34	44	33
2,4,6-Me_3-man	→ 3)-D-Man*p*-(1→	22	20	21	18	19	19	15	13	11	17	16	15
3,4,6-Me_3-man	→ 2)-D-Man*p*-(1→	20	21	22	31	35	33	42	48	43	40	34	44
3,4-Me_2-man	→ 2,6)-D-Man*p*-(1→	24	25	24	17	15	16	17	10	19	9	6	8
Total		28.9	38.0	31.6	34.1	42.1	34.4	12.0	10.5	12.4	24.7	27.5	24.3
Terminal/ branched		0.93	0.87	0.86	1.39	1.19	1.02	1.32	1.21	0.98	1.21	1.52	1.35

[1] 2,3,4-Me_3-rha = 2,3,4-tri-O-methyl-1,5-di-O-acetyl-rhamnitol, etc.
[2] Relative mole percent of rhamnose methyl ethers within the rhamnose family, etc.
[3] Relative mole percent of each parent sugar family (sum of ethers from one sugar type) within total sugars.

1980), sustained this statement. An average structure is presented in Fig. 4. Such polysaccharides are widely distributed in the plant kingdom often associated with proteic moieties (CLARKE *et al.* 1979). Fractions II A, III A and IV A exhibited more complex patterns, arabinan-like structures (strong proportions of 5-linked and, to a lesser extent, of 3,5-linked arabinose) (ASPINALL 1980; SAULNIER *et al.* 1988 b) coexisting with type II arabinogalactans (distribution of galactose methyl ethers) and rhamnogalacturonans (emergence of uronic acids and of strong proportions of 2-linked rhamnose) (ASPINALL 1980; SAULNIER *et al.* 1988 b). According to their respective elution volume (Kav > 0.5), it is likely that they correspond to degraded forms of grape pectic substances due to endogenous pectinases since these native pectins would be excluded (Kav = 0) from the gel under similar conditions (SAULNIER and THIBAULT 1987 a, 1987 b). Although some structural variations were detected at the mannan level, their overall structure fitted rather well with that described formerly by VILLETTAZ *et al.* (1980) for a mannan from a rosé wine.

The terminal/branched unit ratio, which must be equal to unity in a well conducted methylation structural analysis, was close to 1 for fraction I and tended to increase with elution volume which reflected the decrease in molecular weight: indeed when the degree of polymerisation decreases, the proportion of terminal non-reducing unit of the main chain (usually negligible in polymers of high MW) becomes significant and increases this ratio artificially.

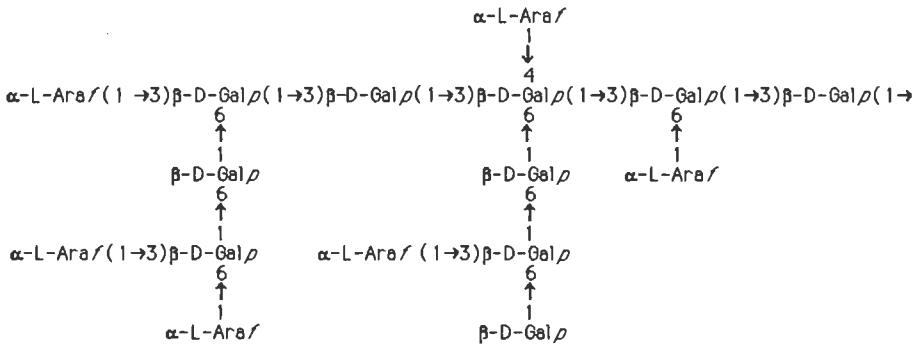

Fig. 4: Average structure of a type II arabinogalactan.

Structure moyenne d'un arabinogalactane de type II.

α-L-Araf = α-L-arabinofuranose; β-D-Gal*p* = β-D-galactopyranose.

When considering the relative distribution of methyl ethers for each parent sugar, it becomes obvious that the overall structure of each class of polysaccharides (I B—IV B) remained unchanged by the microfiltration process. Similarly, the backflush pulse did not affect the structure of reintroduced polysaccharides (I C—IV C) which were similar in all aspects to the native ones.

However, the proportion of each parent sugar was altered after microfiltration in fractions I B and II B. The mannose content was increased by ≈ 31 and 23 % in I B and II B, respectively, as compared to the corresponding fractions from the starting wine. The sugars from grape polysaccharides were concomitantly decreased. No such variations were observed in fractions III B and IV B where the mole ratio distribution remained almost unaltered after microfiltration, except a slight increase in rhamnose in IV B.

After application of a back-flush pulse, a recovery of the proportions occurring in the starting wine was observed.

So the cross-flow microfiltration process did not induce any structural alteration of the polysaccharides passing through the membrane but modified their balance at least in the high molecular weight range. Moreover, it can be concluded that for polysaccharides having similar hydrodynamic volumes (identical Kav) membrane exhibited some selectivity, grape arabinogalactans being little more retained than yeast mannans. Since no data are available on the molecular size and shape of these polysaccharides, it was difficult to relate this differential behaviour to the molecular characteristics of the considered polymers. However, since polysaccharides in fractions I and II were selected on the basis of their common elution volumes, the preferential losses in arabinogalactans in comparison with mannans (for a given fraction) could not be related to their hydrodynamic volume at least in dilute solution. It is, however, likely that when wine polysaccharides reach the concentration polarisation layer at the surface of the membrane, their molecular shape and mutual interactions change dramatically due to hyperconcentration phenomenon, which will modify their respective ability to pass the pores of the membrane.

Summary

Cross-flow microfiltration of a young red wine through a mineral membrane of zirconium oxide (average pore size 0.2 μm) laid over a support of agglomerated microporous carbon reduced by 44 % the concentration of the starting wine in soluble polysaccharides. These carbohydrate polymers were mainly constituted of mannose, arabinose, galactose and galacturonic acid associated with minor amounts of rhamnose, glucose, xylose and fucose. The polysaccharides from starting wine and final permeate were separated by gel filtration on Ultrogel AcA 34 (exclusion limit for globular proteins 750,000) in at least four fractions (I—IV) of respective Kav 0.22, 0.50, 0.75 and 0.90. Each polysaccharidic population contained various proportions of yeast mannans, while grape polysaccharides were unequally distributed, fraction I containing neutral type II arabinogalactans and fractions II to IV being complex mixtures of type II arabinogalactans, arabinans and degraded forms of acidic rhamnogalacturonans (pectins). Losses due to microfiltration were positively correlated to hydrodynamic volume (molecular weight) of molecules: (I) ≈ 79 %, (II) ≈ 58 %, (III) ≈ 38 % and (IV) no loss. Yeast and grape polysaccharides coexisting in a given fraction (having the same Kav) were not equally affected by the microfiltration process, yeast mannans passing preferentially the membrane, while grape polymers were more retained. This differential retention was only observed in fractions of high molecular weights (I and II) and was discussed in relation with possible modifications at the molecular level (size and shape of polysaccharides) occurring in the concentration polarisation layer. Application of a back-flush pulse destined to unplug the membrane resulted in a reenrichment of the permeate in the polysaccharides present in the starting wine at a 82 % level.

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