CHARACTERIZATION OF MANNOPROTEINS DURING WHITE WINE (*VITIS VINIFERA* L. CV. ENCRUZADO) AGEING ON LEES WITH STIRRING IN OAK WOOD BARRELS AND IN A STAINLESS STEEL TANK WITH OAK STAVES

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Abstract

Aim : To compare the evolution of mannoproteins in a white wine submitted to two different stirring of lees processes : in French oak barrels (BA) and in a stainless steel tank equipped with French oak staves (IN).

Methods and results : Total colloids were obtained by ultrafiltration and ethanol precipitation. Mannoproteins were isolated by adsorption on concanavalin-A and molecular weight separation. Sugar residues were characterized by GC analysis of their alditol acetates and protein content was determined according to Lowry method. Wines were submitted to sensory analysis.

Conclusion : Three polysaccharide fractions were isolated from both BA and IN wines. Regarding sensory attributes, the only significant difference between BA and IN wines was the round mouth-feel. The BA wine showed a higher final polysaccharide concentration, which can be related to the rounder mouth-feel.

Significance and impact of the study : This study is a technological approach of a process largely used by white wine producers who want to market a fresh wine produced with stirring of lees with a woody character. It reports the evolution of mannoproteins through four months of ageing on lees with two different stirring processes that can have a direct impact on the cost of the final product.

Résumé

Key words : wine, yeast, stirring of lees, ageing on lees, mannoproteins

Objectif : Comparer l'évolution des mannoprotéines d'un vin blanc soumis à deux processus de bâtonnage : en barriques de chêne Français (BA) et dans une cuve inox avec des douelles de barrique de chêne Français (IN).

Méthodes et résultats : Les colloïdes totaux du vin ont été obtenus par ultrafiltration et précipitation à l'éthanol. Les mannoprotéines absorbées sur une colonne de concanavaline-A ont été séparées par poids moléculaire. Les résidus de sucre ont été caractérisés par l'analyse en chromatographie gazeuse de leurs acétates d'alditol. Les teneurs en protéines ont été déterminées par la méthode de Lowry. Les vins ont été évalués par l'analyse sensorielle.

Conclusion : Trois fractions polysaccharidiques ont été isolées du vin BA et du vin IN. En ce qui concerne l'analyse sensorielle, la seule différence significative entre les vins BA et IN a été la sensation de rondeur au niveau gustatif. Les vins BA ont montré des teneurs finales plus élevées en polysaccharides, ce qui peut être en rapport avec la plus grande sensation de rondeur en bouche.

Signification et impact de l'étude : Cette étude est une approche technologique d'un procédé largement utilisé par les producteurs de vins blancs qui souhaitent mettre sur le marché un vin jeune et frais produit en utilisant le bâtonnage et en montrant un caractère boisé. Elle donne un compte rendu de l'évolution des mannoprotéines pendant quatre mois d'élevage sur lies, avec deux procédés distincts de bâtonnage qui peuvent avoir un impact direct sur le coût des produits obtenus.

Mots clés : vin, levures, bâtonnage, élevage sur lies, mannoprotéines

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INTRODUCTION

White wine fermentation and ageing on lees in barrels is an oenological practice well spread all over the world that is used to achieve wines with an enriched woody aroma and a rounder mouth-feel. When combined with stirring, a process normally called *bâtonnage*, it allows the resuspension of the fine lees and the release of interesting and important compounds into the wine. The fine lees represent 2-4 % of the total wine volume and are composed of 25 % of dry matter, mainly consisting of 25-35 % of tartaric salts, 35-45 % of microorganisms, namely yeasts, and 30-40 % of organic residues (Fornairon-Bonnefond *et al.*, 2002), like amino acids (Sciancalepore *et al.*, 1983), fatty acids and vitamins (Fornairon-Bonnefond *et al.*, 2002). These compounds result from the yeast autolysis, defined by Hernawan & Fleet (1995) as the breakdown of the yeast cell components by the action of endogenous enzymes. It occurs naturally when the yeasts complete their growing cycle and enter the death phase and is characterized by the loss of cell organization, the degradation of cell macromolecules and the release of the breaking products into the extracellular environment. The enzymes responsible for this process are a complex of proteinases and peptidases. The principal products derived from the yeast autolysis are the mannoproteins, which are released into the medium after the enzymatic hydrolysis of the cell wall glucans (Feuillat *et al.*, 1989). As reported by Charpentier & Feuillat (1992), the mannoproteins and peptidomannans released during autolysis have significant effects in the sensory properties of wines as they contribute to the enhancement of the wine's structure, richness and roundness (Feuillat *et al.*, 1998; Feuillat, 2003).

Mannoproteins are glycoproteins and represent 35-40 % of the *Saccharomyces cerevisiae* yeast wall, being highly glycosylated. They are located in the cell wall external layer, where they are covalently linked to an amorphous matrix of β-1,3-glucans (Klis *et al.*, 2002). Mannoproteins are composed by 10-20 % of protein and about 80 % of D-mannose associated to D-glucose and N-acetylglucosamine residues (Pellerin & Cabanis, 1998). The release of mannoproteins into wine can occur via two different processes: during alcoholic fermentation in the yeast growth phase and after yeast autolysis. The latter mannoproteins are similar to those released during fermentation, but they have lower protein content (Saulnier *et al.*, 1991). During alcoholic fermentation, the yeasts release a high quantity of mannose-rich polysaccharides (Llaubères *et al.*, 1987; Doco *et al.*, 1996; Dupin *et al.*, 2000). These mannoproteins are released during the yeast growth phase and they accumulate in wine during the alcoholic fermentation (Llaubères *et al.*, 1987; Doco *et al.*, 1996). The highest production occurs in the transition between the growth phase and the stationary phase, when the yeast walls have a high mannoprotein content (Dupin *et al.*, 2000). According to Llaubères *et al.* (1987) and Saulnier *et al.* (1991), mannoproteins constitute the second most abundant polysaccharide group in wine, reaching quantities around 150 mg/L. The release of mannoproteins from the yeast cell wall during autolysis necessitates the action of the β-1,3-glucanase enzyme (Feuillat, 2003). This enzyme is produced by yeasts or can be added in a pure form isolated from *Trichoderma harzianum* (Dubourdieu *et al.*, 1985). Freyssinet *et al.* (1990) reported a degradation process of the cell wall involving two steps. In the initial phase, the exo- and endo-β-1,3-glucanases hydrolyze the cell wall glucans, releasing the polysaccharides into the medium, including mannoproteins of high molecular weight that are released by the action of α-mannosidases. In the second step, the parietal glucans continue to be degraded by the active β-1,3-glucanase. At the end of autolysis the solubilized exo-β-1,3-glucanase degrades the glucans that are still linked to mannoproteins and that are able to be hydrolyzed by α-mannosidases and proteases, releasing mannoproteins of low molecular weight. The periodic stirring of the wine, during contact with the fine lees, increases the concentration of mannoproteins (Feuillat *et al.*, 1998). Doco *et al.* (2003) have shown that the release of mannoproteins during wine ageing is a progressive and linear phenomenon and that the simple contact between lees and wine is not enough to release mannoproteins, stirring being necessary to resuspend the lees. Concerning the molecular weight, Saulnier *et al.* (1991) found mannoproteins in a range between 5 and 800 kDa.

For some white wines the roundness achieved with these compounds is associated to some particular aromas, namely wood aromas from the contact with wood barrels or wood staves and chips, and is recognized as a high quality factor giving the wine a differentiated style and a high commercial value. This way, several wineries are searching for techniques that can enrich the wines in mannoproteins in an easy and economical way. The aim of this work was to compare the nature and content of mannoproteins in a white wine from Encruzado (*Vitis vinifera* L.), a grape variety with high commercial interest in the Dão Region (Portugal), submitted to two different stirring of lees processes: stirring of lees in French oak wood barrels (225 l) and stirring of lees in a stainless steel tank (10000 L) equipped with French oak wood staves, a less expensive method. To the best of our knowledge, no work comparing the evolution of the mannoprotein profile between ageing on lees in wood barrels and ageing in stainless steel tank has been published.

MATERIALS AND METHODS

All chemicals used were of analytical reagent grade.

1. Wines

Encruzado (*Vitis vinifera* L.) grapes from Dão Sul: Sociedade Vitivinícola, S.A., located in the Dão Region (Portugal) were de-stemmed and crushed into a pneumatic press. The free running juice was cooled at 5ºC and transferred into a stainless steel tank. A commercial preparation of pectinolytic enzymes (Novoclair Speed, Novozymes, Denmark) was added to the must and allowed to settle down for 24 hours. After decantation into a stainless steel tank (10000 L) the must was inoculated with a commercial active dry *Saccharomyces cerevisiae bayanus* var. preparation (Enoferm QA23, Lallemand, Canada), fermenting at 15 to 18ºC for three weeks. When the must-wine density was around 1.060 g/cm^3, part of the must-wine was transferred to French oak wood barrels with medium toast (225 L), where it finished the alcoholic fermentation. The wood contact area was 133.31 cm^2/L. The rest of the must-wine finished the alcoholic fermentation in the stainless steel tank with French oak wood staves with medium toast. The oak wood staves contact area was 12.24 cm^2/L. At the end of the fermentation both wines were submitted to stirring of lees as follows: **stainless steel wine (IN)**: introduction of food grade nitrogen gas through the bottom tap of the tank at 5 L/min flow rate for 5 minutes; **barrel wine (BA)**: manual stirring with a stainless steel stick for 30 seconds per barrel. The stirring of lees was carried out in both wines at 2 to 3-day intervals for one month after the end of alcoholic fermentation. Then the lees were allowed to settle down until being stirred once again 100 days after the end of alcoholic fermentation. Both wines were separated from lees four months after the end of alcoholic fermentation. The samples were collected at days 0, 7, 15, 22, 28, 42, 71 and 128 after the beginning of the stirring of lees process. The wines were homogenized prior to sampling. Wine sampling from the wood barrels was performed by taking equal parts from five barrels. The two wines (BA and IN) were analyzed for ethanol content, pH, volatile acidity, titratable acidity and free and total SO_2 according to the *Organisation International de la Vigne et du Vin* official methods (OIV, 2006). The chemical parameters of the wines were the following: **IN wine**: alcohol content 13.8 % v/v, pH 3.82, volatile acidity 0.3 g/L expressed in acetic acid, titratable acidity 7.2 g/L expressed in tartaric acid, free SO_2 17 mg/L, and total SO_2 88 mg/L; **BA wine**: alcohol content 13.6 % v/v, pH 3.82, volatile acidity 0.4 g/L expressed in acetic acid, titratable acidity 7.5 g/L expressed in tartaric acid, free SO_2 21 mg/L, and total SO_2 87 mg/L.

2. Separation and purification of total colloids

In order to remove salts and small molecules, a volume of 6 L of wine was concentrated by ultrafiltration using an Ultrafiltration Millipore system (Millipore, Massachusetts, USA) equipped with a Masterflex Easy-Load I/PÔ Model 77410-00 pump (Cole-Parmer's Masterflex, Illinois, USA) and a regenerated cellulose cartridge Millipore Prep/ScaleÔ-TFF with 0.23 m^2 membrane area and a 10 kDa cut-off. The program was run in concentration mode at 1.2 bar and room temperature, adding de-salted water to the sample until the sample conductivity dropped 1000 mS/cm and the final volume was 1 L. The process was monitored with a Denver Instrument Model 220 conductivity meter (Denver Instrument, New York, USA). The concentrate of macromolecules was submitted to ethanol precipitation by adding twice the volume of cold ethanol to the concentrate at 4ºC. The mixture was allowed to precipitate for 36 hours. After this time, it was centrifuged at 1500 rpm for 10 minutes. The total colloids were recovered and freeze-dried for 48 hours and the resulting powder was submitted to mannoprotein isolation.

3. Mannoprotein isolation and characterization

A centrifuged solution of total colloids at 1 g/L in water was injected onto a concanavalin-A Sepharose 4B (GE Healthcare Bio-Sciences, Uppsala, Sweden) packed column (100 x 10 mm) and eluted with a sodium acetate-HCL 50 mM pH 5.6, NaCl 150 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM and $MnCl_2$ 1 mM buffer solution at 0.8 mL/min, monitored by a refractive index and a 254 nm wavelength detectors as described by Gonçalves *et al.* (2002). The bounded fraction was eluted with the same buffer solution added with methyl α-D-mannopyranoside 500 mM. The bounded fraction was dialyzed against water for 7 days at 4ºC. After freeze drying of the unbounded fraction, the sample (1 g/L) was injected onto a FPLC system equipped with a Pharmacia LKB Pump P-500, a 6HR 10/30 FPLC size-exclusion column (GE Healthcare Bio-Sciences, Uppsala, Sweden) and eluted with an ammonium acetate 0.3 M buffer solution at 0.3 mL/min and monitored by a Waters 2414 refractive index detector (Waters, Massachusetts, USA) and a Knauer WellChrom Spectro-Photometer K-2501 (Knauer, Berlin, Germany) wavelength detector at 254 nm. Calibration of the system was performed with a P-82 Pullulan standards (Showa Denko K.K., Kanagawa, Japan).

The carbohydrate composition was determined by gas chromatography after derivatization of the samples into their alditol acetates according to Albersheim *et al.* (1967). 100 µL of a myo-inositol solution (1 mg/mL) and 1 mL of trifluoroacetic acid 2 M were added to 1 mL of polysaccharide solution (1 mg/mL). After hydrolysis at 120ºC for 75 minutes, the mixture was washed with 5 mL of water and dried. 500 µL of a saturated sodium borohydride solution in ammonia were added and the mixture reacted for 2 hours at room temperature. The reaction was stopped by adding some drops of glacial

acetic acid and the mixture was washed with 5 mL of a solution of 1 % HCl in methanol and dried. 150 µL of pyridine and 150 µL of acetic anhydride were added to the mixture and allowed to react for 12 hours at room temperature. The reaction was stopped by adding a drop of water in an ice bath. The mixture was washed with 5 mL of water, followed by 1 mL of ethanol, and dried. The alditol acetates were extracted to 200 µL of chloroform and were quantified on a CE Instruments GC 8000 Top gas chromatographer (Thermo Fisher Scientific, Milan, Italy) equipped with a capillary column Zebron ZB-Wax 10 60 x 0.25 mm, 0.25 µm film (Phenomenex, California, USA) and a FID detector. The column temperature was initially set at 220ºC for 4 minutes and raised to 235ºC at 10ºC/min, maintaining this temperature for 5 minutes. Hydrogen was used as carrier gas at 1 mL/min. Myo-inositol was used as internal standard and the quantification of sugars was made after determination of each sugar response factor using pure sugars for this purpose.

The total protein content was determined as described by Lowry *et al.* (1951) using bovine serum albumin fraction V (Sigma-Aldrich, Missouri, USA) for the calibration curve. The total polysaccharide content was determined by the phenol-sulfuric method as described by Dubois *et al.* (1956) using glucose (Panreac, Barcelona, Spain) for the calibration curve.

4. Sensory analysis

The BA and IN wines were submitted to a tasting panel of 11 experts from the Technical University of Lisbon: Instituto Superior de Agronomia and the Dão Sul wine producing company. The following attributes were considered: Color: intensity; Aroma: fruity, floral, intensity, persistency, balance; Mouth-feel: roundness, bitterness, astringency, acidity, persistency, balance; Global appreciation. All parameters were scored on a 5-point scale (discontinuous structured interval scale) from 1 (absent) to 5 (very intense), except for the aroma balance, mouth-feel balance and global appreciation parameters, which were scored on a scale from 1 (bad) to 5 (excellent). Significant differences between results were analyzed using the Statistica 6.0 software (Statsoft, Oklahoma, USA). The values for each tasting descriptor were submitted to a single-way analysis of variance (ANOVA). Differences between samples refer to significant differences at $p < 0.05$.

5. Color intensity determination

The spectrophotometric method described by Somers & Evans (1977) was used to determine wine color intensity. The wines were centrifuged for 10 minutes at 3500 rpm and the absorbances were measured using a Unicam UV – Vis UV4 spectrophotometer (Unicam, Cambridge, UK).

RESULTS

1. Evolution of precipitable colloids and concanavalin-A adsorbed polysaccharides of wines

Precipitable colloid and concanavalin-A isolated polysaccharide content in BA and IN wines as a function of time is presented in figure 1.

The total colloids increased during the first three weeks of the experiment in both modalities. This initial increase was the result of the extraction performed by stirring during this time. The BA wine reached higher total colloid content (489 mg/L) than the IN wine (428 mg/L). This difference in the ability to extract more colloids into the medium in the BA wine was possibly due to the fact that the wood barrel had a smaller capacity (225 L), resulting in a more effective stirring than the one performed in the stainless steel tank (10000 L). After this initial increase, the total colloid content of both wines started to decrease. For the IN wine, the total colloid content remained stable until the last sampling date (day 128), i.e., until the wine was separated from the lees. As for the BA wine, the total colloid content slightly increased at the end of the process, probably due to the stirring performed one month before the wine was separated from the lees. The impact of this last stirring on total colloid content was higher in the BA wine (167 mg/L) than in the IN wine (148 mg/L). Once again, the effectiveness of the stirring was higher in the BA wine due to the smaller volume of wine, allowing a better extraction and resuspension of colloids.

The mannoprotein quantities found in these two wines were lower than those reported by other authors (Llaubères *et al.*, 1987; Saulnier *et al.*, 1991). The final polysaccharide (mannoproteins) content in the IN wine was around 20 mg/L. The BA wine had a decrease in polysaccharide content during the first 20 days of the stirring of lees process, after which it started to increase, reaching higher polysaccharide content (43 mg/L) on the day it was separated from lees.

The concanavalin-A isolated polysaccharides were submitted to molecular weight separation. In both IN (Figure 2) and BA (Figure 3) samples it was possible to consider the existence of three differentiated fractions (FI, FII and FIII) with different molecular weights that were maintained in time.

Comparing the molecular weight distribution profiles of both IN and BA wines it was possible to say that FI and FIII of both wines were similar, possibly corresponding to the same polysaccharides. In the IN samples it was possible to see a well defined second peak, corresponding to FII, which was not so well defined in the BA wine samples. The average molecular weights for each fraction were similar in both wines.

Each fraction was characterized for its protein and polysaccharide content and residual sugar composition (Table 1). The results shown are the averages of four samples (day 0, 22, 42 and 128 after the end of alcoholic fermentation). The analyses were done in duplicate.

The three fractions isolated from the IN wine presented average molecular weights of 745±0, 160±10 and 21±4 kDa for FI, FII and FIII, respectively. FI had a much higher concentration of glucose (61.5 %) than mannose (38.5 %) and protein represented only 3.3 % of the total composition. These results showed that this fraction did not represent a mannoprotein, as mannose was not the main sugar residue in this compound. FII was composed of 96.9 % of mannose and 3.1 % of rhamnose and protein represented 1.8 % of the total composition. This second fraction seemed to be a mannoprotein. FIII had 62.7 % of mannose, 30.6 % of glucose, 4.7 % of arabinose and 2.1 % of rhamnose, with protein corresponding to 2.2 % of the total composition.

The fractions isolated from the BA wine presented the following average molecular weights: 669±44, 160±12 and 22±5 kDa for FI, FII and FIII, respectively. FI presented 66.2 % of glucose, followed by 31.3 % of mannose, 1.7 % of arabinose and 0.8 % of rhamnose. No protein was detected in this fraction regarding the analytical methods used. Once again, and similarly to the results obtained for FI of the IN wine, glucose was the main sugar, suggesting that this fraction was not a mannoprotein. Between the first and the third fraction, there seemed to be another compound in a low quantity but which was not very well differentiated from the other two observed peaks. This FII was composed of 74.7 % of mannose, 20.2 % of glucose, 3.5 % of arabinose and 2.2 % of rhamnose and protein represented 8.5 % of the total composition. FIII had 69.0 % of mannose and 31.0 % of glucose, with 9.1 % of protein.

In Figures 2 and 3, it was possible to see the differences in the evolution profile in stainless steel tank and wood barrels, respectively. It is important to notice that each fraction was always present on each sampling date, although the quantities differed in time.

Figure 4 shows the evolution of the obtained quantities for each fraction and each wine in time.

Comparing the quantities of each fraction of polysaccharides between the two types of wine stirring of lees techniques it was possible to observe the following: the FI polysaccharide content of the IN wine (IN 745 kDa; Figure 1A) tended to diminish in time, with a final 2 µg/L. In the BA wine, it also diminished in time but on the last sampling day it increased to 42 µg/L (BA 669 kDa). Concerning FII of the IN wine (IN 160 kDa; Figure 1B), it increased between the first two sampling dates and then

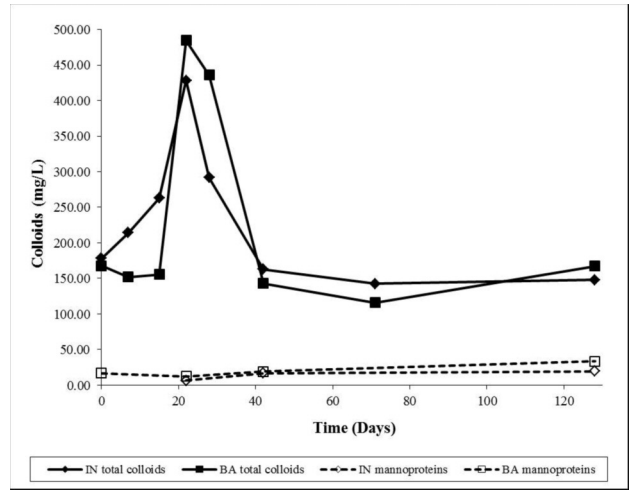

Figure 1 - Evolution of wine total colloid and concanavalin-A isolated polysaccharide content as a function of time for both processes (IN: stirring of lees with N_2 in a 10000 L stainless steel tank and BA: stirring of lees with a stainless steel stick in 225 L French oak wood barrels). Day 0: end of alcoholic fermentation, Day 0 to Day 30: stirring every 2 to 3 days, Day 30 to Day 100: settling down, Day 100: one last stirring and Day 128: end of wine-lees contact.

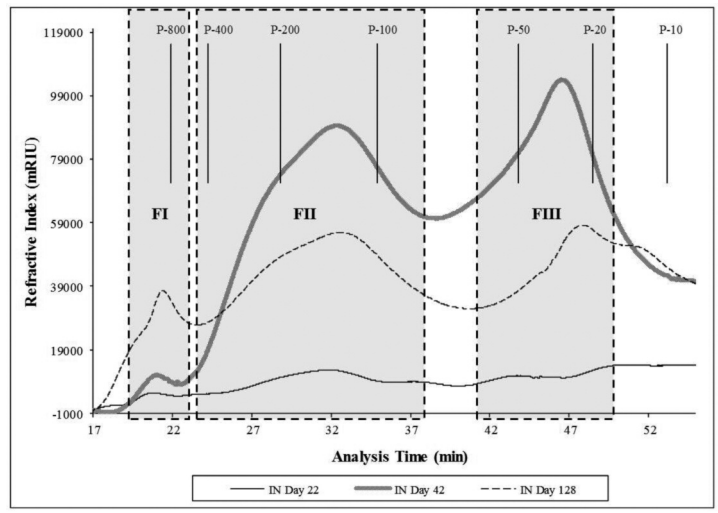

Figure 2 - Stainless steel-aged wine (IN) molecular weight distribution on the three sampling dates. The pullulan retention times are indicated at the top of the graph (P-10 to P-800). FI, FII and FIII identify the isolated fractions of polysaccharides for later chemical analysis. RIU is defined as refractive index unit.

decreased until the end of the experiment, finishing with a polysaccharide content of 18 µg/L. The BA wine sample had the same behavior but in a smaller extent, finishing with 31 µg/L. The FIII polysaccharide content started to decrease at the beginning for the IN wine (IN 21 kDa; Figure 1C) and then increased until the end of the experiment for both IN and BA wines (BA 22 kDa). This increase started earlier in the BA wine, where it finished

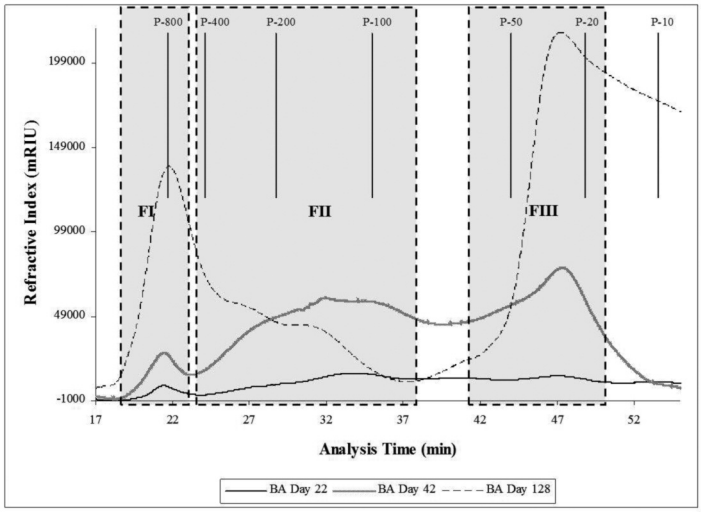

Figure 3 - Wood barrel-aged wine (BA) molecular weight distribution on the three sampling dates. The pullulan retention times are indicated at the top of the graph (P-10 to P-800). FI, FII and FIII identify the isolated fractions of polysaccharides for later chemical analysis. RIU is defined as refractive index unit.

with a polysaccharide content of 146 µg/L, whereas the final content in the IN wine was 34 µg/L. The observed increase was possibly due to the degradation of higher molecular weight molecules.

Sensory evaluation

The sensory evaluation of wines aged in stainless steel tank and wood barrels was made in order to understand the organoleptic differences achieved with the two stirring of lees techniques used in this study. The results are presented in Table 2.

Regarding color intensity, there was a significant difference between samples. The BA wine showed a deeper yellow coloration (score of 3.64 in a scale of 1 to 5). The IN wine presented a color intensity score of 2.64 with a lighter yellow coloration when compared to the BA wine. These results were confirmed by the measurement of color intensity by spectrophotometric method, where IN wine had 0.120 AU and BA 0.970 AU (data not shown). Regarding aroma, no significant differences ($p < 0.05$) were found when comparing both wines. Regarding mouth-feel, there was a significant difference ($p < 0.05$) between wines in the roundness sensation, with the BA wine having a higher score (3.73) than the IN wine (3.00). All the other mouth-feel parameters did not show any significant differences. With respect to global appreciation, the BA wine score was 3.36 and IN wine 3.73. No off-flavor was detected in any of the wine analyzed.

DISCUSSION

Comparing the concanavalin-A adsorbed polysaccharide profile of both wine with respect to molecular weight distribution and chemical characterization, it was possible to see that both IN and BA wines presented a first fraction with a higher percentage of glucose than mannose. In both cases, these fractions corresponded to the highest molecular weight isolated polysaccharide, with an average of 745 kDa for the IN wine and 669 kDa for the BA wine. In the first case, this compound was present in a small quantity throughout the experiment and had a protein part representing 3.3 % of the total polysaccharide content, whereas in the BA wine it did not have a protein part. The fact that glucose was the main sugar was probably due to the fact that this polysaccharide could be a high molecular weight glucan from the yeast cell wall that was also adsorbed by concanavalin-A and that was not yet

Table 1 - FPLC peak characterization : molecular weight (MW), polysaccharides (%), proteins (%) and residual sugars (%) (arabinose (Ara), rhamnose (Rha), mannose (Man) and glucose (Glu)).

Sample		MW (kDa)	Polysaccharides	Proteins	Ara	Rha	Man	Glu
IN	FI	745 ± 0	96,7 ± 0,0	3,3 ± 0,0	0,1 ± 0,0	n.d.	38,5 ± 0,0	61,5 ± 0,0
	FII	160 ± 10	98,2 ± 0,1	1,8 ± 0,1	n.d.	3,1 ± 0,2	96,9 ± 0,2	n.d.
	FIII	21 ± 4	97,8 ± 0,0	2,2 ± 0,0	4,7 ± 0,3	2,1 ± 1,1	62,7 ± 10,8	30,6 ± 11,6
BA	FI	669 ± 44	100,0 ± 0,0	n.d.	1,7 ± 2,4	0,8 ± 1,1	31,3 ± 0,1	66,2 ± 3,6
	FII	160 ± 12	91,5 ± 0,3	8,5 ± 0,3	3,5 ± 2,4	2,2 ± 2,7	74,7 ± 1,8	20,2 ± 2,1
	FIII	22 ± 5	90,9 ± 2,4	9,1 ± 2,4	n.d.	n.d.	69,0 ± 0,0	31,0 ± 0,0

n.d. : not detected
IN : stainless steel tank-aged wine ; BA : wood barrel-aged wine
Values are mean ± SD

totally hydrolyzed by the action of β-1,3-glucanases. Concanavalin-A is a lectin that specifically adsorbs mannose and glucose residues, having a high affinity for polysaccharides and glycoproteins. Feuillat *et al.* (1989) have shown that ethanol-precipitated colloids derived from yeast growth and autolysis are polysaccharides and most of them are mannans associated to proteins. The release of mannoproteins occurs when β-1,3-glucanases hydrolyze mannoproteins-associated glucans from the yeast cell wall. Glucans are then continuously hydrolyzed to oligomers and monomers.

The medium molecular weight polysaccharides of each wine presented exactly the same average molecular weight (160 kDa). The polysaccharide in the IN wine presented 96.9 % of mannose and 1.8 % of protein, indicating that this isolated fraction corresponds to a mannoprotein. In the BA wine, it presented 74.7 % of mannose, 20.2 % of glucose and 8.5 % of protein.

In both modalities, the polysaccharide with the lowest average molecular weight (21 and 22 kDa for IN and BA wine, respectively) was the predominant compound on the last day of lees contact. However, the composition did not allow to determine whether this was the same compound for both modalities, as the protein quantities in each modality were different, the BA wine polysaccharide containing more protein (9.1 %) than the IN wine polysaccharide (2.2 %). For both compounds, mannose was the main sugar, being present in around 70 % of the total composition. These two compounds were probably mannoproteins. The fact that these lowest molecular weight compounds were the most present on the last day of the experiment was probably due to the continuous degradation of higher molecular weight glucans by the action of β-1,3-glucanases. Doco *et al.* (1996) found that mannoproteins released into wine could vary in a wide range of molecular weights, extending to low molecular weight fragments. Gonçalves *et al.* (2002) also found that 32.2 % of the total polysaccharide content of a Loureiro grape variety white wine corresponded to mannoproteins and have identified two different mannoproteins with molecular weights of 53.4 and 560 kDa. Vidal *et al.* (2003) found six different mannoprotein fractions in a red wine with molecular weights varying between 51 and 527 kDa. The decrease in mannoprotein content observed at the beginning of the stirring of lees process was probably also due to some degradation, resulting in smaller oligosaccharides, or to some precipitation phenomena that dragged mannoproteins. In fact, mannoproteins can be hydrolyzed by α-mannosidases and proteases, thereby releasing smaller molecules (Feuillat *et al.*, 1989; Doco *et al.*, 2003).

The mannoprotein content obtained after the purification by concanavalin-A at the end of this experiment demonstrated that the BA wine had a slightly higher quantity of mannoproteins than the IN wine. According to the molecular distribution observed, the BA wine had only two well differentiated polysaccharides but in a higher concentration than the three found in the IN wine. The difference in polysaccharide concentration between the two modalities was probably the main reason why the BA wine showed a significant difference in the mouth-feel evaluation by the tasting panel (rounder mouth-feel than for the IN wine). Anyway, it is important to notice that there were no significant differences between wines with respect to the global appreciation obtained by sensory analysis.

Table 2 - Wines sensory data

Attributes	IN	BA
CI*	2,64 ± 0,67	3,64 ± 0,67
AFr	3,55 ± 0,93	3,00 ± 0,89
AFl	2,91 ± 1,14	2,18 ± 0,75
AI	3,91 ± 0,83	3,45 ± 0,52
AP	3,55 ± 0,82	3,27 ± 0,65
AB	3,64 ± 0,67	3,18 ± 0,60
MRo*	3,00 ± 0,63	3,73 ± 0,65
MBi	2,00 ± 1,10	2,00 ± 0,77
MAs	1,55 ± 0,69	1,73 ± 0,65
MAc	3,73 ± 0,65	3,18 ± 0,60
MP	3,36 ± 0,50	3,45 ± 0,69
MB	3,45 ± 0,52	3,18 ± 0,75

IN: stirring of lees with N2 in a 10000 L stainless steel tank; BA: stirring of lees with a stainless steel stick in 225 L French oak wood barrels. CI: Color intensity; AFr: Aroma fruity; AFl: Aroma floral; AI: Aroma intensity; AP: Aroma persistency; AB: Aroma balance; MRo: Mouth-feel roundness; MBi: Mouth-feel bitterness; MAs: Mouth-feel astringency; MAc: Mouth-feel acidity; MP: Mouth-feel persistency; MB: Mouth-feel balance. Data are mean ± SD; * indicates significant differences at $p < 0.05$.

CONCLUSION

The characteristics (molecular weight, protein content and residual sugar content) of each polysaccharide isolated from IN and BA wines were quite similar, although not exactly the same. It seemed that the degradation evolution of the yeast cell wall mannans and glucans was similar for both modalities, but the final quantities were not the same. BA wine showed a higher concentration of

polysaccharides at the end of the experiment (219 µg/L), which was perceived by the tasting panel with respect to the round mouth-feel sensation, in contrast to the IN wine (53 µg/L). As the global appreciation was similar for both wines, we could assume that both stirring of lees techniques can be used to achieve white wines with a good mouth-feel, as long as "roundness" is not the key measure of wine quality. Each winemaker should evaluate which of these techniques is the most adequate to meet the objectives of the end-product, having in mind the costs and the efforts that are needed to achieve these objectives.

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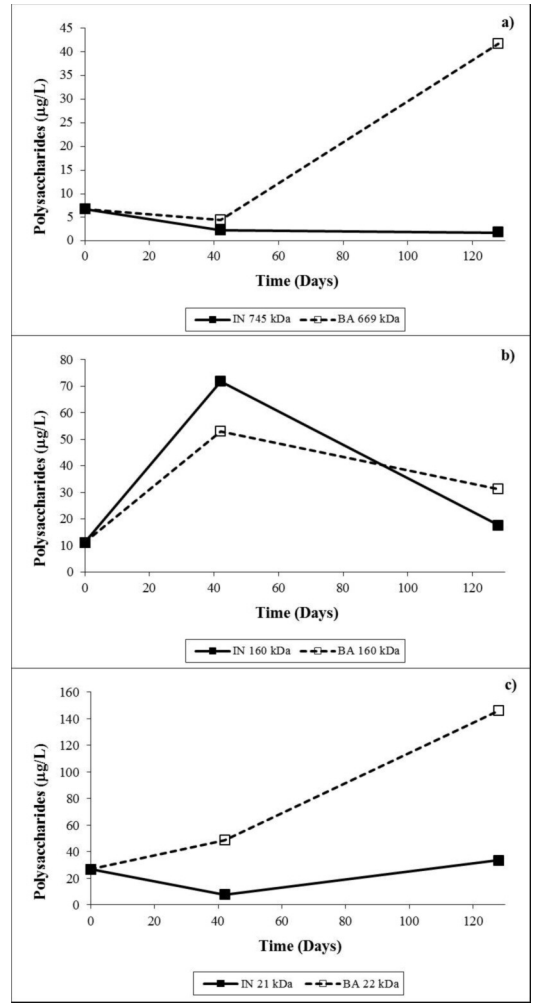

Figure 4 - Polysaccharide concentrations as a function of time in each molecular weight fraction isolated from wines aged in stainless steel (IN) and in wood barrels (BA). (a) Fraction I, (b) Fraction II and (c) Fraction III.

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