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Polysaccharides and Oligosaccharides Produced on Malvar Wines Elaborated with *Torulaspota delbrueckii* CLI 918 and *Saccharomyces cerevisiae* CLI 889 Native Yeasts from D.O. “Vinos de Madrid”

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1 ABSTRACT

2 Polysaccharides and oligosaccharides released into Malvar white wines
3 elaborated through pure, mixed and sequential cultures with *Torulasporea*
4 *delbrueckii* CLI 918 and *Saccharomyces cerevisiae* CLI 889 native yeasts from
5 D.O. "Vinos de Madrid" were studied. Both fractions from different white wines
6 were separated by high-resolution size-exclusion chromatography. Glycosyl
7 composition and linkages of wine polysaccharides were determined by GC-EI-
8 MS chromatography. Molar-mass distributions were determined by SEC-
9 MALLS and intrinsic viscosity by differential viscometer. Yeast species and type
10 of inoculation have a significant impact on wine carbohydrate composition and
11 structure. Mannose residues from mannoproteins were significantly predominant
12 in those cultures where *T. delbrueckii* was present in the fermentation process in
13 comparison with pure culture of *S. cerevisiae*, whereas galactose residues from
14 Polysaccharides Rich in Arabinose and Galactose presented higher values in pure
15 culture of *S. cerevisiae*, indicating that *S. cerevisiae* released less mannoproteins
16 than *T. delbrueckii*. Moreover, we reported structural differences between
17 mannoproteins released by *T. delbrueckii* CLI 918 and those released by *S.*
18 *cerevisiae* CLI 889. These findings help to provide important information about
19 the polysaccharides and oligosaccharides released from cell wall of Malvar
20 grapes and the carbohydrates released from each yeast species.

21

22 **Keywords:** Malvar white wines, native yeast, mannoproteins, polysaccharides,
23 oligosaccharides

24 INTRODUCTION

25 The study of wine polysaccharides has acquired great interest in recent years
26 owing to its role on a number of technological and sensorial properties in wine.
27 Numerous studies have clearly proven that these macromolecules have many
28 positive oenological features such as protection against tartrate salt
29 crystallization;¹ prevention of protein haze formation in white wines;² reduction
30 of astringency;^{3,4} interaction with tannins;⁵ increased sweetness and increased
31 body and mouth feel;³ formation of specific coordination complexes with Pb⁺²
32 ions.⁶ In sparkling wines, foam characteristics have been correlated with the type,
33 the molecular weight and the glycosyl composition of polysaccharides.⁷
34 Polysaccharides found in wine originate from grapes, yeasts and bacteria during
35 the winemaking. Those originating from grape cell walls are Polysaccharides
36 Rich in Arabinose and Galactose [PRAGs, which comprise arabinans,
37 arabinogalactans, and arabinogalactan proteins],⁸ homogalacturonans⁹ and
38 rhamnogalacturonans of type II (RG-II)¹⁰ whereas those released by
39 microorganisms are mainly mannoproteins (MPs) produced by yeasts during the
40 alcoholic fermentation or ageing on lees,¹¹ and glucan-like structures by
41 bacteria.¹²
42 However, the oligosaccharides composition in wine have only recently been
43 studied.¹³⁻¹⁶ These molecules are linked to defensive responses of plants.¹⁷
44 Moreover, oligosaccharides can be found in important medicinal and food
45 applications.¹⁸ According to their influence on the wine, it should be noted their
46 ability to chelate cations which can be important for wines. The structure and the

47 amounts of oligosaccharides, as well as in the case of polysaccharides, depend on
48 several factors, such as the grape origin,¹⁹ the grape cultivar¹⁶ and the
49 winemaking process and can be modified by enzyme treatment.¹⁹ Therefore, it
50 would appear to be necessary to go further with the identification, quantification
51 and composition of oligosaccharides in different wines in order to better
52 understand their influence on technological and organoleptic properties.

53 Recently, Giovani et al.²⁰ have shown the high capacity of non-*Saccharomyces*
54 wine yeasts to release important polysaccharides into wine during alcoholic
55 fermentation. The natural capacity of non-*Saccharomyces* yeasts to release
56 complex carbohydrates could be consider as selection criteria of these yeasts to
57 wine elaboration.²¹ The role of non-*Saccharomyces* yeasts in winemaking has
58 been revised in recent years, the use of controlled mixed fermentations as a
59 biotechnological tool has been promoted in order to enhance special and specific
60 characteristics of a wine thus improve their complexity.²¹ This practice has also
61 been reported as being able to increase some desirable metabolites, such as some
62 acetate esters²² and glycerol.²¹ Moreover, some non-*Saccharomyces* yeasts have
63 been reported as being able to release more polysaccharides than *S. cerevisiae*
64 strains during alcoholic fermentation.²⁰

65 *T. delbrueckii* was one of the first commercially available non-*Saccharomyces*
66 for the winemaking industry. During wine fermentation, *T. delbrueckii* in pure
67 cultures and mixed cultures with *S. cerevisiae* has produced wines with higher
68 sensory complexity and floral and fruity aromas because of its capacity of
69 produce high concentration of higher alcohols, esters, terpenes and phenolic

70 aldehydes as well as other compounds like 2-phenylethanol and linalool.²³ It has
71 also been described its ability to produce wines with lower volatile acidity,
72 acetaldehyde and acetoin.²⁴ Depending on the strain, *T. delbrueckii* can produce
73 low/medium glycerol, succinic acid and polysaccharides.^{20,25,26} Moreover,
74 *Torulaspota* genera is reported good producer of enzymes such as β -
75 glucosidases, pectinases, proteases and those related to xylan degradation but this
76 capacity of enzymes secretion also depends on the yeast strain analyzed.²⁷

77 The Denomination of Origin (D.O.) “Vinos de Madrid”, created in 1990, is
78 located in the centre of Spain (between 40° 16’ N and 40° 24’ N latitude) and
79 covers an area of 8.390 ha. The climate of this agronomy zone is continental with
80 temperatures ranging from -8 °C minimum in winter to 41 °C maximum in
81 summer,²⁸ and the annual rainfall means are between 460 and 660 mm. The most
82 cultivated grape cultivars are Airén and Malvar (white), and Garnacha and
83 Tempranillo (red) (all of them *Vitis vinifera* L. cv.). Malvar is an autochthonous
84 cultivar for this D.O., while Airén, Garnacha and Tempranillo have major
85 extensions all over the Iberian Peninsula. Part of the economic development of
86 this area is based on wine production with winemakers searching for the
87 production of high quality wines with singular identity. The selection of native
88 yeasts is an emerging tool which contributes to elaborate new styles of wines
89 more competitive in the market.²⁹

90 The aim of this study was to determine the polysaccharides and oligosaccharides
91 release into Malvar white wines elaborated with *T. delbrueckii* CLI 918 and *S.*
92 *cerevisiae* CLI 889 native yeasts from D.O. “Vinos de Madrid” in pure, mixed

93 and sequential cultures made with both strains. To date, this is the first report of
94 the polysaccharide and oligosaccharide composition in regional Malvar wines
95 using autochthonous *S. cerevisiae* and non-*Saccharomyces* yeasts from this
96 region.

97 **MATERIALS AND METHODS**

98 **Chemicals**

99 Analytical Reagent grade chemicals were used in the present study. Ammonium
100 sulphate supplied by Panreac Quimica (Barcelona, Spain) and Enozym Altair
101 pectolytic enzymes obtained from Agrovin (Ciudad Real, Spain), were used
102 during must preparation. The following compounds were used by
103 polysaccharides and oligosaccharides isolation and analysis in the studied wines.
104 Sodium chloride, phosphorus pentoxide, hydrogen chloride, trifluoroacetic acid,
105 ammonia, acetone, glacial acetic acid, ethyl acetate, acetic anhydride, perchloric
106 acid 70%, 1-methylimidazole, chloroform, and n-hexane were obtained from
107 Merck (Darmstadt, Germany). Methanol anhydrous, sodium borodeuteride and
108 myo-inositol were purchased from Sigma-Aldrich (St Louis, MO, USA).
109 Polyamide SC6 was supplied by Macherey-Nagel (Düren, Germany).
110 Ammonium formiate was supplied by Acros Organics (Geel, Belgium). Tri-Sil
111 HTP Reagent was obtained from Thermo Scientific (Waltham, MA, USA).

112 **Yeast strains**

113 The yeast strains used for the elaboration of the wines were the *Saccharomyces*
114 CLI 889 strain, selected as native strain from D.O. “Vinos de Madrid” and
115 characterized in the IMIDRA’s laboratories based on some established and

116 desirable oenological criteria³⁰. This strain has been deposited in Spanish Type
117 Culture Collection (CECT 13145). And *Torulaspota delbrueckii* CLI 918,
118 selected according to its biotechnological potential.²⁸ *T. delbrueckii* CLI 918 has
119 been described earlier as a strain with potential interest for its contribution to the
120 aromatic wine profile adding flowery and fruity notes and its use was considered
121 interesting in mixed starter cultures with *S. cerevisiae*,²⁸ and this strain has also
122 shown good fermentative capacity under different stress conditions.³¹

123 **Vinification procedure**

124 Grapes from Malvar cultivar (*Vitis vinifera* L. cv.) were hand-collected from
125 IMIDRA's experimental vineyard located in Madrid winegrowing region, Spain
126 (40° 31' N Longitude, 3° 17' W Latitude, and 610 m Altitude) during the 2010
127 vintage at commercial maturity (21.5 °Brix, equivalent to about 205 g L⁻¹ of
128 sugars). The grapes were gently destemmed and pressed and, the must was
129 racked, homogenized and clarified by pectolytic enzymes (1 g/hL) at 4 °C. Clear
130 must was supplemented with nitrogen by adding ammonium sulphate (NH₄)₂ SO₄
131 up to a level of 250 mgN L⁻¹ before beginning the alcoholic fermentation.

132 Triplicate experiments were carried out in sterile flasks with 1 L of pasteurized
133 (as indicated in García et al., 2017³²) Malvar must with constant agitation (150
134 rpm) in an 18 °C temperature controlled room. Fermentations were divided into
135 pure, mixed and sequential cultures. Pure cultures were independently inoculated
136 with 10⁶ cells mL⁻¹ CLI 889 *S. cerevisiae* strain (culture considered as control)
137 and 10⁶ cells mL⁻¹ of CLI 918 *T. delbrueckii* strain. Mixed fermentation trials
138 were simultaneously inoculated with 10⁶ cells mL⁻¹ of *T. delbrueckii* and 10⁶

139 cells mL⁻¹ of *S. cerevisiae* strain. Sequential fermentation trials, were inoculated
140 with 10⁶ cells mL⁻¹ of the *T. delbrueckii* culture at first and the addition of the *S.*
141 *cerevisiae* strain (10⁶ cells mL⁻¹) took place when the wine contained 5 % alcohol
142 (v/v). The fermentation progress was monitored by automatic weight using the
143 software OPCEX3 (Resolvica Inc., Chanhassen, MN) every 24 h until the end of
144 the fermentation (constant weight). After fermentation, yeast cells and wines
145 were separated by centrifugation (9000 rpm, 10 min, 4 °C); the pellets were
146 discarded and the wines were kept frozen until analysis.

147 Principal oenological parameters of final wines were measured by Fourier
148 transform infrared spectroscopy in the laboratories of Liec Agroalimentaria S. L.
149 (Manzanares, Spain), an accredited laboratory for physico-chemical analysis in
150 wines to conform to UNE-EN ISO/IEC 17025:2005 rules.

151 **Isolation of polysaccharide and oligosaccharide fractions**

152 The polysaccharide and oligosaccharide fractions were isolated as previously
153 described.^{4,7,13,15} Briefly, Malvar white wines (5 mL) were partially depigmented
154 in polyamide CC6 columns, particle size 0.05–0.16 previously equilibrated with
155 1 M NaCl. Wine polysaccharides and oligosaccharides passed through the
156 column and were eluted with two bed volumes of 1 M NaCl. The eluted fraction
157 was concentrated in a centrifugal evaporator (EZ-2, Genevac, Ipswich, UK).
158 High-resolution size-exclusion chromatography (HRSEC) was performed by
159 loading concentrated total wine carbohydrate on a system composed by a
160 rheodyne sampling injector with a loop of 2 mL, an Intelligent pump 301
161 (FLOM, France), and a fraction collector Frac-920 (GE Healthcare Bio-Sciences,

162 Pittsburgh, USA). Elution was performed on a Superdex-30 HR column (60 x 1.6
163 cm, Pharmacia, Sweden) with a precolumn (0.6 x 4 cm) equilibrated at 1 mL
164 min⁻¹ with 30 mM ammonium formate pH 5.6. The elution of polysaccharides
165 and oligosaccharides was monitored with a RI 101 (Shodex Showa Denko,
166 Japan) refractive index combined with Chromeleon software (Dionex,
167 Sunnyvale, CA). The polysaccharide fraction was eluted between 41 and 55 min,
168 while oligosaccharide fraction was collected between 56 and 93 min.^{4,7,16} The
169 isolated fractions were freeze-dried, redissolved in water, and freeze-dried again
170 four times to remove the ammonium salt.

171 **Glycosyl-Linkage determination**

172 The glycosyl-linkage compositions were determined by GC-MS of the partially
173 methylated alditol acetates. One milligram of polysaccharides in 0.5 mL of
174 dimethyl sulfoxide was methylated using methyl sulfinyl carbanion and methyl
175 iodide.³³ Methylated samples were hydrolyzed with 2 M of trifluoroacetic acid
176 for 75 min at 120 °C. The released methylated monosaccharides were converted
177 in their corresponding alditols by treatment with NaBD₄ and then acetylated by
178 adding ethyl acetate, acetic anhydride and perchloric acid.³⁴ Partially methylated
179 alditol acetates were analyzed by GC-EI-MS using a DB-1 capillary column (30
180 m x 0.25 mm i.d., 0.25 μm film), temperature programming 135 °C for 10 min
181 and then 1.2 °C min⁻¹ to 180 °C, with hydrogen as the carrier gas on a Shimadzu
182 GCMS-QP2010SE gas chromatograph (Shimadzu, Kyoto, Japan).³⁵ Myo-inositol
183 was used as internal standard and, to verify the rate of methylation. Response
184 factors of partially methylated alditol acetates are those described by Sweet et

185 al.³⁶ The validity of the used methods and their repeatability were checked
186 according to Vidal et al.³⁵

187 **Oligosaccharides analysis**

188 The neutral and acidic sugar compositions were determined after solvolysis with
189 anhydrous MeOH containing 0.5 M HCl (80 °C, 16h), by GC of their per-O-
190 trimethylsilylated methyl glycoside derivatives.³⁷ The TMS derivatives were
191 separated on two DB-1 capillary columns (30 m x 0.25 mm i.d., 0.25 µm film)
192 (temperature programming 120–145 °C at 1.5 °C min⁻¹, 145–180 °C at 0.9 °C
193 min⁻¹, and 180–230 °C at 50 °C min⁻¹), coupled to a single injector inlet through
194 a two-holed ferrule, with hydrogen as the carrier gas on a Shimadzu GCMS-
195 QP2010SE gas chromatograph. The outlet of the one column was directly
196 connected to a FID at 250 °C, and the second column via a deactivated fused-
197 silica column (0.25 m x 0.11 µm i.d.) was connected to a mass detector. Samples
198 were injected in the pulse split mode with a split ratio of 20:1. The transfer line to
199 the mass was set at 280 °C. Electron ionization mass spectra were obtained from
200 m/z 50 to 400 every 0.2 s in the total ion-monitoring mode using an ion source
201 temperature of 200 °C, a filament emission current of 60 µA, and an ionization
202 voltage of 70 eV.

203 **Determination of molar mass of wine polysaccharides**

204 Molar mass distributions, molar weight and number-average mass (M_w and M_n in
205 g/mol), polydispersity index (M_w/M_n), and intrinsic viscosity ($[\eta]$ in mL g⁻¹) were
206 determined at 25 °C by coupling size exclusion chromatography with a
207 multiangle light scattering device (MALLS), a differential viscometer, and a

208 differential refractive index detector. SEC elution was performed on an OH-pack
209 guard followed by two serial Shodex OH-pack KB-804 and KB805 columns (0.8
210 x 30 cm; Shodex Showa Denko, Japan) at a 1 mL min⁻¹ flow rate in 0.1 M LiNO₃
211 after filtration through a 0.1 μm filter unit. The MALLS photometer, a DAWN-
212 HELEOS from Wyatt Technology Inc. (Wyatt Technology Corporation, Santa
213 Barbara, CA, USA), was equipped with a GA-AS laser (λ = 658 nm). The
214 differential viscometer detector (Viscostar II, Wyatt Technology Inc., USA) was
215 equipped with a 4-capillary bridge design. The concentration of each eluted
216 polysaccharide was determined using the differential refractive index detector
217 (Optilab TrEX, Wyatt Technology Inc., USA). All collected data were analyzed
218 using Astra V 6.0.6 software with the zimm plot (order 1) technique for molar-
219 mass estimation and a differential refractive index increment of the polymer in
220 the solvent used. A dn/dc classical value was employed for polysaccharides
221 (0.146 mL g⁻¹).³⁸

222 **Statistical treatment**

223 One-factor ANOVA and Tukey HSD post-hoc tests were applied to establish the
224 significance of differences between means (α = 0.05). The data were analysed
225 with SPSS Statistics 21.0 Software for Windows (SPSS Inc., Chicago, IL).

226 **RESULTS AND DISCUSSION**

227 **Fermentations**

228 All fermentations finished in 27 days. Mixed culture of *T. delbrueckii* and *S.*
229 *cerevisiae* (m-Td/Sc) presented a similar fermentation rate with regard to pure
230 culture of *S. cerevisiae* (p-Sc, considered as control). In sequential culture of *T.*

231 *delbrueckii*/*S. cerevisiae* (s-Td/Sc), the addition of *S. cerevisiae* (day 6 of
232 fermentation; Sc in graphics) produced a significant increase in the fermentation
233 rate (Figure 1). These co-cultures with *T. delbrueckii* and *S. cerevisiae* as well as
234 the fermentation control p-Sc were characterized by an amount of residual sugar
235 lower to 3 g L⁻¹, which were therefore considered as dry wines. By contrast, *T.*
236 *delbrueckii* in pure culture presented a lower fermentative capacity, did not
237 consume the total quantity of sugars finishing with 33.70 g L⁻¹ and 10.88 % of
238 ethanol. It should also be highlighted the noticeably higher glycerol content
239 found in pure and sequential of *T. delbrueckii* (7.14 and 5.28 g L⁻¹, respectively)
240 in comparison with mixed culture and the control (2.87 and 3.12 g L⁻¹,
241 respectively).

242 **Purification of polysaccharides and oligosaccharides fractions**

243 Malvar wines from pure, mixed and sequential cultures with *T. delbrueckii* and *S.*
244 *cerevisiae* native yeasts were injected on Superdex 30-HR column in order to
245 separate polysaccharides and oligosaccharides. The first two peaks eluted
246 between 40 and 55 min corresponds to the polysaccharide fraction, it is
247 composed of polysaccharides rich in arabinose and galactose (PRAGs),
248 mannoproteins (MPs) and rhamnogalacturonans type II (RG-II).^{6,39} The
249 polysaccharides rich in arabinose and galactose and rhamnogalacturonans come
250 from the pecto-cellulosic cell walls of grape berries.^{4,7,13,16,35} The mannoproteins
251 are released from the yeast during alcoholic fermentation.³⁵ The fraction eluted in
252 the range 56–93 min contained a complex mixture of small sugars which
253 compose the oligosaccharides fraction of the different wines.^{4,13,15,16,19} Because

254 the winemaking process was the same, the profiles would indicate an important
255 influence of yeast strain employed in the fermentation process and, the type of
256 elaboration of the wines through pure, mixed or sequential culture could also be
257 influential. Sequential culture of *T. delbrueckii* and *S. cerevisiae* presented the
258 highest peaks during the purification process, which could suggest that the
259 metabolism of both yeast strains have a cumulative effect (data not shown).

260 **Glycosyl-linkage composition of polysaccharides fraction**

261 The wine polysaccharides fraction was methylated, hydrolysed and analyzed for
262 partially methylated alditol acetates by GC-MS. Their corresponding glycosyl-
263 linkage composition is given in Table 1. Neutral sugars commonly present in
264 wine polysaccharides³⁵ can be found in Malvar wines (Table 1). The
265 predominance of arabinose, galactose and mannose in the total colloids indicates
266 that PRAGs from grape cell walls and mannoproteins from yeast cell walls were
267 the major macromolecules in Malvar wines regardless of the type of inoculation
268 used with *T. delbrueckii* and *S. cerevisiae*.

269 The major proportion of arabinosyl residues were present as terminal non-
270 reducing position (2,3,5-tri-*O*-methyl ether) under furanose form, which may
271 arise from both AGPs and arabinans. Arabinose linked $\rightarrow 5$ is characteristic of
272 branched arabinans on the rhamnogalacturonan chain of the pectins (presence of
273 2- and 2,4-Rha).³⁵ Values obtained for $\rightarrow 5$ arabinose linked are similar to
274 commercial Champagne wines studies⁴⁰ but noticeably lower than other red
275 wines analyzed.^{13,16} It has been also detected arabinose linked at positions 2 and
276 3 with similar values regarding red wines, which origin are AGPs from grapes.

277 It also contained all methyl ether corresponding to the galactose linked at
278 positions $\rightarrow 3$, $\rightarrow 6$, $\rightarrow 3,6$ and $\rightarrow 3,4,6$ linkages that are found in AGPs.^{8,10,35} The
279 presence of 3,6-linked galactose indicates that type II arabinogalactans are side
280 chains component of the RG-I in Malvar wines.^{8,10} The ratio of 3-linked
281 galactose to 3,6-linked galactose residues (p-Sc, 1:3.1; p-Td, 1:2.9; m-Td/Sc,
282 1:2.7; s-Td/Sc, 1:3.0) indicated a highly-branched molecule similar to the AGP
283 isolated from Carignan wine⁸ or Champagne wine.⁴⁰ Vidal et al.³ reported that
284 the addition of a mixture of AGPs and MPs have an influence on wine
285 astringency and fullness. However, the physicochemical and sensorial properties
286 related with PRAGs in wines need to be investigated.

287 Mannose residues were significantly predominant in those cultures where *T.*
288 *delbrueckii* CLI 918 strain has been used in the fermentation process. On the
289 contrary, pure culture of *S. cerevisiae* presented lower value of mannose residues
290 (Table 1). Significant differences in molar percentage of mannose linked in $\rightarrow 2$,
291 $\rightarrow 3$, $\rightarrow 4$ and $\rightarrow 3,4$ positions as well as in non-reducing terminal were observed,
292 particularly for pure culture of *T. delbrueckii* and sequential culture of *T.*
293 *delbrueckii/S. cerevisiae* compared to pure culture of *S. cerevisiae*. The glycosyl-
294 linkage compositions of mannoproteins isolated from the Malvar wine inoculated
295 by pure culture of *S. cerevisiae* is in good agreement with accepted structure for
296 glycosidic moiety of mannoproteins previously isolated from a Carignan red
297 wine.³⁵ The structure of mannoproteins in these fractions consisted of a long 6-
298 linked backbone, highly substituted on position two with 2- and 3- linked
299 mannose chains and attached to protein at asparagine units in the protein. These

300 structural characteristics are in agreement with the model proposed by Ballou⁴¹
301 for mannoproteins from *Saccharomyces*, and coincided with those typically
302 found in yeast mannoproteins⁴² or with those mannoproteins released into the
303 wine.³⁵

304 Nevertheless, it is important to highlight the structural differences of
305 mannoproteins released by *T. delbrueckii* strain in comparison with those
306 released by *S. cerevisiae* strain. In pure and sequential cultures with *T.*
307 *delbrueckii*, the majority of mannoproteins were composed of short 2-linked
308 mannose chains, attached to serine and threonine residues in the protein part
309 (29.7% and 29.3% respectively) forming highly branched molecules. The ratio
310 between the values (Table 1) exhibited for 2-linked mannose and terminal
311 mannose in pure culture of *T. delbrueckii* presented a value of 4, therefore the
312 chains of mannoproteins released by this non-*Saccharomyces* strain contain 4
313 residues of mannose linked in $\rightarrow 2$ for a terminal mannose. Mannose linked at 4-
314 position has only been observed in pure culture of *T. delbrueckii* (Table 1), this
315 2,3,6-Man corresponds to the first mannose unit that it is 4-linked to the di-*N*-
316 acetylchitobiose unit.⁴¹ In pure culture of *S. cerevisiae* (p-Sc) and in pure culture
317 of *T. delbrueckii* (p-Td) the structure of mannoproteins released into the wine are
318 different (Table 1).

319 Besides, it was also observed in co-cultures that the secreted mannoproteins will
320 not be the same according to whether they are inoculated at the same time (m-
321 Td/Sc: mixed culture of *T. delbrueckii* and *S. cerevisiae*) or in a sequential
322 manner (s-Td/Sc: sequential culture of *T. delbrueckii* and *S. cerevisiae*). In mixed

323 culture (m-Td/Sc), mannoproteins have a glycosidic structure similar to that
324 obtained with a pure culture of *S. cerevisiae* (p-Sc). And in sequential culture (s-
325 Td/Sc), the structure of mannoproteins is similar to that of the pure culture of *T.*
326 *delbrueckii* (p-Td) (Mannose linked in 2-position, Table 1). Furthermore, both
327 pure cultures presented a different ability to release polysaccharides into the
328 wine, showing higher ratio of polysaccharide release for *T. delbrueckii* than *S.*
329 *cerevisiae*, these results are in agreement with the results obtained by Domizio et
330 al.²⁵ In the case of sequential culture, the polysaccharide release from *T.*
331 *delbrueckii* CLI 918 could be explained as the result of actively growing cells of
332 this non-*Saccharomyces* yeast strain before the inoculation of *S. cerevisiae* CLI
333 889. In the case of mixed culture of *T. delbrueckii* and *S. cerevisiae*, the
334 fermentation process seems to be dominated by *S. cerevisiae* as the results were
335 similar to the control (p-Sc). In reference to polysaccharides production in mixed
336 cultures, Cominiti et al.⁴³ observed a significant increase in the polysaccharides
337 content only in the mixed fermentations of *T. delbrueckii/S. cerevisiae* at the
338 inoculation ratios of 100:1 and 10,000:1. Other authors,⁴⁴ using a spectroscopic
339 approach to evaluate the polysaccharide/mannoprotein ratio has found
340 differences depending on the yeast strain or species used.

341 Moreover, it is well known the relation of MPs with the retention of aromatic
342 compounds and the increase of body and mouth feel in wines.^{3,45} After physico-
343 chemical, aromatic and sensorial analysis of these wines, pure culture of *T.*
344 *delbrueckii* and sequential culture of *T. delbrueckii* and *S. cerevisiae* contributed
345 to increase the complexity and quality in the final wines. Specifically, sequential

346 culture was distinguished for their higher concentration of β -phenylethyl alcohol
347 (rose aroma) and larger contents of esters such as 2-phenylethyl acetate, ethyl
348 isovalerate and ethyl hexanoate. Sensorial analysis was carried out by tasting
349 panel, tasters valued pure and sequential cultures of *T. delbrueckii* as the best
350 ones due to their fruity and flowery aroma, higher aroma intensity and overall
351 quality.³²

352 **SEC-MALLS analysis of polysaccharides fractions from Malvar wines**
353 **elaborated with *T. delbrueckii* and *S. cerevisiae* strains**

354 The polysaccharides relative index elution profiles show three principal
355 populations (Figure 2). The concentration signal peaks are in the ranges 14.0–
356 16.7 minutes for first population (P1), 16.7–18.8 minutes for second population
357 (P2) and 18.8–20.5 minutes for third population (P3) (Figure 2, DRI signal). The
358 molar mass of the eluting molecules decreased with increased elution volume in
359 agreement with the normal size exclusion separation mechanism (Figure 2, M_w
360 signal). Different molecular parameters as molar mass, polydispersity index
361 (M_w/M_n) and intrinsic viscosity ($[\eta]$) were measured in studied wines (Table 2).
362 The molar mass appeared considerably higher for pure culture of *T. delbrueckii*
363 in all three populations (P1: 682 000 g/mol; P2: 133 500 g/mol; P3: 33 300
364 g/mol) in comparison with other cultures. This result was in good agreement to
365 González-Royo et al.,²⁶ that described the presence of three populations of
366 polysaccharides (HM_w ; 144–1,000 kDa; IM_w ; 40–144 kDa; LM_w ; 5–40 kDa) in
367 white wine fermented by sequential inoculation with *T. delbrueckii* and *S.*
368 *cerevisiae*. The polydispersity index (M_w/M_n) was in general lower in third

369 populations (P3). All first populations (P1) showed higher values than values
370 previously reported in sparkling red wines.⁷ These first populations (P1) (14.0–
371 16.7 minutes) mainly corresponds to mannoproteins which molecular weights
372 have been defined in the range from 50 000 to 560 000 g/mol.³⁵ The intrinsic
373 viscosity was notably lower for pure and sequential cultures of *T. delbrueckii* in
374 the first population (P1), it may be related to the higher proportion of branched
375 mannoproteins found in these two cultures. Previous publications established the
376 influence of polysaccharides degree of branching on the intrinsic viscosity.⁴⁶
377 The molar mass distribution analysis of the polysaccharides fractions from
378 Malvar white wines elaborated with *T. delbrueckii* and *S. cerevisiae* yeasts is
379 shown in Figure 3. Regarding these data, six delimited ranges among different
380 wines can be observed (Molar mass range: range 1 = 2 500–20 000 g/mol; range
381 2 = 20 000–100 000 g/mol; range 3 = 100 000–250 000 g/mol; range 4 = 250
382 000–500 000 g/mol; range 5 = 500 000–1 000 000 g/mol; range 6 = 1 000 000–
383 10 000 000 g/mol). Previous six ranges have been selected due to their
384 correspondence with values obtained from different polysaccharides families by
385 SEC analysis: RG-II monomer, $M_w = 5\ 000$ g/mol; RG-II dimer, $M_w = 10\ 000$
386 g/mol; MP_{0c} , $M_w = 58\ 000$ g/mol; AGP_2 , $M_w = 165\ 000$ g/mol; MP_{0a} , $M_w = 350$
387 000 g/mol; MP_3 , $M_w = 1\ 000\ 000$ g/mol.^{7,35} Also, AGP_0 with a molar mass
388 around 145 000 g/mol was found in Carignan red wine.³⁵
389 In pure culture of *T. delbrueckii* polysaccharides fractions, 0% of mass can be
390 observed in range 1 which corresponds to polysaccharides like mannoproteins of
391 small molar mass (and mainly RG-II in the case of red wines). However, values

392 in the range 1 in pure culture of *S. cerevisiae* (11%), mixed (8%) and sequential
393 (10%) combinations presented values below the described in red wine.⁷ Obvious
394 differences are also observable concerning the ranges 2 and 3. Pure culture of *T.*
395 *delbrueckii* showed the highest values in the range 2 followed by mixed culture,
396 pure culture of *S. cerevisiae* and sequential culture (Figure 3). On the contrary,
397 this sequential culture presented higher percentage of mass in range 3, then pure
398 culture of *T. delbrueckii*, mixed culture and pure culture of *S. cerevisiae*. In
399 contrast, the percentage of mass in range 4, range 5 and range 6 were notably
400 similar between four different types of cultures (Figure 3).

401 **Oligosaccharide composition**

402 There are no published studies about the glycosyl composition of
403 oligosaccharides in Malvar varietal wines. In addition, this is the first time that
404 the glycosyl composition and characteristic ratios of oligosaccharides in pure,
405 mixed and sequential cultures with the native yeasts *T. delbrueckii* CLI 918 and
406 *S. cerevisiae* CLI 889 has been analysed, showing the corresponding results in
407 Table 3. The wines studied contain most of the sugars which take part in the
408 composition of wine carbohydrates.^{4,7,16,35} These sugars are mainly rhamnose,
409 arabinose, galactose, xylose, galacturonic acid and glucuronic acid coming from
410 the pecto-cellulosic cell walls of grapes. The presence of xylose, glucuronic and
411 4-*O*-Me glucuronic acid residues indicated that traces of hemicellulose might be
412 solubilized from grape berry cell walls.^{13,47} Other sugars such as mannose and
413 glucose are released from yeast polysaccharides.

414 In our work, differences were observed in the predominance of several
415 oligosaccharides between the types of cultures. However, the predominant
416 oligosaccharides were glucose (20.0–40.9%), galacturonic acid (14.5–26.8%),
417 xylose (11.1–15.3%), mannose (10.3–15.3%), arabinose (8.2–10.1%) and
418 galactose (4.3–7.9%). 4-*O*-Me glucuronic acid (2.8–3.8%), rhamnose (1.8–2.9%)
419 and xylitol (1.9–2.5%) were also found in all cultures but in lower quantities. The
420 smallest quantities of oligosaccharides corresponded to glucuronic acid (1.1–
421 1.8%) and fucose (1.4–1.6%) in agreement with results obtained in red and white
422 still wines^{4,13,14} and sparkling wines.⁷ As shown in Table 3, glucose content
423 released into the medium was higher (40.9%) in pure culture of *S. cerevisiae*
424 (culture considered as control) compared to the other samples. It could be
425 possible that strains used in this work release a small part of their underlying
426 layer composed of β -glucans, in addition to the external mannoprotein layer.²⁰
427 It could be highlighted the galacturonic acid content in pure culture of *T.*
428 *delbrueckii*. The high value of galacturonic acid has been reported in red wines
429 before, and it has been explained by differences in the pectin composition and in
430 the natural pectinase activities present in grape skins.^{4,7,13,16} In our case, it could
431 be possible that the pectinase activity showed by *T. delbrueckii* CLI 918 strain²⁸
432 also influence this galacturonic acid value. It is well known that pectinase
433 enzymes have a notable influence on technological and sensorial properties of
434 wines.⁴⁸
435 Mannose residues were higher in wines elaborated with different combinations of
436 *T. delbrueckii* and *S. cerevisiae*, in mixed and sequential inoculations (Table 3).

437 Mannose and glucose residues are mainly released from yeast cell walls, our
438 findings regarding these two oligosaccharides are similar to those found by
439 Quijada-Morín et al.⁴ and higher than obtained by others authors,^{7,13} probably
440 due to differences in maturity stages between cultivars at time of the harvest or to
441 different winemaking conditions.

442 The oligosaccharides coming from grape cell walls presented similar proportions
443 in respect to those described in Chardonnay and Grignolino wines,¹⁴ with
444 exception of galacturonic acid and mannose content that was considerably higher
445 in Malvar wines. Oligosaccharides such as arabinose, rhamnose and galactose
446 was larger in Carignan,¹³ Cabernet Sauvignon, Syrah and Monastrell¹⁶ red wines
447 in comparison with Malvar white wines. It could be related to longer contact
448 between grape skins and must during the elaboration of red wines than during the
449 production of white ones.

450 In order to know the oligosaccharide sugar structures, several characteristic ratios
451 have been calculated (Table 3): arabinose to galactose (Ara/Gal), rhamnose to
452 galacturonic acid (Rha/Gal A), arabinose+galactose to rhamnose (Ara+Gal/Rha)
453 and mannose to glucose (Man/Glc).

454 The ratio Ara/Gal is characteristic of the wine PRAGs.^{38,47} The value of this ratio
455 has been previously described in red wines, close to 1.⁴⁸ Other authors obtained
456 ratios 2-fold higher in Carignan and Merlot red wines¹³ and in Tempranillo
457 sparkling wines.⁷ In our work, this ratio showed significantly lower values in the
458 case of sequential culture of *T. delbrueckii* and *S. cerevisiae* and pure culture of
459 *T. delbrueckii* compared to the control (Table 3). The higher ratio Ara/Gal in *S.*

460 *cerevisiae* pure culture and mixed culture suggests a release of arabinose or
461 oligosaccharides rich in arabinose coming from pectic framework. By contrast, it
462 could be suggested that sequential inoculation produced a slight degradation of
463 PRAG structures. This PRAG degradation has been described previously during
464 post maceration and malolactic fermentation⁴⁹ and during wine aging of lees by a
465 partial dearabinosylation.⁴⁷ Moreover, champagne wines presented a much lower
466 ratio Ara/Gal (0.18) in comparison with red wines.⁴⁰

467 The Rha/Gal A ratio provides information on the relative richness of the wine
468 oligosaccharides in homogalacturonans versus rhamnogalacturonans.⁵⁰ There is a
469 slight difference in the sequential culture. Although, these low ratios (between
470 0.1–0.2) for all the samples would suggest homogalacturonan predominance in
471 oligosaccharides from Malvar wines. The results obtained were lower than those
472 found in red still wines^{4,13,16,19} but higher than results obtained in red sparkling
473 wines.⁷ This apparent discrepancy could be explained by the fact that grape
474 variety could impact on wine oligosaccharide structure, as demonstrated by
475 Apolinar-Valiente et al.¹⁶

476 It is assumed that most of the Ara and Gal residues are associated with pectin
477 hairy regions. Therefore, the (Ara+Gal) to rhamnose ratio was calculated to
478 estimate the relative importance of the neutral side chains to the
479 rhamnogalacturonan backbone. This ratio was considerably higher in pure
480 culture of *S. cerevisiae* in comparison with pure culture of *T. delbrueckii*, mixed
481 and sequential cultures (Table 3). It might be concluded that Malvar white wines
482 elaborated from different *T. delbrueckii*/*S. cerevisiae* combinations contain more

483 structures from the hairy regions of pectins (rhamnogalacturonan-like structures
484 carrying neutral lateral chains) as a result of breakdown of grape cell wall berries
485 by pectinases. This degradation could be favoured by pectinase activity presented
486 by *T. delbrueckii* CLI 918 strain.²⁸ Different values for this ratio have been found
487 in other grape varieties, 2.8 and 3.1 in Carignan and Merlot oligosaccharides;¹³
488 2.8 for Syrah oligosaccharides, 4.7 in Cabernet Sauvignon grape variety and 5.1
489 in Monastrell variety.¹⁶ Also, Martínez-Lapuente et al.⁷ showed the change of
490 (Ara+Gal)/Rha ratio in Tempranillo sparkling wines during the aging.

491 The Man/Glc ratio has been related with the effectiveness of mannoproteins for
492 protein stabilization in white wines, being more effective when this proportion is
493 higher.⁴⁵ In our case, glucose was the largely major residue sugar (20.0–40.9%)
494 whereas mannose represented smaller proportions (10.3–15.3%). Glucose is the
495 prevalent sugar in grape berries⁹ since it is the main component of cellulose and
496 hemicellulosic xyloglucans. Furthermore, the presence of glucose in wines may
497 also be related to microbial cell walls (*Botryotinia fuckeliana*, *Oenococcus oeni*)
498 or condensed anthocyanins.³⁹ In this work, grapes were harvested in good
499 sanitary conditions, Malvar white grapes did not contain anthocyanins, and
500 malolactic fermentation was not conducted. Therefore, the glucose content in
501 these Malvar wines would come from yeast glucans released during the
502 fermentation. Because the different chemical composition indicated by the
503 Man/Glc ratio for oligosaccharides could determine a different functional effect
504 on the wine, further analyses will be necessary to determine a possible
505 correlation on the wine attributes derived from the different mannoprotein

506 released through pure, mixed or sequential culture of *T. delbrueckii* CLI 918 and
507 *S. cerevisiae* CLI 889.

508 Taking into account that the grape variety, maturity stage and grape processing
509 were equal, the results of this study highlight that yeast strain and type of
510 inoculation have a significant impact on wine carbohydrate composition and
511 structure. Regarding the composition of polysaccharides fraction, mannose
512 residues from MPs were significantly predominant in those cultures where *T.*
513 *delbrueckii* was present in the fermentation process, whereas galactose residues
514 from PRAGs presented higher values when pure culture of *S. cerevisiae* was
515 employed, indicating that *S. cerevisiae* released less mannoproteins than *T.*
516 *delbrueckii*. Concerning the molecular parameters, the molar mass appeared
517 considerably higher for pure culture of *T. delbrueckii* in comparison with other
518 cultures, appearing also variations with regard to the intrinsic viscosity
519 depending on the population observed by RI technique. Moreover, the molar
520 mass distribution of the polysaccharides fractions from Malvar white wines also
521 showed obvious changes relied on the yeast strain and type of inoculation used.
522 As regards the polysaccharide fractions in pure culture of *T. delbrueckii*, 0% of
523 molar mass can be observed in the range between 2 500 and 20 000 g/mol
524 whereas the control (p-Sc) and the co-cultures presented values between 8.4 and
525 10.9 % for this range. Clear differences were also observable in the range
526 between 20 000 and 100 000 g/mol and the range between 100 000 and 250 000
527 g/mol. The analysis of Malvar wines has also revealed that the oligosaccharide

528 composition and structure could be significantly influenced by the types of
529 cultures.

530 In summary, our results provide relevant information about the polysaccharides
531 and oligosaccharides released from cell wall of Malvar grapes and the
532 carbohydrates released from cell wall of different yeast strains through pure,
533 mixed or sequential cultures of *T. delbrueckii* CLI 918 and *S. cerevisiae* CLI 889.
534 These findings, about the use of different types of cultures, aim to improve the
535 quality of wines from D.O. "Vinos de Madrid", highlighting especially sequential
536 cultures to produce larger amounts of mannoproteins enhancing the complexity
537 and quality of Malvar wines. Analysis should be carried out to deepen our
538 knowledge concerning the capacity of *Saccharomyces* and non-*Saccharomyces*
539 yeast strains to release mannoproteins, which could be considered a selection
540 criterion for wine elaboration because of their reported contribution to wine
541 quality.

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551 **Notes**

552 The authors declare no competing financial interest.

553 **ABBREVIATIONS USED**

554 PRAGs, polysaccharides rich in arabinose and galactose; AGs, type II
555 arabinogalactans; AGPs, type II arabinogalactan-proteins; RG-I,
556 rhamnogalacturonans type I; RG-II, rhamnogalacturonans type II; MPs,
557 mannoproteins; TMS, per-O-trimethylsilylated methyl glycoside; HRSEC, high-
558 resolution size-exclusion chromatography; GC-EI-MS, gas chromatography
559 electron ionization mass spectrometry; SEC-MALLS, size exclusion
560 chromatography-multiangle laser light scattering.

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Table 1. Glycosyl-Linkage Composition (mole percentage) of polysaccharides fractions isolated from Malvar wines elaborated with different types of inoculation of the yeast strains *T. delbrueckii* CLI 918 and *S. cerevisiae* CLI 889. Values are the mean \pm SD of fermentations.

Methyl ether	Linkage	p-Sc ^b	p-Td ^b	m-Td/Sc ^b	s-Td/Sc ^b
234 Rhamnose	Terminal	2.9 \pm 0.8	1.3 \pm 0.1*	2.2 \pm 0.4	1.9 \pm 0.2*
34 Rhamnose	2-Linked	1.8 \pm 0.5	0.8 \pm 0.2*	1.4 \pm 0.4	1.1 \pm 0.6
3 Rhamnose	2,4-Linked	0.4 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.1	0.1 \pm 0.0*
Total rhamnose ^a		5.1 \pm 1.4	2.4 \pm 0.2*	3.8 \pm 0.2	3.1 \pm 0.8*
235 Arabinose	Terminal furanose	13.3 \pm 3.3	11.0 \pm 0.7	15.9 \pm 3.5	12.2 \pm 2.2
25 Arabinose	3-Linked	1.6 \pm 0.4	0.6 \pm 0.1*	0.2 \pm 0.0*	0.6 \pm 0.2*
35 Arabinose	2-Linked	0.4 \pm 0.1	0.4 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.1
23 Arabinose	5-Linked	0.4 \pm 0.2	0.7 \pm 0.1	0.7 \pm 0.1	0.9 \pm 0.3
Total arabinose ^a		15.7 \pm 3.9	12.7 \pm 0.9	17.0 \pm 3.6	13.9 \pm 2.8
2346 Galactose	Terminal	3.3 \pm 0.4	2.5 \pm 0.6	3.0 \pm 0.8	2.8 \pm 0.6
234 Galactose	6-Linked	8.1 \pm 0.6	8.8 \pm 0.1	8.1 \pm 0.1	7.1 \pm 0.8*
246 Galactose	3-Linked	5.6 \pm 0.3	5.6 \pm 0.1	5.9 \pm 1.2	4.3 \pm 0.9
24 Galactose	3,6-Linked	17.7 \pm 1.3	16.3 \pm 0.8	16.4 \pm 2.1	13.1 \pm 0.8*
2 Galactose	3,4,6-Linked	4.2 \pm 0.6	3.5 \pm 0.4	3.2 \pm 0.4	2.7 \pm 0.7*
Total galactose ^a		38.9 \pm 2.9	36.7 \pm 1.8	36.6 \pm 4.0	30.0 \pm 2.4*
2346 Glucose	Terminal	1.4 \pm 0.5	1.0 \pm 0.1	0.4 \pm 0.0	0.3 \pm 0.0
234 Glucose	6-Linked	1.2 \pm 0.9	0.4 \pm 0.1	0.4 \pm 0.1	1.5 \pm 0.8
Total glucose ^a		2.6 \pm 0.4	1.4 \pm 0.4	0.8 \pm 0.6*	1.8 \pm 0.9
2346 Mannose	Terminal	11.8 \pm 0.1	7.0 \pm 0.1*	12.3 \pm 0.8	11.4 \pm 0.4
346 Mannose	2-Linked	7.2 \pm 0.8	29.7 \pm 2.4*	12.3 \pm 3.9*	29.3 \pm 2.3*
246 Mannose	3-Linked	8.3 \pm 0.4	0.0 \pm 0.0*	7.8 \pm 0.2	3.2 \pm 0.7*
236 Mannose	4-Linked	0.0 \pm 0.0	2.6 \pm 0.7*	0.0 \pm 0.0	0.0 \pm 0.0
34 Mannose	2,6-Linked	8.8 \pm 1.6	6.1 \pm 0.5	8.1 \pm 2.8	6.5 \pm 1.7
26 Mannose	3,4-Linked	2.0 \pm 0.5	2.0 \pm 0.1	1.7 \pm 0.2	1.3 \pm 0.1*
Total mannose ^a		38.1 \pm 3.3	47.4 \pm 2.1*	42.2 \pm 7.6	51.7 \pm 0.3*
Ratio terminal/branched		0.9 \pm 0.2	0.7 \pm 0.0	1.0 \pm 0.2	1.1 \pm 0.2

^a Relative molar percent of each parent sugar family (sum of ethers from one sugar type) within total sugars.

^b p-Sc: pure culture of *S. cerevisiae*; p-Td: pure culture of *T. delbrueckii*; m-Td/Sc: mixed culture of *T. delbrueckii* and *S. cerevisiae*; s-Td/Sc: sequential culture of *T. delbrueckii* and *S. cerevisiae*.

* Means statistically different from the control (p-Sc), $p < 0.05$

Table 2. Parameters^a obtained for the polysaccharides isolated from Malvar white wines elaborated with *T. delbrueckii* and *S. cerevisiae* strains using different type of inoculation.

Wine sample ^b	Peak ^c	M_w	M_n	M_w/M_n	Intrinsic Viscosity
		(g/mol)	(g/mol)		(mL/g)
p-Sc	1	539 000	422 200	1.31	43.59
	2	118 850	97 390	1.21	17.34
	3	18 975	16 595	1.14	9.36
p-Td	1	682 000	522 100	1.31	33.32
	2	133 500	108 200	1.23	16.30
	3	33 300	30 300	1.10	7.03
m-Td/Sc	1	679 350	453 600	1.50	51.99
	2	124 200	102 600	1.22	16.81
	3	26 490	22 990	1.15	8.32
s-Td/Sc	1	491 000	387 600	1.27	39.07
	2	129 100	115 550	1.12	17.99
	3	25 620	22 575	1.13	10.76

^a Molar-mass distribution, M_w (Molar weight), M_n (Number-average mass), M_w/M_n (Polydispersity index), and Intrinsic viscosity ($[\eta]$).

^b Abbreviations related with the type of culture employed and the yeast strains are explained in Table 1.

^c Peak 1: ranges 14.0 – 16.7 min (first population, P1); peak 2: ranges 16.7 – 18.8 min (second population, P2); peak 3: ranges 18.8 – 20.5 min (third population, P3).

Table 3. Glycosyl composition (mole percentage) and characteristics ratios of oligosaccharides isolated from Malvar wines elaborated with *T. delbrueckii* CLI 918 strain and *S. cerevisiae* CLI 889 strain using different inoculation strategies. Values are the mean \pm SD of fermentations.

	p-Sc ^b	p-Td ^b	m-Td/Sc ^b	s-Td/Sc ^b
Rha ^a	1.8 \pm 0.9	2.9 \pm 0.3*	2.7 \pm 0.1*	2.7 \pm 0.1*
Fuc	1.0 \pm 0.3	1.4 \pm 0.1*	1.4 \pm 0.1*	1.6 \pm 0.1*
Ara	8.2 \pm 1.0	10.0 \pm 2.2	10.1 \pm 0.2	9.6 \pm 0.4
Gal	4.3 \pm 1.4	7.9 \pm 0.4*	6.6 \pm 0.4*	7.9 \pm 0.3*
Glc	40.9 \pm 1.1	20.0 \pm 1.4*	21.6 \pm 2.9*	26.4 \pm 0.9*
Man	10.3 \pm 0.3	12.4 \pm 2.2	15.1 \pm 1.6*	15.3 \pm 0.8*
Xyl	11.1 \pm 0.8	12.2 \pm 2.8	15.3 \pm 0.1*	15.1 \pm 0.3*
Gal A	16.7 \pm 1.8	26.8 \pm 3.0*	19.9 \pm 0.7	14.5 \pm 1.1
Glc A	1.1 \pm 0.1	1.3 \pm 0.2	1.4 \pm 0.1	1.8 \pm 0.8
4-O-MeGlc A	2.9 \pm 0.2	3.4 \pm 0.1*	3.8 \pm 0.0*	2.8 \pm 0.0
Xylitol	1.9 \pm 0.4	1.9 \pm 0.3	2.1 \pm 0.4	2.5 \pm 0.2*
Ratio				
Ara/Gal	2.0 \pm 0.4	1.3 \pm 0.5*	1.6 \pm 0.1	1.2 \pm 0.1*
Rha/Gal A	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0	0.2 \pm 0.0*
(Ara+Gal)/Rha	7.9 \pm 2.8	6.2 \pm 0.4	6.2 \pm 0.4	6.5 \pm 0.4
Man/Glc	0.2 \pm 0.0	0.6 \pm 0.1*	0.7 \pm 0.2*	0.6 \pm 0.1*

^a Rha, Rhamnose; Fuc, Fucose; Ara, Arabinose; Gal, Galactose; Glc, Glucose; Man, Mannose; Xyl, Xylose; Gal A, Galacturonic acid; Glc A, Glucuronic acid; 4-O-MeGlc A, 4-O-methyl Glucuronic acid.

^b Abbreviations related with the type of culture employed and the yeast strains are explained in Table 1.

* Means statistically different from the control (p-Sc), $p < 0.05$

FIGURE CAPTIONS

Figure 1. Fermentation kinetics of pure (p), mixed (m) and sequential (s) cultures in Malvar must with *T. delbrueckii* (Td) and *S. cerevisiae* (Sc) yeast strains. Values are the means from triplicate fermentations.

Figure 2. SEC-MALLS chromatograms and weight-average molar mass distributions of the polysaccharide fraction in pure (p), mixed (m) and sequential (s) cultures made with *T. delbrueckii* (Td) and *S. cerevisiae* (Sc) strains. Molar weight distribution (M_w ; g/mol; continuous line) and refractive index (DRI; relative scale; dashed line).

^a Peak 1: ranges 14.0 – 16.7 min (first population, P1); peak 2: ranges 16.7 – 18.8 min (second population, P2); peak 3: ranges 18.8 – 20.5 min (third population, P3).

Figure 3. Distribution analysis determined by light scattering ($dn/dc = 0.146$ mL/g) of polysaccharides fractions isolated from Malvar white wines elaborated with *T. delbrueckii* and *S. cerevisiae* yeast strains with different type of inoculation.

^a Abbreviations related with the type of culture employed and the yeast strains are explained in Table 1.

Figure 1

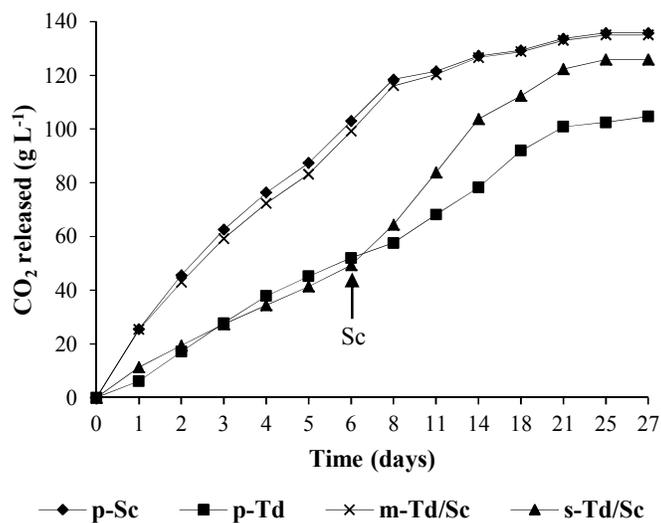


Figure 2

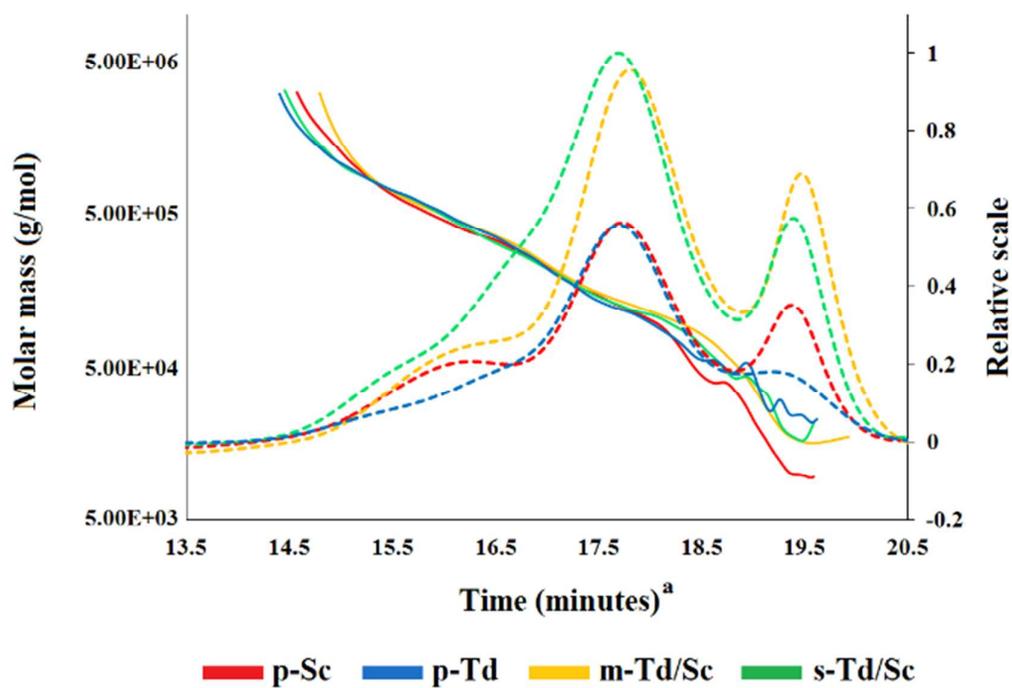
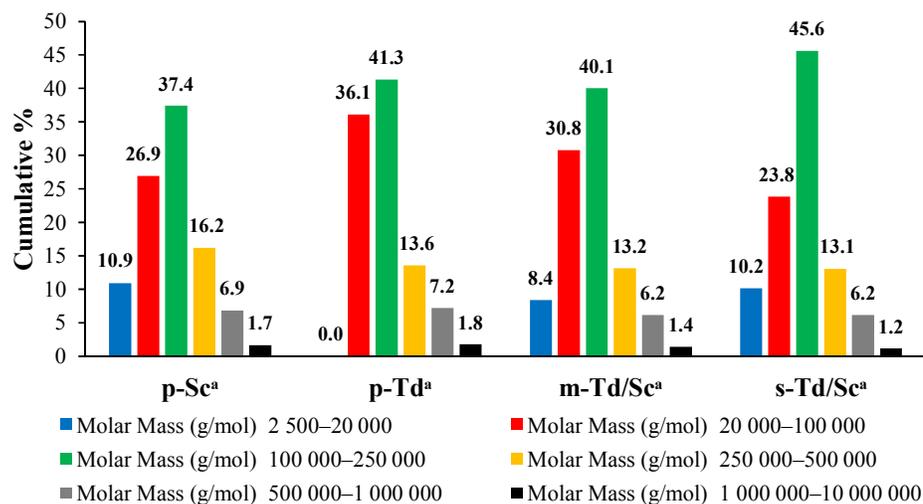


Figure 3



TOC graphic

