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# Effect of mannoproteins from different oenological yeast on pigment composition and color stability of red wine

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ABSTRACT

In this work, MP-rich extracts from *Saccharomyces* and non-*Saccharomyces* yeasts were obtained by cell sonication and were characterized. The extracts were added to a red wine and color and pigment composition modifications were evaluated by CIELAB parameters and HPLC-DAD-MS, respectively, after cold treatment (to provoke colloidal instability) and storage at room temperature (to accelerate wine aging). Results indicate that the MP-rich extracts showed differences in their composition and in the structure of the MPs depending on the yeast species. They also had different effects on the stability of wine pigments, being the extract obtained from *Torulaspora delbrueckii* the one that provided the best results, by contributing to the colloidal and chemical stability of the coloring matter. Wines added with this extract showed concentrations of *p*-coumaroylated and caffeoylated anthocyanins 33.40% higher than the control wine after 4 days of storage at 4 °C.

## 1. Introduction

The grapevine phenological development is deeply dependent on the atmospheric conditions, having air temperature a prominent role. Therefore, grapevine is a crop particularly susceptible to climate change. The increasing temperatures can accelerate the phenology of grapevine, resulting in the modification of the accumulation in the grapes of quality-related compounds such as sugars, acids and phenolic compounds (Droulia & Charalampopoulos, 2021; Santos et al., 2020). In this sense, high temperatures can favor the accumulation of sugars and slow down the biosynthesis of anthocyanins (Arrizabalaga et al., 2018; Sadras & Moran, 2012) leading to an imbalance of these two types of compounds in grapes and, consequently, to wines with poor and/or unstable color. Given the projection on future temperature trends, this decoupling of technological and phenolic maturity of grapes represents a serious problem for many winegrowing regions, particularly for the Southern European regions (Venios et al., 2020).

In view of the negative effects that climate change consequences can exert on grape composition and wine quality, new oenological tools are required in order to mitigate this problem. In this way, the addition to wine of mannoproteins (MPs) could have a protective effect on wine color, since some studies have reported that MPs can contribute to the colloidal stabilization of the pigments (Alcalde-Eon et al., 2014, 2019; Escot et al., 2001). In fact, there are many MP preparations commercialized for this purpose (Escribano-Bailón et al., 2019).

MPs released by yeasts are the second most abundant group of polysaccharides in wine (Vidal et al., 2003). They are highly glycosylated proteins located in the outermost layer of the yeast cell wall in which the polysaccharide fraction can represent up 50–95% (*w/w*) (Lipke & Ovalle, 1998). In spite of the studies showing the protective role of MPs with regard to wine color, not all the MPs seem to have this positive effect. For example, Guadalupe and Ayestarán (2007 and 2008) found that the addition of commercial MPs and/or the use of MP over-producing strains did not contribute to stabilize wine color. The same was found by Rodrigues et al. (2012), who reported that the addition of three commercial MPs had no effect on wine color stabilization. Del Barrio-Galán et al. (2011 and 2015) showed that neither the use of yeast derivative products rich in parietal polysaccharides nor the use of a high producing polysaccharide *S. cerevisiae* strain contributed to stabilize wine color. This discrepancy could be due to differences in the composition and structure of the MPs. However, the studies that relate these factors to the ability of MPs to stabilize wine color are still scarce.

MPs are naturally released by yeast when they are actively growing during alcoholic fermentation or by yeast autolysis during aging on lees (Guadalupe et al., 2014). In wine, other yeast species besides *Saccharomyces cerevisiae* (such as those belonging to the genres *Hanseniaspora*, *Pichia*, *Torulaspora*, *Lachancea* or *Metschnikowia*) can be found. These non-*Saccharomyces* yeasts can govern the initial phases of spontaneous

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fermentations, when the ethanol concentration is still low (Vicente et al., 2020). Given that some non-*Saccharomyces* yeasts have a high capacity to release MPs during alcoholic fermentation (Domizio et al., 2014; Giovani et al., 2012), wines can naturally present a great diversity of MPs derived from different yeast species. However, most studies are focused on the MPs released from *S. cerevisiae* cell wall and, currently, there are few studies addressing the characterization of MPs derived from the cell wall of non-*Saccharomyces* yeasts, as well as their possible role in modulating wine organoleptic properties, such as color. Furthermore, commercial MP preparations are obtained from *S. cerevisiae* (Pozo-Bayón et al., 2009). The use of different yeast species as new sources of MPs could provide the oenological industry with a greater diversity of these compounds that can help address the challenges present during the winemaking process. Thus, the objective of this work was to obtain and characterize MP-rich extracts from *S. cerevisiae* and non-*Saccharomyces* yeasts and evaluate their effect on wine color and pigment composition.

## 2. Materials and methods

### 2.1. Yeast strains

Four Active Dry Yeast (ADY) from Lallemand Inc. (Montreal, Canada) were used in this study: *S. cerevisiae* (EC1118 Organic), *Lachancea thermotolerans* (LAKTIA), *Torulaspora delbrueckii* (BIODIVA) and *Metschnikowia pulcherrima* (FLAVIA). Rehydration of the cells was performed following the manufacturer instructions: ADY was rehydrated in 10 times its weight of water at 30 °C and, after 15 min, the suspension was gently agitated for 15 min at the same temperature.

### 2.2. Extraction and purification of MP-rich extracts

The MP-rich extracts were obtained by the mechanical disruption of the yeasts. After the rehydration of 12 g of ADY, cell lysis was performed on ice by the application of 10 cycles of ultrasounds (30 s, output power of 11 W) (Microson ultrasonic cell disruptor XL, Misonix Inc., Farmingdale, NY, USA), waiting 1 min between cycles. Then, the samples were centrifuged (10 min, 12000 rpm) and the supernatants were separated and freeze-dried.

The lyophilized extracts were dissolved in water at a concentration of 10 mg/mL and submitted to 70 °C for 2 h in order to denaturalize and precipitate the cellular proteins extracted in the rupture of the cells. Then, the samples were centrifuged (10 min, 12000 rpm) and the supernatants were dialyzed at 12–14 kDa molecular weight (MW) cut-off membranes to remove low molecular weight compounds. Dialysis was carried out in 5 L of distilled water for 48 h with periodic water changes. Finally, the purified extracts were freeze-dried.

### 2.3. Characterization of the MP-rich extracts

The four MP-rich extracts were characterized as follows. The protein and glycoprotein profiles were determined by SDS-PAGE on 4–15% linear gradient Mini-PROTEAN® TGX™ precast gels (Bio-Rad Laboratories Inc., Hercules, CA, USA). Samples were dissolved in water at a concentration of 37.5 mg/mL and then diluted 1:1 with sample loading buffer 2X (100 mM Tris-HCl pH 6.8, 20% (*v/v*) glycerol, 4% (*v/v*) SDS, 25 mM DTT and traces of bromophenol blue). Then, samples were heated for 5 min at 100 °C and 16 μL were loaded into the gel. The running buffer used was Tris-Glycine SDS buffer (24.8 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3, Bio-Rad Laboratories Inc.). The electrophoretic conditions included a constant voltage of 180 V for 50 min at room temperature. Resolved proteins were stained with Coomassie Brilliant Blue G-250 (Alfa Aesar, ThermoFisher Scientific, Germany) and glycoproteins were detected following the Periodic Acid-Schiff (PAS) staining method. The result of both staining processes was scanned using a Lexmark XC4140 scanner (Lexington, KY, USA). Molecular weights were estimated using a protein ladder (PageRuler™ Plus Prestained Protein Ladder, 10–250 kDa, ThermoFisher Scientific, Germany).

The protein concentration was determined by Lowry method (Lowry et al., 1951) using the DC Protein assay kit (Bio-Rad Laboratories Inc.) and a bovine serum albumin calibration curve. From the concentration data, total protein content (*w/w*) of each extract was calculated.

The MW distribution of the MP-rich extracts was analyzed by HRSEC-RID following the method described by Manjón et al. (2020) using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with two serial Shodex OHpak SB-803 HQ and SB-804 HQ columns (8 mm × 300 mm) (Showa Denko Europe GmbH, Germany) and a refractive index detector (RID). The MW calibration curve was constructed using several pullulan standards of MW between 342 Da and 805 000 Da.

Finally, the MPs contained in the extracts were hydrolyzed and derivatized following a modification of the procedure proposed by Ruiz-Garcia et al. (2014) (see Supplementary Material for further details). The monosaccharide derivatives were analyzed by HPLC-DAD-MS in an Agilent 1200 Series HPLC equipped with an Agilent Poroshell 120 EC-C18 column (2.7 μm, 4.6 mm × 150 mm) (Agilent Technologies, Waldbronn, Germany) coupled with a mass spectrometer API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an electrospray ionization source and a triple quadrupole-ion trap mass analyzer and controlled by Analyst 5.1 software. The details of the HPLC-DAD-MS analysis are explained in the Supplementary Material. For the quantification of the monosaccharide derivatives, chromatograms were registered at 250 nm. A calibration curve was constructed using glucose, mannose, xylose and fucose standards, purchased from Sigma-Aldrich (St Louis, MO, USA) and AlfaAesar (Kandel, Germany).

### 2.4. Evaluation of the effect of MP-rich extracts on color and pigment composition

To evaluate the effect of the extracts on wine color and pigment composition, 500 mg/L of each MP-rich extract were added to a Tempranillo red wine (D.O. Ribera de Duero) aged for 6 months in barrel. This resulted in four different samples named S wine, L wine, M wine, T wine (for the wines added with *S. cerevisiae*, *L. thermotolerans*, *M. pulcherrima* and *T. delbrueckii* MP-rich extracts, respectively) and the control wine (same wine but not supplemented). All samples were made in triplicate and kept for 24 h at room temperature and darkness and, then, each sample was divided into two aliquots that were submitted to two different treatments: i) cold treatment and ii) storage at room temperature.

*Experiment 1. Cold treatment.* With the aim of studying the effect of the MP-rich extracts on the colloidal stability of the pigments, wine samples were cold-treated to provoke colloidal instability, following the procedure proposed by Alcalde-Eon et al. (2019). Wine samples were kept at 4 °C in darkness for 4 (sampling point C1) and 11 days (sampling point C2). Before the analysis of wine color and pigment composition, samples were centrifuged for 10 min at 5000 rpm and 5 °C.

*Experiment 2. Room temperature.* To evaluate if the addition of the MP-rich extracts could affect the evolution of the coloring matter that takes place during wine oxidative aging, samples were stored in darkness at room temperature (22 °C) for 7 (sampling point R1) and 18 days (sampling point R2). Then, wine samples were centrifuged for 10 min at 5000 rpm and color and pigment composition were analyzed.

### 2.5. Colorimetric measurements

Before the measurement, samples were filtered with a 0.22 μm pore size filter. Absorption spectra (190–770 nm) were registered in a Hewlett-Packard 8453 UV–Vis spectrophotometer (Agilent Technologies, Waldbronn, Germany) utilizing 2 mm path length quartz cells and synthetic wine as blank (5 g/L tartaric acid, 12% (*v/v*) ethanol, 11.65 g/L NaCl, pH 3.6). CIELAB parameters were calculated from the visible

spectrum (380–770 nm) using the software CromaLab® (Heredia et al., 2004). As references, the CIE standard illuminant D65 and the CIE 1964 standard observer were used. Color differences ($\Delta E^*_{ab}$) between samples were calculated employing the following equation:

$$\Delta E^*_{ab} = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

### 2.6. HPLC-DAD-MS analysis of pigment composition

The analysis of anthocyanins and derivative pigments was carried out by HPLC-DAD-MS using an Agilent 1100 Series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a C18 reversed-phase column (5 μm, 150 mm × 4.6 mm) (Aqua®, Phenomenex, Torrance, CA) thermostatted at 35 °C. HPLC-DAD conditions described in the work by Alcalde-Eon et al. (2006) were used for the analysis. Previously, the samples were diluted 1:1 with HCl 0.1 N and filtered through a 0.45 μm Millex syringe-drive filter unit (Millipore Corp.). Chromatograms were obtained at 520 nm. Mass spectrometry detection was performed in an API 3200 Qtrap (Applied Biosystems) mass spectrometer. The conditions of the mass spectrometer employed in this study are described in detail by Alcalde-Eon et al. (2019). A total of 37 pigments were identified and their concentrations were determined from the chromatographic peaks registered at 520 nm using a malvidin 3-*O*-monoglucoside calibration curve.

### 2.7. Statistical analysis

Statistical analysis was performed using the IBM-SPSS Statistics 26 software. The significance of the differences in protein content and in monosaccharide composition among extracts as well as the significance of the differences in pigment composition and CIELAB color parameters among treated samples was determined by a one-way analysis of variance (ANOVA) followed by post-hoc Tukey-b test. As to the pigment composition of wine samples, the significance of the differences between the control and the added wines were assessed by Student's t-test. In all cases, differences were considered significant when $p < 0.05$. Finally, to observe patterns between wine samples, a Principal Component Analysis (PCA) was performed. In each sample, the mean values ($n = 3$) of the concentrations of each group of pigments and of CIELAB parameters were considered.

## 3. Results and discussion

### 3.1. Characterization of the MP-rich extracts

The protein and glycoprotein profiles of the yeast extracts were characterized by SDS-PAGE (see Fig. S1 in the Supplementary Material). The purification process resulted in a great protein loss (data not shown), although some non-glycosylated proteins remained in the extracts. The glycoprotein bands, situated above the 250 kDa standard, suggest that the glycoproteins released during the sonication of the cells were of high molecular mass.

Total protein quantification by Lowry method showed that the protein content varied significantly among extracts (Table 1). These differences could be explained by differences in the content of non-glycosylated proteins and in the protein fraction of the MPs. The extract from *M. pulcherrima* contained the lowest quantity of soluble proteins (Table 1). Given that there are few non-glycosylated protein bands in the gel (Fig. S1 in the Supplementary Material), the 10% protein present in this extract could be attributed, mostly, to the protein fraction of the MPs obtained. Conversely, the highest protein content was found in the extract from *S. cerevisiae*, whereas the extracts obtained from *L. thermotolerans* and *T. delbrueckii* showed similar protein percentages (Table 1).

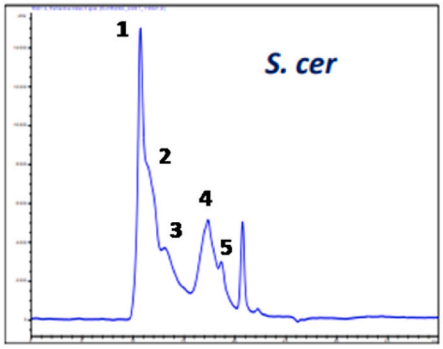
**Table 1**
Protein content (%) determined in the four yeast extracts and average MW (kDa) of the MPs obtained. Different letters indicate significant differences ($p < 0.05$).

| | Protein content (%) | Average MW (kDa) |
|---|---|---|
| *S. cerevisiae* | 49 ± 7 a | 335.62 |
| *L. thermotolerans* | 25 ± 2 b | 88.30 |
| *M. pulcherrima* | 10.0 ± 0.2 c | 206.37 |
| *T. delbrueckii* | 33 ± 5 b | 195.76 |

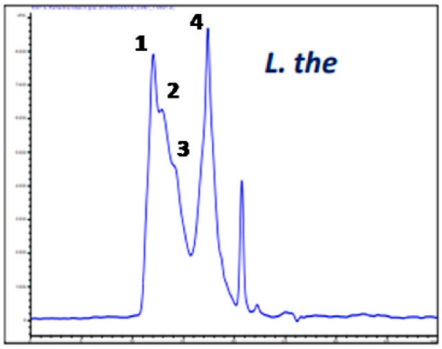
The HRSEC-RID analysis allowed the identification in each extract of several chromatographic peaks corresponding to polysaccharides of different MW. The chromatographic profile of the MPs contained in each extract was different (Fig. 1) and so it was their MW distribution. This suggests important structural differences in the MPs extracted from the different yeasts. Furthermore, the wide range of MW observed for the extracted MPs showed their great polydispersity and agrees with the literature. For example, Doco et al. (2003) showed that the MPs released by yeast autolysis during aging on lees had a MW between 5 and 800 kDa.

The MPs from *L. thermotolerans* were the smallest ones, showing an average MW of 88 kDa (Table 1). Unlike this extract, the *S. cerevisiae* MP-rich extract contained the largest MPs, since the average MW of this extract was 336 kDa. Regarding the MPs from *S. cerevisae* and *T. delbrueckii*, their MW distribution was similar. However, the percentage that each chromatographic peak represents of the total was different: the largest MPs (1988-445 kDa) from *S. cerevisiae* represented an important part (28.56%), whereas in *T. delbrueckii* extract, the 35.47% corresponded to smaller MPs (145-11 kDa). The chromatographic profile of the MPs obtained from *M. pulcherrima* differed from the rest because it was divided into two peaks, one of MW 1254-15 kDa and other of 15–0.40 kDa.

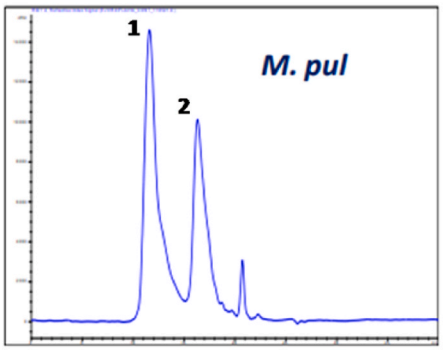
Finally, monosaccharide composition analysis by HPLC-DAD-MS showed important variation between the extracts (Table 2). Mannose and glucose were the main constituents, accounting for more than 94% of the total monosaccharide content. Xylose and fucose were also detected, although in low proportion (<1%). Ribose was also detected, but only in the extract from *L. thermotolerans*. The predominance of mannose and glucose was expected, given that mannoproteins and β-glucan are the major components of the yeast cell wall (Klis et al., 2002). However, the percentages of mannose and glucose varied significantly among extracts. The higher glucose content present in the extracts from *S. cerevisiae* and *T. delbrueckii* could suggest that the fragmentation of the cell wall of these two yeasts by ultrasounds resulted in the solubilization of MPs that are connected to a portion of the cell wall β-glucan. This hypothesis is supported by the high MW of the polysaccharides contained in these two extracts (especially in that from *S. cerevisiae*, as aforementioned), since these high MW molecules could be attributed to MPs linked to a fragment of β-glucan. On the contrary, the extracts obtained from *L. thermotolerans* and *M. pulcherrima* showed low glucose contents. The fact that the same extraction method applied to different yeasts led to these important compositional differences could be explained by the different relative abundance of the cell wall elements in the yeast used in this study. In fact, some authors have shown that yeast cell wall composition can vary at genre, species and strain level (Nguyen et al., 1998). The differences in the monosaccharide profiles of the extracts could also be due to differences in the strength of the linkages between the cell wall elements. In this sense, the application of ultrasounds did not seem capable to break the bond between the MPs and β-glucan of *S. cerevisiae* and *T. delbrueckii* cell wall. Therefore, the application of ultrasounds seems to be useful for obtaining extracts with high mannose content from the used strains of *L. thermotolerans* and *M. pulcherrima*. However, when this method was applied to *T. delbrueckii* and *S. cerevisiae*, resulted in extracts with a relevant content of glucose (especially in the case of *S. cerevisiae*) possibly due to the liberation of MPs connected to a fragment of cell wall β-glucan.



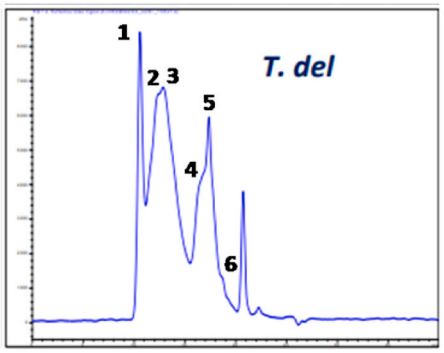

| *S. cerevisiae* | | |
|---|---|---|
| Peaks | M (kDa) | % |
| 1 | 1988-445 | 28.56 |
| 2 | 445-125 | 23.30 |
| 3 | 125-10 | 19.01 |
| 4 | 10-1 | 23.90 |
| 5 | 1-0.5 | 5.23 |




| *L. thermotolerans* | | |
|---|---|---|
| Peaks | Mw (kDa) | % |
| 1 | 825-139 | 24.43 |
| 2 | 139-45 | 20.84 |
| 3 | 45-9 | 16.60 |
| 4 | 9-0.5 | 38.14 |




| *M. pulcherrima* | | |
|---|---|---|
| Peaks | Mw (kDa) | % |
| 1 | 1254-15 | 60.55 |
| 2 | 15-0.4 | 39.45 |




| *T. delbrueckii* | | |
|---|---|---|
| Peaks | Mw (kDa) | % |
| 1 | 1739-505 | 14.73 |
| 2 | 505-145 | 19.27 |
| 3 | 145-11 | 35.47 |
| 4 | 11-3 | 13.27 |
| 5 | 3-0.8 | 15.38 |
| 6 | 0.8-0.5 | 1.88 |

**Fig. 1.** Chromatographic profiles of the MPs contained in the yeast extracts and percentage (%) that each chromatographic peak represents.

### *3.2. Evaluation of the effect of MP-rich extracts on color and pigment composition*

Cold stabilization is a method used to improve the tartaric and colloidal stability of wines, avoiding the formation of haze and sediments in the bottle. However, cold treatments can provoke the precipitation of coloring matter (Ribéreu-Gayon et al., 2006a). MPs, as well as other polysaccharides, are considered colloidal stabilizers (Ribéreu-Gayon et al., 2006b) whose addition could enhance pigment colloidal stability, but, as aforementioned, there is controversy surrounding the role of MPs in wine color stability.

To evaluate the effect of the MP-rich extracts on color and pigment stability, wine samples were kept at 4 °C during 4 (C1) and 11 days (C2). At both sampling points, wine color and pigment composition were analyzed. 37 pigments were identified (Table S2 in the Supplementary Material) and grouped in: anthocyanin monoglucosides (5 compounds), acetyl derivatives (5 compounds), caffeoyl and *p*-coumaroyl derivatives (7 compounds), flavanol-anthocyanin direct condensation products (F-$A^+$, 6 compounds), flavanol-anthocyanin acetaldehyde condensation products (F-et-$A^+$, 5 compounds), A-type and B-type vitisins (Vit A+Vit

**Table 2**
Monosaccharide composition (%) of the yeast extracts. Different letters within each row indicate significant differences ($p < 0.05$). Man: mannose; Rib: ribose; Glc: glucose; Xyl: xylose; Fuc: fucose.

| % | *S. cerevisiae* | *L. thermotolerans* | *M. pulcherrima* | *T. delbrueckii* |
|---|---|---|---|---|
| Man | 36 ± 1 d | 85.4 ± 0.6 b | 92.9 ± 0.4 a | 62.3 ± 0.2 c |
| Rib | – | 3.9 ± 0.1 | – | – |
| Glc | 63 ± 1 a | 8.7 ± 0.3 c | 5.4 ± 0.3 d | 36.2 ± 0.2 b |
| Xyl | 0.37 ± 0.04 c | 0.9 ± 0.1 a | 0.98 ± 0.04 a | 0.64 ± 0.04 b |
| Fuc | 0.77 ± 0.03 bc | 1.00 ± 0.06 a | 0.73 ± 0.08 c | 0.88 ± 0.02 b |

B, 4 compounds) and vinylphenol-type vitisins (Vit VF, 5 compounds). Total pigment concentration was calculated as the sum of the concentrations of the above-mentioned compounds.

After a short cold treatment (C1), total pigment content was significantly higher in L, M and T wines (Fig. 2A) compared to the control, suggesting that the addition of *L. thermotolerans*, *M. pulcherrima* and *T. delbrueckii* extracts could increase the colloidal stability of the coloring matter.

Considering the groups of pigments separately, some differences can be observed. The main effect of the extracts was produced in Vit A+Vit B, being the differences with the control significant in all cases (Fig. 2A). This agrees with Alcalde-Eon et al. (2014 & 2019), who reported a stabilizing effect of a commercial MP on Vit A+Vit B after a cold treatment. However, the stabilization effect of *T. delbrueckii* extract was significantly higher than the produced by *S. cerevisiae* extract. On the contrary, no significant differences were observed in the effect on Vit A+Vit B of these two extracts and those obtained from *L. thermotolerans* and *M. pulcherrima*. The MP-rich extracts seemed also able to stabilize Vit VF, but their effect was less important than that observed for Vit A+Vit B (Fig. 2A). The extract obtained from *T. delbrueckii* seemed, again, the most effective. The fact that the effect of the extracts was higher for Vit A+Vit B than for Vit VF could imply that the lack of the hydroxyphenyl group bound to the pyran ring in Vit A+Vit B could favor the interaction of these pigments with the MPs and, thus, their higher stability in solution. However, further studies about the interaction between MPs and vitisins at a molecular level will be necessary to understand the observed effects.

A tendency towards a higher stability of F-A$^+$ and F-et-A$^+$ in the treated samples was also observed, being greater in the latter group (Fig. 2A). The higher stabilization of F-et-A$^+$ when compared to F-A$^+$ could result from the presence of the ethyl bridge that could favor a molecular conformation that facilitates the interaction with the MPs. Concerning F-et-A$^+$, the stabilizing effect of *T. delbrueckii* extract was also significantly higher than that of *S. cerevisiae* extract.

The concentrations of acylated and non-acylated anthocyanin monoglucosides were also higher in the treated wines at the first sampling point (C1, Fig. 2A). Thus, the addition of the MP-rich extracts seemed to increase the colloidal stability of anthocyanins. This is in accordance with the work of Alcalde-Eon et al. (2019) that showed a protective effect of a commercial MP against the cold-induced precipitation of anthocyanins. Although all the extracts assayed had a positive effect, it is important to note some differences. The addition of *T. delbrueckii* extract had an important effect on the stability against cold of *p*-coumaroylated and caffeoylated anthocyanins, since the concentration of these compounds in T wine was 33.40% higher than in the control. In addition, this extract was significantly more effective than those obtained from the other yeasts. Gonçalves et al. (2018) have demonstrated the interaction of MPs with acylated and non-acylated anthocyanins and found that this interaction was stronger with coumaroylated derivatives possibly due to their higher hydrophobicity. Therefore, the high retention of *p*-coumaroylated and caffeoylated anthocyanins by *T. delbrueckii* extract could be due to a higher propensity of this extract to form hydrophobic interactions with wine pigments. On the contrary, *S. cerevisiae* extract was the least effective, mainly for the colloidal stabilization of

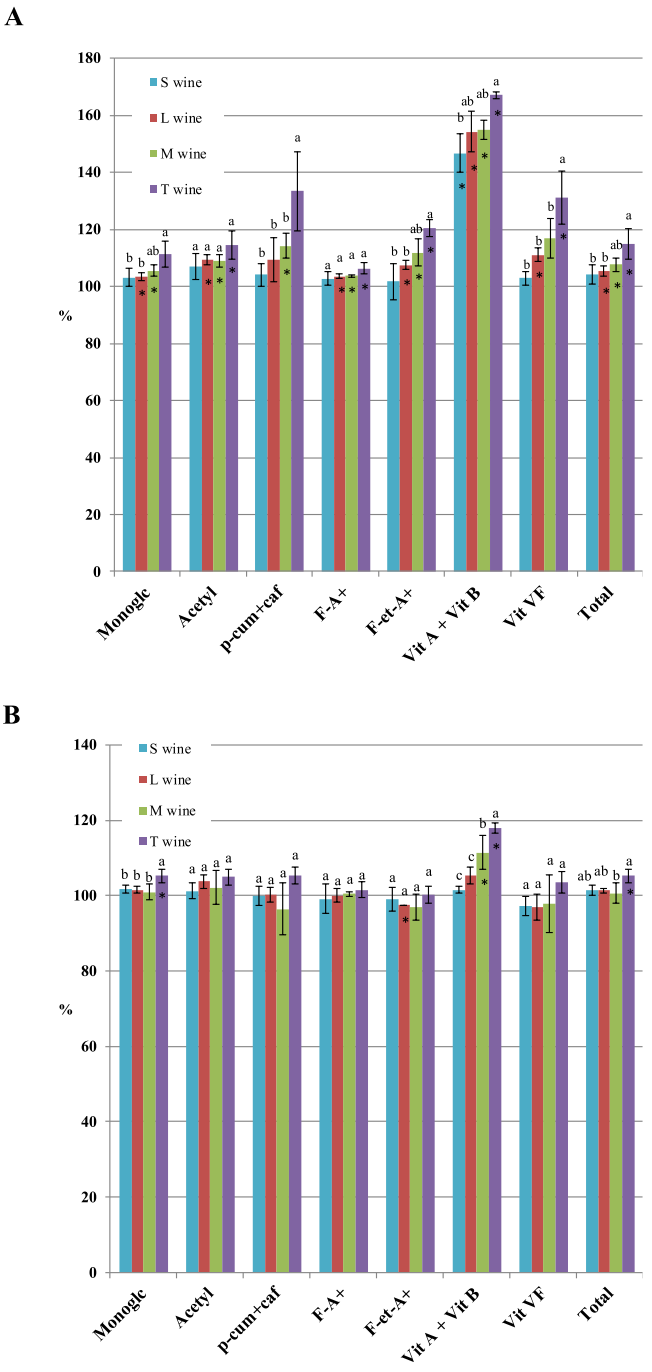



**Fig. 2.** Percentage (%) of each pigment group with respect to the control at C1 (A) and C2 (B) sampling points. Monoglc: anthocyanin monoglucosides; Acetyl: acetylated anthocyanins; p-coum+caf: p-coumaroylated and caffeoylated anthocyanins; F-A$^+$: flavanol-anthocyanin direct condensation products; F-et-A$^+$: flavanol-anthocyanin acetaldehyde-mediated condensation products; Vit A+Vit B: A-type and B-type vitisins; Vit VF: vinylphenol-type vitisins. Different letters indicate significant differences ($p < 0.05$) among added samples. Significant differences ($p < 0.05$) between the control and the corresponding added samples are indicated with an asterisk.

grape native pigments. The extracts from *L. thermotolerans* and *M. pulcherrima* seemed to have a greater protective effect of anthocyanins than the obtained from *S. cerevisiae*. However, the effect of *L. thermotolerans* and *M. pulcherrima* extracts was less important than the observed for *T. delbrueckii* extract.

Thus, the observed differences in the effect of the extracts seemed to

be dependent on the yeast of origin and could be related to differences in the composition and structure of the MPs. *S. cerevisiae* extract contained the highest MW MPs and was the least effective for the protection of the pigments. This suggests that high MW MPs could be less effective as colloidal stabilizers. Furthermore, the higher presence of β-glucan in the *S. cerevisiae* extract did not seem to help stabilize wine pigments. On the other hand, the lowest MW MPs were found in *L. thermotolerans* extract, which seemed to be more effective than the extract from *S. cerevisiae*, but less than *M. pulcherrima* and *T. delbrueckii* extracts. The MPs in the extracts from *M. pulcherrima* and *T. delbrueckii* had an average MW of ~200 kDa. Thus, these MP-rich extracts could be more effective for the colloidal stabilization of the coloring matter. Poncet-Legrand et al. (2007) studied the effect of the MW of MPs on the colloidal stabilization of tannins and found that smaller MPs of ~62 kDa and ~51 kDa were more effective at inhibiting the aggregation of tannin colloids than high MW MPs (~337 kDa). This is not found in the present study, where smaller MPs (like those from *L. thermotolerans*) are less effective for the colloidal stabilization of the pigments than larger ones (*i. e.*, those from *M. pulcherrima* and *T. delbrueckii*). However, there were differences in the protective effects of *M. pulcherrima* and *T. delbrueckii* extracts, being the latter one the most efficient. Consequently, there must be other factors besides the MW of the MPs that also affect the stabilization. In this sense, the extract from *T. delbrueckii* presented higher glucose content (36.19%) than the extract from *M. pulcherrima* (5.38%). Therefore, the moderate presence of β-glucan in *T. delbrueckii* extract, unlike the high content (62.58%) present in the extract from *S. cerevisiae*, could constitute a contributing factor for the stabilization of wine pigments.

Conversely, given that the protein content of *T. delbrueckii* (32.57%) and *L. thermotolerans* (25.01%) extracts was similar, but their effects on pigment composition were significantly different, the protein content of the extracts did not seem an important factor to explain their different stabilizing effects of wine pigments.

The prolongation of cold treatment (C2) seemed to diminish the positive effect of the MP-rich extracts on pigment stability, since the differences in total pigment content with the control were only significant in T wine (Fig. 2B). This suggests that the extracts assayed lost efficacy when the magnitude of the colloidal instability increased. Therefore, the extracts seemed more effective against a moderate colloidal destabilization (*i.e.* a short treatment with cold, like that used by wineries for the tartaric stabilization of wine) but their effect was limited when the degree of instability increased (*i.e.* when cold treatment was prolonged). Nevertheless, the effect of *M. pulcherrima* and *T. delbrueckii* extracts in the stability of Vit A+Vit B was still significant as well as the effect of *T. delbrueckii* extract in the stability of anthocyanin monoglucosides (Fig. 2B). Contrarily to what happened in C1, in C2 there was a slight tendency towards a less content of F-et-A+ in added wines (Fig. 2B). Despite the decrease in the effect of the MP-rich extracts under conditions of high colloidal instability, the addition of *T. delbrueckii* extract still resulted in a greater stability of all pigment families.

The results of the colorimetric analysis showed some significant differences in the CIELAB parameters of the samples (Table 3). The decrease in chroma ($C^*_{ab}$) of the added wines in C1 could be related to a slight increase in the proportion of Vit A+Vit B, Vit VF and F-et-A$^+$ (see Table S1 in the Supplementary Material), whose color is orange and blue, respectively, which implies a loss in color purity. In addition, the lower hue ($h^*_{ab}$) of T wine could be explained by the important stabilization observed for *p*-coumaroylated and caffeoylated anthocyanins (which are bluish). Conversely, the higher hue ($h^*_{ab}$) of S wine may be due to the fact that the stabilization produced by this extract was only important for Vit A+Vit B (orange-colored pigments) resulting in a slight increase of the proportion of this group of pigments (see Table S1 in the Supplementary Material). After the long cold treatment (C2), the differences in CIELAB parameters were smaller. This correlates well with

**Table 3**
CIELAB parameters of wine samples determined in C1 and C2 sampling points. Different letters within each column indicate significant differences ($p < 0.05$).

| | C1 | | | C2 | | |
|---|---|---|---|---|---|---|
| | $L^*$ | $C^*_{ab}$ | $h_{ab}$ | $L^*$ | $C^*_{ab}$ | $h_{ab}$ |
| Control | 55.0 ± 0.1 b | 47.7 ± 0.1 a | 1.1 ± 0.2 ab | 54.4 ± 0.1 a | 48.3 ± 0.2 a | 2.2 ± 0.1 a |
| S wine | 55.4 ± 0.2 ab | 47.2 ± 0.2 ab | 1.30 ± 0.03 a | 55.2 ± 0.4 a | 48.1 ± 0.3 a | 1.6 ± 0.3 b |
| L wine | 56.0 ± 0.4 a | 47.0 ± 0.4 b | 1.1 ± 0.2 ab | 54.9 ± 0.3 a | 48.2 ± 0.2 a | 1.7 ± 0.1 ab |
| M wine | 55.5 ± 0.5 ab | 47.2 ± 0.3 ab | 1.0 ± 0.1 ab | 55.0 ± 0.5 a | 47.9 ± 0.1 a | 1.7 ± 0.4 ab |
| T wine | 55.4 ± 0.3 ab | 47.1 ± 0.3 ab | 0.9 ± 0.1 b | 55.0 ± 0.6 a | 47.9 ± 0.4 a | 1.4 ± 0.2 b |

the loss in the efficacy of the MP-rich extracts at C2. In spite of the fact that the addition of the extracts seemed to contribute to the stability of the pigments against cold, the color of the wine was not substantially modified, since color differences with the control were not perceptible by human eye ($\Delta E^*_{ab} < 3$) at the sampling points studied. The fact that the main effect of the extracts was produced in Vit A+Vit B, which are pigments found in a low proportion in a young wine like the used in the present study, could be the reason why the increase in the stability of these compounds did not impact substantially the color of the wine.

To evaluate whether the addition of the MP-rich extracts could affect the evolution of the coloring matter during wine oxidative aging, wine samples were stored at room temperature for 7 (R1) and 18 days (R2). At R1 and R2 sampling points, wine color and pigment composition were analyzed as in previous section. The addition of the extracts seemed to lead to slight lower total pigment content at R1 and R2 (Fig. 3). Indeed, a general decrease in the contents of grape native pigments in added samples can be observed in both sampling points. Although at R1 there were no significant differences between the effect of the extracts on the stability of these pigments (Fig. 3A), at R2, the content of anthocyanin monoglucosides was the lowest in T wines (Fig. 3B). In T wine, slightly higher contents of acylated anthocyanins were observed, that were significantly different in the case of *p*-coumaroylated and caffeoylated anthocyanins when compared to the control at R2 (Fig. 3B). In relation to derivative pigments, the addition of the extracts led to a lower content of F-A$^+$ at R1 and R2. T wine showed the lowest content of this family of pigments at both sampling points. Conversely, the addition of the extracts led to a higher content of F-et-A$^+$. This is in accordance with the work of Alcalde-Eon et al. (2019), where the addition of a commercial MP led to an increase in the content of F-et-A$^+$ after 6 days of storage at 19 °C. In R1, the content of this type of pigments was significantly higher in M and T wines when compared to the control (Fig. 3A). In R2, the effect of *M. pulcherrima* extract seemed to decrease, since the differences with the control wine were no longer significant (Fig. 3B). In relation to *L. thermotolerans* extract, its addition led to a significantly higher content of F-et-A$^+$ in this last sampling point. Finally, there was a tendency towards a higher concentration of Vit A+Vit B after a short period of storage (R1), especially in M and T wines, which appeared to diminish over time (R2). However, at R2 sampling point, the higher content of these pigments was still significant in T wine. Contrarily, the effect of the addition of this extract on the content of Vit VF seemed to increase over time, being the differences in the content of these pigments only significant at R2 for T wine.

Thus, the addition of the extracts did not lead to a higher content of grape native pigments and this decrease in anthocyanin content seemed to be associated to an increase in the content of Vit A+Vit B, Vit VF and F-et-A$^+$, which require oxidative conditions for their formation. Thus, the addition of the yeast extracts could favor the transformation of anthocyanins to derivative pigments through a modification of wine redox state. In fact, Yue et al. (2021) found that the post-fermentative addition of MPs reduced wine antioxidant capacity. Other authors have also

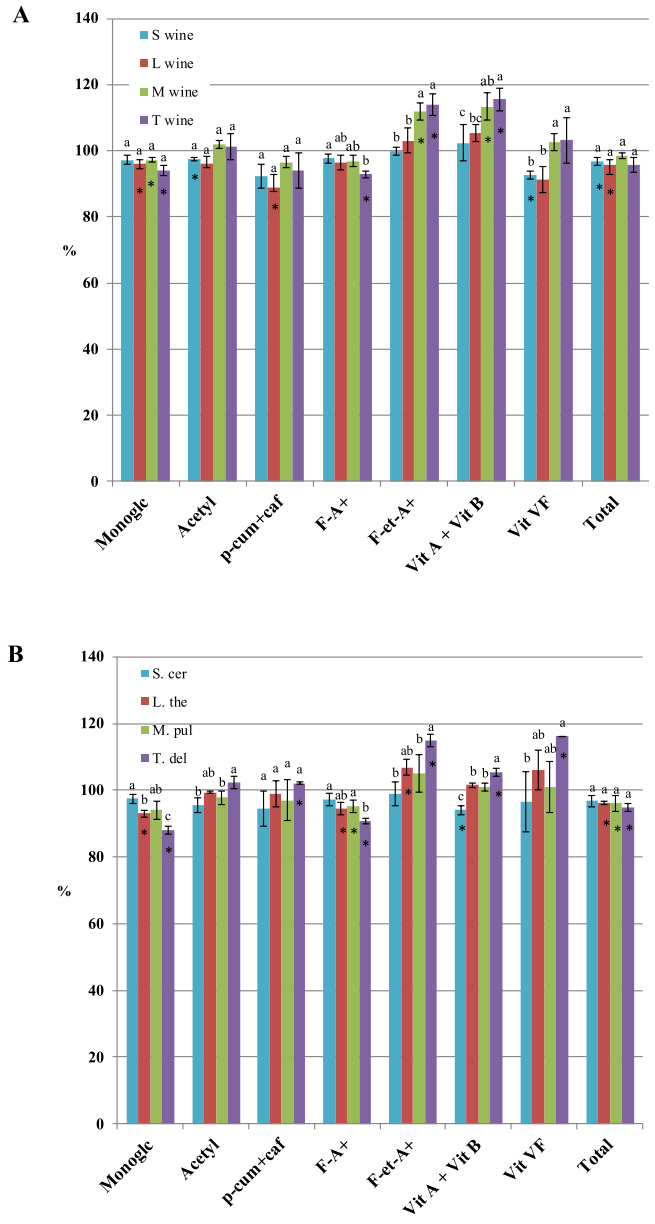



**Fig. 3.** Percentage (%) of each pigment group with respect to the control at R1 (A) and R2 (B) sampling points. Monoglc: anthocyanin monoglucosides; Acetyl: acetylated anthocyanins; p-coum+caf: p-coumaroylated and caffeoylated anthocyanins; F-A$^+$: flavanol-anthocyanin direct condensation products; F-et-A$^+$: flavanol-anthocyanin acetaldehyde-mediated condensation products; Vit A+Vit B: A-type and B-type vitisins; Vit VF: vinylphenol-type vitisins. Different letters indicate significant differences ($p < 0.05$) among added samples. Significant differences ($p < 0.05$) between the control and the added samples are indicated with an asterisk.

reported an increase in the formation of derivative pigments during wine aging after the addition of MPs and commercial yeast derivatives (del Barrio-Galán et al., 2012). In this sense, Rinaldi et al. (2019) proposed that MPs could accelerate pigmented polymers formation by favoring multiple interactions between anthocyanins and flavanols. Therefore, the extracts seem to accelerate wine aging, which could occur by a modification of wine redox state and/or by favoring the interaction of anthocyanins and other wine compounds, which could facilitate their reactivity and the formation of new pigments.

Finally, it is important to note some differences among the extracts assayed. The extracts obtained from *L. thermotolerans*, *M. pulcherrima* and *T. delbrueckii* (especially the latter one) seemed to favor the evolution of wine coloring matter towards a more aged profile. For this reason, the addition of these extracts, particularly, that obtained from *T. delbrueckii* could favor the chemical stability of wine color, since derivative pigments such as pyranoanthocyanins are more resistant to changes in pH, oxidation and $SO_2$ bleaching (He et al., 2012). However, after the addition of the extract from *S. cerevisiae*, it can be observed a slight decrease in the content of all pigment families at R1 and R2 sampling points (Fig. 3). Therefore, given the little ability of this extract for maintaining the pigments in solution observed in the cold treatment, it seems that this extract could have a negative effect in the chemical stability of the pigments after a time of storage.

The changes in pigment composition in treated wines did not lead to a significant modification of CIELAB color parameters (Table 4), being the color differences with the control wine not perceptible by human eye ($\Delta E^*_{ab} < 3$). This indicates that, at the sampling points studied, the increase in the content of derivative pigments does not translate into a more aged color, probably due to the low proportion of this type of pigments in the wine used in the present study.

### 3.3. Principal Component Analysis (PCA)

Fig. 4 shows the results of the PCA performed to observe differences between wine samples in relation to pigment composition and CIELAB color parameters. The two treatments conducted (cold treatment and storage at room temperature) were evaluated together. PC1 and PC2 explained 92.2% of data variability. Regarding cold treatment, it can be observed a clear differentiation between wine samples at C1 along PC2. This indicates that the addition of the MP-rich extracts generated important differences among the samples after a short cold treatment. The distance between wine samples decreased at C2, meaning that the differences between the extracts decreased at this sampling point, which correlates well with the decrease in the effect of the extracts after the prolonged application of cold. At C1, T wine was the sample that showed the greatest separation from the control, which could be explained by the highest effect as colloidal stabilizer of the extract from *T. delbrueckii*. S wine was the sample located more closely to the control, which agrees with the lesser changes in pigment composition and color produced after the addition of *S. cerevisiae* extract. L and M wines were close and located in an intermediate position between the other added samples. This indicates that the addition of *L. thermotolerans* and *M. pulcherrima* extracts had similar effects and were more effective than the extract from *S. cerevisiae* but less than the extract from *T. delbrueckii*. At C2, T wine also showed the greatest separation from the control, whereas S, L and M wines were located in a similar position. This correlates with the notable effect of *T. delbrueckii* extract under conditions of high colloidal instability. In relation to room temperature treatment, the main separation of the samples was produced along PC1. Thus, data variability was essentially explained by the time of storage, meaning that the addition of the extracts had little effect in wine color and pigment composition when

**Table 4**
CIELAB parameters of wine samples determined in R1 and R2 sampling points. Different letters within each column indicate significant differences ($p < 0.05$).

| | R1 | | | R2 | | |
|---|---|---|---|---|---|---|
| | $L^*$ | $C^*_{ab}$ | $h_{ab}$ | $L^*$ | $C^*_{ab}$ | $h_{ab}$ |
| Control | 53.4 ± 0.1 a | 48.3 ± 0.1 a | 1.70 ± 0.04 a | 51.1 ± 0.02 a | 48.0 ± 0.2 a | 5.2 ± 0.3 a |
| S wine | 53.5 ± 0.9 a | 47.8 ± 0.2 a | 2.3 ± 0.5 a | 51.7 ± 0.3 a | 47.7 ± 0.1 a | 5.0 ± 0.3 a |
| L wine | 53.7 ± 0.3 a | 47.9 ± 0.3 a | 1.9 ± 0.1 a | 51.6 ± 0.4 a | 48.0 ± 0.2 a | 4.9 ± 0.2 a |
| M wine | 53.3 ± 0.4 a | 48.2 ± 0.1 a | 1.9 ± 0.2 a | 51.4 ± 0.3 a | 48.0 ± 0.1 a | 4.8 ± 0.1 a |
| T wine | 53.9 ± 0.1 a | 48.2 ± 0.5 a | 1.9 ± 0.2 a | 51.7 ± 0.2 a | 47.90 ± 0.02 a | 4.9 ± 0.2 a |

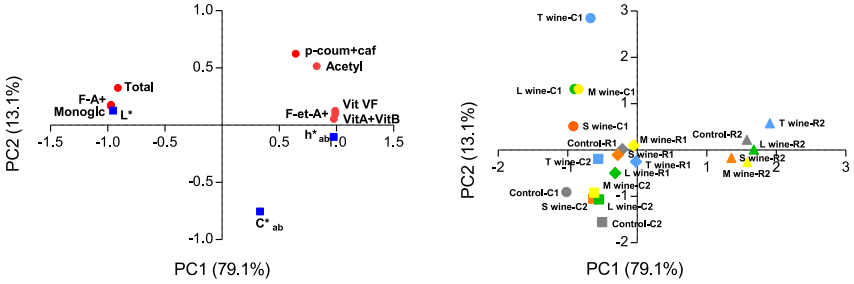



**Fig. 4.** Loadings (left) and scores (right) obtained in the PCA performed using pigment and color variables of all the samples analyzed in this study.

compared to the effect of time itself.

## 4. Conclusions

The addition of the yeast extracts seemed to have an effect in pigment composition. In the cold treatment, it was observed that the addition of the extracts contributed to the colloidal stability of wine pigments. In addition to stabilizing wine coloring matter from a colloidal point of view, the addition of the extracts seemed to favor the transformation of anthocyanins to derivative pigments and, therefore, it could also lead to a greater chemical stability of color.

Finally, the differences in the effect of the four extracts were evident. This could be related to differences in their composition and/or in the structure of the MPs, which, in turn, seemed to depend on the yeast of origin. The extract obtained from *T. delbrueckii* had the greatest impact on wine pigment composition in both treatments. Thus, the fact that the extracts obtained from other yeast different than *S. cerevisiae* and, primarily those from *T. delbrueckii*, had a greater effect on wine coloring matter could open the possibility to the use of non-*Saccharomyces* yeasts as a new source for the development of MP-based oenological products indicated for the modulation of wine color.

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## CRediT authorship contribution statement

**María Oyón-Ardoiz:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Elvira Manjón:** Conceptualization, Methodology, Formal analysis, Validation, Supervision, Writing – review & editing, Funding acquisition. **María Teresa Escribano-Bailón:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Project administration, Funding acquisition. **Ignacio García-Estévez:** Conceptualization, Methodology, Formal analysis, Validation, Supervision, Writing – review & editing, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare no competing interests.

## Data availability

The data that has been used is confidential.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.114219.

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