https://doi.org/10.17113/ftb.60.04.22.7777 original scientific paper

Yeast Multi-Enzymatic Systems for Improving Colour Extraction, Technological Parameters and Antioxidant Activity of Wine

Running title: Yeast Multi-Enzyme Systems to Improve Wine Quality

Sara Jaquelina Longhi[1,2], María Carolina Martín[1,2]*, María Gabriela Merín[1,2] and Vilma Inés Morata de Ambrosini[1,2]*

[1]National University of Cuyo/Faculty of Applied Sciences to Industry, Bernardo de Irigoyen 375, 5600 San Rafael, Mendoza, Argentina
[2]National Scientific and Technical Research Council (CONICET), Ciudad Autónoma de Buenos Aires, Argentina

Received: 29 April 2022
Accepted: 14 July 2022

SUMMARY

Research background. Wine yeasts are a heterogeneous microbial group with high enzymatic potential that makes them a useful tool in winemaking. With a better understanding of their oenological properties, selection procedures can be optimised to obtain more efficient strains. The present study aimed to isolate and select yeasts from wine grape surface by studying their production of enzymes that hydrolyse plant cell wall polymers and by linking them to different technological parameters and antioxidant activity of wines.

Experimental approach. Yeasts that were able to produce carbohydrolases and related enzymes of oenological importance were firstly selected on plates and subsequently identified. Then, a secondary selection of yeasts was carried out according to technological effects of their extracellular enzyme extracts on short macerations. In this way, the colour extraction, total polyphenol content, clarification, filterability and antioxidant activity were studied. This approach makes it possible to relate

*Corresponding authors:
Phone: +542604421947
Fax: +542604430673
E-mail: mcmartin@fcai.uncu.edu.ar (M.C. Martín); vmorata@fcai.uncu.edu.ar (V.I. Morata de Ambrosini)

the microorganism capacity to produce cell wall depolymerizing-enzymes with the technological effects produced.

Results and conclusions. From 366 isolates, 26.2 % (96 strains) showed at least one of the polysaccharidase activities and 57.3 % (55 strains) of them exhibited a multi-enzyme blend active against plant cell wall polymers. Sixteen strains were selected and identified as *Aureobasidium*, *Candida, Debaryomyces*, *Hanseniaspora, Metschnikowia, Pichia, Saccharomyces* and *Torulaspora*. Pectinolytic enzymes displayed the highest hydrolytic activity levels. *Aureobasidium pullulans* showed a more comprehensive enzyme blend and higher activity levels, dominated by pectinases, and followed by xylanases and cellulases. Moreover, the *Torulaspora delbrueckii* m7-2 strain exhibited high polysaccharidase production levels and this was strain-dependent. Strains that produced the most comprehensive enzyme extracts and the highest levels of activity also exhibited the best chromatic and technological properties. Cluster analysis confirmed that *A. pullulans* R-22, m11-2, m86-1 and m86-2 and *T. delbrueckii* m7-2 could be grouped for a better effect on filterability, clarification and extraction of bioactive compounds, encouraging future studies regarding their application in winemaking.

Novelty and scientific contribution. The study of yeast multi-enzymatic systems impacting in the grape maceration process results in a proper selection criterion for wine yeasts to improve colour extraction, technological parameters and antioxidant activity of Malbec wine. This work shows that *A. pullulans* and *T. delbruekii* have high enzymatic potential for oenological purposes.

Keywords: antioxidant activity; colour extraction; technological parameters; plant cell wall depolymerizing-enzymes; winemaking; yeast

INTRODUCTION

Winemaking is a dynamic microbiological process with a succession of yeast genera, species and strains. *Saccharomyces cerevisiae* is the species that is responsible to carry out the alcoholic fermentation. Other yeast types present in the early stages of winemaking are so-called non-*Saccharomyces* species, which provide special characteristics to the final product because of their ability to produce extracellular metabolites and enzymes such as pectinases, cellulases, xylanases, glycosidases, proteases or esterases (*1–4*). Some unconventional species in winemaking worth mentioning are presented as epiphytic grape microbiota, which provide novel enzyme activities of oenological interest that will help the wine industry face technical and consumer challenges (*5*).

The colour of red wine is an easily perceived quality attribute which is affected by various viticultural and wine-producing factors. Quality wines usually contain high levels of non-volatile phenolic compounds, which are important contributors to flavour, colour, mouth-feel (astringency and bitterness) and health promoting properties (e.g. antioxidant and anti-inflammatory metabolites) of wine (*6*). The major phenolic compounds found in grapes and wines are hydroxycinnamic acids, stilbenes, anthocyanins and tannins (*7*). Currently there is great interest in the application of technological strategies that increase the phenolic composition and hence antioxidant activity of musts and wines. Maceration process is crucial for the extraction of these compounds. In particular, cold pre-fermentation macerations carried out between 5 and 15 °C improve the colour and stability of red wine, and produce an increase in the production and retention of aromatic compounds with the consequent enhancement of the flavour of the wines (*8*).

However, low temperatures reduce the extraction of phenolic compounds due to a decrease in molecular mobility. This reduced extraction can be compensated and/or enhanced with the use of *cold-active* enzyme extracts, as previously demonstrated (*9–11*). These enzyme extracts consist of a pool of depolymerizing enzymes, mainly pectinases, which play an essential role in the degradation of cell wall polymers, alongside secondary enzyme activities that specifically act on other components of wine must (*12,13*).

Pectinolytic enzymes are a heterogeneous group of enzymes that degrade pectins present in the middle lamella and primary cell walls of plants, affecting both the sensory and technological properties of wines (*10,14*). Pectinases can help improve clarification and filtration by releasing more colour and flavour compounds contained in the grape skin, and making the release of phenolic compounds more effective (*1*). Cellulases and hemicellulases degrade polysaccharides from the cell walls of the skin and the pulp of the grape, a process that contributes to the extraction of juice, improves clarification of the wine and increases the fruity aroma of the wines through the release of aromatic precursors (*15*). Furthermore, glycosidases and proteases are some of the enzymes of oenological interest produced by epiphytic yeasts. The former ones improve the aromatic fraction of wines by releasing aromatic terpenes present in the must in the form of glycosylated precursors. Brandolini *et al*. (*16*) and Gaensly *et al*. (*17*) also showed that yeasts with β-glucosidase activity were able to increase the concentration of free resveratrol in wine and increase its antioxidant capacity. Proteases, for their part, improve the nutritional content of musts, contribute to the production of aromatic precursors and reduce protein levels, thus facilitating protein stability of the wine (*1*). Contribution of enzymes that are provided by natural yeasts on the grape carposphere and adapted to their ecological environment would release essential pigments and compounds. This process would

not produce any changes other than those related to the natural course of the process, and would thus increase the typicality of the wines, a trend increasingly valued by consumers.

Regarding the addition of enzymes to red winemaking and based on the results of previous studies (*3,4,9*), the activities of different polysaccharidases and those of related enzymes that constitute enzyme preparations perform different hydrolytic actions on the cell walls and produce complementary effects to efficiently breakdown cell walls, resulting in an enrichment of the composition of wine must. Recently, scientific advances have been reported regarding the chemical and structural composition of the grape berry cell wall, and the hydrolytic action on its polysaccharide components by commercial enzymes. The results revealed different cell wall compositions in the different cell strata and the need for unique enzyme activities to achieve its complete degradation (*12,18*). These findings indicate the need to search for complementary enzyme activities to effectively and completely degrade grape berry cell walls.

In previous own studies, Merín *et al.* reported the occurrence of pectinolytic yeasts isolated from grape berries, grape must fermentation and winery equipment (*3,4,19,20*), however these studies mainly focused on cold-active pectinolytic activity and not based on the pool of carbohydrolases and related enzymes of oenological importance. In the present study, the objective was to select microorganisms from the wine grape surface that produce multi-enzyme systems based on the physicochemical and technological performance during short macerations of Malbec must. Therefore, a selection was carried out with broader criteria than those used in previous studies. The pool of hydrolytic activities degrading plant cell wall polymers was assayed at two maceration temperatures, and compared with different technological effects, colour extraction as well as the antioxidant activity of the resulting macerations.

MATERIALS AND METHODS

Study area, sampling and microorganism isolation

Grape samples were taken from the San Rafael wine region, Mendoza, Argentina. Isolation was carried out from the surface of Bonarda, Cabernet Sauvignon and Malbec cvv. (*Vitis vinífera* L.) wine grape berries. Twenty (20) grape berries were taken, placed in a container with 20 mL of sterile 0.1 % peptone water and stirred for 1 h at 165 rpm. Samples were sown by surface depletion on solid WL (Wallerstein Laboratory) medium and nutritive agar and MEA (malt extract agar). The incubation time was 5 and 7 d at 28 and 15 °C, respectively. Culture media were purchased from Sigma-Aldrich Co. Ltd., Merck KGaA (Darmstadt, Germany).

Primary selection

Plate screening for selection of microorganisms producing grape berry cell wall-depolymerizing enzymes

For detection of pectinase activity, isolates were inoculated in mineral medium with citric pectin as the sole carbon source according to the method by Moyo *et al*. (*21*) with modifications and with the following composition (g/L): citric pectin, 2.0; yeast extract, 1.0; agar, 15.0; KH_2PO_4, 0.2; $CaCl_2$, 0.05; $(NH_4)_2SO_4$, 1.0; $MgSO_4.7H_2O$, 0.8; $MnSO_4$, 0.05; pH=4.5. To demonstrate xylanase, cellulase and amylase activity, isolates were plated onto selective medium containing 6.7 g/L Yeast Nitrogen Base (YNB) and 20 g/L agar, plus 0.2 % birchwood xylan for xylanase, 0.5 % carboxymethylcellulose for cellulase or 2 % starch for amylase activity. All reagents and YNB medium were from Sigma-Aldrich Co. Ltd., Merck KGaA (Darmstadt, Germany). Enzyme production was detected using the qualitative method that consists of the development of hydrolysis halos. Pectinase, xylanase and amylase were detected with a lugol solution (*22*) and cellulase activity with a 0.2 % (*m*/*V*) Congo Red solution. Activity was detected when a clarification halo was formed around the colonies against a brown-purple background of the medium with non-degraded polymers after all polysaccharidases had been developed, except for amylase, which had a blue background (*23*).

Plate screening for other hydrolytic activities of oenological importance

Extracellular protease activity was assayed qualitatively by point-inoculation of yeasts on plates with skim milk agar and gelatine agar (Sigma-Aldrich Co. Ltd., Merck KGaA Darmstadt, Germany) at pH=4.5, according to Charoenchai *et al.* (*24*). Skim milk agar plates were directly examined for clear zones surrounding yeast growth after incubation, whereas gelatine agar plates were flooded with 10 mL acetic acid (50 g/L) prior to examination of clear zones around the yeast cells.

β-Glucosidase activity was assayed following Villena *et al.* (*25*) in a medium containing 0.5 % cellobiose (4-O-β-D-glucopyranosyl-D-glucose, (Sigma-Aldrich Co. Ltd., Merck KGaA, Darmstadt, Germany), 0.67 % YNB and 2 % agar. Prior to incubation, a pure isolated colony was suspended in 1 mL of sterile distilled water in an Eppendorf tube. The yeast suspension was then centrifuged at 2,500x*g* for 5 min at 4 °C (Rolco Srl, Buenos Aires, Argentina), the supernatant discarded and the sediment washed twice with sterile distilled water to eliminate remaining nutrients from the initial medium. Finally, the sediment was suspended in sterile distilled water and seeded with a loop onto solid medium containing cellobiose. Positive activity was determined by colony growth. *Torulaspora delbrueckii* BTd259 and *Debaryomyces vanrijiae* BDv566 were used as positive control strains for β-glucosidase (*2,13*).

Pheno- and genotype identification of selected yeasts

Yeasts demonstrating highest hydrolytic enzyme production were identified at species level following the taxonomic criteria described by Kurtzman *et al.* (*26*) based on their morphological and physiological characteristics as well as PCR-RFLP analysis of the ITS1-5.8S–ITS2 region from the rRNA gene. PCR was carried out according to protocols described by Esteve-Zarzoso *et al.* (*27*) with some modifications using universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′; Integrated DNA Technologies, Iowa, USA) and already described by White *et al.* (*28*), using a thermal cycler TC-312 (Techne, Waltham, MA, USA). PCR products were digested with *Cfo*I, *Hinf*I and *Hae*III restriction enzymes following the supplier's instructions (Promega Co., Madison, USA). Amplified products and their restriction fragments were electrophoresed on 1.4 and 2.2 % agarose gels, respectively, in 1×TAE (Tris-acetic acid-EDTA) buffer, using a horizontal electrophoresis cell (Labnet International, Inc., New Jersey, USA). Gels were stained with ethidium bromide, visualized, and photographed under UV light (NYX Technyk Inc., San Diego, USA). Fragment sizes were estimated by comparison against a DNA standard (100-bp ladder). All PCR-RFLP reagents were purchased from Promega Co., Madison, USA).

Pectinolytic yeasts used as reference strains

In addition to the microorganisms isolated in the present study, the following pectinolytic microorganisms were obtained from the "Biodiversidad San Rafael (Mendoza)" culture collection of the FCAI-UNCUYO belonging to the SCCM-AAM (Argentine Association of Microbiology) and affiliated to the FELACC (Latin-American Federation of Culture Collections): *Aureobasidium pullulans* GM-R-22 (in this manuscript referred to as “Ap-R-22”), *Cryptococcus saitoi* GM-4 (Crs-GM-4), *Filobasidium capsuligenum* B-13 (Fc-B-13), *Rhodotorula dairenensis* GM-15 (Rd-GM-15) and *S. cerevisiae* B-17 (Sc-B-17). These strains had been selected in previous own works for their pectinolytic activity with oenological importance (*3,4,19,20*) and were included in the present study to expand analyses of unexplored aspects.

Production of extracellular enzyme extracts

The yeasts under study were inoculated in liquid medium according to Moyo *et al.* (*21*) with modifications and with the following composition (in g/L of 50 mM citric-citrate buffer, pH=3.8): $CaCl_2$, 0.05; KH_2PO_4, 0.2; $MnSO_4$, 0.05; $(NH_4)_2SO_4$, 1.0; citric pectin 1.0; dextrose, 1.0.; Sigma-Aldrich Co. Ltd., Merck KGaA (Darmstadt, Germany). Cultures were incubated under shaking conditions (130 rpm) at 28 °C for 3 d, using a wather bath shaker (SHZ-88, Semedix, Buenos Aires, Argentina. Cells

were separated by centrifugation (10,000x *g* for 15 min at 4 °C, Presvac EPF-12R, Buenos Aires, Argentina) and cell-free supernatants were used as enzyme extracts.

Quantitative evaluation of enzyme activities under oenological-like conditions

Pectinolytic activity was assayed by measuring the amount of reducing sugars released from a pectin dispersion (0.25 % pectin in 50 mM citric-citrate buffer, pH=3.8) using 3,5-dinitrosalicylic acid (DNS) reagent (*29*). Galacturonic acid was used as standard. All reagents were purchased from Sigma-Aldrich Co. Ltd., Merck KGaA (Darmstadt, Germany). Reaction mixtures (enzyme extract-substrate ratio: 1/10) were incubated at 15 and 28 °C for 20 min. One pectinase unit (U) was defined as the enzyme activity that released 1 μmol of reducing sugars per min under the given assay conditions.

The conditions for determination of cellulase, xylanase and amylase activities were the same as those described for pectinolytic activity but using 0.25 % (w/v) carboxymethyl cellulose, 0.25 % (*m*/*V*) birchwood xylan and 1 % (*m*/*V*) soluble starch (Sigma-Aldrich Co. Ltd., Merck KGaA, Darmstadt, Germany), respectively, dissolved in 50 mM citric-citrate buffer (pH=3.8). The reaction mixture was incubated for 20 min at 15 and 28 °C. One unit of cellulase, xylanase or amylase activity was defined as the activity necessary to produce 1 μmol of reducing sugars (glucose or xylose) per min under the assay conditions.

β-Glucosidase activity was assayed by incubating 100 μL of enzyme extract with 100 μL of 15 mM D-(+)-cellobiose (Sigma-Aldrich Co. Ltd., Merck KGaA, Darmstadt, Germany), solution in 50 mM citric-citrate buffer (pH 3.8) at 15 and 28 °C for 30 min. Glucose production was quantified using the enzymatic colorimetric GOD-POD method (Wiener lab., Rosario, Argentina) (*25*). One β-glucosidase unit was defined as the enzyme activity necessary to release 2 μmol of glucose from cellobiose per min under the assay conditions.

Secondary selection: Technological effects of extracellular enzyme extracts on short macerations with grape must

A secondary selection of yeasts according to technological criteria was made by evaluating the effects of extracellular enzyme extracts on short macerations with grape must. Short macerations were carried out in 50 mL Falcon tubes containing 40 g of Malbec grape must and supplemented with 1 mL of the corresponding enzyme extracts (1 U/mL). Control treatments without enzyme (reaction blank) were obtained by replacing the enzyme extracts with 1 mL of citric-citrate buffer, and a reference treatment, using a commercial enzyme (Extrazyme, Institut Oenologique de Champagne,

Épernay, Marne, France) supplemented at a concentration of identical net enzyme units (EU), were also performed. All maceration assays were incubated for 6 h at 15 and 28 °C. All experiments were carried out in triplicate.

Colour parameters

Colour extraction was assayed by examining classic vinification parameters. Colour intensity (CI) was determined spectrophotometrically (HACH DR6000, Onelab, Buenos Aires, Argentina) by summarizing the absorbances at 620, 520 and 420 nm of undiluted must, using 1 mm optical path cuvettes, according to Glories (*30*). The hue was calculated as the quotient between the absorbances at 420 and 520 nm according to Sudraud (*31*). The total polyphenol index (TPI) was determined with a 100x diluted must sample with distilled water; absorbance was determined at 280 nm according to Glories (*30*) and Ribéreau-Gayon *et al*. (*32*). CIELAB coordinates (*L**, *a**, and *b**) were determined according to the standard method by the Commission Internationale de l'Éclairage (*33*). The CIELAB color difference (ΔE*) was calculated by using the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad /1/$$

where the difference (Δ) is calculated for each independent variable between the enzymatically treated macerations and its respective controls. Finally, total polyphenol content (TPC) was determined according to the classic Folin-Ciocalteu method (*34*) and expressed as mg gallic acid equivalents per L of sample (mg GAE/L).

Clarification and filterability

An appropriate amount of enzyme extract was added to 10 mL of Chardonnay white grape must. After enzyme treatment, clarification was evaluated by determining the transmittance percentage as a measure of clarification at 650 nm, with distilled water as a reference, using a spectrophotometer (Metrolab, Lima, Perú).

After the enzyme treatment described above, the must was vacuum filtered (25 mm diameter filter; 14 µm pore size; 0.9 bar, Sartoruis, Gotinga, Germany) as described by Belda *et al*. (*1*) with modifications. Filterability was expressed as the seconds needed to filter 1 mL of clarified must.

Antioxidant activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl radical capture method (DPPH•), according to the modified technique by Brand-Williams *et al.* (*35*). The reaction mixture was prepared with 100 µL of sample (1:10 dilution) and 2.9 mL of DPPH• solution (0.03

mg/*mL*), the reaction time was set at 25 min and spectrophotometric readings were performed at 515 nm. All determinations were carried out at room temperature, and results are expressed as antioxidant capacity in gallic acid equivalents (mg GAE/L). Reagents were purchased from Sigma-Aldrich Co. Ltd., Merck KGaA, Darmstadt, Germany.

Statistical analysis

All experimental data are the average of three repetitions ± standard deviation. Analysis of variance (ANOVA) was applied to these data, and comparison of the mean values was carried out by means of Fisher's test of significant differences, with a level of significance of $p<0.05$, and using Statgraphics Plus software version 5.1. (*36*). In addition, R Software version x64 3.6.3 (*37*), Rcmdr and FactoMindeR was used for principal component analysis (PCA).

RESULTS AND DISCUSSION

Screening of enzyme activities and identification of wine grape yeasts

A total of 366 yeast and yeast-like microorganisms was isolated and 96 of them showed pectinase, xylanase and cellulase activities with the plate technique, indicating that 26.2 % of the isolates presented at least some of the enzyme activities assayed. Of the 96 isolates demonstrating enzyme activity, 28 only presented pectinase and 13 only xylanase activity, while no isolate showed cellulase activity without being positive for the other two activities. This suggests that cellulolytic activity would be an adjunct to pectinolytic and xylanolytic activity during degradation of plant organic matter. The remaining isolates presented at least two of the assayed activities, indicating that 57.3 % of the isolates with enzyme activity presented a multi-enzyme blend necessary to degrade a material as complex as the plant cell wall.

A primary isolate selection was carried out based on the halo diameter vs. colony diameter ratio (Dh/Dc) at 15 and 28 °C, selecting isolates that presented Dh/Dc ratios between 2 and 7 and a colony diameter greater than 5 mm. Additionally, other enzyme activities complementary to polysaccharidases such as proteases, amylases and β-glucosidases were assayed for their positive effect on various technological and sensorial parameters. As can be seen in Table 1, 9 yeast and yeast-like isolates were selected at 15 ºC and 7 at 28 ºC.

Table 1

Table 2 shows identification of the selected strains based on PCR-RFLP analyses of the ITS1-5.8S-ITS2 region of the rRNA gene, defining them as *Aureobasidium* (3 strains), *Candida* (1), *Debaryomyces* (2), *Hanseniaspora* (2), *Metschnikowia* (3), *Pichia* (1), *Saccharomyces* (1) and

Torulaspora (3) genera. It can be seen that the ability to produce polysaccharidases, necessary to survive in such a complex niche, was present in various microbial genera. This result differs from that reported by Belda *et al.* (*1*), who assayed 462 yeast isolates for different enzyme activities of oenological interest and observed that only the *Metschnikowia* and *Aureobasidium* genera were positive for polygalacturonase activity, while cellulase activity was only observed in *Aureobasidium*.

Table 2

According to Barata *et al.* (*38*), the different genera found on the grape surface can be classified into three main groups, which are successively detected as maturation progresses: (i) oligotrophic oxidative basidiomycetous yeasts, the yeast-like fungus *A. pullulans* and lactic acid bacteria; (ii) copiothrophic oxidative ascomycetes (several *Candida* spp.), weakly fermentative apiculate yeasts (*Hanseniaspora* spp.), film forming yeasts (*Pichia* spp.) and fermentative yeasts (*C. zemplinina*, *Metschnikowia* spp.); (iii) copiotrophic strong fermentative yeasts (*Saccharomyces* spp., *Torulaspora* spp.). In agreement with this classification and because the samples were ripe grapes our results revealed a great diversity of strains belonging to the three microbial groups on the grape surface. The ability to produce cell wall degrading enzymes may be closely related to these population dynamics. Consequently, during the first stages, oligotrophic microorganisms that are able to survive with few available nutrients are more abundant. These microorganisms produce enzymes that make gaps in the plant cell walls, thus creating favourable conditions like a greater availability of nutrients for the development of copiotrophic species. The latter species do not require their own enzyme activities because the enzymes for the degradation of cell walls would already be present in the environment.

Evaluation of hydrolytic enzyme activities under oenological-like conditions

Fig. 1 shows the different activities assayed in the enzyme extracts and produced by the strains under study at two temperatures, 15 and 28 °C. Pectinolytic activity was the main enzyme activity observed, reaching significant levels compared with the other two activities assayed. This activity was particularly predominant at low temperature and was the only activity detected in several of the strains studied, with the exception of *A. pullulans* and *T. delbrueckii*, which demonstrated a very comprehensive enzyme profile.

Fig. 1

As observed in previous studies, *A. pullulans* is the most abundant species on the grape surface, in fresh grape juice and during very early fermentation stages (*4,19,39,40*). This species has been reported to produce a great diversity of extracellular enzymes such as pectinases, cellulases,

xylanases, glycosidases and proteases (*1,3,19*). In fact, in the present study it was the only species that showed an enzyme pool containing all five activities aforementioned, confirming the presence of a very comprehensive polysaccharidase enzyme system, at least consisting of the activities assayed (pectinase, xylanase, cellulase and amylase) as well as other complementary activities such as proteases and β-glucosidases. Within this species, the Ap-R-22 and Td-m11-2 strains were remarkable, as they presented the highest values of pectinolytic activity at both assay temperatures (1.149 and 0.954 U/mL at 28 °C, and 0.765 and 0.722 U/mL at 15 °C, respectively). *A. pullulans* is a yeast-like euascomycete fungus, meaning that despite presenting yeast stages, it is multicellular and, as such, an excellent producer of lytic enzymes. Given the trophic-ecological role of these fungi, degradation of organic matter, they release enzymes for primary degradation to the environment, and during later stages they provide yeasts responsible to finish the degradation. Based on the present results and according to previous reports (*3*), *A. pullulans* demonstrated the best activity profiles of oenological importance. This yeast-like fungus is very common in the phyllosphere and carposphere of fruit and vegetable crops and has a potential action against phytopathogenic fungi (*41*). According to Onetto *et al.* (*42*), the genus *Aureobasidium* is a central component of the microbial community of grape must, and their studies revealed the potential of the species to affect the composition of grape must through the production of polymers and extracellular enzymes, as well as through modulation of the fermentation kinetics by competition for trace elements.

Continuing with the screening of carbohydrolase-secreting microorganisms, and in agreement with Merín and Morata de Ambrosini (*3*), basidiomycetes were the second-most abundant isolates, especially those belonging to the genera *Cryptococcus* and *Rhodotorula*. Only *Cr. saitoi* produced elevated levels of enzyme activity and a complete enzyme pool considering the activities assayed. Strain *Cr. saitoi* Crs-GM-4 reached a pectinolytic activity of 0.634 U/mL at 15 °C and 0.974 U/mL at 28 °C, while R. dairenensis Rd-GM-15 showed a different enzyme composition: no xylanase or β-glucosidase activity, little pectinase activity and cellulase was the main carbohydrase activity at 15 and 28 °C (0.197 and 0.394 U/mL, respectively). *F. capsuligenum* Fc-B-13 produced a very complete enzyme blend with high pectinolytic activity (0.715 U/mL at 15 °C and 0.901 U/mL at 28 °C). Previously, Merín *et al.* (*20*) studied *F. capsuligenum* strains and they found good activity at 12 and 28 °C (0.77 and 1.15 U/mL, respectively). Their report was the first mentioning the ability of this species to produce pectinases.

Subsequently, the oxidative and weakly fermentative hemiascomycete yeasts belonging to the genera *Candida*, *Debaromyces*, *Hanseniaspora*, *Metschnikowia* and *Pichia* presented less complete enzyme systems, with lower levels of enzyme activities. Previously, production of polysaccharidase

activities such as pectinases, cellulases, amylases, as well as proteases in other fruits or fermentation processes by the genera *Debaryomyces* (*43*), *Metschnikowia* (*1*), *Hanseniaspora*, *Candida* and *Pichia* (*44*) had been reported. The findings for strain *D. vanrijiae* Dv-m87-3 were particularly interesting, as it showed significant levels of pectinase (0.656 and 0.841 U/mL at 15 and 28 °C, respectively), xylanase (0.167 and 0.390 U/mL at 15 and 28 °C, respectively), cellulase (0.058 and 0.228 U/mL at 15 and 28 °C, respectively) and β-glucosidase activity (0.062 U/mL at 28 °C), with a spectrum similar to that of basidiomycete yeasts. There are reports on pectinolytic activity by *Debaromyces hansenii* and *Debaromyces polymorphus* strains present in tropical fruits, whereas xylanolytic activity was only observed in *D. hansenii* and *D. vanrijiae* strains from oenological origin (*45*). Cellulolytic activity is not abundant in wine yeasts, although this activity has been reported in *D. hansenii* isolated from decayed wood (*46*).

Likewise, the apiculated yeast *H. uvarum*/*K. apiculata* seems to be the most common grape berry species worldwide, which is consistent with its predominance in the beginning of spontaneous must fermentations (*38*). It should be mentioned that *Hanseniaspora sp.* H-m27-2 had a particular enzyme profile, almost exclusively consisting of pectinolytic activity, and reaching significantly high levels (0.677 and 0.840 U/mL at 15 and 28 °C, respectively).

Finally, two strongly fermentative hemiascomycete yeasts, *S. cerevisiae* and *T. delbrueckii*, were also observed in the present isolation and selection study. These strains produce lytic enzymes, which are of great interest because they are able to produce plant cell wall-degrading enzymes, while carrying out alcohol fermentation. However, these results differ from a previous study by Merín *et al.* (*4*), who did not detect pectinolytic activity among ascomycetous yeasts isolated from grapes and during fermentation.

The species *S. cerevisiae* is rarely isolated from grapes using traditional sampling techniques (*47*). Most *S. cerevisiae* strains normally used in winemaking do not show the ability to degrade pectin substrates. Only a few wild strains have been reported to possess the ability to degrade pectin during wine fermentation (*48*). In the present study, two *S. cerevisiae* strains, one isolated from the grape surface (Sc-m79-1) and one from winery equipment (Sc-B-17) (*20*), were able to secrete pectinases, reaching enzyme activities of 0.264 and 0.321 U/mL at 15 °C, and 0.476 and 0.543 U/mL at 28 °C, respectively. These findings could be related to the fact that some grapes used for isolation had an advanced state of maturity.

Regarding *T. delbrueckii*, strain Td-m7-2 showed significantly higher pectinolytic activity at both assay temperatures (0.682 and 0.723 U/mL at 15 and 28 °C, respectively), and xylanase (0.195 and 0.241 U/mL), cellulase (0.212 and 0.250 U/mL) and β-glucosidase activity (0.113 and 0.185

U/mL) were also excellent. This species is characterized by its decent oenological characteristics such as low acetic acid production, a higher glycerol concentration and a better composition of volatile aromatic compounds in wines (*49*). Recent reports indicate its ability to promote malolactic fermentation in wines with a high polyphenol content (*50*). However, the ability of this species to produce extracellular enzymes has been little studied (*2,51*). *T. delbrueckii* Td-m30-1 showed a different behaviour, as it only presented pectinase activity, but less than strain Td-m7-2, and it was negative for the other activities assayed, suggesting that production of polysaccharidases is strain-dependent.

On the other hand, amylase production was observed in all basidiomycetes and all *A. pullulans* strains assayed, while it was negative in hemiascomycetes, with the exception of *S. cerevisiae* Sc-B-17.

Technological effects of extracellular enzyme extracts on short macerations with grape must

Table 3 shows the effects of microbial enzyme extracts on different technological parameters evaluated after short macerations with Malbec must at 15 and 28 °C. Musts treated with enzymes showed differences in the extraction of total polyphenols during the maceration. All enzymatically treated macerations presented TPI values that were higher than that of the control treatment without enzymes (C1) at both assay temperatures. *A. pullulans* Ap-m11-2 demonstrated the highest TPI values (59.1±0.4 and 58.6±0.0 at 15 and 28 °C, respectively). However, the other *A. pullulans* extracts presented similar values, which were highest for macerations carried out at 15 °C. Similarly, the *T. delbrueckii* Td-m7-2 extract produced a TPI value during maceration which was higher at 15 than at 28 °C, and also higher than the three *M. pulcherrima* extracts. The remaining extracts produced slightly lower TPI values, probably as a result of the effect of the lower molecular mobility due to the low temperature. Our results are in agreement with those observed by Belda *et al.* (*1*) for the same parameter. In particular, this study indicates the potential of *M. pulcherrima* in co-culture with *S. cerevisiae* on a semi-industrial scale to improve the colour properties in red wine owing to its pectinolytic activities at 12 and 28 °C. Likewise, the TPC was highest for must treated with Ap-m11-2 extract at both maceration temperatures (750.5±0.6 and 746.8±1.2 at 15 and 28 °C, respectively). Moreover, the TPC for all *M. pulcherrima* extracts and for those of *A. pullulans* Ap-R-22, *T. delbrueckii* Td-m7-2, *D. vanrijiae* Dv-m87-3 and *R. dairenensis* Rd-GM-15 was significantly higher at 15 than at 28 °C.

Table 3

Quantification of the antioxidant activity (AA) by means of DPPH• radical scavenging is based on the capacity of the sample to reduce free radicals, which is proportional to the antioxidant content. In our case, the AA values of all enzymatically treated macerations were higher at 15 than at 28 °C. All *A. pullulans* enzyme extracts presented a high antioxidant capacity, particularly Ap-R-22 and Ap-m11-2 which showed a significantly higher activity at 15 °C compared with the other strains (553.3 and 548.0, respectively). Other prominent enzyme extracts regarding their high antioxidant activity at low temperatures were Td-m7-2 (528.3), Fc-B-13 (506.1) and the two *M. pulcherrima* extracts: Mp-ts-2 and Mp-ts-1 (514.1 and 505.3, respectively).

Therefore, macerations with highest TPI, TPC and antioxidant capacity values correspond to the most comprehensive multi-enzyme systems. The antioxidant capacity is related to the content of anthocyanins and total polyphenols. Each phenolic compound contributes proportionately and differently to this activity. Several authors have observed a strong linear correlation between antioxidant activity and TPI values (*52*).

The colour of a sample is defined by the colour intensity (CI) and the hue, with the former one as a measure of the intensity of the colour and the latter one representing the relative importance of yellow over red. According to the results shown in Table 3, a significant decrease in the hue was observed for all macerations with enzymes, even with the commercial enzyme (C2), compared with the control (C1). There were no statistically significant differences in CI between the two Ap-R-22 and Ap-m11-2 extracts, but for the hue there were, which would indicate that the Ap-m11-2 sample extracted more red pigments. Ap-m86-1 had a lower CI than the other extracts at 15 and 28 °C, but the hue was also lower. In the case of Crs-GM-4, there were no significant differences in CI between both temperatures, but the hue was significantly lower at 15 than at 28 °C. This would be a favourable effect of the low temperature on the colour composition. Additionally, the microbial extracts with the most comprehensive enzyme systems produced the highest values of the CIELAB parameters and highest ΔE.

Filterability and clarification of the macerated musts was also assessed at 15 and 28 °C (Table 4). At both temperatures and for all enzyme extracts assayed, filtration time was shorter than that of control without enzyme. Furthermore, for all enzyme extracts the filtration time at 15 °C was less than that recorded at 28 °C. In particular, macerations with Td-m7-2 and Ap-R-22 extracts produced the shortest filtration times at 15 °C (249.2 and 250.4 s/mL, respectively), followed by those with Ap-m11-2 and Dv-m87-3 (256.3 and 255.0 s/mL, respectively). Regarding clarification, the formation of pectin flocs facilitates the production of a clear supernatant through elimination of the colloidal portion of the must. Hence, musts treated with Td-m7-2 and Ap-m86-2 were the most efficient, with an increase in

transmittance of more than 4 times at both maceration temperatures. Other extracts with good clarification performance at low temperature were those of *D. vanrijiae* Dv-m87-3, *A. pullulans* Ap-R-22 and *Hanseniaspora* sp. H-m27-2. These results demonstrate that filtration and clarification would also be positively correlated with strains with very comprehensive hydrolytic enzyme activity profiles for the degradation of plant cell wall polymers.

Table 4

Principal component and cluster analyses

In order to select strains with multi-enzyme systems of great oenological importance based on the effects observed with macerations carried out with Malbec must, principal component analysis (PCA) was applied. At 28 °C (Fig. 2a), the first two dimensions explain 80.4 % of the variability, with component one (PC1) representing 71.4 % and component two (PC2) 9.0 %. At 15 °C, PC1 represents 73.4 % and PC2 8.6 % of the variability (Fig. 2b). At both temperatures, PC1 positively correlated with the following parameters: clarification, TPI, TPC, CI, a^*, ΔE and AA. All *A. pullulans* enzyme extracts together with Td-m7-2, Dv-m87-3 and Mp-ts-2 showed a similar behaviour regarding their relationship with the variables mentioned in component one. In turn, enzyme extracts presenting negative values for PC1 show an inverse correlation with these parameters. In this case, they correspond to strains with little enzyme activity and with enzyme systems comprising only one or two of the activities assayed. Therefore, extracts that present a more comprehensive multi-enzyme blend are represented by positive PC1 values, while those that show lower levels and types of enzyme activity are negative, and the intersection of the components is occupied by extracts with intermediate enzyme activities.

Fig. 2

PC2 was represented with significant weight by the following parameters: filterability, hue, clarity (L^*) and component b^*. The hue, L^* and b^* are variables that are negatively related to the colour of red wine, and hence Pg-m28-1, H-m82, Cs-m89-1, Tp-m29 and Td-m30 extracts had a negative impact on the colour of the maceration. Filterability in the negative quadrant indicates a low efficiency of this parameter, resulting in longer filtration times.

Cluster Analysis (Fig. 3) demonstrates that all *A. pullulans* extracts (Ap-R-22, Ap-m11-2, Ap-m86-1 and Ap-m86-2) as well as those of *T. delbrueckii* Td-m7-2, *D. vanrijiae* Dv-m87-3 and M. *pulcherrima* Mp-ts-2 generated the same cluster at 28 °C. At 15 °C, however, only the Ap-R-22, Ap-m11-2, Ap-m86-2 and Td-m7-2 extracts generated a cluster which was different from all the others, showing the ability to improve technological parameters assessed at the two assay temperatures.

Consequently, these strains would present the best multi-enzyme systems to improve the chromatic characteristics, clarification and filterability as well as extraction of bioactive compounds during maceration of the grape must.

Fig. 3

CONCLUSIONS

The present study assessed the enzymatic potential of epiphytic grape yeasts selected for their ability to produce polysaccharidases and belonging to 13 species of oenological interest. The majority of the strains assayed presented a multi-enzyme system necessary to degrade a material as complex as the plant cell wall. A great difference in the activity profile could be observed among the yeast species, even within the same species, which indicates the importance to select optimal strains for the production of enzymes. All *A. pullulans* strains were able to produce carbohydrolases of oenological importance -pectinases, xylanases and cellulases- in addition to amylases, proteases and β-glucosidases. Besides, two strains belonging to the species *T. delbrueckii* and *M. pulcherrima* presented an enzyme spectrum that was very different from the other isolated strains of the same species. Two *S. cerevisiae* strains (Sc-m79-1 and Sc-B-17) were effective pectinase producers under oenological conditions, which represents an advantage of their use as starter cultures to improve particular wine properties. Strains that produced the most comprehensive enzyme extracts and the highest levels of activity also exhibited the best chromatic and technological properties. The present study shows the enzyme capacity of some non-*Saccharomyces* yeast strains suggesting their potential use as a co-culture with *S. cerevisiae* to improve the sensorial and technological properties assayed, thus enhancing the wine quality. Four *A. pullulans* strains (Ap-R-22, Ap-m11-2, Ap-m86-2, Ap-m86-1) plus *T. delbrueckii* m7-2, *D. vanrijiae* m87-3 and *M. pulcherrima* ts-2 showed the highest enzyme activities and they were associated with remarkable effects on colour, clarification, filterability and antioxidant activity of the must. The performance of the selected strains and their extracts still has to be examined in winemaking to confirm the technological effects and rule out possible negative effects. These microorganisms can be granted a GRAS status, because they are isolated from their natural habitat and because of their identity. Hence, besides the use of their enzyme extracts it is also possible to propose application of the microorganism itself to constitute a mixed culture able to produce *in situ* maceration enzymes. Therefore, the present study allows expansion of previous findings by our research group, thus contributing to a better understanding of the effect of multi-enzyme systems on compound transformations during the maceration process and produced by indigenous yeasts and yeast-like organisms on the grape berry and in the must ecosystem.

ACKNOWLEDGEMENTS

The authors are very grateful to CASA BIANCHI winery for Malbec grape must supply.

FUNDING

This work was supported by SIIP-UNCUYO [grant number 06/L150]; PICTO-UNCUYO [grant number BID Loan 2016-0048]; PICT-MINCYT [grant number BID Loan 2019-03446].

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTION

Sara J. Longhi: This author participed in data collection, experiment performing, data analysis and interpretation, and drafting the article. María C. Martín: This author participed in conception or design of the work, as methodology assistent, supervision, data analysis and interpretation, and writing -review & editing- the article. María G. Merín: This author participed in data analysis and interpretation, critical revision and writing -review & editing- the article. Vilma I. Morata: This author participed in conception or design of the work, data analysis and interpretation, critical revision, project administration, and supervision.

ORCID ID

S.J. Longhi https://orcid.org/0000-0001-9325-9561

M.C. Martín https://orcid.org/0000-0003-1163-0676

M.G. Merín https://orcid.org/0000-0001-9893-794X

V.I. Morata de Ambrosini https://orcid.org/0000-0001-9597-7508

REFERENCES

1. Belda I, Conchillo LB, Ruiz J, Navascués E, Marquina D, Santos A. Selection and use of pectinolytic yeasts for improving clarification and phenolic extraction in winemaking. Int J Food Microbiol. 2016;223:1–8. https://doi.org/10.1016/j.ijfoodmicro.2016.02.003
2. Maturano YP, Rodríguez Assaf LA, Toro ME, Nally MC, Vallejo M, Castellanos de Figueroa LI, et al. Multi-enzyme production by pure and mixed cultures of *Saccharomyces* and non-*Saccharomyces* yeasts during wine fermentation. Int J Food Microbiol. 2012;155:43–50.

https://doi.org/10.1016/j.ijfoodmicro.2012.01.015

3. Merín M, Morata de Ambrosini VI. Highly cold-active pectinases under wine-like conditions from non-*Saccharomyces* yeasts for enzymatic production during winemaking. Lett Appl Microbiol. 2015;60(5):467–74. https://doi.org/10.1111/lam.12390
4. Merín MG, Martín MC, Rantsiou KK, Cocolin L, Morata de Ambrosini VI. Characterization of pectinase activity for enology from yeasts occurring in Argentine Bonarda grape. Brazilian J Microbiol. 2015;46(3):815–23. https://doi.org/10.1590/s1517-838246320140160
5. Claus H, Mojsov K. Enzymes for Wine Fermentation: Current and Perspective Applications. Fermentation. 2018;4(3):52. https://doi.org/10.3390/fermentation4030052
6. Yacco RS, Watrelot AA, Kennedy JA. Red Wine Tannin Structure–Activity Relationships during Fermentation and Maceration. J Agric Food Chem. 2016;64(4):860–9. https://doi.org/10.1021/acs.jafc.5b05058
7. Garrido J, Borges F. Wine and grape polyphenols–A chemical perspective. Food Res Int. 2013;54(2):1844–58. https://doi.org/10.1016/j.foodres.2013.08.002
8. Molina AM, Swiegers JH, Varela C, Pretorius IS, Agosin E. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Appl Microbiol Biotechnol. 2007;77(3):675–87. https://doi.org/10.1007/s00253-007-1194-3
9. Martín MC, Morata de Ambrosini VI. Effect of a cold-active pectinolytic system on colour development of Malbec red wines elaborated at low temperature. Int J Food Sci Technol. 2014;49(8):1893–1901. https://doi.org/10.1111/ijfs.12498
10. Merín MG, Morata de Ambrosini VI. Kinetic and metabolic behaviour of the pectinolytic strain *Aureobasidium pullulans* GM-R-22 during pre-fermentative cold maceration and its effect on red wine quality. Int J Food Microbiol. 2018;285:18–26. https://doi.org/10.1016/j.ijfoodmicro.2018.07.003
11. Merín MG, Morata de Ambrosini VI. Application of a grape surface majority pectinolytic species, *Aureobasidium pullulans*, to low-temperature red winemaking: development and stability of wine colour. J Wine Res. 2020;31(3):218–39.

https://doi.org/10.1080/09571264.2020.1816534

12. Benucci I, Río Segade S, Cerreti M, Giacosa S, Paissoni MA, Liburdi K, et al. Application of enzyme preparations for extraction of berry skin phenolics in withered winegrapes. Food Chem. 2017;237:756–65. https://doi.org/10.1016/j.foodchem.2017.06.003
13. Maturano YP, Assof M, Fabani MP, Nally MC, Jofré V, Rodríguez Assaf LA, et al. Enzymatic activities produced by mixed *Saccharomyces* and non-*Saccharomyces* cultures: relationship with wine volatile composition. A Van Leeuw. 2015;108(5):1239–56. https://doi.org/10.1007/s10482-015-0578-0
14. Fratebianchi D, González M, Tenorio C, Cavalitto S, Ruiz-Larrea F. Characterization and winemaking application of a novel pectin-degrading enzyme complex from *Aspergillus sojae* ATCC 20235. Vitis–J Grapevine Res. 2017;56(2):85–93. DOI: 10.5073/vitis.2017.56.85-93
15. Romero-Cascales I, Fernández-Fernández JI, Ros-García JM, López-Roca JM, Gómez-Plaza E. Characterisation of the main enzymatic activities present in six commercial macerating enzymes and their effects on extracting colour during winemaking of Monastrell grapes. Int J Food Sci Technol. 2008;43(7):1295–305. https://doi.org/10.1111/j.1365-2621.2007.01608.x
16. Brandolini V, Fiore C, Maietti A, Tedeschi P, Romano P. Influence of *Saccharomyces cerevisiae* strains on wine total antioxidant capacity evaluated by photochemiluminescence. World J Microbiol Biotechnol. 2007;23(4):581–6. https://doi.org/10.1007/s11274-006-9268-4
17. Gaensly F, Agustini BC, da Silva GA, Picheth G, Bonfim TMB. Autochthonous yeasts with β-glucosidase activity increase resveratrol concentration during the alcoholic fermentation of *Vitis labrusca* grape must. J Funct Foods. 2015;19:288–95. https://doi.org/10.1016/j.jff.2015.09.041
18. Gao Y, Zietsman AJJ, Vivier MA, Moore JP. Deconstructing Wine Grape Cell Walls with Enzymes During Winemaking: New Insights from Glycan Microarray Technology. Molecules. 2019;24(1):165. https://doi.org/10.3390/molecules24010165
19. Merín MG, Mendoza LM, Farías ME, Morata de Ambrosini VI. Isolation and selection of yeasts from wine grape ecosystem secreting cold-active pectinolytic activity. Int J Food Microbiol. 2011;147(2):144–8.

https://doi.org/10.1016/j.ijfoodmicro.2011.04.004

20. Merín MG, Mendoza LM, Morata de Ambrosini VI. Pectinolytic yeasts from viticultural and enological environments: Novel finding of *Filobasidium capsuligenum* producing pectinases. J Basic Microbiol. 2014;54(8):835–42. https://doi.org/10.1002/jobm.201200534
21. Moyo S, Gashe BA, Collison EK, Mpuchane S. Optimising growth conditions for the pectinolytic activity of *Kluyveromyces wickerhamii* by using response surface methodology. Int J Food Microbiol. 2003;85:87–100. https://doi.org/10.1016/s0168-1605(02)00503-2
22. Fernandes-Salomão TM, ACR A, VM C-A, JLC C, DO S, EF A. Isolation of pectinase hyperproducing mutants of *Penicillium expansum*. Rev Microbiol. 1996;27(1):15–8.
23. Arcuri SL, Pagnocca FC, da Paixão Melo WG, Nagamoto NS, Komura DL, Rodrigues A. Yeasts found on an ephemeral reproductive caste of the leaf-cutting ant *Atta sexdens rubropilosa*. A Van Leeuw. 2014;106(3):475–87. https://doi.org/10.1007/s10482-014-0216-2
24. Charoenchai C, Fleet GH, Henschke PA, Todd BEN. Screening of non-*Saccharomyces* wine yeasts for the presence of extracellular hydrolytic enzymes. Aust. J. Grape Wine Res. 1997;3(1):2–8. https://doi.org/10.1111/j.1755-0238.1997.tb00109.x
25. Villena MA, Iranzo JFÚ, Pérez AIB. β-Glucosidase activity in wine yeasts: Application in enology. Enzyme Microb Technol. 2007;40(3):420–5. https://doi.org/10.1016/j.enzmictec.2006.07.013
26. Kurtzman CP, Fell JW, Boekhout T., editors. The Yeasts: A Taxonomic Study. (5th Ed.) Amsterdam: Elsevier Science Publisher; 2011.
27. Esteve-Zarzoso B, Belloch C, Uruburu F, Querol A. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int J Syst Evol Microbiol. 1999;49(1):329–37. https://doi.org/10.1099/00207713-49-1-329
28. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungi ribosomal RNA gene for phylogenetics. In: Innis, M.A., Gelfalnd, D.H., Sninsky, J.J., White, T.T., editors. PCR protocols. A guide of methods and applications. San Diego, USA: Academic Press; 1990. pp. 315–22. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

29. Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal Chem. 1959;31(3):426–8. https://doi.org/10.1021/ac60147a030

30. Glories Y. The color of red wines. Part 2. Measurement, origin, and interpretation Connaiss. Vigne Vin. 1984;18:253–71.

31. Sudraud P. Interpretation des courbes d'absortion des vins rouges. Ann Technol Agric. 1958;7:203–8.

32. Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D. In: Handbook of Enology. Chichester, UK: John Wiley & Sons, Ltd; Chichester, West Sussex, England. 2006. https://doi.org/10.1002/0470010398

33. Robertson A. Recent CIE Work on Color Difference Evaluation. In: Review and Evaluation of Appearance: Methods and Techniques ASTM International; 1986. https://doi.org/10.1520/stp18344s

34. Chen LY, Cheng CW, Liang JY. Effect of esterification condensation on the Folin–Ciocalteu method for the quantitative measurement of total phenols. Food Chem. 2015;170:10–5. https://doi.org/10.1016/j.foodchem.2014.08.038

35. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT–Food Sci Technol. 1995;28(1):25–30. https://doi.org/10.1016/s0023-6438(95)80008-5

36. Statgraphics Plus software v.5.1. Manugistic, Rockville, MD, USA; Statpoint Technologies Inc., 2001. Available from: https://www.statgraphics.com

37. R language software version x64 3.6.3, survival library. (2020-02-29). Running under: Windows 10 x64. Available from: https://cran.r-project.org/bin/windows/base/old/3.6.3/

38. Barata A, Malfeito-Ferreira M, Loureiro V. The microbial ecology of wine grape berries. Int. J. Food Microbiol. 2012;153:243–59. https://doi.org/10.1016/j.ijfoodmicro.2011.11.025

39. Pinto C, Pinho D, Cardoso R, Custódio V, Fernandes J, Sousa S, et al. Wine fermentation microbiome: a landscape from different Portuguese wine appellations. Front. Microbiol. 2015;6:905. https://doi.org/10.3389/fmicb.2015.00905

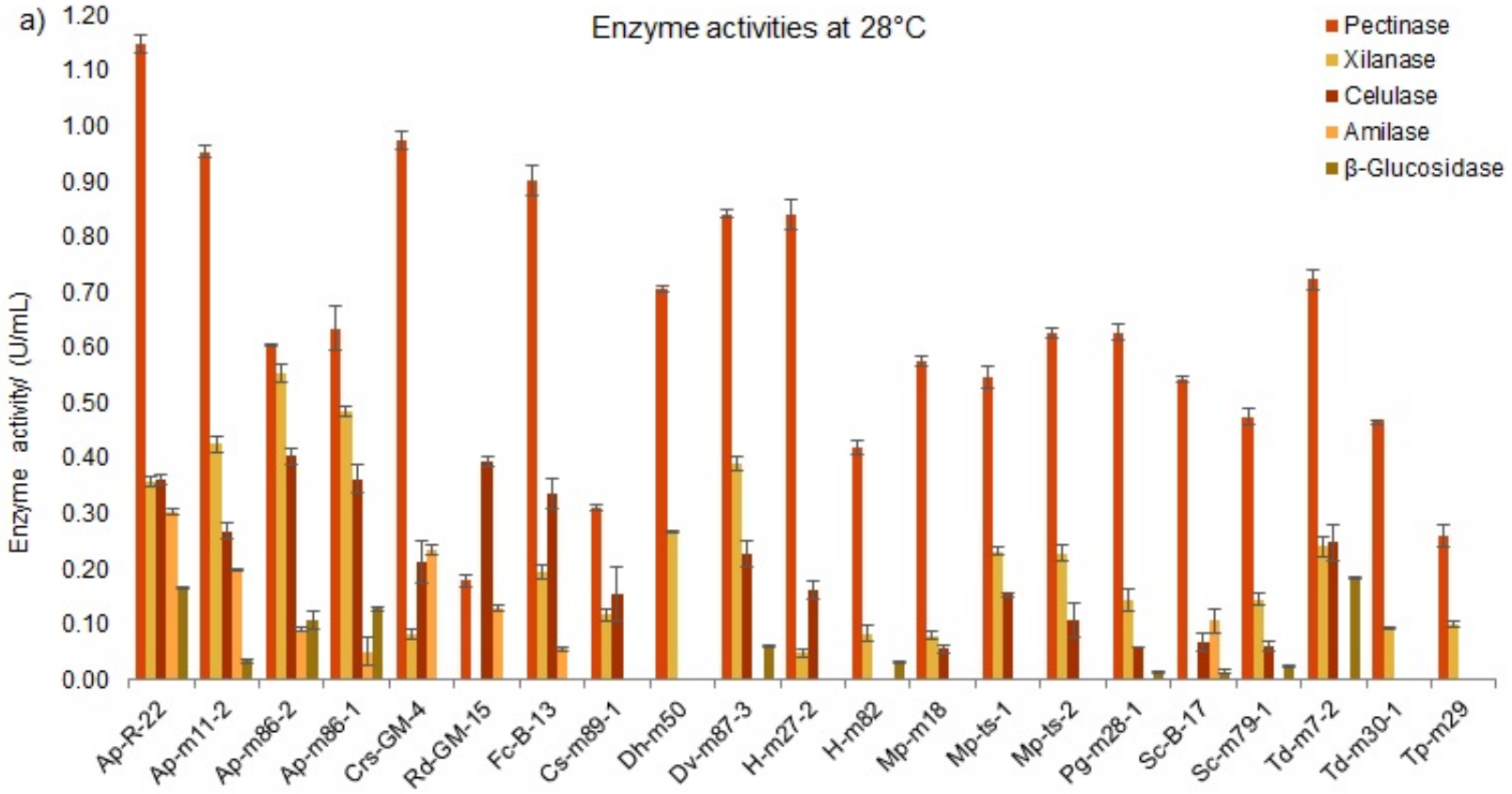
40. Sternes PR, Lee D, Kutyna DR, Borneman AR. A combined meta-barcoding and shotgun metagenomic analysis of spontaneous wine fermentation. GigaScience. 2017;6(7):gix040. https://doi.org/10.1093/gigascience/gix040

41. Rathnayake RMSP, Savocchia S, Schmidtke LM, Steel CC. Characterisation of *Aureobasidium pullulans* isolates from *Vitis vinifera* and potential biocontrol activity for the management of bitter rot of grapes. Eur J Plant Pathol. 2018;151(3):593–611. https://doi.org/10.1007/s10658-017-1397-0
42. Onetto CA, Borneman AR, Schmidt SA. Investigating the effects of *Aureobasidium pullulans* on grape juice composition and fermentation. Food Microbiol. 2020;90: 103451. https://doi.org/10.1016/j.fm.2020.103451
43. Gummadi SN, Kumar S, Aneesh CNA. Effect of salts on growth and pectinase production by halotolerant yeast, *Debaryomyces nepalensis* NCYC 3413. Curr Microbiol. 2007;54(6):472–6. https://doi.org/10.1007/s00284-007-0060-y
44. Elhalis H, Cox J, Frank D, Zhao J. Microbiological and biochemical performances of six yeast species as potential starter cultures for wet fermentation of coffee beans. LWT. 2021;137:110430. https://doi.org/10.1016/j.lwt.2020.110430
45. da Silva EG, Borges MF, Medina C, Piccoli RH, Schwan RF. Pectinolytic enzymes secreted by yeasts from tropical fruits. FEMS Yeast Res. 2005;5:859–65. https://doi.org/10.1016/j.femsyr.2005.02.006
46. Giese EC, Dussán KJ, Pierozzi M, Chandel AK, Pagnocca FC, Da Silva SS. Cellulase Production by *Trichosporon laibachii*. Orbital–Electron J Chem. 2017;9(4*):271*–78. https://doi.org/10.17807/orbital.v9i4.1024
47. van der Westhuizen TJ, Augustyn OPH, Pretorius IS. Geographical distribution of indigenous *Saccharomyces cerevisiae* strains isolated from vineyards in the coastal regions of the western cape in South Africa. South African J Enol Vitic. 2016;21(1):*3*–9. https://doi.org/10.21548/21-1-2179
48. Blanco P, Thow G, Simpson CG, Villa TG, Williamson B. Mutagenesis of key amino acids alters activity of a *Saccharomyces cerevisiae* endo-polygalacturonase expressed in Pichia pastoris. FEMS Microbiol Lett. 2002;210(2):187–91. https://doi.org/10.1111/j.1574-6968.2002.tb11179.x
49. Belda I, Ruiz J, Beisert B, Navascués E, Marquina D, Calderón F, et al. Influence of *Torulaspora delbrueckii* in varietal thiol (3-SH and 4-MSP) release in wine sequential fermentations. Int J Food Microbiol. 2017;257:183–91. https://doi.org/10.1111/j.1574-6968.2002.tb11179.x
50. Balmaseda A, Rozès N, Bordons A, Reguant C. *Torulaspora delbrueckii* promotes malolactic fermentation in high polyphenolic red wines. LWT. 2021;148.

https://doi.org/10.1016/j.lwt.2021.111777

51. Escribano R, González-Arenzana L, Garijo P, Berlanas C, López-Alfaro I, López R, et al. Screening of enzymatic activities within different enological non-*Saccharomyces* yeasts. J Food Sci Technol. 2017;54(6):1555–64. https://doi.org/10.1007/s13197-017-2587-7

52. Di Carlo BM, Pérez NP, Gómez de Díaz R, Navia F, Salomón R, Moyano F, *et al.* Antioxidant characteristics of red wines from Calchaquíes Valleys in Salta. J. Pharm. Pharmacol. 2017;5:907–12. https://doi.org/10.17265/2328-2150/2017.12.010



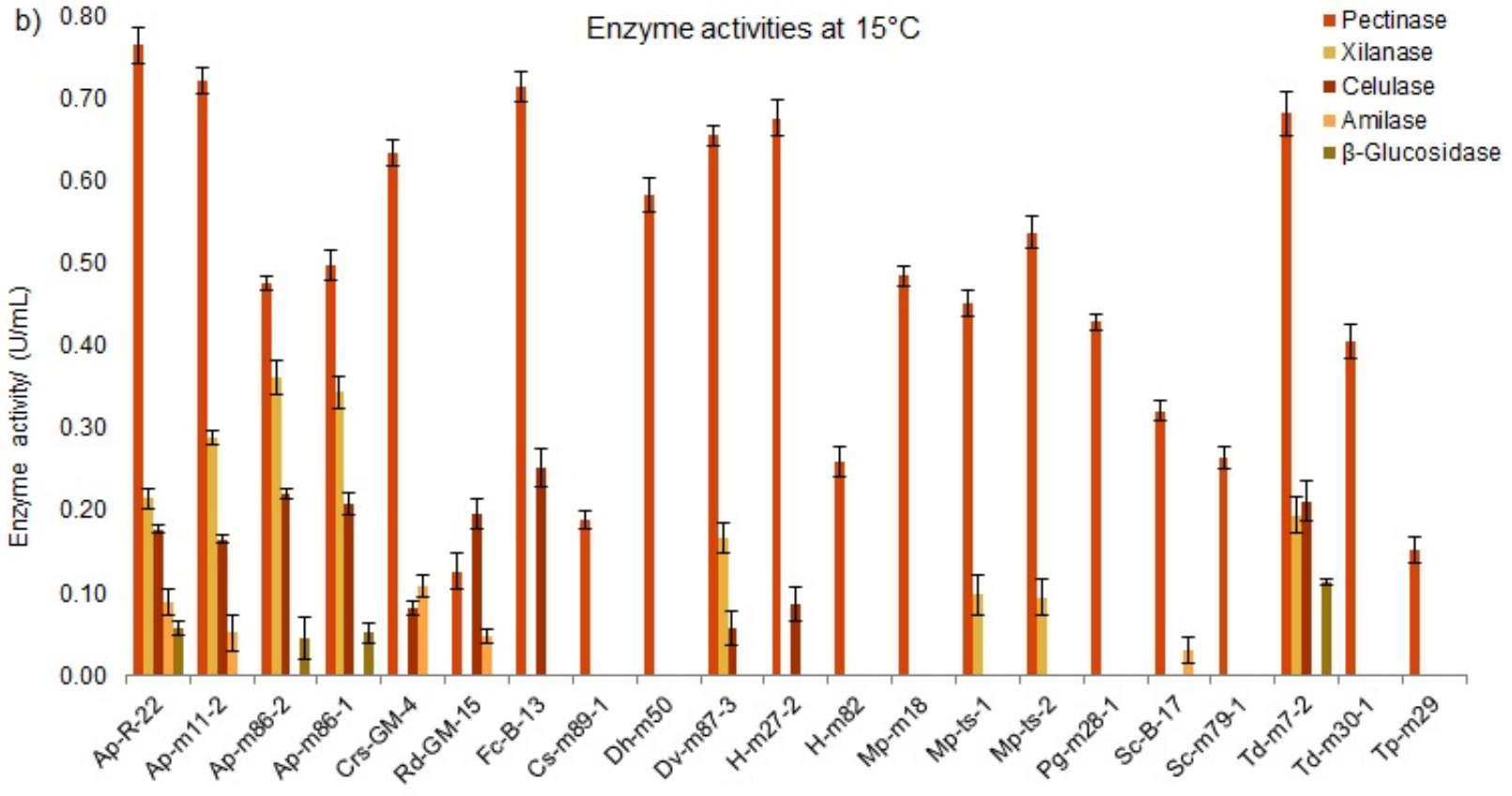
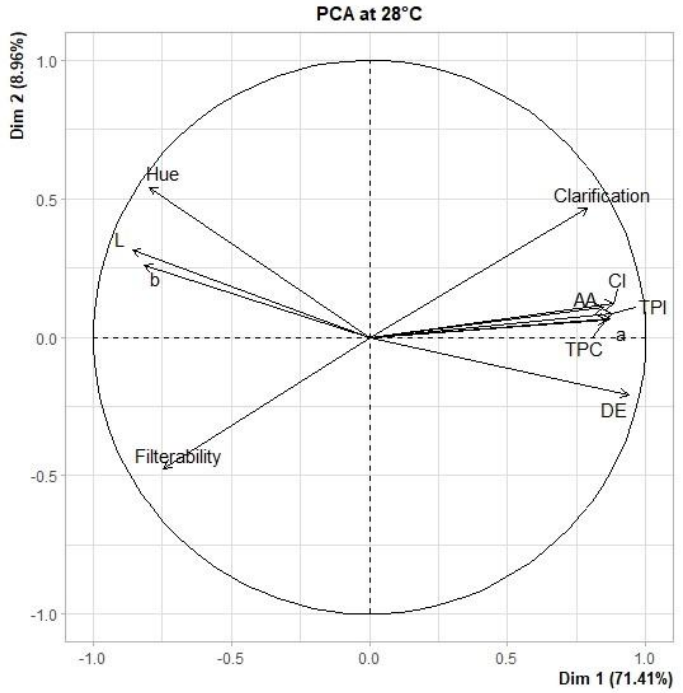
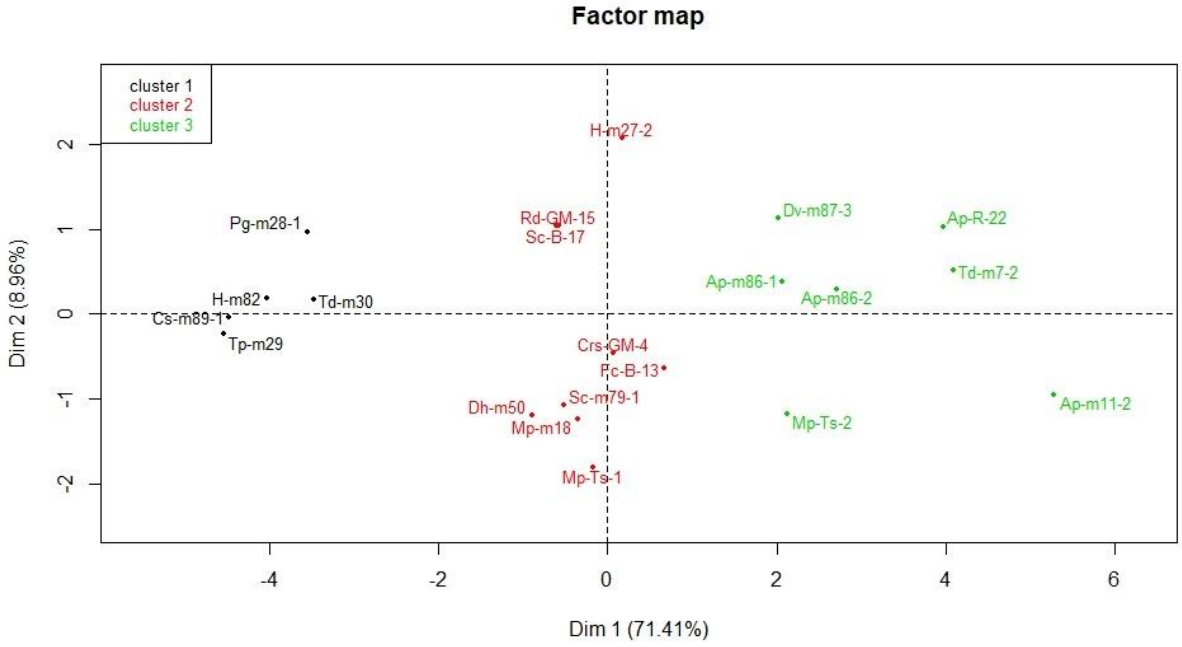


Fig. 1. Enzyme activities of oenological importance at a) 28 °C and b) 15 °C of selected strains (means ± SD, n=3). One unit of enzyme activity (U) was defined as the amount of enzyme required to release 1 μmol of reducing sugar per min at pH=3.8

a)





b)

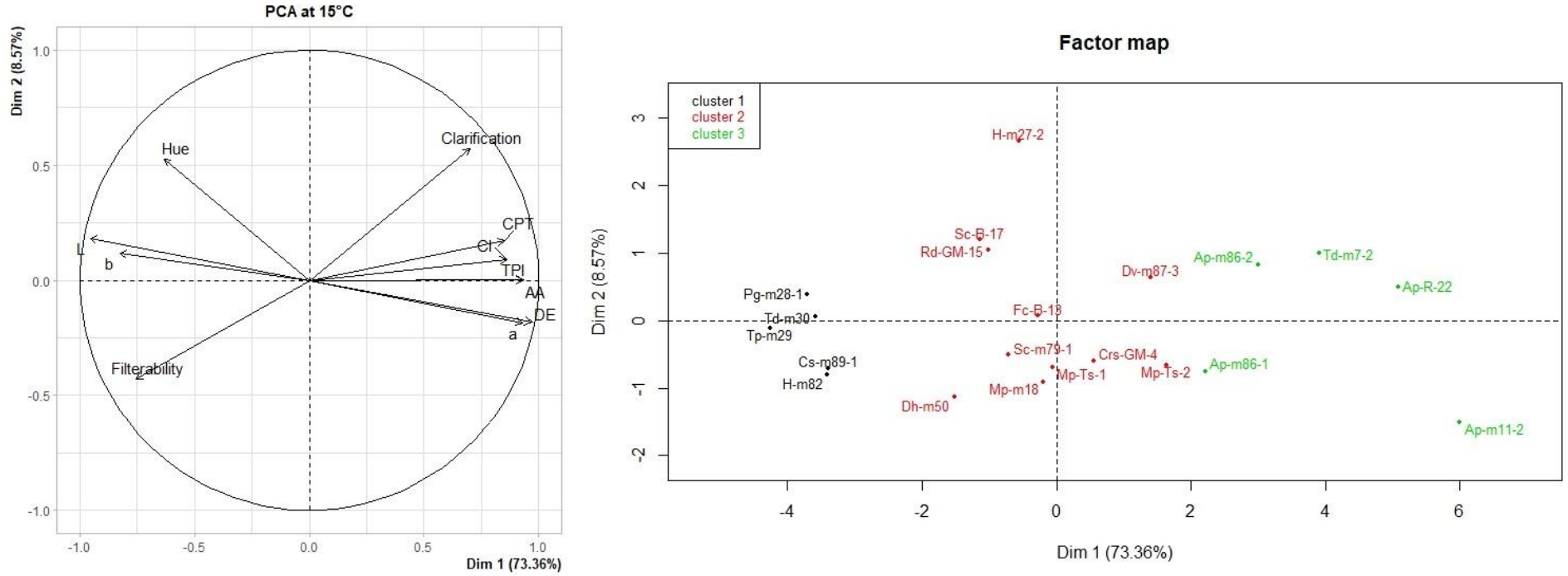


Fig. 2. Biplot graphs of the first two principal components using PCA and Factor Map for the effects of microbial enzyme extracts on technological parameters assayed for short maceration with Malbec must at a) 28 °C and b) 15 °C

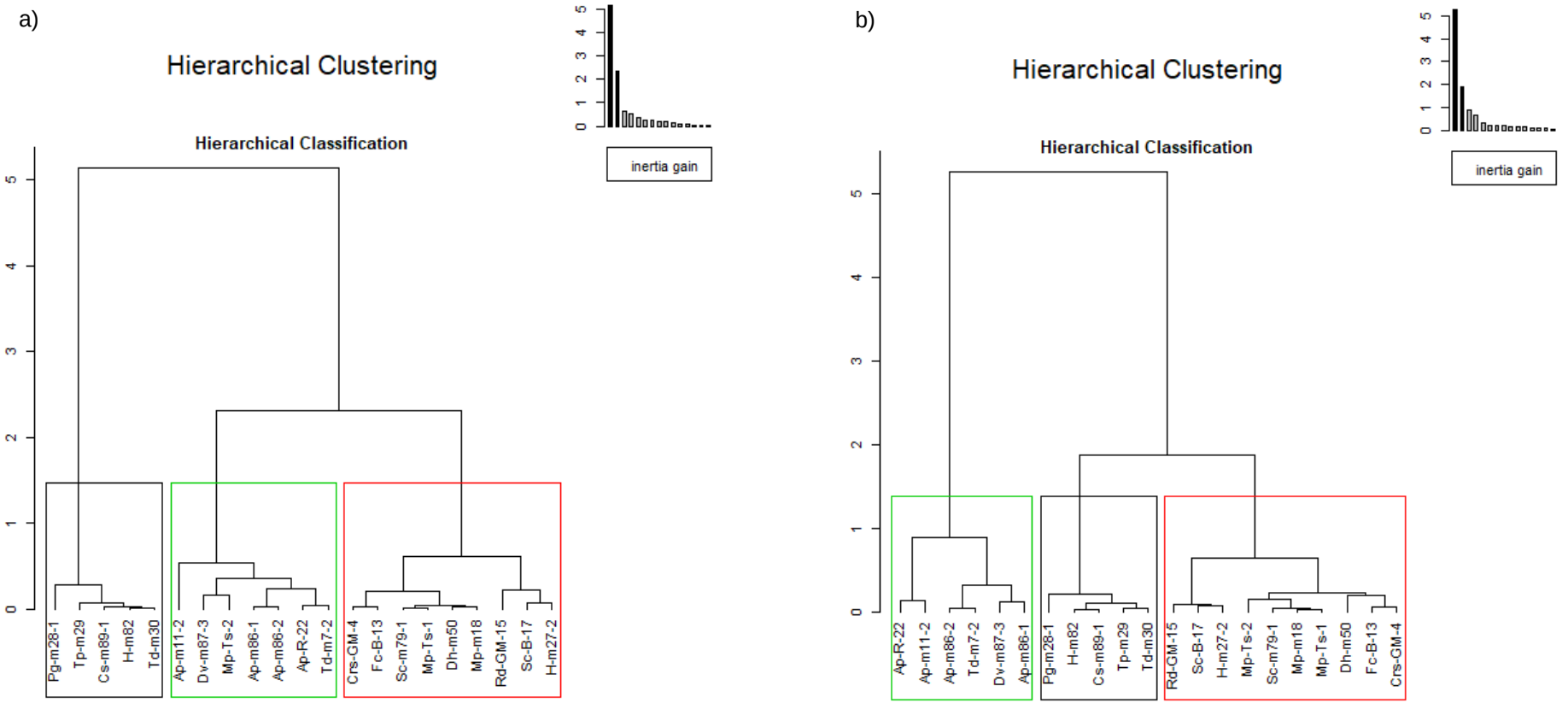


Fig. 3. Cluster analysis of microbial enzyme extracts analysed after their technological effects on maceration of Malbec grape must at a) 28 °C and b) 15 °

Table 1. Strains selected at 15 and 28 °C based on the halo diameter/colony diameter ratio (Dh/Dc) and substrate degradation, resulting from screening on solid medium

Strain	Pectinase	Xylanase	Cellulase	Amylase	Protease	β-Glucosidase
	Dh/Dc ratio					
15 °C						
m86-2	3.50	2.45	3.91	1.50	+	+
m86-1	2.36	2.00	2.20	1.80	-	+
m89-1	4.00	2.00	2.20	nd	-	-
m50	4.25	3.33	2.22	nd	-	-
m87-3	2.00	4.50	2.80	nd	+	+
m82	3.00	3.50	2.00	nd	-	+
ts-1	3.00	2.88	2.20	nd	+	-
ts-2	3.50	2.29	2.80	nd	+	-
m79-1	3.36	2.09	2.10	nd	-	+
28 °C						
m11-2	3.80	3.20	4.55	2.10	+	+
m27-2	6.67	6.67	2.75	nd	-	-
m18	4.25	2.88	2.20	nd	+	-
m28-1	2.50	7.00	2.50	nd	+	+
m7-2	4.00	3.38	2.40	nd	-	+
m30-1	2.00	3.50	2.50	nd	-	-
m29	2.30	4.29	2.20	nd	-	-

nd: not detected

Table 2. Identification of selected strains based on PCR-RFLP analyses of the ITS1-5.8S-ITS2 region of the rRNA gene

Strain	Species[e]	Taxonomy	AP (bp)[a]	Restriction length (bp)		
				*Cfo*I[b]	*Hae*III[c]	*Hinf*I[d]
m11-2	*Aureobasidium pullulans*	*Euascomycete*	600	190+180+100	450+150	290+180+130
m86-2	*Aureobasidium pullulans*		600	190+180+100	450+150	290+180+130
m86-1	*Aureobasidium pullulans*		600	190+180+100	450+150	290+180+130
m89-1	*Candida stellata*	Oxidative or weakly fermentative *Hemiascomycete*	500	220+130	470	250+230
m50	*Debaryomyces hansenii*		650	320+300+50	420+150+100	330+320
m87-3	*Debaryomyces vanrijiae*		650	310+300+50	420+140+90	320+320
m27-2	*Hanseniaspora* sp.		750	330+320+100	760	330+180+150+80
m82	*Hanseniaspora* sp.		750	320+310+100	740	330+180+150+70
m18	*Metschnikowia pulcherrima*		400	210+100+80	280+100	200+180
ts-1	*Metschnikowia pulcherrima*		400	210+100+80	280+100	200+180
ts-2	*Metschnikowia pulcherrima*		400	210+100+80	280+100	200+180
m28-1	*Pichia guilliermondii*		630	300+260+80	400+110+80	320+300
m79-1	*Saccharomyces cerevisiae*	Fermentative *Hemiascomycete*	880	390+360	320+220+180+150	360+150
m7-2	*Torulaspora delbrueckii*		800	330+220+150+100	800	410+380
m30-1	*Torulaspora delbrueckii*		800	330+220+150+100	800	410+380
m29	*Torulaspora pretoriensis*		825	380+330+110	800	380+290+125

[a]AP 5.8S-ITS-amplified product size; [b,c,d]Restriction enzymes used; [e]Species assigned according to Esteve-Zarzoso *et al.* (*27*) and/or www.yeast-id.com data base

Table 3. Effect of microbial enzyme extracts on extraction of pigments, polyphenol content and antioxidant activity of Malbec grape must macerations at 28 and 15 °C. Grape musts were incubated under shaking (100 rpm) for 6 h and supplemented with enzyme extracts, except for control without enzymes (C1). A reference treatment with a commercial enzyme supplemented at identical net enzyme units (EU) to those of the enzyme extracts (C2) was also included

Assays 28 °C	TPI	CI	Hue	*L**	*a**	*b**	ΔE	TPC	AA
C1	47.5±0.1	9.70±0.10	0.760±0.002	73.2±1.6	26.9±1.0	4.1±1.5	-	624.1±1.0	420.2±2.1
C2	53.6±0.2	10.50±0.04	0.702±0.004	70.3±1.5	30.2±1.4	1.2±1.3	5.3	719.1±1.4	473.8±1.4
Ap-R-22	(55.4±0.0)^{ab}	(11.71±0.03)^{ab}	(0.671±0.003)^{ab}	(66.8±1.1)^{ab}	(32.8±1.2)^{a}	(1.0±1.2)^{a}	9.2	(741.2±0.7)^{ab}	(527.2±1.3)^{ab}
Ap-m11-2	(58.6±0.0)^{ab}	(12.61±0.05)^{ab}	(0.572±0.001)^{ab}	(64.5±1.3)^{ab}	(30.7±1.0)^{a}	(0.5±1.6)^{a}	10.2	(746.8±1.2)^{ab}	(526.9±1.1)^{ab}
Ap-m86-2	(56.6±0.2)^{ab}	(11.53±0.04)^{ab}	(0.643±0.002)^{ab}	(67.2±1.4)^{ab}	(31.5±0.9)^{a}	(1.8±0.8)^{a}	7.9	(744.1±1.4)^{ab}	(516.1±0.8)^{ab}
Ap-m86-1	(55.6±0.4)^{ab}	(10.91±0.02)^{ab}	(0.670±0.003)^{ab}	(66.4±1.2)^{ab}	(30.8±0.9)^{a}	(2.0±1.4)^{a}	8.1	(742.7±1.1)^{ab}	(511.6±2.3)^{ab}
Crs-GM-4	(54.2±0.2)^{ab}	(11.50±0.02)^{ab}	(0.650±0.003)^{ab}	(67.1±1.5)^{ab}	(28.9±1.5)^{a}	(2.9±1.3)	6.5	(722.3±0.6)^{a}	(484.2±1.3)^{ab}
Rd-GM-15	(49.1±0.1)^{ab}	(11.20±0.02)^{ab}	(0.750±0.004)^{b}	(68.3±0.9)^{a}	(31.0±2.3)^{a}	(1.9±1.4)^{a}	6.8	(670.9±0.5)^{ab}	(478.6±1.9)^{a}
Fc-B-13	(56.2±0.2)^{ab}	(11.10±0.03)^{ab}	(0.673±0.001)^{ab}	(66.3±1.2)^{ab}	(30.2±1.3)^{a}	(2.7±1.8)^{a}	7.8	(728.2±1.7)^{ab}	(489.0±0.9)^{ab}
Cs-m89-1	(47.7±0.5)^{b}	(9.90±0.02)^{ab}	(0.773±0.004)^{b}	(68.9±1.0)^{a}	(27.1±1.1)	(3.2±1.1)^{b}	4.4	(633.4±1.1)^{ab}	(472.6±1.7)^{a}
Dh-m50	(51.2±0.2)^{ab}	(10.70±0.01)^{ab}	(0.641±0.004)^{ab}	(68.5±1.0)^{ab}	(30.5±2.1)^{a}	(2.1±1.0)^{a}	6.2	(689.5±0.6)^{ab}	(480.6±1.0)^{a}
Dv-m87-3	(54.6±0.4)^{a}	(11.81±0.01)^{ab}	(0.670±0.006)^{ab}	(65.9±1.4)^{ab}	(30.8±1.3)^{a}	(2.8±1.1)	8.4	(718.9±0.7)^{a}	(484.7±1.1)^{ab}
H-m27-2	(55.1±0.3)^{ab}	(11.55±0.01)^{ab}	(0.730±0.006)^{ab}	(68.1±1.3)^{ab}	(29.5±2.0)	(3.4±1.7)^{b}	5.8	(729.3±0.4)^{ab}	(485.4±1.7)^{ab}
H-m82	(48.0±0.3)^{b}	(10.42±0.04)^{b}	(0.750±0.004)^{b}	(69.5±0.9)^{a}	(27.8±1.4)	(3.5±1.3)^{b}	3.8	(646.6±1.0)^{ab}	(478.3±0.7)^{a}
Mp-m18	(51.9±0.1)^{ab}	(10.90±0.02)^{ab}	(0.672±0.005)^{ab}	(67.0±1.8)^{ab}	(30.8±1.8)	(2.3±1.2)^{a}	7.5	(706.3±1.1)^{ab}	(480.3±0.8)^{a}

Mp-Ts-1	(53.1±0.1)^{a}	(10.80±0.03)^{ab}	(0.640±0.002)^{ab}	(66.2±1.9)^{ab}	(28.9±1.1)	(2.0±0.9)^{a}	7.6	(710.8±1.0)^{ab}	(481.9±0.6)^{ab}
Mp-Ts-2	(54.4±0.1)^{ab}	(11.50±0.04)^{ab}	(0.611±0.003)^{ab}	(65.3±1.7)^{ab}	(31.3±1.0)	(1.6±1.7)^{a}	9.4	(715.0±0.8)^{a}	(484.7±1.0)^{ab}
Pg-m28-1	(53.4±0.2)^{b}	(10.81±0.02)^{ab}	(0.790±0.003)^{ab}	(71.1±0.8)	(27.4±1.9)	(3.1±1.1)^{b}	2.4	(711.7±0.6)^{a}	(477.5±1.8)^{a}
Sc-B-17	(53.5±0.4)^{a}	(11.13±0.04)^{ab}	(0.721±0.005)^{a}	(68.8±0.9)^{a}	(30.5±1.9)	(3.1±1.2)^{b}	5.8	(717.3±0.1)^{a}	(483.1±2.1)^{ab}
Sc-m79-1	(51.0±0.4)^{ab}	(11.10±0.02)^{ab}	(0.660±0.003)^{ab}	(67.2 ± 1.9)^{ab}	(29.8±1.3)	(2.5±1.4)^{a}	6.8	714.1 ± 0.5^{*1}	(482.1±2.3)^{ab}
Td-m7-2	(57.9±0.1)^{ab}	(11.90±0.03)^{ab}	(0.631±0.002)^{ab}	(66.2±1.1)^{ab}	(31.9±1.5)^{a}	(0.9±1.1)^{a}	9.2	(735.5±0.6)^{ab}	(508.3±1.4)^{ab}
Td-m30	(48.4±0.3)^{b}	(10.70±0.01)^{ab}	(0.735±0.003)^{ab}	(69.3±1.6)^{a}	(28.1±2.1)	(3.2±1.7)^{b}	4.2	(649.4±1.0)^{ab}	(475.5±1.9)^{a}
Tp-m29	(48.0±0.5)^{b}	(10.61±0.02)^{a}	(0.724±0.003)^{ab}	(71.4±1.8)	(27.8±1.4)	(2.9±1.3)	2.3	(644.3±1.0)^{ab}	(471.2±1.1)^{a}
Assays 15 °C									
C1	47.7±0.2	9.80±0.01	0.760±0.005	74.1±1.4	26.2±1.2	3.8±1.0	-	628.7±1.2	440.6±2.2
C2	54.1±0.1	10.90±0.03	0.612±0.005	70.6±1.1	31.0±1.5	-1.0±1.1	7.6	724.8±0.5	482.9±1.9
Ap-R-22	(56.6±0.1)^{ab}	(12.10±0.03)^{ab}	(0.610±0.003)^{a}	(67.2±1.0)^{ab}	(34.0±1.1)^{ab}	(-1.2±0.8)^{a}	11.5	(749.1±0.5)^{ab}	(553.3±1.9)^{ab}
Ap-m11-2	(59.1±0.4)^{ab}	(12.71±0.03)^{ab}	(0.553±0.003)^{ab}	(65.0±1.1)^{ab}	(35.1±1.0)^{ab}	(-0.5±0.9)^{a}	13.4	(750.5±0.6)^{ab}	(548.0±1.4)^{ab}
Ap-m86-2	(57.1±0.1)^{ab}	(11.55±0.08)^{ab}	(0.621±0.008)^{a}	(67.9±1.7)^{ab}	(32.3±1.4)^{a}	(2.0±1.1)	8.9	(747.2±0.3)^{ab}	(537.6±0.9)^{ab}
Ap-m86-1	(55.9±0.3)^{ab}	(10.72±0.01)^{ab}	(0.550±0.002)^{ab}	(68.0±0.9)^{a}	(31.9±1.7)^{a}	(2.7±1.2)	8.4	(747.0±0.7)^{ab}	(531.9±1.5)^{ab}
Crs-GM-4	(53.9±0.1)^{a}	(11.50±0.03)^{ab}	(0.581±0.001)^{ab}	(69.4±1.4)^{a}	(29.9±1.4)^{a}	(2.1±0.9)	6.2	(720.2±0.9)^{ab}	(496.9±1.3)^{ab}
Rd-GM-15	(50.8±0.1)^{ab}	(11.20±0.01)^{ab}	(0.650±0.002)^{ab}	(70.4±1.4)^{a}	(27.8±0.9)	(2.3±1.4)	4.3	(675.3±0.4)^{ab}	(490.3±1.2)^{a}
Fc-B-13	(56.2±0.2)^{ab}	(11.40±0.03)^{ab}	(0.610±0.006)^{a}	(69.7±1.4)^{a}	(27.1±1.0)^{b}	(3.7±1.1)^{b}	4.5	(726.3±0.7)^{a}	(506.1±1.7)^{ab}
Cs-m89-1	(48.7±0.2)^{b}	(10.30±0.06)^{ab}	(0.620±0.002)^{a}	(71.2±2.1)	(26.5±1.2)^{b}	(3.4±0.8)^{b}	2.9	(631.3±1.0)^{b}	(480.2±0.8)^{a}
Dh-m50	(52.8±0.2)^{ab}	(10.73±0.01)^{ab}	(0.572±0.002)^{ab}	(71.1±1.6)	(28.2±0.9)	(3.4±0.8)^{b}	3.6	(688.5±0.1)^{ab}	(489.9±1.8)^{a}
Dv-m87-3	(55.2±0.1)^{ab}	(11.10±0.04)^{ab}	(0.570±0.004)^{ab}	(68.6±1.1)^{a}	(30.1±0.8)^{a}	(3.0±1.3)^{b}	6.8	(721.9±0.7)^{a}	(498.4±0.9)^{ab}

H-m27-2	(52.5±0.2)^{ab}	(11.43±0.04)^{ab}	(0.680±0.001)^{ab}	(72.0±0.9)	(28.0±1.2)	(2.8±0.9)^{b}	2.9	(724.5±1.3)^{a}	(499.2±2.1)^{ab}
H-m82	(48.9±0.2)^{b}	(10.32±0.03)^{ab}	(0.634±0.001)^{a}	(72.8±1.7)	(28.2±1.7)	(3.3±0.9)^{b}	2.4	(642.9±0.9)^{ab}	(488.1±0.7)^{a}
Mp-m18	(52.6±0.1)^{ab}	(10.10±0.08)^{a}	(0.610±0.004)^{a}	(69.9±1.1)^{a}	(31.3±1.2)^{a}	(2.2±0.8)	6.8	(711.3±1.8)^{ab}	(493.5±1.4)^{ab}
Mp-Ts-1	(54.4±0.1)^{a}	(11.10±0.03)^{ab}	(0.635±0.001)^{ab}	(69.5±1.4)^{a}	(30.2±1.6)^{a}	(2.5±1.2)	6.2	(714.0±0.6)^{ab}	(505.3±0.8)^{ab}
Mp-Ts-2	(55.1±0.2)^{ab}	(11.81±0.05)^{ab}	(0.624±0.002)^{a}	(68.1±1.6)^{a}	(32.0±0.9)^{a}	(1.7±1.5)	8.6	(720.0±0.9)^{ab}	(514.1±1.0)^{ab}
Pg-m28-1	(51.3±0.2)^{ab}	(10.51±0.06)^{ab}	(0.690±0.008)^{ab}	(73.2±1.7)^{b}	(25.8±1.8)^{b}	(3.9±0.9)^{b}	1.0	(707.6±0.7)^{ab}	(488.4±2.4)^{a}
Sc-B-17	(51.6±0.2)^{ab}	(11.10±0.03)^{ab}	(0.660±0.004)^{ab}	(71.0±1.9)	(29.2±1.4)^{b}	(4.1±1.1)^{a}	4.3	(713.6±0.1)^{ab}	(494.9±1.4)^{ab}
Sc-m79-1	(51.0±0.4)^{ab}	(11.20±0.03)^{ab}	(0.631±0.007)^{ab}	(70.7±1.5)^{a}	(30.2±1.7)^{a}	(1.7±0.6)	5.7	(709.1±1.1)^{ab}	(494.0±0.6)^{ab}
Td-m7-2	(58.5±0.1)^{ab}	(11.94±0.02)^{ab}	(0.610±0.001)^{ab}	(67.3±1.0)^{ab}	(32.5±1.7)^{a}	(1.0±1.0)^{a}	9.7	(739.5±0.6)^{ab}	(528.3±1.3)^{ab}
Td-m30	(48.1±0.2)^{b}	(11.01±0.02)^{ab}	(0.660±0.002)^{ab}	(72.5±1.3)	(26.6±1.2)^{b}	(3.5±1.3)^{b}	1.7	(648.3±0.6)^{ab}	(484.4±0.4)^{a}
Tp-m29	(48.2±0.3)^{b}	(10.20±0.03)^{ab}	(0.690±0.001)^{ab}	(72.1±1.1)	(26.8±1.5)^{b}	(3.3±1.2)^{b}	2.2	(643.5±0.2)^{ab}	(478.7±1.6)^{a}

Ap: *Aureobasidium pullulans*, Crs: *Cryptococcus saitoi*, Rd: *Rhodotorula dairenensis*, Fc: *Filabasidium capsuligenum*, Cs: *Candida stellate,* Dh: *Debaryomyces hansenii,* Dv: *Debaryomyces vanrijiae,* H: *Hanseniaspora sp.,* Mp: *Metschnikowia pulcherrima,* Pg: *Pichia guilliermondii,* Sc: *Saccharomyces cerevisiae,* Td: *Torulaspora delbrueckii,* Tp: *Torulaspora pretoriensis.* C1: Control without enzymes; C2: Control with commercial enzyme (1*U*/*mL*). ^{ab}, there are significant differences with respect to both controls C1 and C2; ^{a}, there are significant differences with respect to the control without enzyme C1; ^{b}, there are significant differences with respect to the control with enzyme treatment C2; Without letters, there are no significant differences with respect to the controls.

Table 4. Effect of microbial enzyme extracts on filterability and clarification of white grape must macerations at 28 and 15 °C

Assays	Filterability (s/mL)		Clarification (T650)	
	28 °C	15 °C	28 °C	15 °C
C1	388.0±0.4	380.1±0.1	12.9±1.2	12.8±1.2
C2	238.0±0.4	210.4±0.2	65.0±1.3	67.5±1.3
Ap-R-22	(266.6±1.1)^{ab}	(250.4±1.0)^{ab}	(48.9±1.1)^{ab}	(55.4±1.0)^{ab}
Ap-m11-2	(268.5±0.9)^{ab}	(256.4±1.0)^{ab}	(40.8±1.3)^{ab}	(42.5±0.8)^{ab}
Ap-m86-2	(354.0±1.4)^{ab}	(283.2±1.0)^{ab}	(53.0±1.4)^{ab}	(55.2±1.2)^{ab}
Ap-m86-1	(312.3±0.8)^{ab}	(296.6±0.8)^{ab}	(46.7±1.4)^{ab}	(47.9±1.3)^{ab}
Crs-GM-4	(378.0±1.4)^{b}	(361.0±1.1)^{ab}	(42.6±1.8)^{ab}	(48.9±0.6)^{ab}
Rd-GM-15	(315.7±1.6)^{ab}	(282.5±0.8)^{ab}	(45.4±1.6)^{ab}	(46.4±1.0)^{ab}
Fc-B-13	(378.3±0.9)^{ab}	(355.0±1.4)^{ab}	(40.5±1.8)^{ab}	(45.0±1.0)^{ab}
Cs-m89-1	(370.5±2.1)^{ab}	(351.9±0.9)^{ab}	(31.7±1.2)^{ab}	(37.5±0.7)^{ab}
Dh-m50	(365.5±0.6)^{ab}	(327.8±1.3)^{ab}	(33.4±1.5)^{ab}	(35.3±1.0)^{ab}
Dv-m87-3	(272.5±0.7)^{ab}	(255.0±1.2)^{ab}	(50.5±1.3)^{ab}	(54.4±0.9)^{ab}
H-m27-2	(299.0±1.5)^{ab}	(264.3±1.4)^{ab}	(48.9±1.3)^{ab}	(53.4±0.7)^{ab}
H-m82	(363.0±1.5)^{ab}	(346.5±0.9)^{ab}	(29.7±1.4)^{ab}	(32.3±0.9)^{ab}
Mp-m18	(379.9±0.8)^{b}	(368.2±0.9)^{ab}	(34.4±1.3)^{ab}	(43.1±1.1)^{ab}
Mp-Ts-1	(375.5±0.8)^{ab}	(322.5±1.5)^{ab}	(33.5±1.0)^{ab}	(34.3±0.8)^{ab}
Mp-Ts-2	(340.3±0.9)^{ab}	(336.6±0.8)^{ab}	(43.5±1.7)^{ab}	(43.0±1.0)^{ab}
Pg-m28-1	(390.0±1.4)^{b}	(379.6±0.9)^{b}	(30.7±1.1)^{ab}	(33.0±1.5)^{ab}
Sc-B-17	(324.3±0.9)^{ab}	(309.2±1.3)^{ab}	(39.4±1.6)^{ab}	(46.5±0.9)^{ab}
Sc-m79-1	(368.0±1.5)^{ab}	(335.6±0.8)^{ab}	(33.4±0.7)^{ab}	(36.0±1.1)^{ab}
Td-m7-2	(271.1±1.3)^{ab}	(249.3±1.1)^{ab}	(51.6±2.0)^{a}	(57.0±1.5)^{a}
Td-m30	(383.7±0.9)^{b}	(370.5±0.9)^{b}	(36.4±1.5)^{ab}	(38.2±1.0)^{ab}
Tp-m29	(380.7±1.1)^{b}	(366.2±1.4)^{ab}	(26.1±1.7)^{ab}	(33.2±1.1)^{ab}

C1: Control without enzymes; C2: Control with commercial enzyme (1*U*/*mL*). ^{ab}, there are significant differences with respect to both controls C1 and C2; ^{a}, there are significant differences with respect to the control without enzyme treatment C1; ^{b}, there are significant differences with respect to the control with enzyme treatment C2; Without letters, there are no significant differences with respect to the controls.