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Impact of high hydrostatic pressure and ultrasounds technologies in the autolytic process of *Saccharomyces cerevisiae* in a model wine system

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ABSTRACT

Autolysis plays a crucial role as a technological tool in the ageing process of specific wines. This includes white, red and sparkling wines. During ageing on the lees, yeasts release different compounds that positively modify the composition of the wine. However, traditional autolytic methods can be time-consuming. This work evaluates the use of different ultrasound (US) and high hydrostatic pressure (HHP) treatments to expedite the autolytic process in *Saccharomyces cerevisiae* in a model wine system. The results suggest that treating yeast cells with US resulted in a faster release of nucleic acids, proteins, and total polysaccharides compared to HHP treatment. The environmental scanning electron microscopy (ESEM) of the treated lees demonstrated that the impact on the yeast cell surface was more pronounced after exposure to US compared to treatments involving HHP. In conclusion, under these conditions the US treatment effectively triggered autolysis in the wine yeast strain, facilitating the release of macromolecules in a model wine.

1. Introduction

Ageing on the lees is a traditional method extensively employed in the production of both white and red wines, along with its application in sparkling wines. In this type of ageing, the wine continues interacting with its fermentation lees, mainly composed of yeasts, tartaric acid crystals and plant material from the grapes, during a period of varying length (Fornairon-Bonnefond et al., 2002). Along the ageing on the lees, a phenomenon called yeast autolysis takes place. The yeasts are self-degraded by their own enzymes, modifying their structure and enhancing the wine with elements from the yeast's cytoplasm and cell wall, primarily glycoproteins, polysaccharides, nucleic acids, and lipids (Comuzzo et al., 2022). These compounds released into the wine increase its proteic, tartaric and colour stability, as well as improve its physicochemical and sensory properties (Alexandre, 2022). It has been described that this winemaking technique reduces the wine astringency and greenness, increases its roundness and volume in mouth (Rinaldi et al., 2019), as well as provides a greater aromatic complexity to the wine (Masino et al., 2008).

Despite these positive effects on wine, ageing on lees is a very expensive process as it consumes a considerable amount of time and effort in the winery. In still wines, periodic lees stirring is achieved through "bâtonnage" for 3–6 months, which raises the risk of microbial spoilage. (Alexandre & Guilloux-Benatier, 2006). In the traditional method of producing sparkling wines, the ageing period on lees must last a minimum of 9–12 months (Buxaderas et al., 2022) causing a significant impact on production costs. Therefore, the exploration of novel technological approaches to expedite yeast autolysis and consequently shorten the ageing period of wines in contact with lees is highly interesting (Morata et al., 2019).

Novel techniques to accelerate yeast autolysis, such as ultrasounds (US), microwaves (MW), pulsed electric fields (PEF) and high pressure homogenization (HPH) have been explored in winemaking. Cavitation generated by high-frequency US waves (above 20 kHz) has been employed as a technique to break down cellular structures (Cárcel et al., 2012). This treatment mainly promoted the liberation of macromolecules from yeast lees, encompassing cell wall glycoproteins and polysaccharides from cytosolic content (Cacciola et al., 2013; del Fresno et al., 2019). MW treatment has been also tested to accelerate the autolytic process in wine yeasts. It was recently proved that MW can cause a morphological disruption of the yeasts (Gnoinski et al., 2021) and an increased extraction of the cell components (Liu et al., 2016). The

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application of high-intensity intermittent electric fields by PEF for brief durations on yeasts has been reported as an effective technology to cause local pores in their cytoplasmic membranes and change their cell wall structure, which triggers cell lysis and the release of mannoproteins (Martínez et al., 2016, 2018).

Regarding techniques based on the application of pressure, HPH has been studied as a strategy to induce yeast autolysis, observing that its application in a wine yeast suspension enhances the liberation of proteins, glucidic colloids and amino acids from cells (Comuzzo et al., 2015, 2017; Dimopoulos et al., 2020). Cell disruption by HPH relies on cavitation, shear, and turbulence phenomena when a yeast suspension is compelled to flow through a tight gap in a homogenizer valve (Chevalier-Lucia & Picart-Palmade, 2019; Comuzzo & Calligaris, 2019).

High hydrostatic pressure (HHP) is another attractive technique that involves applying pressures ranging from 100 to 600 MPa to the sample. A fluid, usually water, acts as a medium to transmit the pressure to the sample contained in a vessel. It has been reported that HHP causes a damage to the outer layer of the cell wall of *S. cerevisiae*, leading to an opening and an absence of cell wall on the yeast surface (Marx et al., 2011). Nevertheless, there has been relatively little focus on employing HHP to accelerate the yeast autolytic process in winemaking, as the applications reported are mainly limited to the microbiological stabilization of must and wine (Buzrul, 2012; Tomašević et al., 2020).

Based on literature results, HHP technology could be an attractive technique to enhance the autolytic process in wine yeasts, nevertheless there is no information about the effect of HHP on the release of macromolecules from pressure-treated wine yeasts.

Therefore, the novelty of this research is the comparison between two emerging technologies (HHP and US) on inducing yeast autolysis with the possibility to accelerate this process and consequentially the period of wine aging on lees. Another new feature is the possibility to improve wine quality and stability in shorter times and to reduce times and costs of the overall production process of aged wines.

Thus, the aim of this work was to evaluate comparatively the effect of different HHP and US treatments to accelerate the autolytic process of *S. cerevisiae* in a model wine system. The release of macromolecules during ageing on lees and the changes in the morphological structure of yeasts at the end of this process were assessed.

2. Material and methods

2.1. Chemical reagents

All chemicals were analytical quality grade and purchased from Panreac, S.A. (Madrid, Spain).

2.2. Yeast strain

The *S. cerevisiae* strain utilized in this study was a wine yeast commercially available as active dried yeast (Lalvin EC1118, Lallemand S.A., Montréal, QC, Canada).

2.3. Model wine preparation

The ageing on lees process was simulated in a model wine composed of water and ethanol 14% (v/v), tartaric acid (5 g/L), pH adjusted to 3.8 with 1 M NaOH and sulphited to 90 mg/L of total SO_2 using $K_2S_2O_5$ (del Fresno et al., 2018).

2.4. High hydrostatic pressure (HHP) treatments

The model wine with 1% (w/v) of yeasts was treated in a discontinuous high hydrostatic pressure unit (Hiperbaric 55, Hiperbaric S.A., Burgos, Spain) equipped with a cylinder of 0.20 m of diameter and 2.0 m of length. The operating conditions of HHP treatments (pressure applied and time) were chosen based on the recommendations of the company's technicians (Hiperbaric S.A). Polyethylene terephthalate (PET) bottles of 330 mL were completely filled with the suspension of yeasts and placed into a cylindrical container of 55 L. The pressure fluid was water, the rise time to the designated pressure was less than 180 s, and the depressurisation was less than 3 s. The initial temperature was 21 °C, and it increased about 3 °C per 100 MPa due to adiabatic heating. The following treatments were carried out on the model wine with yeasts: H4-3 (400 MPa for 3 min), H4-5 (400 MPa for 5 min), H4-10 (400 MPa for 10 min), H5-3 (500 MPa for 3 min), H5-5 (500 MPa for 5 min), H5-10 (500 MPa for 10 min), H6-3 (600 MPa for 3 min), H6-5 (600 MPa for 5 min), H6-10 (600 MPa for 10 min) and an untreated control sample (C) was used.

2.5. Ultrasound (US) treatments

An UP400S ultrasonic processor (400 W and 24 kHz) (Hielscher Ultrasonics, Teltow, Germany) equipped with a S24d22D sonotrode made of titanium (22 mm of diameter, submerged depth of 30 mm) at 80% on-off pulse was used to carry out the sonication. The model wine with 1% of yeasts (330 mL) was treated in a glass jacket containing circulating water from a water bath to set the target temperature under 25 °C and keep it constant during the different treatments (Ferraretto et al., 2013). Three different amplitude levels (30%, 60% and 90%) and three processing times (3, 5 and 10 min) were modified based on scientific literature (Cacciola et al., 2013; Ferraretto et al., 2013). The following treatments were carried out on the model wine with yeasts: U3-3 (30% for 3 min), U3-5 (30% for 5 min), U3-10 (30% for 10 min), U6-3 (60% for 3 min), U6-5 (60% for 5 min), U6-10 (60% for 10 min), U9-3 (90% for 3 min), U9-5 (90% for 5 min), U9-10 (90% for 10 min) and an untreated control sample (C) was used.

2.6. Simulated ageing on lees process

After US and HHP processing, samples (330 mL) were divided in Falcon tubes and the simulated ageing on lees was carried out by triplicate during 42 days at 33 °C in an orbital shaker (150 rpm) (Orbital Shaker SO1, Stuart Scientific, Stone, UK). Control samples were also incubated. Samples were chemically analysed at 0, 14, 28 and 42 days. Before the analysis, the samples were centrifuged at 3260×*g* during 5 min (Sorvall ST 8R Centrifuge, Osterode am Harz, Germany).

2.7. Analytical methods

Protein and nucleic acid content of the samples were analysed for their maximum absorbances at 280 and 260 nm, respectively, (Martínez et al., 2018), using a Genesys 150 spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). Total polysaccharide content at the conclusion of the ageing period was quantified according to phenol-sulfuric acid method (Segarra et al., 1995). All the analyses were performed in triplicate.

2.8. Environmental scanning electron microscopy (ESEM)

After 42 days of ageing on lees, high hydrostatic pressure-treated (H4-10, H5-10 and H6-10) and ultrasonic-treated (U3-10, U6-10 and U9-10) yeasts were subjected to freeze-drying at −40 °C for dehydration (Lyoquest-55 ECO, Telster, Barcelona, Spain). To preserve the samples with minimal distortion, they were examined in a Quanta 200FEG ESEM (Hillsboro, Oregon, USA) operated under low vacuum (LV) conditions using a large field electron detector (LFD) at a landing energy of 3.0 keV.

2.9. Statistical analysis

IBM SPSS Statistical version 26.0 (IBM Corp. in Armonk, New York, USA) and Statgraphics Centurion XIX (StatPoint Technologies, Inc., Warrenton, Virginia, USA) were used for all statistical data processing.

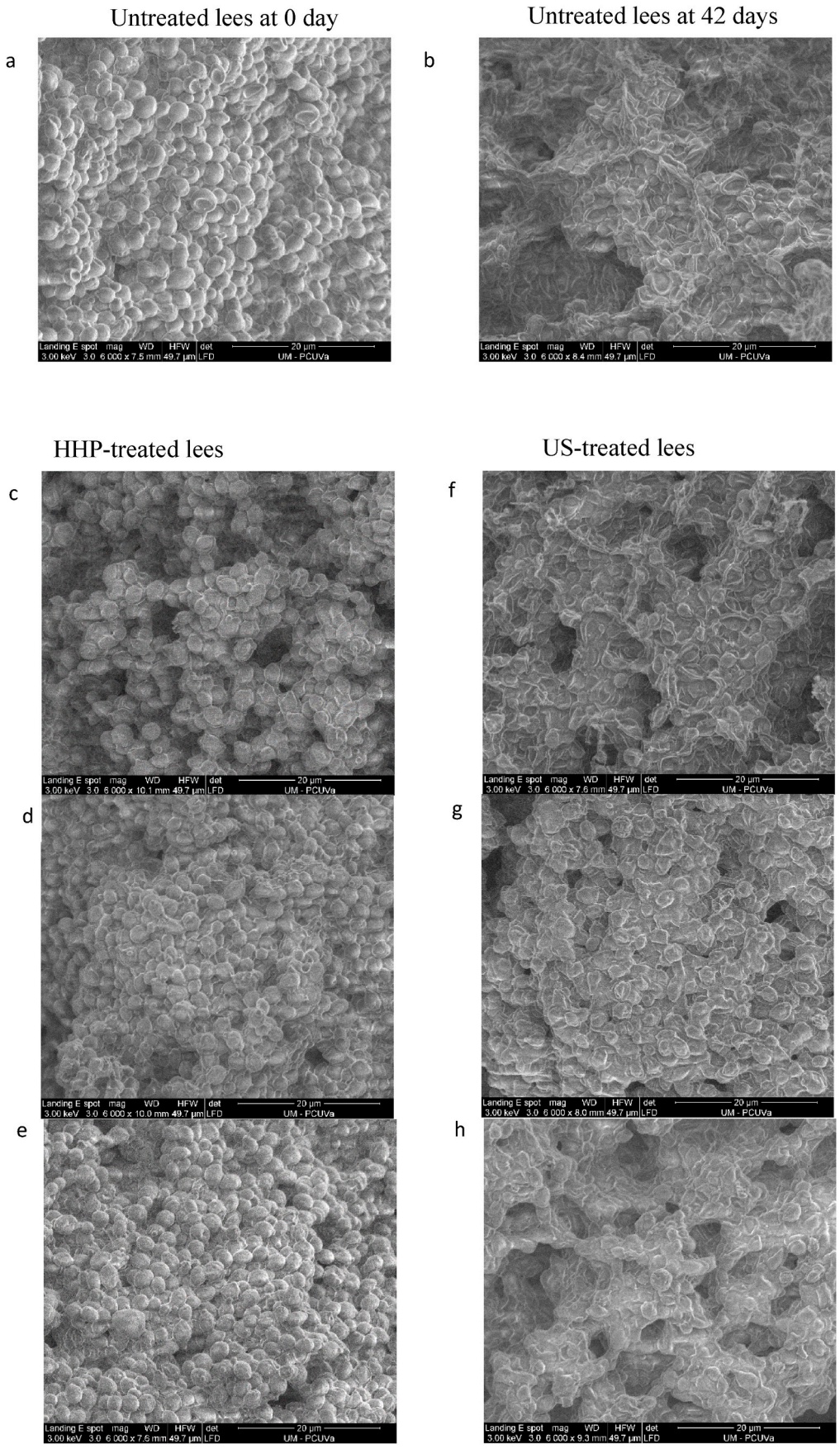

Fig. 1. ESEM images of untreated lees at time 0 (a) and after 42 days of lysis (b), lees treated with HHP 400 MPa (c), 500 MPa (d), and 600 MPa (e) for 10 min after 42 days of lysis, and US-treated lees 30% (f), 60% (g), and 90% (h) for 10 min after 42 days of lysis.

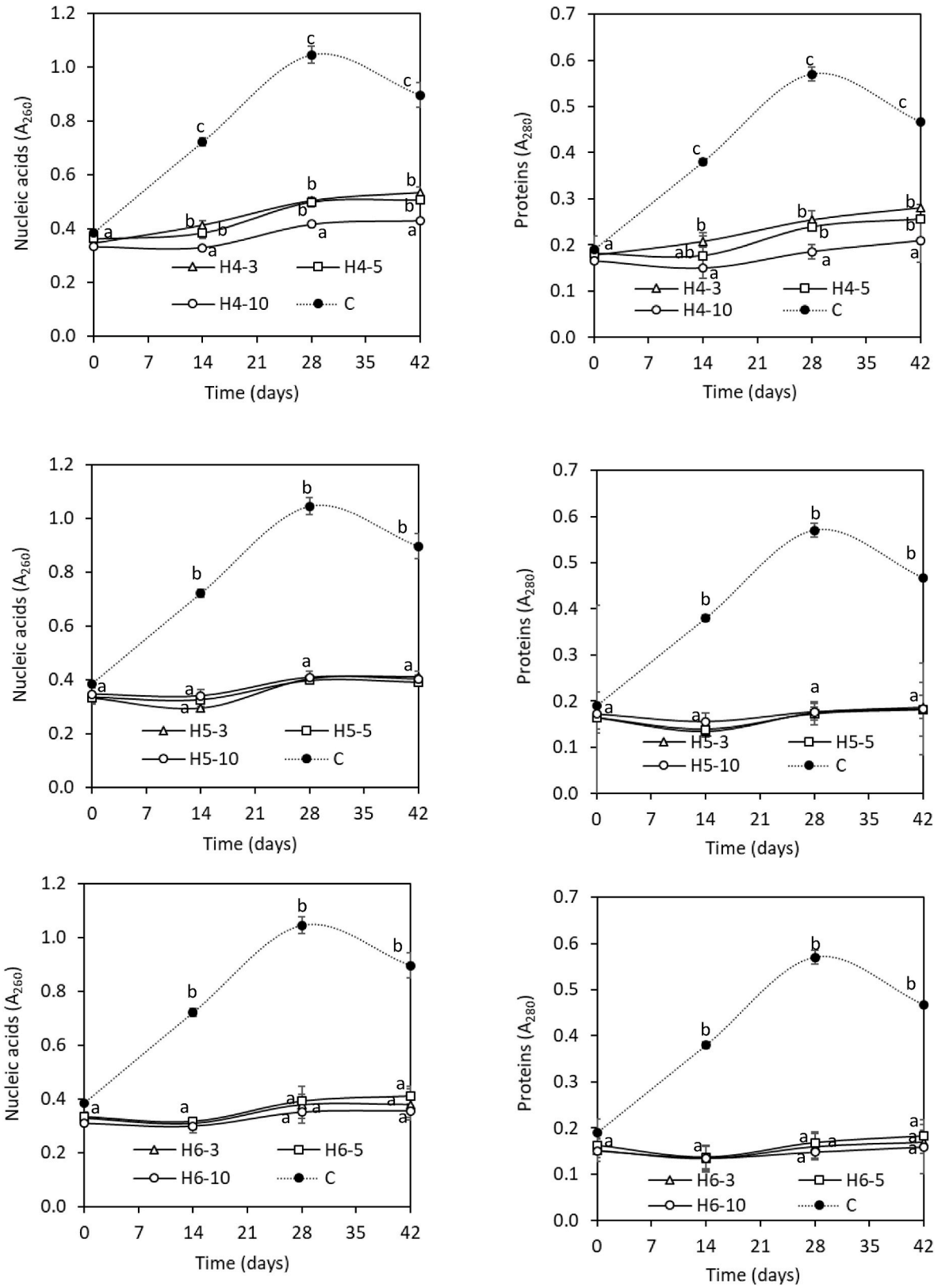

Fig. 2. Evolution of nucleic acids and proteins of the model wine during ageing on lees treated by HHP. C (control), H4-3 (400 MPa for 3 min), H4-5 (400 MPa for 5 min), H4-10 (400 MPa for 10 min), H5-3 (500 MPa for 3 min), H5-5 (500 MPa for 5 min), H5-10 (500 MPa for 10 min), H6-3 (600 MPa for 3 min), H6-5 (600 MPa for 5 min), H6-10 (600 MPa for 10 min).

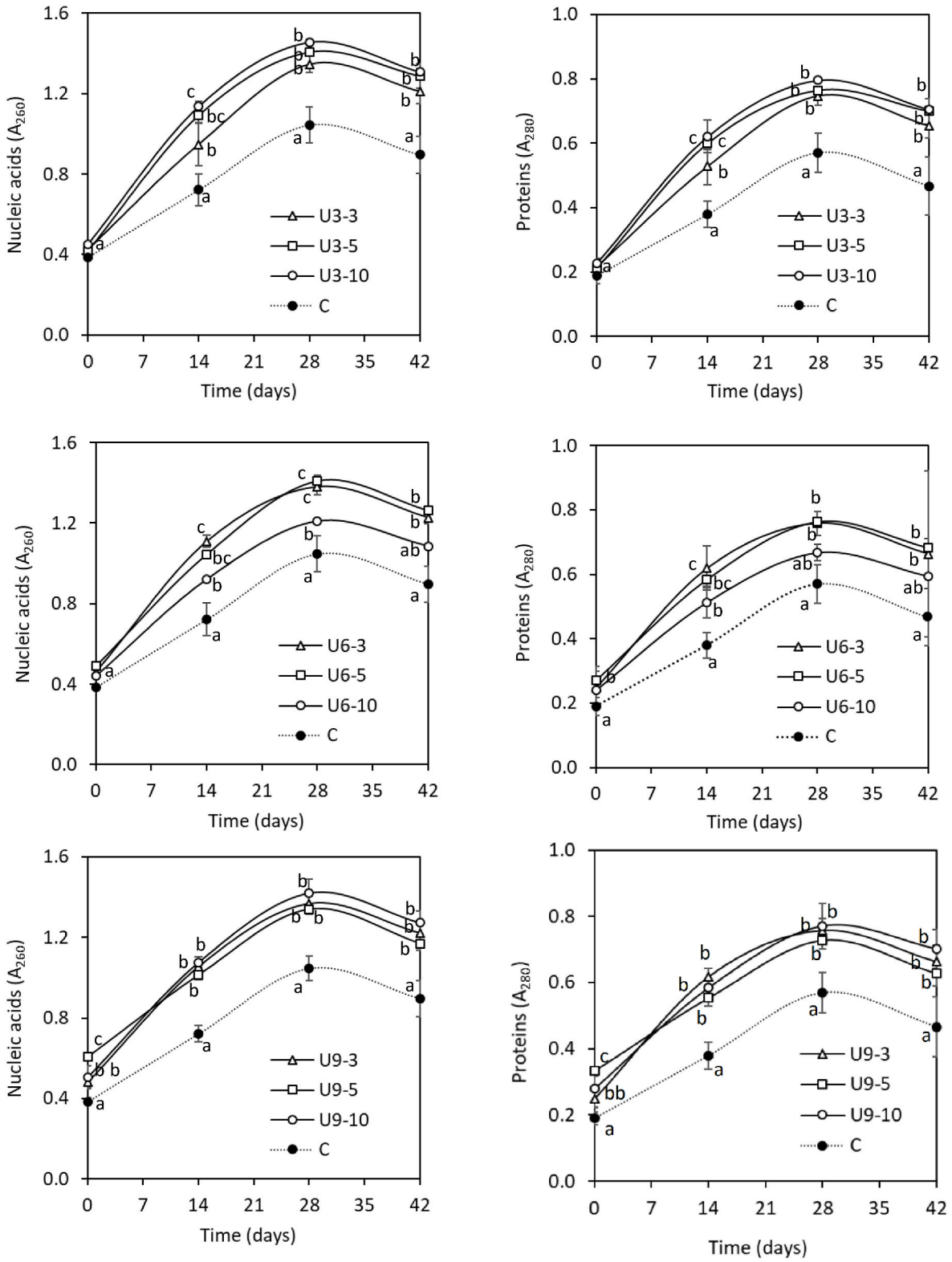

Fig. 3. Evolution of nucleic acids and proteins of the model wine during ageing on US-treated lees. C (control), U3-3 (30% for 3 min), U3-5 (30% for 5 min), U3-10 (30% for 10 min), U6-3 (60% for 3 min), U6-5 (60% for 5 min), U6-10 (60% for 10 min), U9-3 (90% for 3 min), U9-5 (90% for 5 min), U9-10 (90% for 10 min).

Statistical analysis, including analysis of variance (ANOVA) and the Tukey test, was conducted at a confidence level of 95% to identify significant differences among the various treatments. Principal component analysis (PCA) was performed using nucleic acids, proteins and total polysaccharides at 42 days of the ageing period as variables, and HHP treatments (400, 500 and 600 MPa) and US treatments (30%, 60% and 90% of amplitude) for 10 min and the control (C) as samples.

3. Results and discussion

3.1. Environmental scanning electron microscopy (ESEM)

ESEM was performed to examine the cell surfaces with the purpose of understanding the effect of the different treatments on the morphology of the yeasts. Fig. 5 shows the ESEM images of yeasts after 0 and 42 days of the ageing, and yeasts treated by HHP (400, 500 and 600 MPa) and US (30%, 60% and 90% of amplitude) for 10 min after 42 days of the ageing.

The distinctive cell envelope of *S. cerevisiae* comprises three primary components: the cell wall, the plasma membrane, and the periplasm. The cell wall primarily consists of 80%–90% polysaccharides, with the remaining composition comprising chitin, proteins, lipids, and inorganic phosphate (Marx et al., 2011). Moreover, the cell wall serves as a robust structure crucial for regulating the passage of substances into and out of the cell. It also safeguards the plasma membrane, preserving additional proteins and enabling cell adhesion (Brul et al., 2000). Comparisons between untreated lees revealed that at 0-day (Fig. 1a) the cell had a characteristic ellipsoidal shape with a smooth and uniform appearance and a continuous cell wall as other authors described (Marx et al., 2011), that is, budding cells with primary growth characteristics were observed. However, after 42 days of the ageing period (Fig. 1b), changes were observed in the structure of the wall, and the yeast cells were broken down showing more disordered structures and irregular fragments. Throughout autolysis, the gradual degradation of cell walls occurs as a result of the breakdown of glucan and chitin chains, facilitated by enzymes inherent to the dead yeast cells (Charpentier & Freyssinet, 1989). Autolysis results in a reduction in the rigidity of yeast cell walls. This process leads to the liberation of their polysaccharides (Feuillat, 2003) and hydrolyzed proteins, consequently elevating the content of nitrogenous compounds in the wine (Martínez-Rodriguez et al., 2001).

When subjected to an environmental pressure greater than 10 MPa, *S. cerevisiae* does not exhibit growth, signifying its inability to withstand high pressure (Walker, 1998). The HHP treatments (400, 500 and 600 MPa) after 42 days provoke less cell damage (Fig. 1c, d and 1e), than that of the untreated lees in the same period of time, since oval-shaped yeast cells with visibly smooth surfaces are noticeable in HHP treatments, especially at 600 MPa for 10 min. Denaturation of the enzymes causing cell autolysis is likely to occur in the HHP-treated samples, with the most significant effect at 600 MPa. A negative impact of pressure on enzyme activity related to irreversible changes of their native secondary and tertiary structure has been published (Kunugi & Tanaka, 2002; Rivalain et al., 2010). These results conflict with those found by Marx et al. (2011), as they applied 600 MPa and observed a disruption of the cell membrane with scars on the surface of the pressurized cells, perforation, and release of the cell wall. Goh et al. (2007) reported changes on the shape of the cells and the existence of surface wrinkles on *S. cerevisiae* cells subjected to a pressure of 600 MPa. Ross et al. (2003) explained that the cell death induced by HHP is associated with alterations in cell membrane proteins, along with compression of the membrane bilayer resulting from changes in permeability. Some studies have shown that pressure can cause elimination or denaturation of the mannoproteins present in the cell wall (Brul et al., 2000). Moreover, it has been found that the structural impacts of HHP differ depending on the type of microorganism (Marx et al., 2011), including yeasts, where changes have been observed due to the use of low pressures (100 and 200 MPa) (Earnshaw et al., 1995; Osumi et al., 1996).

The ESEM pictures of the US-treated lees are quite similar between them. There exist completely US-damaged cells at 30%, 60% and 90% amplitude (Fig. 1f, g and 1h). These results are in agreement with those reported by Marx et al. (2011), who observed the breakdown and perforation of the cell wall with a processing condition of 100% amplitude, 60 °C and 30 min. Earlier researchers (Ross et al., 2003) also documented that the effects of US on cells are linked to the degradation of cell walls, the breakdown and thinning of cell membranes, and subsequent DNA alterations caused by the generation of free radicals. The cavitation induced by US produces strong implosions and explosions into the cell and these violent collapses of bubbles are responsible of the final breakdown of the membrane, of the cell wall and the structures (Earnshaw et al., 1995). Additionally, the implosion of bubbles near the cell walls leads to cell disruption, thereby enhancing the release of compounds contained within the cells (Osete-Alcaraz et al., 2019). Other authors (Wu et al., 2015) suggest that the measurement of the released polysaccharides can be used to assess physical damage to the cell wall, because most of the yeast polysaccharides are found in the cell wall. In addition, Balasundaram et al. (2009) indicate that few proteins are present in the cell wall and they are more common within the cell membrane, so protein release can be used to assess physical damage to the cell membrane. Therefore, our results suggested that the yeast cell wall and the cell membrane at those amplitudes (30%, 60% and 90%) were damaged.

3.2. Nucleic acids and proteins during the ageing period

To track the release of intracellular components and demonstrate cell lysis, the levels of nucleic acids and proteins were measured throughout the storage period (Martínez et al., 2018). Fig. 2 illustrates the progression of nucleic acids and proteins in the model wine during the ageing on lees treated by HHP. The evolution of nucleic acids and proteins in this wine was similar for all the samples treated with HHP. The sample without HHP treatment (C) showed the highest values of nucleic acids and proteins during all the ageing. It must be noticed a rise in the concentration of nucleic acids and proteins in the control sample (C) until the 28th day of the ageing. Significant statistical differences were noted with HHP in every analysis from the 7th day of the ageing. Besides that, no significant differences were found among the HHP treated samples in nucleic acids and proteins. Just the H4-10 (400 MPa for 10 min) showed significantly lower nucleic acids and proteins values.

Nonetheless, throughout the lees ageing process, the levels of nucleic acids and proteins were consistently higher in the samples treated with US compared to the control sample (C) (Fig. 3). From day 28 to day 42 of the ageing, a decrease in nucleic acids and proteins was observed for all the US treatments as well as for the control sample. In all the analysis carried out, statistically significant differences were found between the US treatments and the control sample, except at day 0 on the content of nucleic acids and proteins for treatments US at 30% amplitude and in the nucleic acids of the treatments at 60% amplitude. Therefore, the US treatment influenced the levels of particular intracellular compounds, with absorption peaks at 260 nm (nucleic acids) and 280 nm (proteins). Along ageing on lees, nucleic acids and proteins release increased rapidly in sonicated samples, in comparison to those with untreated lees (C). After 28 days of ageing, the control sample absorbance at 260 nm was 1.046. Nevertheless, U3-10 treatment (30% amplitude for 10 min) was 1.457, U6-5 (60% amplitude for 5 min) was 1.408 and U9-10 (90% amplitude for 10 min) was 1.421. The same behavior was observed for the absorbance at 280 nm (proteins). After 28 days of ageing, the control sample absorbance at 280 nm was 0.570, while U3-10 (30% amplitude for 10 min) was 0.796, U6-5 (60% amplitude for 5 min) was 0.764 and U9-10 (90% amplitude for 10 min) was 0.771. These data revealed a significantly quicker increase in nucleic acids and proteins content for the yeast treated with US, reaching higher values compared to the untreated samples.

The autolysis process is linked to cellular demise, an essential step that triggers the degradation of the cell's components through the activity of its own enzymes. As the inherent autolysis of yeast due to aging occurs gradually, different authors (Comuzzo et al., 2017; Martín et al., 2013) have experimented with various physical methods as catalysts for this process inducing microbial inactivation. It has been shown that certain innovative physical techniques expedite a sequence of events in yeast autolysis, including the release of compounds absorbing at 260 and 280 nm, i.e. nucleic acids and proteins, into the extracellular

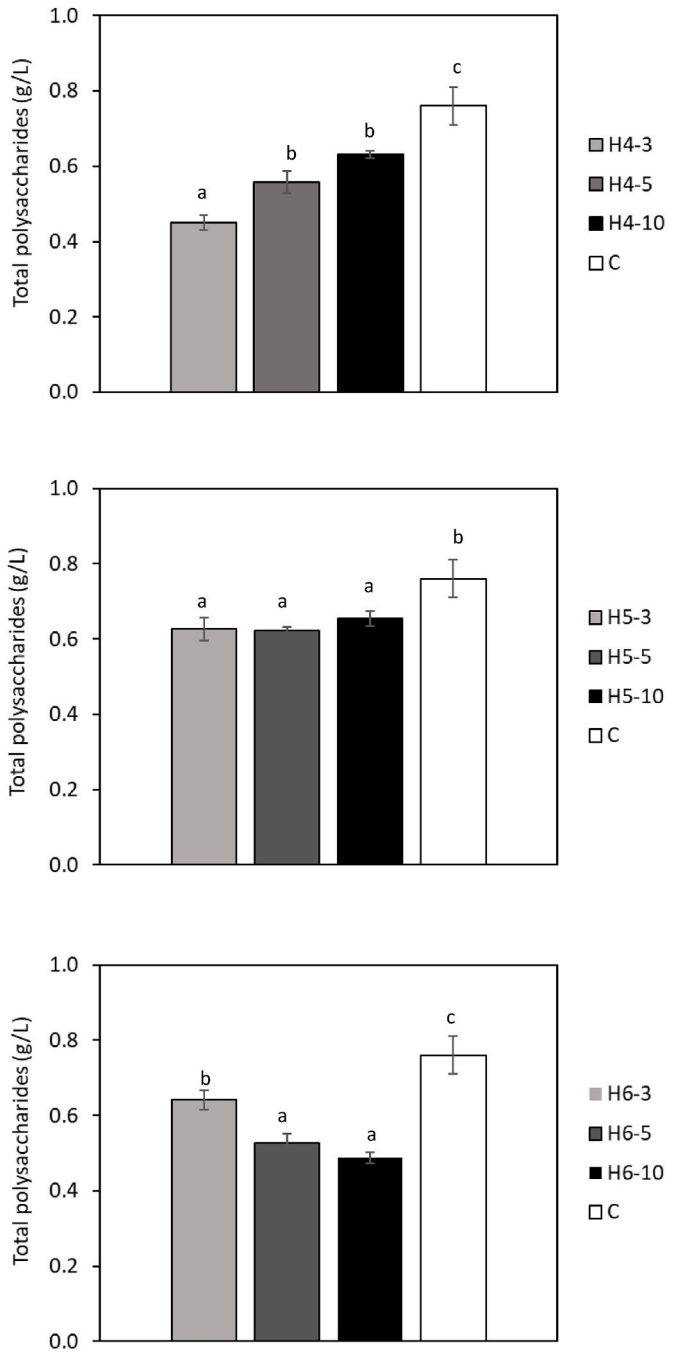

Fig. 4. Polysaccharide concentration in the model wine after 42 days of ageing on lees treated by HHP. C (control), H4-3 (400 MPa for 3 min), H4-5 (400 MPa for 5 min), H4-10 (400 MPa for 10 min), H5-3 (500 MPa for 3 min), H5-5 (500 MPa for 5 min), H5-10 (500 MPa for 10 min), H6-3 (600 MPa for 3 min), H6-5 (600 MPa for 5 min), H6-10 (600 MPa for 10 min).

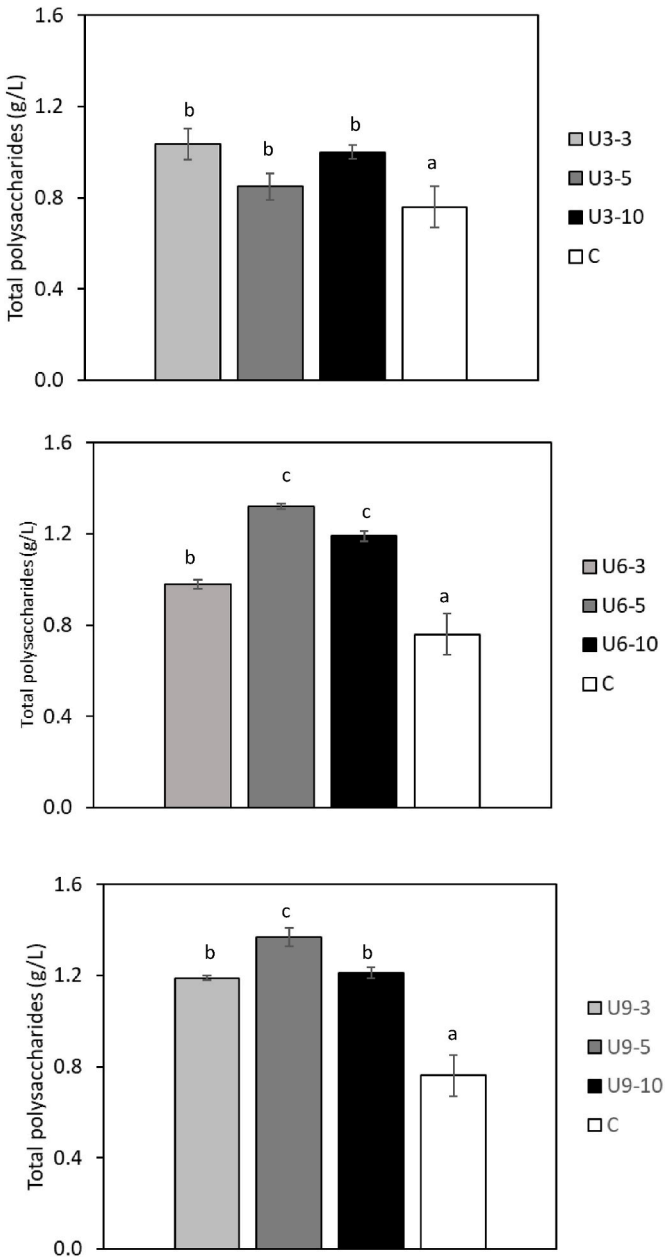

Fig. 5. Polysaccharide concentration in the model wine after 42 days of ageing on US-treated lees. C (control), U3-3 (30% for 3 min), U3-5 (30% for 5 min), U3-10 (30% for 10 min), U6-3 (60% for 3 min), U6-5 (60% for 5 min), U6-10 (60% for 10 min), U9-3 (90% for 3 min), U9-5 (90% for 5 min), U9-10 (90% for 10 min).

medium (Martínez et al., 2016, 2018). In conclusion, in this research the release of nucleic acids and proteins was faster when the yeast cells were treated with US compared to HHP, indicating a faster autolytic process.

3.3. Total polysaccharides at the end of the ageing period

Polysaccharides are the major components released during autolysis. In addition, mannoproteins are the main components of the extracellular polysaccharides in yeasts (Fornairon-Bonnefond et al., 2002). Therefore, the analysis of the polysaccharide's concentration was conducted to assess the treatments' ability to release polysaccharides, the primary goal of the lees ageing process. After 42 days of ageing, the control sample (C) released a significantly higher amount of total polysaccharides than the samples treated with the HHP (Fig. 4) regardless of the pressure. Therefore, the HHP treatment induced a lower release in the total polysaccharides in all the samples in comparison to the control sample. These results coincided with those obtained when studying the liberation of nucleic acids and proteins. At a pressure of 400 MPa, there was an observed rise in the polysaccharide content with the duration of the application. Nevertheless, it remained practically constant in samples subjected to 500 MPa for 3, 5 and 10 min (0.626, 0.623 and 0.655 g/L, respectively), no statistically significant differences were observed for any time. Under a pressure of 600 MPa, a decline in polysaccharide content was noticed as the application time increased. The concentration of polysaccharides decreased significantly when the HHP treatment

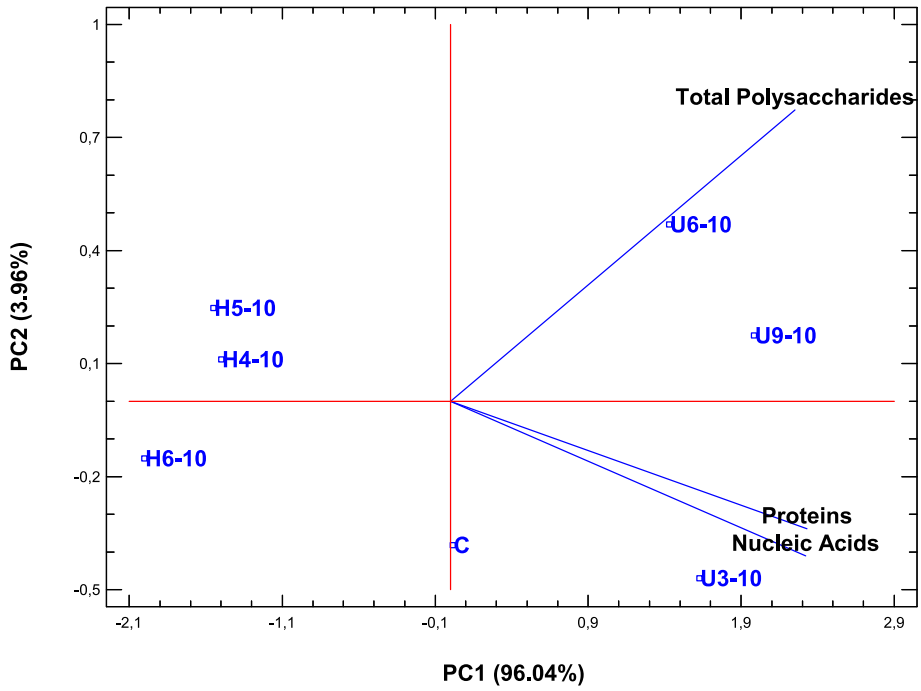

Fig. 6. Principal component analysis biplot of loadings (nucleic acids, proteins and total polysaccharides) and samples after 42 days of ageing on lees treated by HHP for 10 min: H4-10 (400 MPa), H5-10 (500 MPa), and H6-10 (600 MPa); and by US for 10 min: U3-10 (30% amplitude), U6-10 (60% amplitude), and U9-10 (90% amplitude) and control (C).

was applied, especially in model wines subjected to 400 MPa for 3 min (H4-3) (0.450 g/L) and those subjected to 600 MPa for 10 min (H6-10) (0.487 g/L). These findings suggest that HHP treatment might have determined a lower release of polysaccharides compared to the control sample, because this treatment may inactivate enzymes involved in autolysis as can be seen in the ESEM images (Fig. 1c, d and 1e).

Nonetheless, after 42 days of ageing, the US-treated samples showed significantly greater amounts of total polysaccharides compared to the control sample (C) for all amplitudes studied (Fig. 5). Research has demonstrated that US can significantly improve the extraction of polysaccharides from an aqueous solution of fungi without altering their molecular weight profiles (Cheung et al., 2013). A similar behavior was found in previous studies (Del Fresno et al., 2018), where samples sonicated in a model medium released higher content of polysaccharides through ageing. Regarding the treatments with US at 30% amplitude, statistically significant differences were observed only between the control and the sonicated samples (U3-3, U3-5, and U3-10). As shown in Fig. 5, no significant differences were observed for any sonication time at 30% amplitude, nevertheless significant distinctions were noted among the various application times at amplitudes of 60% and 90%. However, for the US treatments at 60% amplitude, samples U6-5 and U6-10 presented significantly higher polysaccharide values (1.321 and 1.191 g/L, respectively) than U6-3 (0.981 g/L). Finally, for the US treatments at 90% amplitude, the sample sonicated for 5 min (U9-5) showed significantly higher polysaccharide concentration (1.368 g/L) than the samples sonicated for 3 (U9-3) and 10 (U9-10) minutes, with a polysaccharide concentration of 1.188 and 1.211 g/L, respectively. According to Cacciola et al. (2013), the crucial factor in US treatment for the extraction of soluble colloids is the duration of the treatment. They studied three times of treatment (1, 3 and 5 min) and three amplitudes (30%, 60% and 90%) and they obtained that the duration of treatment had a more significant impact on the extraction of soluble colloids compared to the percentage of amplitude. In our study, the values of total polysaccharides of the samples treated for 5 min at 60% and 90% amplitude almost duplicated that of the control sample; however, in the samples treated for 10 min at 60% and 90% amplitude, the values did not duplicate that of the control sample (0.76 g/L), despite of being significantly higher. Other authors (Osete-Alcaraz et al., 2022) found that short-time US treatments (30, 60 or 120 min) released nearly equivalent quantities of soluble polysaccharides compared to the longer-term control sample (24 h). In addition, they demonstrated that the combination of US (in short times), glucanase and pectolytic enzymes had an additive effect in comparison to individual treatments, releasing a significant quantity of low molecular weight polysaccharides.

3.4. Principal component analysis (PCA)

Finally, multivariate statistics were employed to assess the similarities among samples: untreated lees (C), treated by HHP (400, 500 and 600 MPa) and US-treated lees (30%, 60% and 90% of amplitude) for 10 min after 42 days of the ageing. In Fig. 6, the outcomes of PCA are presented using the value of nucleic acids, proteins and polysaccharide concentration after 42 days of ageing, which were plotted in the plane formed by principal components 1 (PC1) and 2 (PC2). PC1 accounted for 96.04% of the variance, while PC2 explained 3.96% of the variance. Both PCs allowed differentiation between treatments. The US-treated samples were located at positive values of PC1 and characterised by high levels of total polysaccharides, nucleic acids and proteins. However, the HHP-treated samples were positioned in the negative part of PC1. Nucleic acids and proteins were highly related to the US-treated at 30% amplitude samples (U3-10), so according to Balasundaram et al. (2009) it can be said that these samples showed greater physical damage to the cell membrane. The sample US-treated at 60% amplitude (U6-10) was highly related to the total polysaccharides, which means that this sample also presented a greater damage in the cell wall (Wu et al., 2015).

4. Conclusions

The content of nucleic acids, proteins and total polysaccharides was positively affected by US treatments; however, HHP treatment has

determined a lower release of such compounds compared to the control samples. Cell surface investigations obtained by ESEM revealed shrinkage of the yeasts under both treatments (HHP and US), while US exposure had a more pronounced impact on the cell surface compared to HHP treatments. Results showed morphological differences among the studied US treatments suggesting cell surface modifications like cavitation, breakage and shrinkage. Hence, US emerges as a dependable method to expedite the yeast autolytic process and augment the release of polysaccharides by altering cell wall structure. In any case, determining the optimal amplitude and duration for US treatment is crucial. Therefore, further research is necessary to fully understand the optimal conditions for US treatment and its potential applications in wine industry to accelerate the ageing on lees. The preliminary experience of the study, which was conducted on a model wine solution, suggests that new technologies may be able to accelerate the process of yeast autolysis. With all this, it will be attainable to enhance the quality and stability of wine in a shorter timeframe, with the potential to decrease both time and costs in the overall production of aged wines.

CRediT authorship contribution statement

Coro Blanco-Huerta: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **José Manuel Rodríguez-Nogales:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Josefina Vila-Crespo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Violeta Ruipérez:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Encarnación Fernández-Fernández:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing 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.

Data availability

Data will be made available on request.

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