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The role of the membrane lipid composition in the oxidative stress tolerance of different wine yeasts

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ABSTRACT

Oxidative stress is a common stress in yeasts during the stages of the winemaking process in which aerobic growth occurs, and it can modify the cellular lipid composition. The aim of this study was to evaluate the oxidative stress tolerance of two non-conventional yeasts (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) compared to *Saccharomyces cerevisiae*. Therefore, their resistance against H_2O_2, the ROS production and the cellular lipid composition were assessed. The results showed that the non-*Saccharomyces* yeasts used in this study exhibited higher resistance to H_2O_2 stress and lower ROS accumulation than *Saccharomyces*. Regarding the cellular lipid composition, the two non-*Saccharomyces* species studied here displayed a high percentage of polyunsaturated fatty acids, which resulted in more fluid membranes. This result could indicate that these yeasts have been evolutionarily adapted to have better resistance against the oxidative stress. Furthermore, under external oxidative stress, non-*Saccharomyces* yeasts were better able to adapt their lipid composition as a defense mechanism by decreasing their percentage of polyunsaturated fatty acids and squalene and increasing their monounsaturated fatty acids.

1. Introduction

Our understanding of the response and adaptation of yeasts to external environmental changes is very important within the biotechnological, pharmaceutical, food and beverage industries. Changes in the temperature, pH and osmotic pressure, nutrient starvation, ethanol toxicity, prolonged anaerobiosis, exposure to chemical preservatives and oxidative stress are the primary causes for the decrease in yeast viability and vitality in industrial processes (Walker and Dijck, 2006; Gibson et al., 2007).

Oxidative stress is the result of an imbalance between the presence of reactive oxygen species (ROS) and the capacity of cells to detoxify these reactive intermediates of molecular oxygen, or to repair the resulting damage. Disturbances in the normal redox state of cells can damage all of their components, including lipids, carbohydrates, proteins and nucleic acids, and they may even induce programmed cell death (Costa and Moradas-Ferreira, 2001; Gibson et al., 2008; Moradas-Ferreira et al., 1996). Under normal physiological conditions, yeasts are able to effectively defend themselves against the direct consequences of stress exposure and damage by immediate cellular enzymatic and non-enzymatic responses, and finally, the adapted cells can resume proliferation (Herrero et al., 2008; Jamieson, 1998; Moradas-Ferreira and Costa, 2000).

Biological membranes are primarily made of proteins and phospholipids, and they form the first barrier that separates yeast cells and their organelle compartments from their external environment. Fatty acids (FAs), both free and as part of complex lipids, play a number of key roles in metabolism. They can be incorporated into phospholipids (PLs), which are considered as primary structural elements of biological membranes and sphingolipids, or they can serve as an energy reservoir in the form of triacylglycerols and steryl esters (Klug and Daum, 2014). Another important and essential group of lipids for maintaining the membrane integrity is the sterols, and ergosterol is the primary sterol in yeast (Daum et al., 1998; Klug and Daum, 2014). Membrane dysfunction can be associated with a loss of viability (Avery, 2011). Excessive ROS production can overwhelm the detoxifying mechanism and initiate changes in the lipid layers composition, resulting in a lipid peroxidation process, in which unsaturated lipids are converted into polar lipid hydroperoxides. PLs are particularly susceptible to oxidative damage mediated by ROS due to their content of polyunsaturated FAs (PUFAs),

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which are more sensitive to peroxidation than monounsaturated FAs (MUFAs) (Ayala et al., 2014; Howlett and Avery, 1997). Extensive lipid peroxidation has been correlated with membrane disintegration and cell death. However, lethal consequences on membranes are not systematically observed because yeasts are able to sense and adapt to environmental changes by modifying the membrane fluidity and phase transitions and by activating the cellular control of the chemical membrane composition. These changes in lipid composition are used by yeast as a defense mechanism, and they are important for conferring resistance to oxidative stress. (Beney and Gervais, 2001; Los and Murata, 2004).

Yeast species, and even different strains of the same species, can exhibit variations in their membrane lipid composition (Hunter and Rose, 1972). In fact, yeast membranes are structurally and functionally dependent on the growth conditions, e.g., *Saccharomyces cerevisiae* is auxotrophic for oleic acid and ergosterol under strict anaerobic conditions (Walker and Dijck, 2006). Thus, the lipid composition should not be considered a fixed and static characteristic of a single yeast strain (Beltran et al., 2008; Hunter and Rose, 1972; Torija et al., 2003).

S. cerevisiae is the primary yeast species involved in wine fermentation (Ribereau-Gayon, 1985; Fleet and Heard, 1993); however, many other yeast species can participate in different stages of the process (Beltran et al., 2002). Currently, non-*Saccharomyces* yeasts are used to produce final products with improved organoleptic characteristics (González-Royo et al., 2015; Jolly et al., 2014). In general, these yeasts are not able to complete the alcoholic fermentation, several studies have demonstrated that some non-*Saccharomyces* yeasts used with sequential inoculation techniques, can positively contribute to the aroma profile, sensory complexity and color stability of the resulting product (Fleet, 2008; González-Royo et al., 2015; Mas et al., 2016; Pretorius, 2000). Thus, non-*Saccharomyces* species can influence the organoleptic properties of wines, increasing the volatile compounds or secondary metabolites such as glycerol, aromatic alcohols, esters and acetates (Belda et al., 2017; Jolly et al., 2014; Romano et al., 2003). For instance, *Torulaspora delbrueckii* has been proposed to reduce the volatile acidity produced by *Saccharomyces* (Bely et al., 2008), whereas *Metschnikowia pulcherrima* is recommended for the release of some volatile thiols and terpenes in white wines, increasing the aromatic intensity (Belda et al., 2017). Nevertheless, despite the importance of these yeasts, there is still a lack of knowledge about non-*Saccharomyces* species compared with *S. cerevisiae*. Therefore, studies on the effect of oxidative stress on non-*Saccharomyces* yeasts are required, not only for the investigating their cellular physiology but also to acquire a better understanding of the adaptations of non-conventional yeasts in response to the changes imposed by oxidative stress.

The goal of this study was to compare the effects of oxidative stress between *S. cerevisiae* and two species of non-*Saccharomyces* (*T. delbrueckii* and *M. pulcherrima*) on the yeast composition. To accomplish this goal, we evaluated the H_2O_2 resistance, intracellular ROS production and the lipid composition (FAs, PLs and sterols) in these species before and after oxidative stress exposure via H_2O_2.

2. Materials and methods

2.1. Yeast strains and growth conditions

The yeast strains used in this study were as follows: two strains of *S. cerevisiae* (the laboratory strain BY4742, (EUROSCARF collection, Frankfurt, Germany) and a commercial wine strain (QA23®)), two strains of *Torulaspora delbrueckii* (BIODIVA® (TdB) and Tdp) and two strains of *Metschnikowia pulcherrima* (FLAVIA® (MpF) and Mpp). Commercial *Saccharomyces* and non-*Saccharomyces* wine strains QA23, FLAVIA and BIODIVA were provided by Lallemand S.A. (Montreal, Canada), and the other two non-*Saccharomyces* strains (Tdp and Mpp) were isolated from natural musts that were taken from the Priorat Appellation of Origin (Catalonia, Spain) (Padilla et al., 2017, 2016) and deposited in the Spanish Type Culture Collection (CECT) as CECT 13135 and CECT 13131, respectively.

The commercial strains were in active dry yeast form and were rehydrated according to the manufacturer's instructions. For all experiments, precultures for biomass propagation were prepared in YPD liquid medium (2% (w/v) glucose, 2% (w/v) peptone and 1% (w/v) yeast extract (Panreac, Barcelona, Spain)) and incubated for 24 h at 28 °C with orbital shaking (120 rpm).

2.2. Effect of hydrogen peroxide on yeast growth

Yeast cells were pre-cultured for 24 h and then inoculated into YPD broth (25 mL) to obtain an initial population of 5×10^5 cells/mL. After 6 h (early exponential phase), sublethal oxidative stress was induced in each strain by adding 2 mM H_2O_2 to the yeast culture. Yeast growth was followed in both conditions (control and stressed cells) by measuring the optical density at 600 nm (OD_{600}) every 30 min for 24 h, using a microplate reader (Omega Polarstar, BMG Labtech Gmbh, Ortenberg, Germany). Microplate wells were filled with 250 µL of inoculated media. A control well containing medium without inoculum was used to determine the background signal. Measurements were taken every 30 min after pre-shaking the microplate for 30 s at 500 rpm. All assays were performed in triplicate.

2.3. Resistance to hydrogen peroxide (H_2O_2)

Yeast resistance to H_2O_2 was assessed using the agar diffusion method (Bauer et al., 1966; Acar, 1980). Approximately 5×10^6 cells were seeded with glass beads on YPD plates, and 6 mm blank disks were impregnated with 10 µL of 30% (v/v), 15% (v/v), 3% (v/v) or 0.3% (v/v) H_2O_2 (Perdrogen™, Sigma-Aldrich, MO, USA) and placed on the agar surface after drying. One disk impregnated with 10 µL of H_2O was used as the negative control. After 48 h of incubation at 28 °C, the diameter of the inhibition haloes, including the disk, was measured with a ruler and photographed using a ProtoColHr automatic colony counter (Microbiology International, Frederick, USA). The means of three biological replicates were calculated.

2.4. Determination of reactive oxygen species (ROS)

The effect of H_2O_2 on the intracellular ROS concentration was evaluated in the six yeast strains. Yeast cells were inoculated into 50 mL of YPD broth (5×10^5 cells/mL) and grown for 6 h (early exponential phase) at 28 °C with orbital shaking at 120 rpm. The cells were then exposed to different concentrations of H_2O_2 (from 2 mM to 1000 mM) for 1 h, and the ROS were determined and compared to the control (sample without exposure to H_2O_2). Three biological replicates were set up for each condition. Determination of ROS was performed according to the method described by Vázquez et al. (2017) using dihydrorhodamine 123 (DHR 123; Sigma-Aldrich) as an ROS indicator. DHR 123 is an uncharged and non-fluorescent compound that can passively diffuse across membranes being oxidized to cationic rhodamine 123, exhibiting green fluorescence that can be measured by flow cytometry. The mean fluorescence index (MFI) was calculated according to Boettiger et al. (2001): [(geometric mean of the positive fluorescence) – (geometric mean of the control)]/(geometric mean of the control). Additionally, cell viability was evaluated in cells previously exposed to 100 and 1000 mM of H_2O_2 using LIVE/DEAD™ BacLight Viability kit (Molecular Probes, Eugene, OR, USA). Briefly, 1 mL of sample was stained with 1 µL propidium iodide (PI)/SYTO 9 (50:50) during 15 min in darkness, each sample was washed with 1 mL of PBS to eliminate the excess dye and analyzed immediately in the flow cytometer.

2.5. Experimental conditions for lipid analysis

Cells from each of the six yeast strains were inoculated into 450 mL

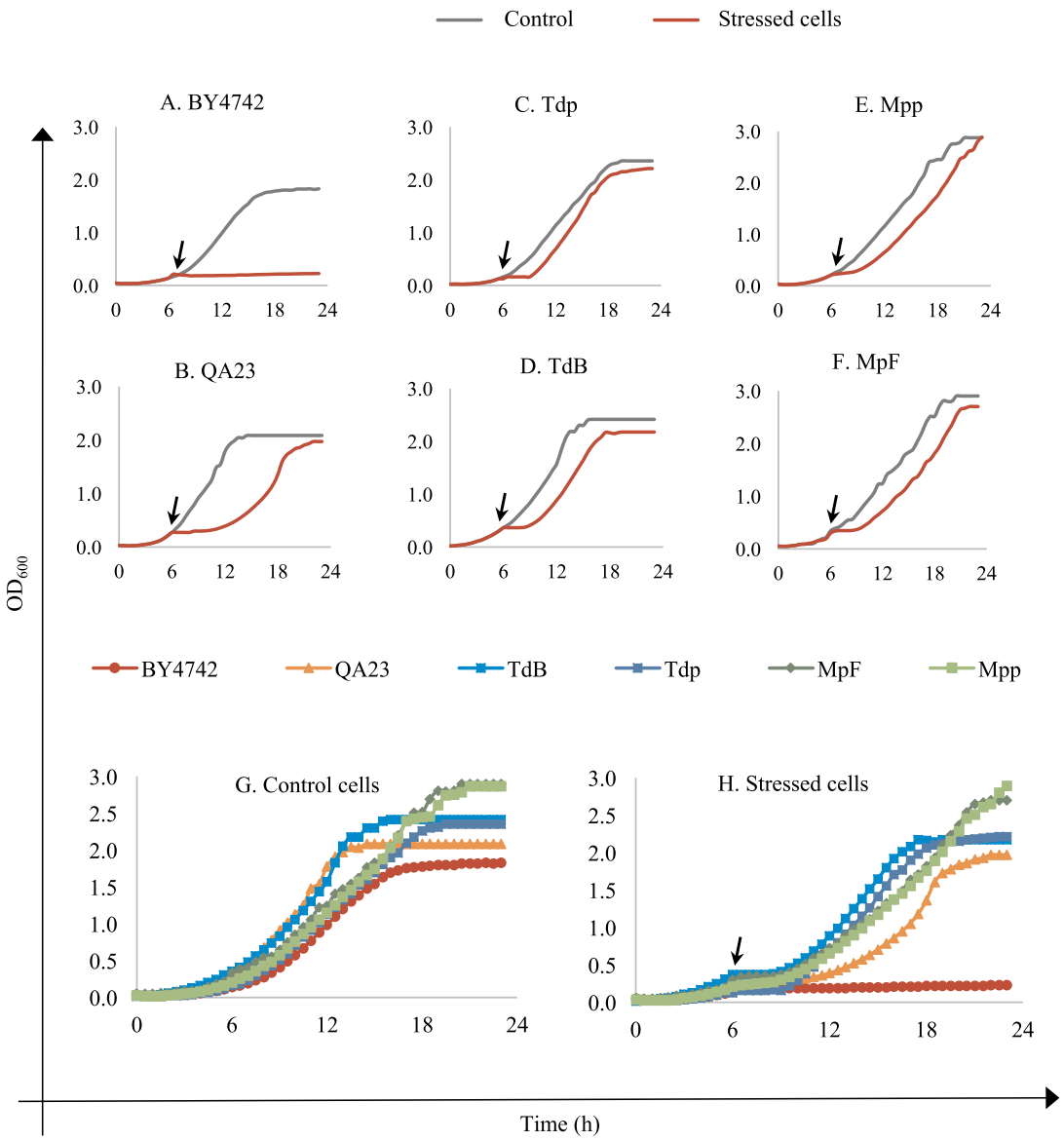

Fig. 1. Effect of H_2O_2 (2 mM) on the cell growth of different yeast strains, compared with their control condition (without oxidative stress). Arrows indicate cell exposition to oxidative stress at 6 h. *Saccharomyces cerevisiae*: (A) BY4742 and (B) QA23 strains, *Torulaspora delbrueckii*: (C) TdB and (D) Tdp strains and *Metschnikowia pulcherrima*: (E) MpF and (F) Mpp strains. Growth curves of all control (G) and stressed cells (H).

of YPD broth to obtain an initial population of 5×10^5 cells/mL and grown at 30 °C with orbital shaking at 130 rpm. After 6 h (early exponential phase), sublethal oxidative stress was induced in each strain by adding 2 mM H_2O_2 to the yeast culture. Cells were harvested for subsequent lipid analysis (OD_{600} ~ 10) at 6 h (before stress) and 18 h after the stress exposure (thus, 24 h from the beginning of the experiment), in order to allow the cells to respond/adapt to this stress. Two biological replicates were set up for each strain.

2.6. Lipid analysis

2.6.1. Cell homogenates, protein quantification and lipid extraction

Homogenates of the yeast cells were obtained using glass beads and a Disruptor Genie® (Scientific Industries, Inc., NY, USA) at 4 °C for 10 min. Aliquots of homogenates were precipitated with 10% (v/v) of trichloroacetic acid to quantify the protein amount with the Folin phenol reagent (Lowry et al., 1951). Total lipids were extracted from cell yeast homogenates corresponding to 1 mg, 3 mg or 0.5 mg of total cell protein, in order to analyze FA, PL or sterol assays, respectively, according to Folch et al. (1957).

2.6.2. Fatty acids

The cell FA composition was analyzed by gas liquid chromatography (GLC) according to Rußmayer et al. (2015). In brief, the total FAs from lipid extracts (1 mg of total cell protein) were converted to methyl esters by methanolysis with sulfuric acid (2.5% in methanol (v/v)) and heating at 80 °C for 90 min. These FA methyl esters were then extracted twice with light petroleum and water (3:1; v/v) by shaking on

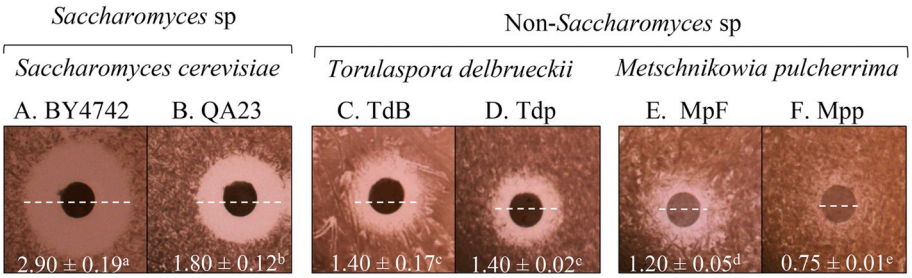

Fig. 2. Resistance to H_2O_2 (10 µL from H_2O_2 3% (v/v)) by disk diffusion method from six yeast strains grown on YPD plates over 48 h, expressed as the mean size of inhibition haloes (cm) and ± SD of n = 3. *Saccharomyces cerevisiae*: (A) BY4742 and (B) QA23 strains. *Torulaspora delbrueckii*: (C) TdB and (D) Tdp strains. *Metschnikowia pulcherrima*: (E) MpF and (F) Mpp strains. Different letters in superscripts (a to e) indicate values significantly different between strains by Tukey's post-test ($P < 0.05$).

a Vibrax® orbital shaker (IKA, Staufen, Germany) for 30 min, and separated by GLC on a Hewlett-Packard 6890 gas-chromatograph (Agilent Technologies, CA, USA) using an HP-INNOWax capillary column (15 m × 0.25 mm x 0.50 µm film thickness) with helium as a carrier gas. Finally, the FAs were identified by comparing with a commercial FA methyl ester standard mix (NuCheck, Inc., MN, USA) and quantified using pentadecanoic acid (C15:0, Sigma-Aldrich) as an internal standard. Two analytical replicates were used for each biological replicate.

2.6.3. Phospholipids

The PLs were separated by two-dimensional thin layer chromatography (TLC) on Silica Gel 60 plates (Merck) using chloroform: methanol: ammonia solution (25%) (65:35:5; per vol.) as the first dimension solvent and chloroform: acetone: methanol: acetic acid: water (50:20:10:10:5; per vol.) as the second dimension solvent (Athenstaedt et al., 1999). Individual PLs were visualized on TLC plates by staining with iodine vapor and then scraping the spots off the plate, which were quantified by measuring the amount of phosphate (Broekhuyse, 1968). The phosphate quantity was calculated as a relative amount of the total phosphate (%), which was estimated as the sum of all PL spots. Two analytical replicates were taken for each biological replicate.

2.6.4. Sterols

The individual sterol composition was determined by gas-liquid chromatography-mass spectrometry (GC-MS) after the alkaline hydrolysis of the lipid extracts (0.5 mg of total cell protein) (Quail and Kelly, 1996). GLC-MS was performed on a Hewlett-Packard 5690 Gas Chromatograph equipped with an HP 5972 mass selective detector using a capillary column (HP 5-MS; 30 m × 0.25 mm i.d. × 0.25 µm film thickness). The injection was set at 270 °C using helium as the carrier gas with a constant flow rate set to 0.9 mL min^{-1}. To identify the mass fragmentation pattern of each sterol, a cholesterol solution was used as an internal standard. The determinations were performed in duplicate. Free sterols and steryl esters were quantified from the homogenates. The sum of total sterols includes squalene, ergosterol precursor, and the sterol intermediates (zymosterol, 4-methylzymosterol, fecosterol, 14-methylfecosterol, episterol, lanosterol and ergosterol).

2.7. Data analysis

The data were subjected to a one-way analysis of variance (ANOVA) and Tukey's post-hoc test to evaluate the effect of each treatment. The results were considered statistically significant at a p-values less than 0.05 (IBM SPSS Inc, XLSTAT Software). A Principal Component Analysis (PCA) was performed to visualize a 2D plot of the first two principal components (PCs) and heatmap of relative changes in lipid composition using XLSTAT Software.

3. Results

3.1. Yeast growth and resistance to hydrogen peroxide

The six yeast strains were grown in YPD medium, and oxidative stress (H_2O_2 2 mM) was applied at early exponential phase. The effect of oxidative stress on cell growth differed depending on the yeast strain, being the growth of both *S. cerevisiae* strains the most affected (Fig. 1). The laboratory *S. cerevisiae* strain (BY4742) showed a strong inhibition of its growth after the stress, indicating that this strain was unable to adapt itself to the new stress conditions (Fig. 1A). On the other hand, all the wine yeast strains were able to grow and achieved similar OD_{600} values at 24 h than their control conditions, although with a growth delay just after the stress exposure (Fig. 1B–F), specially *S. cerevisiae* QA23 (Fig. 1B).

Furthermore, all strains were plated on YPD medium, and the inhibition haloes around the disks that had been previously soaked with 3% (v/v) H_2O_2 were measured. The inhibition haloes for the *S. cerevisiae* strains (BY4742 and QA23) were 2.90 ± 0.19 cm and 1.80 ± 0.12 cm, respectively (Fig. 2A and B). By contrast, the size of the inhibition haloes was significantly smaller for all the non-*Saccharomyces* strains. The *M. pulcherrima* strains, and especially Mpp, had the highest resistance against 3% (v/v) of H_2O_2 (Fig. 2E and F; 1.2 ± 0.05 cm (MpF) and 0.75 ± 0.01 cm (Mpp)). Both *T. delbrueckii* strains exhibited similar inhibition haloes, with an intermediate size between the *S. cerevisiae* and *M. pulcherrima* strains (TdF, 1.40 ± 0.17; Tdp, 1.40 ± 0.02; Fig. 2C and D).

At lower concentrations of H_2O_2 (0.3%), only BY4742 showed a small inhibition halo, while exposure to higher concentrations of H_2O_2 (15% and 30%) resulted in an increase in the sizes of inhibition haloes for all the strains (Fig. S1). As with the 3% (v/v) H_2O_2, the *S. cerevisiae* strains were the most affected yeasts by high concentrations of this oxidant.

3.2. Determination of reactive oxygen species

For all the yeast species, the intracellular ROS levels were measured with and without H_2O_2 stress at the early exponential phase. Under these stress conditions, *S. cerevisiae* strains accumulated higher amounts of ROS than *non-Saccharomyces* species (Fig. 3). BY4742 was the least H_2O_2-resistant strain (Figs. 1A and 2A), and it showed the highest levels of ROS (Fig. 3B) followed by QA23 (Fig. 3A) and the non-*Saccharomyces* strains (Fig. 3C–F), with *M. pulcherrima* Mpp having the lowest levels of endogenous ROS (Fig. 3F). Exposure to increasing concentrations of H_2O_2, from 50 to 1000 mM, resulted in an increase in ROS accumulation for all yeast species (Fig. S2 A-D). Both *S. cerevisiae* strains showed the maximal fluorescence intensity at 50 mM of H_2O_2, whereas the non-*Saccharomyces* strains reached this maximum at 500 mM. After that, the fluorescence intensity, or ROS levels, declined in all strains (Fig. S2 A-D), coinciding with the decrease on its cell viability for each strain (Fig. S2 E).

3.3. Lipid composition before and after stress exposure

First, the FA, PL and sterol compositions in the six strains in this study were evaluated after 6 h of growth in a rich medium to study the differences in lipid composition between the three species used here (*S. cerevisiae*, *T. delbrueckii* and *M. pulcherrima*). The cells were then subjected to oxidative stress (2 mM of H_2O_2), and the lipid composition of these six strains was analyzed after 18 h to determine how the different

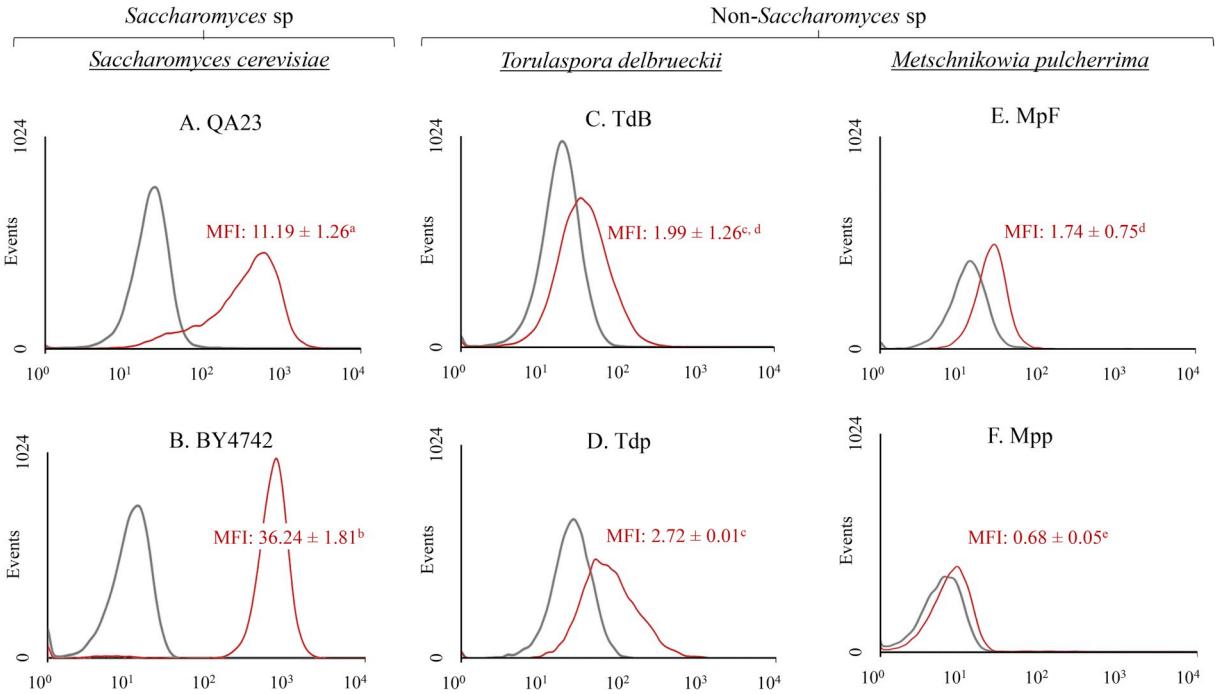

Fig. 3. Effect of H_2O_2 on ROS accumulation as evaluated in six yeast strains with and without stress. The flow cytometry histogram profile expressed as the number of events with 0 mM (—) and 2 mM (—) of H_2O_2. The mean fluorescence index (MFI) and ± SD of n = 3 was calculated according to Boettiger et al. (2001) as follows: [(geometric mean of the positive fluorescence) – (geometric mean of the control)]/(geometric mean of the control). Different letters in superscripts (a to e) indicate values of MFI significantly different between strains by Tukey's post-test ($P < 0.05$).

species could modify their lipid composition to better resist the oxidative stress exposure.

3.3.1. Fatty acid, phospholipid and sterol composition before stress exposure

FAs typically make up parts of complex lipids, and they are important structural components of biological membranes. FA analysis of the total cell extracts showed differences between the species (Table 1). In the *S. cerevisiae* strains (QA23 and BY4742), MUFAs (palmitoleic (C16:1) and oleic (C18:1) acids) and palmitic acid (C16:0) represented almost 90% of the FA in the cell extracts. By contrast, the non-*Saccharomyces* strains contained a lower percentage of C16:1 (especially in the *M. pulcherrima* strains), which was compensated by the presence of linoleic acid (C18:2), a PUFA. Moreover, in the case of the *M. pulcherrima* strains, a low percentage of linolenic acid (C18:3) was also present. As a result of this fatty acid pattern, the *T. delbrueckii* strains presented higher UFA/SFA ratios than the other studied species.

PLs are major structural components of cell membranes and are essential for vital cellular processes. The PL percentages of the homogenates showed a similar composition in all the studied yeasts, with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) representing approximately 50% and 24% of the total PLs, respectively (Table 1). However, there were also small shifts in some PLs between different yeast species. In general, all the non-*Saccharomyces* strains showed a significantly lower percentage of dimethyl phosphatidylethanolamine (DMPE), and the *T. delbrueckii* strains had the lowest amounts of lysophospholipids (LP). Of the strains studied here, Mpp showed the most different PL composition, resulting in the highest PC/PE and the lowest phosphatidylinositol/phosphatidylserine (PI/PS) ratios. In fact, the highest PI/PS ratio was found in the QA23 strain.

Sterols are essential lipid constituents present in yeasts as free membranous sterols and as steryl esters. Although only the free sterol fraction is important for membrane properties or functions, esterification of sterols helps to keep the level of free sterols balanced (Korber et al., 2017). The total sterol content (Table 1) was significantly lower in both *M. pulcherrima* strains (4–8 μg sterol/mg total protein) compared to the other studied strains (approximately 15–25 μg sterol/mg total protein). The primary sterol in all the strains was ergosterol, although the percentages varied markedly between species (38–96%). In the *M. pulcherrima* strains, practically the only sterol that was quantified was ergosterol. Instead, the *T. delbrueckii* strains exhibited the lowest percentage of ergosterol (38–40%) and the highest levels of squalene (33–36%) and lanosterol (6–11%). For *S. cerevisiae*, the strains used in this study showed significant differences in their sterol compositions. Thus, without accounting for the ergosterol, QA23 had a higher percentage of squalene and zymosterol, whereas BY4742 contained a higher proportion of fecosterol than the other strains.

3.3.2. Fatty acid, phospholipid and sterol composition after stress exposure

Differences in the cellular lipid compositions before and after stress exposure are shown in Fig. 4. QA23 and BY4742 showed only a few changes in the FA composition (Fig. 4). In QA23, slightly decreased amounts of C16:0 and C18:0 and increased amounts of C16:1 were found, leading to an increase in the unsaturated FA/saturated FA (UFA/SFA) ratio and in the unsaturation index (UI) (Fig. 4). By contrast, non-*Saccharomyces* species experienced highly modified FA compositions after stress. The percentage of PUFAs (C18:2, and for Mp also C18:3) and SFA (C16:0 and C18:0) decreased, whereas the MUFAs (C16:1 and C18:1) strongly increased. In fact, under these stress conditions, the percentages of C18:1 in non-*Saccharomyces* strains were higher than they were in *S. cerevisiae* (Table 2), unlike what we observed under the control conditions. These variations resulted in a higher UFA/SFA ratio and a lower UI in non-*Saccharomyces* species (Fig. 4 and Table 2).

The PL composition was slightly affected by stress, but the total PL profile remained similar between species (Table 2). The PC and PE persisted as the primary PLs in all the yeast strains, and although all the strains showed increased PC/PE ratios after stress (Fig. 4), this increase was statistically higher in the TdB and Mpp strains (Table 2). Moreover, the PI/PS ratio decreased in non-*Saccharomyces* strains but increased greatly in *S. cerevisiae* (Fig. 4) due to the increased PI and the decreased PS in QA23 and BY4742. Notably, there was a significant decrease in

Table 1
Fatty acids (FAs), phospholipids (PLs) and sterol composition of different strains after 6 h of growth in YPD medium. QA23 and BY4742 strains belong to *S. cerevisiae* species, TdB and Tdp strains to *T. delbrueckii,* and MpF and Mpp strains to *M. pulcherrima*. Different letter superscripts (a to e) indicate, for each studied compound, values significantly different between strains, by Tukey's post-test ($P < 0.05$).

% Lípid composition		Yeast strain					
		QA23	BY4742	TdB	Tdp	MpF	Mpp
Fatty acids (FAs)	Myristic (C14:0) acid	2.58 ± 0.34^{a}	2.45 ± 0.59^{a}	1.30 ± 0.34^{b}	1.55 ± 0.09^{b}	0.98 ± 0.02^{c}	0.89 ± 0.30^{c}
	Palmitic (C16:0) acid	23.17 ± 1.75^{a}	23.55 ± 0.67^{a}	18.30 ± 0.30^{b}	20.03 ± 0.26^{c}	23.27 ± 1.47^{a}	21.76 ± 0.80^{a,d}
	Palmitoleic (C16:1) acid	36.29 ± 3.14^{a}	42.64 ± 0.63^{b}	21.14 ± 2.28^{c}	19.81 ± 0.21^{c}	2.95 ± 0.09^{d}	3.69 ± 0.72^{d}
	Stearic (C18:0) acid	7.64 ± 1.57^{a}	8.36 ± 1.16^{a}	8.09 ± 0.19^{a}	7.40 ± 0.56^{a}	9.37 ± 0.34^{a}	8.62 ± 0.19^{a}
	Oleic (C18:1) acid	30.32 ± 0.53^{a}	22.99 ± 1.14^{b}	24.21 ± 0.52^{b}	25.59 ± 0.86^{b,c}	17.21 ± 0.61^{d}	26.43 ± 1.10^{b,c}
	Linoleic (C18:2) acid	n.d.	n.d.	26.95 ± 2.21^{a}	25.63 ± 0.26^{a}	40.17 ± 1.29^{b}	34.67 ± 1.05^{d}
	Linolenic (C18:3) acid	n.d.	n.d.	n.d.	n.d.	6.05 ± 0.16^{a}	3.95 ± 0.15^{b}
	Total FAs #	86.25 ± 1.14^{a}	87.84 ± 1.48^{a}	76.53 ± 9.34^{a}	91.9 ± 2.72^{a,b}	65.75 ± 6.17^{c}	80.49 ± 0.80^{a,d}
	C16:1/C18:1	1.20 ± 0.08^{a}	1.86 ± 0.12^{b}	0.87 ± 0.11^{c}	0.77 ± 0.03^{c}	0.17 ± 0.00^{d}	0.14 ± 0.03^{e}
	UFA/SFA	2.01 ± 0.33^{a}	1.91 ± 0.04^{a}	2.61 ± 0.06^{b}	2.45 ± 0.05^{b}	1.98 ± 0.16^{a}	2.20 ± 0.13^{a}
	UI*	0.67 ± 0.03^{a}	0.66 ± 0.00^{a}	0.99 ± 0.03^{b}	0.97 ± 0.00^{b}	1.19 ± 0.03^{c}	1.11 ± 0.02^{d}
Phospholipids (PLs)	PI (Phosphatidylinositol)	12.49 ± 1.31^{a}	12.19 ± 0.61^{a}	14.63 ± 2.01^{a}	12.93 ± 1.13^{a}	10.10 ± 0.46^{b}	8.94 ± 1.44^{b}
	PS (Phosphatidylserine)	4.01 ± 0.21^{a}	6.19 ± 0.76^{b}	5.82 ± 1.61^{a,b}	5.22 ± 1.29^{a,b}	6.45 ± 1.42^{b}	6.91 ± 0.63^{b,c}
	PC (Phosphatidylcoline)	43.47 ± 0.51^{a}	40.63 ± 1.07^{b}	44.23 ± 1.55^{a}	46.65 ± 2.00^{a}	44.10 ± 4.56^{a}	51.53 ± 0.93^{c}
	PE (Phosphatidylethanolamine)	24.61 ± 1.38^{a}	24.07 ± 1.66^{a}	23.34 ± 2.64^{a}	23.32 ± 4.19^{a}	21.71 ± 0.17^{a,b}	20.39 ± 1.03^{b}
	CL (Cardiolipin)	5.66 ± 0.23^{a}	2.86 ± 0.18^{b}	6.02 ± 1.33^{a}	6.87 ± 1.24^{a}	7.03 ± 0.23^{a,c}	4.71 ± 0.94^{a}
	DMPE (Dimethyl-phosphatidylethanolamine)	3.76 ± 1.12^{a}	5.39 ± 0.10^{b}	0.99 ± 0.25^{c}	1.18 ± 0.62^{c}	1.60 ± 0.37^{c}	1.02 ± 0.36^{c}
	PA (Phosphatidic acid)	2.00 ± 0.28^{a}	4.48 ± 2.57^{a}	4.18 ± 1.27^{a,b}	3.39 ± 1.37^{a}	4.67 ± 0.67^{a,b}	3.75 ± 0.11^{a,b}
	LP (Lysophospholipids)	4.01 ± 0.21^{a}	4.19 ± 2.06^{a}	0.79 ± 0.25^{b}	0.43 ± 0.54^{b}	4.35 ± 1.48^{a}	2.75 ± 0.29^{a,c}
	PI/PS	3.11 ± 0.16^{a}	2.00 ± 0.34^{b}	2.56 ± 0.36^{b}	2.52 ± 0.40^{b}	1.61 ± 0.43^{b,c}	1.31 ± 0.33^{c}
	PC/PE	1.77 ± 0.12^{a}	1.69 ± 0.16^{a}	1.90 ± 0.14^{a}	2.02 ± 0.27^{a}	2.03 ± 0.19^{a}	2.53 ± 0.17^{b}
Sterols	Squalene	18.51 ± 2.41^{a}	2.91 ± 0.52^{b}	33.15 ± 3.19^{c}	36.37 ± 3.93^{c}	6.24 ± 0.09^{d}	n.d.
	Zymosterol	16.78 ± 0.80^{a}	8.39 ± 0.29^{b}	4.16 ± 0.56^{c}	6.15 ± 1.19^{d}	0.91 ± 0.33^{e}	n.d.
	4-methylzymosterol	1.21 ± 0.08^{a}	n.d.	n.d.	1.71 ± 0.87^{a}	n.d.	n.d.
	Fecosterol	6.48 ± 0.05^{a}	14.11 ± 0.32^{b}	9.36 ± 0.37^{c}	6.02 ± 0.07^{d}	n.d.	2.24 ± 1.16^{e}
	14-methylfecosterol	n.d.	n.d.	n.d.	1.17 ± 0.10	n.d.	n.d.
	Episterol	1.38 ± 0.06^{a}	n.d.	1.08 ± 0.07^{b}	4.51 ± 0.89^{c}	n.d.	n.d.
	Lanosterol	2.75 ± 0.04^{a}	3.72 ± 0.22^{b}	11.26 ± 1.31^{c}	5.67 ± 0.89^{d}	3.91 ± 1.32^{a,b,d}	1.22 ± 1.03^{e}
	Ergosterol	52.89 ± 1.58^{a}	70.88 ± 0.77^{b}	40.99 ± 2.00^{c}	38.39 ± 1.70^{c}	88.95 ± 1.56^{d}	96.54 ± 1.89^{d}
	Total sterols #	26.92 ± 0.69^{a}	16.52 ± 0.40^{b}	27.94 ± 0.87^{a}	24.97 ± 4.69^{a}	3.78 ± 0.86^{c}	8.16 ± 0.50^{d}
	Ergosterol/Squalene	2.88 ± 0.46^{a}	24.78 ± 4.71^{b}	1.24 ± 0.18^{c}	1.07 ± 0.16^{c}	13.95 ± 0.05^{d}	–

(μg/mg protein).
* UI, unsaturation index. The unsaturation index was defined as follows: ((percentage of C16:1 + percentage of C18:1) + 2 (percentage of C18:2) + 3(percentage of C18:3))/100 (Rodríguez-Vargas et al., 2007).

the cardiolipin (CL) content under stress exposure in all the strains except for Mpp, the most H_2O_2-resistant strain, which increased the CL content under these conditions (Fig. 4). Moreover, both *M. pulcherrima* strains showed significantly decreased amounts of lysophospholipids (LP), whereas the *S. cerevisiae* strains, especially BY4742, were the strains with higher LP content after stress (Table 2).

After the stress, ergosterol remained the primary sterol in all the studied yeasts and the only one in Mpp (Table 2). However, a different behavior was observed between the wine yeast strains and laboratory strain BY4742. All the wine yeasts showed increased ergosterol contents and decreased squalene contents under stress (resulting in an increase of the ergosterol/squalene ratio; Fig. 4). However, BY4742 showed the opposite behavior, with increasing squalene and decreasing ergosterol contents, resulting in a decrease in the ergosterol/squalene ratio (Fig. 4). In fact, BY4742 showed the highest value for this ratio before stress and the lowest after stress (Table 2). However, the *T. delbrueckii* strains showed the highest ergosterol/squalene ratios under stress (mostly due to the drop in squalene content), and they were the strains that had more diverse sterol compounds and the only species that exhibited methyl fecosterol. In fact, whereas both *S. cerevisiae* strains experienced decreases in their zymosterol and fecosterol percentages under stress, the *T. delbrueckii* strains increased their component (Fig. 4).

3.4. Principal component analysis (PCA)

PCA was applied to correlate the different variables (lipid composition, inhibition haloes and ROS levels (MFI)) and highlight some grouping patterns within the different species under different conditions. Before stress (Fig. 5A), the species were clearly separated into three groups by their lipid composition (Table 1), with *M. pulcherrima* being the most diverse compared to *S. cerevisiae* and *T. delbrueckii*. Both *M. pulcherrima* strains (MpF and Mpp) were different from the other strains in that they exhibited higher ergosterol and PS percentages and lower PI and PE. Furthermore, the percentage of total PUFAs (C18:2 and C18:3) was clearly higher in the *M. pulcherrima* strains (the *T. delbrueckii* only showed low levels of C18:2, and *S. cerevisiae* had no PUFAs in its lipid composition). Both *S. cerevisiae* strains were characterized by high levels of myristic acid (C14:0) and oleic acid (C16:1), DMPE, PI/PS ratios and zymosterol and low UI values. The lowest LP, C16:0 and ergosterol contents and the highest squalene content and UFA/SFA ratio were characteristics of the *T. delbrueckii* species, which showed similarities with the other non-*Saccharomyces* but also with *Saccharomyces*.

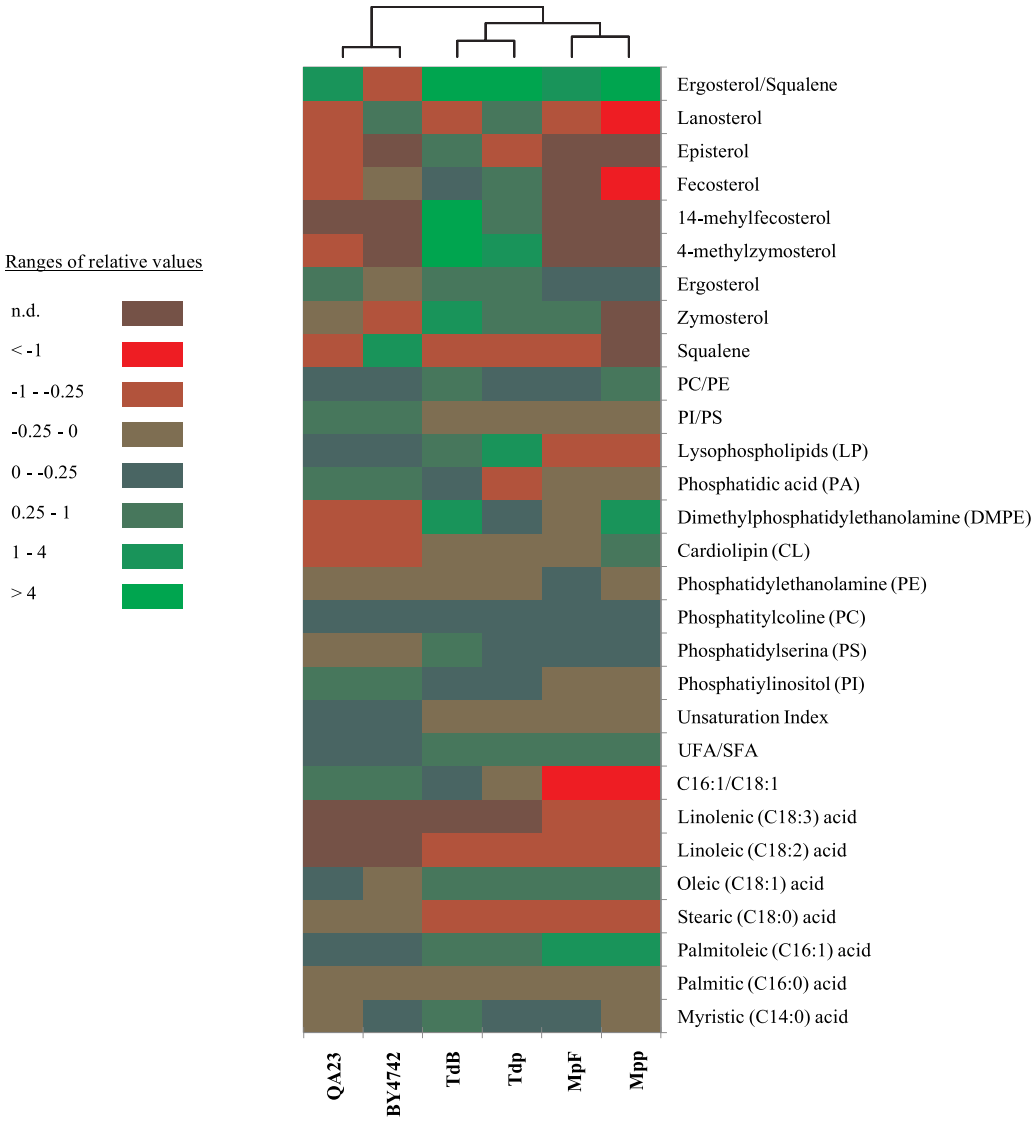

Fig. 4. Heatmap representing the fold changes in the lipid composition of cells following stress exposure to the cells before stress exposure. *S. cerevisiae* strains: QA23 and BY4742; *T. delbrueckii* strains: TdB and Tdp; and *M. pulcherrima* strains: MpF and Mpp.

The key indicative features of oxidative stress were the higher ROS accumulation (Fig. 3) and higher inhibition haloes (Fig. 2), which were positively correlated with the percentage of C14:0, stearic acid (C18:0), C16:1, PI, PE, DMPE, LP and squalene, and the PI/PS and C16:1/C18:1 ratios (Fig. 5B, positive component 1 and Table S1). However, high ROS and inhibition haloes were negatively correlated with the UFA (C18:1, C18:2 and C18:3), UI, CL, PC and ergosterol contents, and with the ratios of PC/PE and ergosterol/squalene (Fig. 5B, negative component 1 and Table S1). Thus, both *S. cerevisiae* strains were clearly different from both non-*Saccharomyces* species because they were grouped on the positive side of component 1, which is indicative of less stress tolerance (with BY4742 having higher positive values). *M. pulcherrima* strains were placed on the opposite side (negative component 1), with Mpp being the strain that exhibited more negatives values, indicating a higher resistance to stress. Thus, the component 1 places the strains according to their resistance to oxidative stress, with the less H_2O_2-tolerant strain BY4742 on one side, and Mpp on the opposite/negative side, which was the most resistant to this stress.

Although the differences between species in terms of lipid composition increased under stress, it is important to highlight that the primary differences were already observed before stress exposure (Fig. S1 and Table S2).

4. Discussion

Although *S. cerevisiae* is the wine yeast par excellence due to its fermentative capacity, there is currently a strong interest that is being driven by consumer and industry demand for wines with improved characteristics to study the possibility of using non-conventional yeasts with peculiar features in industrial fermentations. Under these conditions, yeasts are exposed to a variety of stresses, such as the oxidative stress. For instance, *S. cerevisiae*, used as starter in biotech and food industries in active dry yeast (ADY) form, can suffer oxidative stress during the biomass propagation and dehydration steps of their production, which could negatively affect yeast performance (reviewed by Matallana and Aranda, 2017). The oxidative stress response is a potential target for wine yeast improvement (Gamero-Sandemetrio et al., 2014). Many studies on stress resistance have been performed in *S. cerevisiae*, but few have addressed other yeast species, which have also shown a significant impact on food and beverage production (Pretorius,

Table 2
Fatty acids (FAs), phospholipids (PLs) and sterols composition of different strains after stress exposure at 24 h of growth in YPD medium. QA23 and BY4742 strains belong to *S. cerevisiae* species, and TdB, and Tdp strains to *T. delbrueckii* and MpF and Mpp strains to *M. pulcherrima*. Different letter superscripts (a to e) indicate, for each studied compound, values significantly different between strains (by Tukey's post-test, $P < 0.05$), and asterisk indicate values significantly different between cells before (Table 1) and after stress exposure (by Tukey's post-test, $P < 0.05$).

% Lípid composition		Yeast strain					
		QA23	BY4742	TdB	Tdp	MpF	Mpp
Fatty acids (FAs)	Myristic (C14:0) acid	2.03 ± 0.71^{a}	2.35 ± 0.35^{a}	1.83 ± 0.19^{a}	1.68 ± 0.09^{a,b}	1.12 ± 0.07^{c}	0.68 ± 0.16^{d}
	Palmitic (C16:0) acid	19.88 ± 0.20^{a,*}	23.63 ± 0.73^{b}	13.94 ± 2.11^{c,*}	15.18 ± 0.08^{c,*}	21.75 ± 0.93^{b}	17.17 ± 0.63^{c,*}
	Palmitoleic (C16:1) acid	40.44 ± 0.33^{a,*}	43.28 ± 0.89^{b}	33.32 ± 0.58^{c,*}	31.07 ± 0.02^{d,*}	10.81 ± 0.45^{e,*}	8.59 ± 0.29^{f,*}
	Stearic (C18:0) acid	6.61 ± 0.86^{a}	8.47 ± 1.05^{a}	3.91 ± 0.41^{b,*}	4.03 ± 0.02^{b,*}	4.14 ± 0.61^{b,*}	3.48 ± 0.08^{b,c*}
	Oleic (C18:1) acid	30.53 ± 0.32^{a}	22.27 ± 1.25^{b}	39.96 ± 1.24^{c,*}	41.98 ± 0.73^{c,*}	31.28 ± 1.16^{a,*}	52.23 ± 0.56^{d,*}
	Linoleic (C18:2) acid	n.d.	n.d.	7.03 ± 0.89^{a,*}	6.07 ± 0.76^{a,*}	30.00 ± 0.80^{b,*}	17.32 ± 0.39^{c,*}
	Linolenic (C18:3) acid	n.d.	n.d.	n.d.	n.d.	0.90 ± 0.16^{a,*}	0.54 ± 0.05^{b,*}
	Total FAs #	86.26 ± 3.70^{a}	68.12 ± 4.15^{b,*}	101.08 ± 0.43^{c,*}	93.42 ± 3.53^{a}	88.02 ± 0.34^{a,*}	74.2 ± 1.43^{d,*}
	C16:1/C18:1	1.33 ± 0.00^{a,*}	1.95 ± 0.07^{b}	0.83 ± 0.01^{d}	0.74 ± 0.01^{e}	0.35 ± 0.03^{f,*}	0.16 ± 0.00^{g}
	UFA/SFA	2.49 ± 0.02^{a,*}	1.91 ± 0.18^{b}	4.13 ± 0.71^{c,*}	3.79 ± 0.00^{c,*}	2.70 ± 0.03^{d,*}	3.69 ± 0.16^{c,*}
	UI *	0.71 ± 0.01^{a,*}	0.66 ± 0.02^{a}	0.87 ± 0.04^{b,*}	0.85 ± 0.01^{b,*}	1.05 ± 0.01^{c,*}	0.97 ± 0.01^{d,*}
Phospholipids (PLs)	PI (Phosphatidylinositol)	15.86 ± 3.40^{a}	15.83 ± 2.37^{a,*}	14.52 ± 2.82^{a}	13.48 ± 0.50^{a}	7.71 ± 0.46^{b,*}	8.37 ± 0.19^{a}
	PS (Phosphatidylserine)	3.46 ± 0.21^{a,*}	5.09 ± 0.63^{b}	7.32 ± 1.69^{b}	5.76 ± 1.65^{a,b}	6.35 ± 0.54^{b}	7.37 ± 0.42^{b}
	PC (Phosphatidylcoline)	46.55 ± 2.85^{a,*}	41.38 ± 1.80^{b}	45.90 ± 2.37^{a}	51.73 ± 1.87^{b,*}	51.12 ± 1.69^{b,*}	53.90 ± 3.05^{b}
	PE (Phosphatidylethanolamine)	23.06 ± 0.54^{a}	23.82 ± 0.67^{a}	19.73 ± 2.88^{b,*}	21.36 ± 2.97^{a,b}	22.13 ± 1.11^{b}	17.01 ± 1.01^{c,*}
	CL (Cardiolipin)	3.44 ± 0.45^{a,*}	1.57 ± 0.07^{b,*}	4.67 ± 0.12^{c,*}	4.29 ± 0.22^{c,*}	5.93 ± 0.50^{c,*}	6.64 ± 1.48^{c}
	DMPE (Dimethyl-phosphatidylethanolamine)	2.34 ± 0.15^{a,*}	3.63 ± 0.82^{b,*}	1.61 ± 0.73^{c}	1.28 ± 0.28^{c}	1.10 ± 0.44^{c}	1.62 ± 0.74^{c}
	PA (Phosphatidic acid)	2.87 ± 0.60^{a}	4.54 ± 0.76^{a,b}	3.97 ± 2.08^{a,b}	1.33 ± 1.37^{a}	3.90 ± 0.70^{a,b}	3.65 ± 0.19^{a,b}
	LP (Lysophospholipids)	2.42 ± 0.27^{a,*}	4.14 ± 0.11^{b}	1.28 ± 1.07^{c}	0.75 ± 0.80^{c}	1.77 ± 0.06^{c,*}	1.44 ± 0.16^{c,*}
	PI/PS	4.64 ± 0.35^{a,*}	3.10 ± 0.26^{b,*}	1.99 ± 0.07^{c,*}	2.33 ± 0.13^{d}	1.21 ± 0.03^{e}	1.13 ± 0.09^{e}
	PC/PE	2.02 ± 0.08^{a,*}	1.74 ± 0.03^{b}	2.36 ± 0.16^{c,*}	2.40 ± 0.22^{c}	2.32 ± 0.19^{c}	3.18 ± 0.36^{d,*}
Sterols	Squalene	6.37 ± 1.75^{a,*}	11.01 ± 0.86^{b,*}	3.06 ± 0.15^{c,*}	3.34 ± 1.52^{c,*}	2.51 ± 0.49^{c,*}	n.d.
	Zymosterol	13.90 ± 0.57^{a,*}	6.10 ± 1.16^{b,*}	11.48 ± 1.56^{a,*}	9.09 ± 0.27^{c,*}	1.28 ± 0.02^{d,*}	n.d.
	4-methylzymosterol	0.90 ± 0.07^{a,*}	n.d.	2.29 ± 0.53^{b,*}	2.77 ± 0.86^{b,*}	n.d.	n.d.
	Fecosterol	4.06 ± 0.17^{a,*}	11.09 ± 1.30^{b,*}	11.78 ± 0.21^{b,*}	9.63 ± 0.77^{b,*}	n.d.	n.d.
	14-methylfecosterol	n.d.	n.d.	1.91 ± 0.34^{a,*}	1.77 ± 0.38^{a,*}	n.d.	n.d.
	Episterol	0.31 ± 0.44^{a,*}	n.d.	1.59 ± 0.08^{b,*}	1.73 ± 0.30^{c,*}	n.d.	n.d.
	Lanosterol	1.63 ± 0.17^{a,*}	6.05 ± 0.34^{b,*}	7.88 ± 0.23^{c,*}	8.97 ± 1.38^{c,*}	1.76 ± 0.59^{a}	n.d.
	Ergosterol	72.83 ± 1.48^{a,*}	65.15 ± 1.94^{b,*}	60.89 ± 3.34^{b,*}	62.69 ± 2.81^{b,*}	94.47 ± 1.69^{c,*}	100 ± 0.00^{d,*}
	Total sterols #	40.91 ± 2.10^{a,*}	20.00 ± 2.17^{b,*}	43.41 ± 2.48^{a,*}	27.80 ± 1.60^{c}	8.52 ± 1.00^{d,*}	9.99 ± 1.02^{d,*}
	Ergosterol/Squalene	11.90 ± 3.49^{a,*}	5.62 ± 0.71^{b,*}	19.92 ± 2.04^{c,*}	21.13 ± 2.46^{c,*}	38.26 ± 2.46^{d,*}	–

(μg/mg protein).
* UI, unsaturation index. The unsaturation index was defined as follows: ((percentage of C16:1 + percentage of C18:1) + 2 (percentage of C18:2) + 3(percentage of C18:3))/100 (Rodríguez-Vargas et al., 2007).

2000). In this study, we evaluated oxidative stress tolerance in selected non-*Saccharomyces* wine strains, namely, *T. delbrueckii* and *M. pulcherrima*, and we compared it to the *S. cerevisiae* response.

Our findings clearly indicated that these non-conventional yeasts are more tolerant to external oxidative stress than *S. cerevisiae*. As reported elsewhere (Jamieson, 1998; Moradas-Ferreira et al., 1996; Moradas-Ferreira and Costa, 2000), exposing yeast to H_2O_2 was associated with a rapid ROS generation and a loss of viability, at least until the yeast manages to adapt to the new environmental conditions, i.e., after the activation of defense mechanisms to maintain a proper redox state. Under our conditions, the *M. pulcherrima* species, and especially the autochthonous strain (Mpp), exhibited the greatest resistance to oxidative stress (low ROS generation and higher H_2O_2 tolerance). Both *T. delbrueckii* strains, also showed a higher oxidative resistance compared with *S. cerevisiae* as reported by Alves-Araújo et al. (2004) in a baking industry study. Furthermore, all the wine yeasts tested here were clearly more resistant to oxidative stress than the laboratory strain, probably due to their adaptive evolution to adverse stress conditions (Guillamón and Barrio, 2017; Querol et al., 2003). In fact, the BY4742 strain grew poorly after stress was applied, achieving less than one more generation after stress exposure in liquid medium. BY4742 is part of a set of deletion strains derived from S288C (Brachmann et al., 1998) with very poor fermentation capacity (Rossouw et al., 2013). Unlike wine yeast strains, BY4742 is not adapted to withstand adverse growth conditions such as those found during the fermentation process (Carrasco et al., 2001).

The cell's first barrier against stress is the cellular membrane, and lipids are one of its primary components. In this study, we evaluated the differences in lipid composition between the species before and after stress exposure. Our results showed that the cellular lipid composition differed widely between species, and thus it may be involved with their different abilities to resist and tolerate oxidative stress. Regardless of stress, the primary feature was the high fatty acid unsaturation rate observed in both non-*Saccharomyces* species, which was basically due to the presence of PUFAs, resulting in high membrane fluidity. It is well known that *S. cerevisiae* cannot synthesize PUFAs because it only contains one desaturase, Δ9 fatty acid desaturase (*OLE1*), which can only produce MUFAs of 16- and 18-carbon compounds (Stukey et al., 1990). However, *S. cerevisiae* can incorporate exogenous PUFAs into its cell membranes (Beltran et al., 2008; Rosi and Bertuccioli, 1992). Instead, in yeasts such as *Kluyveromyces lactis*, oleic acid (C18:1) is subsequently desaturated to linoleic acid (C18:2) and then to α-linolenic acid (C18:3) by Δ12 and omega (Δ15) fatty acid desaturases, respectively (Ratledge and Evans, 1989; Kainou et al., 2006; Santomartino et al., 2017). In our

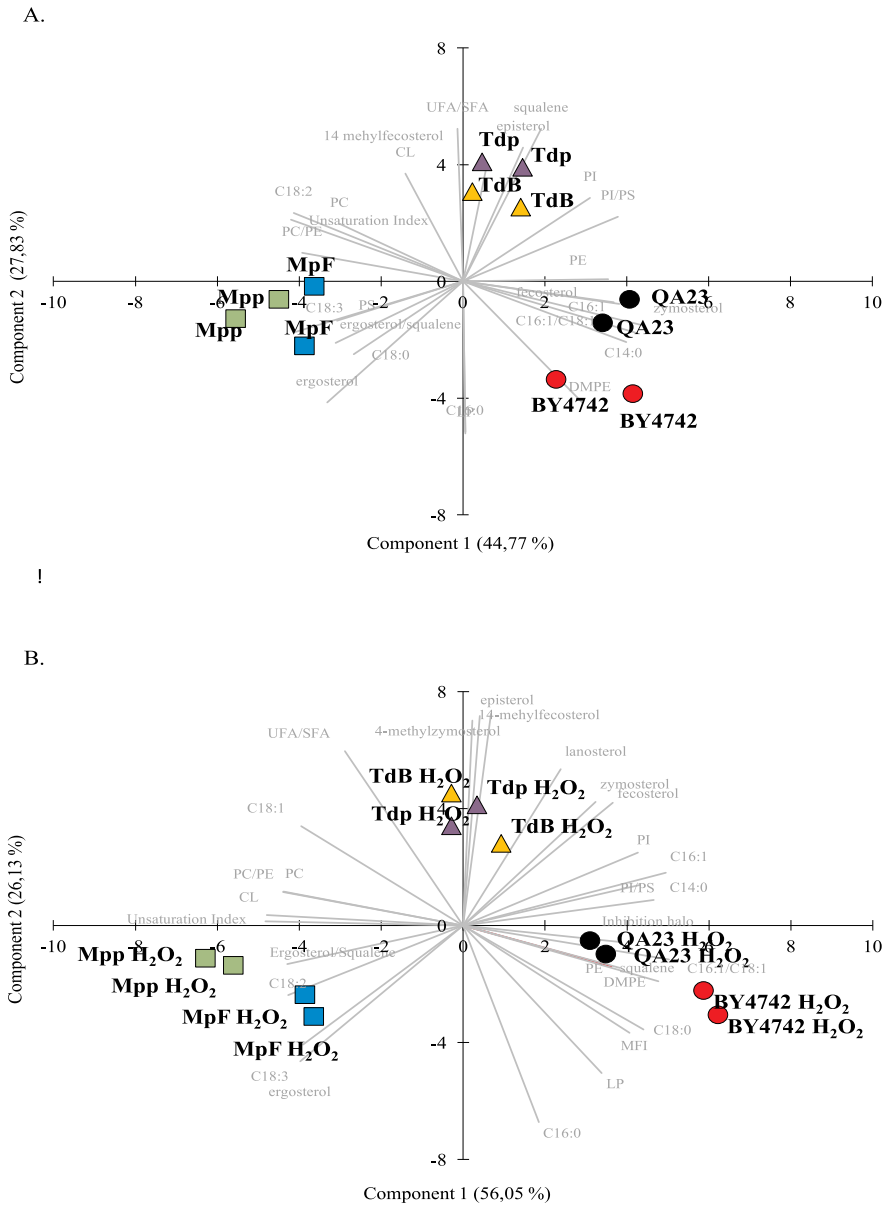

Fig. 5. Biplots of principal components analysis (PCA) using fatty acids (FAs), phospholipids (PLs), sterols, inhibition halo measures and ROS accumulation markers (MFI) as variables. *S. cerevisiae* strains: QA23 (●) and BY4742 (●); *T. delbrueckii* strains: TdB (▲) and Tdp (▲); *M. pulcherrima* strains: MpF (■) and Mpp (■). The explicative variables were distributed along the PCA as follows: (A) Biplot with 72.60% of the variance before the oxidative stress was applied. Component 1: (+); phosphatidylinositol (PI), phosphatidylethanolamine, (PE), PI/phosphatidylserine (PI/PS) ratio, mirystic (C14:0) and palmitoleic (C16:1) acids, C16:1/oleic (C16:1/C18:1) ratio, fecosterol and zymosterol. (−); PS, phosphatidylcoline (PC), PC/PE ratio, stearic (C18:0), linoleic (C18:2) and linolenic (C18:3) acids, ergosterol, unsaturated index and ergosterol/squalene ratio. Component 2: (+); unsaturated/saturated (UFA/SFA) ratio, cardiolipin (CL), squalene, 14-methylfecosterol and episterol. (−); dimethylphosphatidylethanolamine (DMPE), lysophospholipids (LP) and palmitic (C16:0) acid. (B) Biplot with 82.18% of the variance after oxidative stress (2 mM H_2O_2) was applied. Component 1: (+); inhibition halos, MFI, C14:0, C16:0, C16:1, C18:0, C16:1/C18:1 ratios, squalene, fecosterol, zymosterol, PI, PE, DMPE and PI/PS. (−); C18:1, C18:2, C18:3, ergosterol, ergosterol/squalene ratio, PC, CL, PC/PE ratio and unsaturation index. Component 2: (+); 4-methilzymosterol, 14-methylfecosterol, episterol, lanosterol and UFA/SFA ratio. (−); LP and C16:0.

case, both non-*Saccharomyces* species presented PUFAs in their lipid compositions, although linolenic acid (C18:3) was a unique feature of the *M. pulcherrima* strains. By contrast, the ratio $C_{16:1}/C_{18:1}$ ratio was higher in the *S. cerevisiae* strains, with the highest content of palmitoleic acid (C16:1), the primary UFA in aerobically grown *S. cerevisiae* strains (Steels et al., 1994), which is correlated with higher membrane rigidity (Redón et al., 2009). Many *S. cerevisiae* studies have reported a correlation between an increase in membrane fluidity (due to an increase in the degree of unsaturation) and a higher tolerance to various types of stresses, such as cold or ethanol stress (Beltran et al., 2008; Casey and Ingledew, 1986; Guerzoni et al., 1997; Suutari and Laakso, 1994). Therefore, according to the unsaturation degree, the studied non-*Saccharomyces* species were positively correlated with higher oxidative stress resistance. In fact, the introduction of the gene encoding the Δ12 fatty acid desaturase gene (*FAD2*) in the *S. cerevisiae* strains reportedly resulted in a higher resistance to ethanol (Kajiwara et al., 1996), and NaCl and freezing (Rodríguez-Vargas et al., 2007). Moreover, the introduction of both desaturases (*FAD2* and *FAD3* (ω3 fatty acid desaturase) from *K. lactis*) into a strain of *S. cerevisiae* has been reported to increase the alkaline pH tolerance (Yazawa et al., 2009). Therefore, although PUFAs seem to increase yeast tolerance to stress, they can also be toxic to cells because of their susceptibility to peroxidation (Cipak et al., 2006; Johansson et al., 2016). In fact, the heterologous production of PUFAs in *S. cerevisiae* has been shown to increase oxidative stress (Ruenwai et al., 2011), and in non-*Saccharomyces* strains, a higher proportion of C18:2 acid does not assure increased tolerance to ethanol stress (Aguilera et al., 2006; Archana et al., 2015). Under our conditions, i.e., under oxidative stress, high levels of C18:2 acid were positively correlated with low ROS generation and high H_2O_2 tolerance. Nevertheless, the amounts of PUFAs decreased in all the non-*Saccharomyces* strains after stress exposure, probably indicating that the strategy of these species was a reduction of the PUFA content due to their high sensitivity to peroxidation (Ayala et al., 2014; Johansson et al., 2016). This effect could be a mechanism in non-conventional yeasts to withstanding the oxidative stress better without compromising membrane integrity. The other principal mechanism used by non-*Saccharomyces* yeasts to cope with oxidative stress was the modulation of their FA composition, by raising the proportion of MUFAs, such as palmitoleic acid and oleic acid, and by decreasing the amounts of SFA, such as palmitic acid and stearic acid. Oleic acid has been suggested as

a membrane fluidity sensor, and it seems to be the most important UFA for counteracting the toxic nature of ethanol by increasing the membrane stability and antagonizing the fluidity caused by ethanol (You et al., 2003). Furthermore, palmitoleic acid is induced by stress in high-density fermentations, and it has a protective function against damage (Ding et al., 2009). According to Redón et al. (2009), the supplementation of palmitoleic acid in wine yeast culture has a positive effect on the yeast viability and the fermentation kinetics. However, although the UFA/SFA ratio increased, the results showed a decrease in the unsaturation index in non-*Saccharomyces* species, indicating how yeasts try to maintain their membrane fluidity.

Regarding the phospholipid composition, PC and PE are the primary PLs of yeast membranes, representing up to 60–70% of total PLs (Schneiter et al., 1999). The PC/PE ratio is an important parameter for the biophysical status of the membrane (Flis et al., 2015). An increase in the PC/PE ratio has been reported as one of the yeast adaptation mechanisms to oxidative stress, leading to a reorganization of the plasma membrane lipid composition, and a decrease of the membrane permeability against H_2O_2 (Pedroso et al., 2009). On the other hand, a decrease of PC/PE ratio, in combination with low UFA/SFA and high amounts of ergosterol, leads to higher transition temperature of a lipid bilayer, and membranes may become more rigid (Flis et al., 2015). Thus, the large quantities of PC in non-conventional yeasts could lead to a decrease of cellular permeability to H_2O_2 (Pedroso et al., 2009), enhancing oxidative stress tolerance, in a similar way to what has been observed in *S. cerevisiae* ethanol tolerance (Chi and Arneborg, 1999; Vendramin-Pintar et al., 1995). The PI/PS ratio is another important parameter for cell growth potential and essential for maintaining cellular viability in *S. cerevisiae* (Xia et al., 2011). The synthesis of these PLs is closely correlated, because both require the same precursor cytidyldiphosphate diacylglycerol (CDP-DAG) precursor. Moreover, PS can be a precursor for the synthesis of PE and PC (Voelker and Frazier, 1986). However, PI is considered essential for *S. cerevisiae* because the lack of this PL can reduce cell viability (Becker and Lester, 1977; De Kroon et al., 2013). Our results show that the *S. cerevisiae* strains exhibited a high PI/PS ratio, especially after stress exposure, whereas the *M. pulcherrima* strains, which had the highest resistance to H_2O_2 stress, exhibited the lowest values, especially after stress exposure. In fact, this ratio was negatively correlated with tolerance to oxidative stress.

Mitochondria are both the source and the site for the detoxification of reactive oxygen species in yeast (Chevtzoff et al., 2010; Rhoads et al., 2006). Therefore, normal mitochondrial function is required for resistance to oxidative stress (Grant et al., 1997), and the maintenance of a stable respiratory chain strongly prevents the generation of mitochondrial ROS (Barros et al., 2003). CL, a mitochondrial PL found in the inner mitochondrial membrane (De Kroon et al., 2013), plays a key role in the stabilization of electron transport chain complexes and the resistance against oxidative stress during respiratory growth (Chen et al., 2008). Most of the strains in this study, and especially those of *S. cerevisiae*, decreased their CL content after stress exposure, which could indicate a destabilization of the respiratory chain. However, despite this decrease, non-*Saccharomyces* species displayed higher CL values, suggesting a better maintenance of functional mitochondria during H_2O_2 stress as previously described for ethanol stress (Chi and Arneborg, 1999).

Sterols are necessary for maintaining membrane integrity and essential for cell viability (Daum et al., 1998). Consistent with the results obtained by Murakami et al. (1996) in freezing-tolerant strains, our study demonstrated that the sterol content was low in the most H_2O_2-tolerant strains (*M. pulcherrima* species). However, a direct correlation between the sterol content and H_2O_2 tolerance was not observed because, e.g., BY4742 was the most sensitive strain, but it also contained low levels of sterols. Ergosterol, the primary yeast sterol and the end product of the yeast sterol biosynthetic pathway, is responsible for structural membrane features such as higher fluidity and other physiological functions (Parks, 1978; Daum et al., 1998; Klug and Daum, 2014). Although *M. pulcherrima* showed the highest ergosterol percentage (but the lowest content), this parameter could not be correlated with the oxidative stress either. Nevertheless, *M. pulcherrima* might be compensating for fluidization effect elicited by the oxidative stress. The overall fluidity/rigidity of a membrane is the result of a combination of all parameters mentioned (Ding et al., 2009). Unsaturated fatty acids, such as oleic acid, increase cell membrane fluidity. On the other hand, low amounts of ergosterol would facilitate membrane rigidity, allowing yeast to maintain membrane functionality.

The growth of the BY4742 strain was clearly affected after stress exposure, and it showed the highest squalene content and the lowest ergosterol/squalene ratio after stress exposure, being both parameters positively correlated with less tolerance to stress. It has to be taken into account that squalene, the precursor of the synthesis of ergosterol, is a highly hydrophobic molecule, which lacks the amphipathic character provided by the hydroxyl group at C3 atom present in sterols. However, even if under standard conditions it is stored in lipid droplets, it can also be found in organelle membranes (e.g in yeast cells grown anaerobically or in strains lacking *HEM1* gene) without causing deleterious effects (Spanova et al., 2010, 2012). Therefore, although squalene is not a typical membrane lipid, it may be considered as a mild modulator of biophysical membrane properties (Spanova et al., 2012).

High ratios of UFA/SFA and high PC/PE ratios in membranes are known to lead to high membrane fluidity (Flis et al., 2015). In the natural strains of this study, these parameters also seem to lead to higher tolerance against to H_2O_2.

5. Conclusions

In conclusion, our results suggest that non-conventional yeasts are best at resisting induced oxidative stress. The highest stress tolerance was associated with the non-conventional yeasts' abilities to maintain a high proportion and level of unsaturated fatty acids, particularly linolenic acid and linoleic acid. Furthermore, the large variability in the fatty acid composition can result from adaptive responses to changes in external physico-chemical parameters.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2018.10.001.

References

Acar, F., 1980. The disc susceptibility test. In: Baltimore, L.V. (Ed.), Antibiotics in Laboratory Medicine, London, pp. 24–54.

Alves-Araújo, C., Almeida, M.J., Sousa, M.J., Leão, C., 2004. Freeze tolerance of the yeast *Torulaspora delbrueckii*: cellular and biochemical basis. FEMS Microbiol. Lett. 240, 7–14. https://doi.org/10.1016/j.femsle.2004.09.008.

Aguilera, F., Peinado, R.A., Millán, C., Ortega, J.M., Mauricio, J.C., 2006. Relationship between ethanol tolerance, H+ -ATPase activity and the lipid composition of the plasma membrane in different wine yeast strains. Int. J. Food Microbiol. 110, 34–42. https://doi.org/10.1016/j.ijfoodmicro.2006.02.002.

Archana, K.M., Ravi, R., Anu-Appaiah, K.A., 2015. Correlation between ethanol stress and cellular fatty acid composition of alcohol producing non-*Saccharomyces* in comparison with *Saccharomyces cerevisiae* by multivariate techniques. J. Food Sci. Technol. 52, 6770–6776. https://doi.org/10.1007/s13197-015-1762-y.

Athenstaedt, K., Zweytick, D., Jandrositz, A., Kohlwein, S.D., Daum, G., 1999. Identification and characterization of major lipid particle proteins of the yeast

Saccharomyces cerevisiae. J. Bacteriol. 181, 6441–6448.
Avery, S.V., 2011. Molecular targets of oxidative stress. Biochem. J. 434, 201–210. https://doi.org/10.1042/BJ20101695.
Ayala, A., Muñoz, M.F., Arguelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. 2014. https://doi.org/10.1155/2014/360438.
Bauer, A.W., Kirby, W.M.M., J.C, Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45, 493–496.
Barros, M.H., Netto, L.E.S., Kowaltowski, A.J., 2003. H_2O_2 generation in *Saccharomyces cerevisiae* respiratory pet mutants: effect of cytochrome c. Free Radic. Biol. Med. 35, 179–188.
Becker, G.W., Lester, R.L., 1977. Changes in phospholipids of *Saccharomyces cerevisiae* associated with inositol-less death. J. Biol. Chem. 252, 8684–8691.
Belda, I., Ruiz, J., Esteban-Fernández, A., Navascués, E., Marquina, D., Santos, A., Moreno-Arribas, M.V., 2017. Microbial contribution to wine aroma and its intented use for wine quality improvement. Molecules 22, 1–29. https://doi.org/10.3390/molecules22020189.
Beltran, G., Novo, M., Guillamón, J.M., Mas, A., Rozès, N., 2008. Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. Int. J. Food Microbiol. 121, 169–177. https://doi.org/10.1016/j.ijfoodmicro.2007.11.030.
Beltran, G., Torija, M.J., Novo, M., Ferrer, N., Poblet, M., Guillamon, J.M., Rozes, N., Mas, A., 2002. Analysis of yeast populations during alcoholic fermentation: a six year follow-up study. Syst. Appl. Microbiol. 25, 287–293. https://doi.org/10.1078/0723-2020-00097.
Bely, M., Stoeckle, P., Masneuf-Pomarède, I., Dubourdieu, D., 2008. Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. Int. J. Food Microbiol. 122, 312–320. https://doi.org/10.1016/j.ijfoodmicro.2007.12.023.
Beney, L., Gervais, P., 2001. Influence of the fluidity of the membrane on the response of microorganisms to environmental stresses. Appl. Microbiol. Biotechnol. 57, 34–42. https://doi.org/10.1007/s002530100754.
Boettiger, D., Huber, F., Lynch, L., Blystone, S., 2001. Activation of $\alpha_v\beta3$-vitronectin binding is a multistage process in which increases in bond strength are dependent on Y747 and Y759 in the cytoplasmic domain of β3. Mol. Biol. Cell 12, 1227–1237. https://doi.org/10.1091/mbc.12.5.1227.
Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., Boeke, J.D., 1998. Designer deletion strains. Yeast 14, 115–132. https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2.
Broekhuyse, R.M., 1968. Phospholipids in tissues of the eye I. Isolation, characterization and quantitative analysis by two-dimensional thin-layer chromatography of diacyl and vinyl-ether phospholipids. Biochim. Biophys. Acta Lipids Lipid Metab. 152, 307–315. https://doi.org/10.1016/0005-2760(68)90038-6.
Carrasco, P., Querol, A., Del Olmo, M., 2001. Analysis of the stress resistance of commercial wine yeast strains. Arch. Microbiol. 175, 450–457. https://doi.org/10.1007/s002030100289.
Casey, G.P., Ingledew, W.M., 1986. Ethanol tolerance in yeasts. Crit. Rev. Microbiol. 13, 219–280. https://doi.org/10.3109/10408418609108739.
Chen, S., He, Q., Greenberg, M.L., 2008. Loss of tafazzin in yeast leads to increased oxidative stress during respiratory growth. Mol. Microbiol. 68, 1061–1072. https://doi.org/10.1111/j.1365-2958.2008.06216.x.
Chevtzoff, C., Yoboue, E.D., Galinier, A., Casteilla, L., Daignan-Fornier, B., Rigoulet, M., Devin, A., 2010. Reactive oxygen species-mediated regulation of mitochondrial biogenesis in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 285, 1733–1742. https://doi.org/10.1074/jbc.M109.019570.
Chi, Z., Arneborg, N., 1999. Relationship between lipid composition, frequency of ethanol-induced respiratory deficient mutants, and ethanol tolerance in *Saccharomyces cerevisiae*. J. Appl. Microbiol. 86, 1047–1052. https://doi.org/10.1046/j.1365-2672.1999.00793.x.
Cipak, A., Hasslacher, M., Tehlivets, O., Collinson, E.J., Zivkovic, M., Matijevic, T., Wonisch, W., Waeg, G., Dawes, I.W., Zarkovic, N., Kohlwein, S.D., 2006. *Saccharomyces cerevisiae* strain expressing a plant fatty acid desaturase produces polyunsaturated fatty acids and is susceptible to oxidative stress induced by lipid peroxidation. Free Radic. Biol. Med. 40, 897–906. https://doi.org/10.1016/j.freeradbiomed.2005.10.039.
Costa, V., Moradas-Ferreira, P., 2001. Oxidative stress and signal transduction in *Saccharomyces cerevisiae*: insights into ageing, apoptosis and diseases. Mol. Aspect. Med. 22, 217–246. https://doi.org/10.1016/S0098-2997(01)00012-7.
Daum, G., Lees, N.D., Bard, M., Dickson, R., 1998. Biochemistry, cell biology and molecular biology of lipids of *Saccharomyces cerevisiae*. Yeast 14, 1471–1510. https://doi.org/10.1002/(SICI)1097-0061(199812)14:16<1471::AID-YEA353>3.0.CO;2-Y.
De Kroon, A.I., Rijken, P.J., De Smet, C.H., 2013. Checks and balances in membrane phospholipid class and acyl chain homeostasis, the yeast perspective. Prog. Lipid Res. 52, 374–394. https://doi.org/10.1016/j.plipres.2013.04.006.
Ding, J., Huang, X., Zhang, L., Zhao, N., Yang, D., Zhang, K., 2009. Tolerance and stress response to ethanol in the yeast *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 85, 253. https://doi.org/10.1007/s00253-009-2223-1.
Fleet, G.H., Heard, G.M., 1993. Yeasts-growth during fermentation. In: Fleet, G.H. (Ed.), Wine Microbiology and Biotechnology. Harwood Academic Publishers, Switzerland, pp. 27–54.
Fleet, G.H., 2008. Wine yeasts for the future. FEMS Yeast Res. 8, 979–995. https://doi.org/10.1111/j.1567-1364.2008.00427.x.
Flis, V.V., Fankl, A., Ramprecht, C., Zellnig, G., Leitner, E., Hermetter, A., Daum, G., 2015. Phosphatidylcholine supply to peroxisomes of the yeast *Saccharomyces cerevisiae*. PLoS One 10, 1–19. https://doi.org/10.1371/journal.pone.0135084.
Folch, J., Lees, M., Sloane, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.
Gamero-Sandemetrio, E., Gomez-Pastor, R., Matallana, E., 2014. Antioxidant defense parameters as predictive biomarkers for fermentative capacity of active dried wine yeast. Biotechnol. J. 9, 1055–1064. https://doi.org/10.1002/biot.201300448.
Gibson, B.R., Lawrence, S.J., Boulton, C.A., Box, W.G., Graham, N.S., Linforth, R.S.T., Smart, K.A., 2008. The oxidative stress response of a lager brewing yeast strain during industrial propagation and fermentation. FEMS Yeast Res. 8, 574–585. https://doi.org/10.1111/j.1567-1364.2008.00371.x.
Gibson, B.R., Lawrence, S.J., Leclaire, J.P.R., Powell, C.D., Smart, K.A., 2007. Yeast responses to stresses associated with industrial brewery handling. FEMS Microbiol. Rev. 31, 535–569. https://doi.org/10.1111/j.1574-6976.2007.00076.x.
González-Royo, E., Pascual, O., Kontoudakis, N., Esteruelas, M., Esteve-Zarzoso, B., Mas, A., Canals, J.M., Zamora, F., 2015. Oenological consequences of sequential inoculation with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii* or *Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. Eur. Food Res. Technol. 240, 999–1012. https://doi.org/10.1007/s00217-014-2404-8.
Grant, C.M., Maclever, F.H., Dawes, I.W., 1997. Mitochondrial function is required for resistance to oxidative stress in the yeast *Saccharomyces cerevisiae*. FEBS Lett. 410, 219–222. https://doi.org/10.1016/S0014-5793(97)00592-9.
Guerzoni, M.E., Ferruzzi, M., Sinigaglia, M., Criscuoli, G.C., 1997. Increased cellular fatty acid desaturation as a possible key factor in thermotolerance in *Saccharomyces cerevisiae*. Can. J. Microbiol. 43, 569–576.
Guillamón, J.M., Barrio, E., 2017. Genetic polymorphism in wine yeasts: mechanisms and methods for its detection. Front. Microbiol. 8, 806. https://doi.org/10.3389/fmicb.2017.00806.
Herrero, E., Ros, J., Bellí, G., Cabiscol, E., 2008. Redox control and oxidative stress in yeast cells. Biochim. Biophys. Acta 1780, 1217–1235. https://doi.org/10.1016/j.bbagen.2007.12.004.
Howlett, N.G., Avery, S.V., 1997. Induction of lipid peroxidation during heavy metal stress in *Saccharomyces cerevisiae* and influence of plasma membrane. Fatty Acid Unsaturation 63, 2971–2976.
Hunter, Rose, 1972. Lipid composition of *Saccharomyces cerevisiae* as influenced by growth temperature. Biochim. Biophys. Acta 260, 639–653.
Jamieson, D.J., 1998. Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. Yeast 14, 1511–1527. https://doi.org/10.1002/(SICI)1097-0061(199812)14:16<1511::AID-YEA356>3.0.CO;2-S.
Johansson, M., Chen, X., Milanova, S., Santos, C., Petranovic, D., 2016. PUFA-induced cell death is mediated by Yca1p-dependent and -independent pathways, and is reduced by vitamin C in yeast. FEMS Yeast Res. 16, 1–9. https://doi.org/10.1093/femsyr/fow007.
Jolly, N.P., Varela, C., Pretorius, I.S., 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Res. 14, 215–237. https://doi.org/10.1111/1567-1364.12111.
Kainou, K., Kamisaka, Y., Kimura, K., Uemura, H., 2006. Isolation of Δ12 and ω3-fatty acid desaturase genes from the yeast *Kluyveromyces lactis* and their heterologous expression to produce linoleic and α-linolenic acids in *Saccharomyces cerevisiae*. Yeast 23, 605–612. https://doi.org/10.1002/yea.1378.
Kajiwara, S., Shirai, A., Fujii, T., Toguri, T., Nakamura, K., Ohtaguchi, K., 1996. Polyunsaturated fatty acid biosynthesis in *Saccharomyces cerevisiae*: expression of ethanol tolerance and the FAD2 gene from Arabidopsis thaliana. Appl. Environ. Microbiol. 62, 4309–4313.
Klug, L., Daum, G., 2014. Yeast lipid metabolism at a glance. FEMS Yeast Res. 14, 369–388. https://doi.org/10.1111/1567-1364.12141.
Korber, M., Klein, I., Daum, G., 2017. Steryl ester synthesis, storage and hydrolysis: a contribution to sterol homeostasis. Biochim. Biophys. Acta 1862, 1534–1545. https://doi.org/10.1016/j.bbalip.2017.09.002.
Los, D.A., Murata, N., 2004. Membrane fluidity and its roles in the perception of environmental signals. Biochim. Biophys. Acta 1666, 142–157. https://doi.org/10.1016/j.bbamem.2004.08.002.
Lowry, H., Nira, J., Rosebrough, A., Farr, Lewis, Randall, J., 1951. Protein measurement with the Folin phenol reagent. Anal. Biochem. 217, 220–230. https://doi.org/10.1016/0304-3894(92)87011-4.
Mas, A., Guillamón, J.M., Beltran, G., 2016. Editorial: non-conventional yeast in the wine industry. Front. Microbiol. 7, 1494. https://doi.org/10.3389/fmicb.2016.01494.
Matallana, E., Aranda, A., 2017. Biotechnological impact of stress response on wine yeast. Lett. Appl. Microbiol. 64, 103–110 doi:10.111/lam.12677.
Moradas-Ferreira, P., Costa, V., 2000. Adaptive response of the yeast *Saccharomyces cerevisiae* to reactive oxygen species: defences, damage and death. Redox Rep. 5, 277–285. https://doi.org/10.1179/135100000101535816.
Moradas-Ferreira, P., Costa, V., Piper, P., Mager, W., 1996. The molecular defences against reactive oxygen species in yeast. Mol. Microbiol. 19, 651–658. https://doi.org/10.1046/j.1365-2958.1996.403940.x.
Murakami, Y., Yokoigawa, K., Kawai, F., Kawai, H., 1996. Lipid composition of commercial bakers' yeasts having different freeze-tolerance in frozen dough. Biosci. Biotechnol. Biochem. 60, 1874–1876. https://doi.org/10.1271/bbb.60.1874.
Padilla, B., García-Fernández, D., González, B., Izidoro, I., Esteve-Zarzoso, B., Beltran, G., Mas, A., 2016. Yeast biodiversity from DOQ priorat uninoculated fermentations. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00930.
Padilla, B., Zulian, L., Ferreres, À., Pastor, R., Esteve-Zarzoso, B., Beltran, G., Mas, A., 2017. Sequential inoculation of native non-*Saccharomyces* and *Saccharomyces cerevisiae* strains for wine making. Front. Microbiol. 8, 1–12. https://doi.org/10.3389/fmicb.2017.01293.
Parks, L.W., 1978. Metabolism of sterols in yeast. Crit. Rev. Microbiol. 6, 301–340.
Pedroso, N., Matias, A.C., Cyrne, L., Antunes, F., Borges, C., Malhó, R., Rodrigo, F.M., Herrero, E., Marinho, H., 2009. Modulation of plasma membrane lipid profile and

microdomains by H2O2 in *Saccharomyces cerevisiae*. Free Radic. Biol. Med. 46, 289–298. https://doi.org/10.1016/j.freeradbiomed.2008.10.039.
Pretorius, I.S., 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16, 675–729. https://doi.org/10.1002/1097-0061(20000615)16:8<675::AID-YEA585>3.0.CO;2-B.
Quail, M.A., Kelly, S.L., 1996. In: Evans, I.H. (Ed.), The Extraction and Analysis of Sterols from Yeast BT - Yeast Protocols: Methods in Cell and Molecular Biology. Humana Press, Totowa, NJ, pp. 123–131. https://doi.org/10.1385/0-89603-319-8:123.
Querol, A., Fernández-Espinar, M.T., Del Olmo, M., Barrio, E., 2003. Adaptive evolution of wine yeast. Int. J. Food Microbiol. 86, 3–10. https://doi.org/10.1016/S0168-1605(03)00244-7.
Ratledge, C., Evans, C.T., 1989. Lipids and their metabolism. In: In: Rose, A.H., Harrison, J.S. (Eds.), The Yeasts, vol. 3. Academic Press, London, pp. 367–455.
Redón, M., Guillamón, J.M., Mas, A., Rozès, N., 2009. Effect of lipid supplementation upon *Saccharomyces cerevisiae* lipid composition and fermentation performance at low temperature. Eur. Food Res. Technol. 228, 833–840. https://doi.org/10.1007/s00217-008-0996-6.
Rhoads, D.M., Umbach, A.L., Subbaiah, C.C., Siedow, J.N., 2006. Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol. 141, 357–366. https://doi.org/10.1104/pp.106.079129.
Ribereau-Gayon, P., 1985. New developments in wine microbiology. Am. J. Enol. Vitic. 36, 1–10.
Rodríguez-Vargas, S., Sánchez-García, A., Martínez-Rivas, J.M., Prieto, J.A., Randez-Gil, F., 2007. Fluidization of membrane lipids enhances the tolerance of *Saccharomyces cerevisiae* to freezing and salt stress. Appl. Environ. Microbiol. 73, 110–116. https://doi.org/10.1128/AEM.01360-06.
Romano, P., Fiore, C., Paraggio, M., Caruso, M., Capece, A., 2003. Function of yeast species and strains in wine flavour. Int. J. Food Microbiol. 86, 169–180. https://doi.org/10.1016/S0168-1605(03)00290-3.
Rosi, I., Bertuccioli, M., 1992. Influences of lipid addition on fatty acid composition of *Saccharomyces cerevisiae* and aroma characteristics of experimental wines 1. J. Inst. Brew. 98, 305–314. https://doi.org/10.1002/j.2050-0416.1992.tb01113.x.
Rossouw, D., Heyns, E.H., Setati, M.E., Bosch, S., Bauer, F.F., 2013. Adjustment of trehalose metabolism in wine *Saccharomyces cerevisiae* strains to modify ethanol yields. Appl. Environ. Microbiol. 79, 5197–5207. https://doi.org/10.1128/AEM.00964-13.
Ruenwai, R., Neiss, A., Laoteng, K., Vongsangnak, W., Dalfard, A.B., Cheevadhanarak, S., Petranovic, D., Nielsen, J., 2011. Heterologous production of polyunsaturated fatty acids in *Saccharomyces cerevisiae* causes a global transcriptional response resulting in reduced proteasomal activity and increased oxidative stress. Biotechnol. J. 6, 343–356. https://doi.org/10.1002/biot.201000316.
Rußmayer, H., Buchetics, M., Gruber, C., Valli, M., Grillitsch, K., Modarres, G., Guerrasio, R., Klavins, K., Neubauer, S., Drexler, H., Steiger, M., Troyer, C., Al Chalabi, A., Krebiehl, G., Sonntag, D., Zellnig, G., Daum, G., Graf, A.B., Altmann, F., Koellensperger, G., Hann, S., Sauer, M., Mattanovich, D., Gasser, B., 2015. Systems-level organization of yeast methylotrophic lifestyle. BMC Biol. 13, 80. https://doi.org/10.1186/s12915-015-0186-5.
Santomartino, R., Riego-Ruiz, L., Bianchi, M.M., 2017. Three, two, one yeast fatty acid desaturases: regulation and function. World J. Microbiol. Biotechnol. 33, 1–12. https://doi.org/10.1007/s11274-017-2257-y.
Schneiter, R., Brugger, B., Sandhoff, R., Zellnig, G., Leber, A., Lampl, M., Athenstaedt, K., Hrastnik, C., Eder, S., Daum, G., Paltauf, F., Wieland, F.T., Kohlwein, S.D., 1999. Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals acyl chain-based sorting/remodeling of distinct molecular species en route to the plasma membrane. J. Cell Biol. 146, 741–754.
Spanova, M., Czabany, T., Zelling, G., Leitner, E., Hapala, I., Daum, G., 2010. Effect of lipid particle biogenesis on the subcellular distribution of squalene in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 285, 6127–6133. https://doi.org/10.1074/jbc.M109.074229.
Spanova, M., Zweytick, D., Lohner, K., Klug, L., Leitner, E., Hermetter, A., Daum, G., 2012. Influence of squalene on lipid particle/droplet and membrane organization in the yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1821, 647–653. https://doi.org/10.1016/j.bbalip.2012.01.015.
Steels, E.L., Learmonth, R.P., Watson, K., 1994. Stress tolerance and membrane lipid unsaturation in *Saccharomyces cerevisiae* grown aerobically or anaerobically. Microbiology 140 (Pt 3), 569–576. https://doi.org/10.1099/00221287-140-3-569.
Stukey, J.E., McDonough, V.M., Martin, C.E., 1990. The OLE1 gene of *Saccharomyces cerevisiae* encodes the delta 9 fatty acid desaturase and can be functionally replaced by the rat stearoyl-CoA desaturase gene. J. Biol. Chem. 265, 20144–20149.
Suutari, M., Laakso, S., 1994. Microbial fatty acids and thermal adaptation. Crit. Rev. Microbiol. 20, 285–328. https://doi.org/10.3109/10408419409113560.
Torija, M.J., Beltran, G., Novo, M., Poblet, M., Guillamón, J.M., Mas, A., Rozès, N., 2003. Effects of fermentation temperature and *Saccharomyces* species on the cell fatty acid composition and presence of volatile compounds in wine. Int. J. Food Microbiol. 85, 127–136. https://doi.org/10.1016/S0168-1605(02)00506-8.
Vázquez, J., González, B., Sempere, V., Mas, A., Torija, M.J., Beltran, G., 2017. Melatonin reduces oxidative stress damage induced by hydrogen peroxide in *Saccharomyces cerevisiae*. Front. Microbiol. 8, 1–14. https://doi.org/10.3389/fmicb.2017.01066.
Vendramin-Pintar, M., Jernejc, K., Cimerman, A., 1995. A comparative study op lipid composition of baker's and brewer's yeasts. Food Biotechnol. 9, 207–215. https://doi.org/10.1080/08905439509549894.
Voelker, D.R., Frazier, J.L., 1986. Isolation and characterization of a Chinese hamster ovary cell line requiring ethanolamine or phosphatidylserine for growth and exhibiting defective phosphatidylserine synthase activity. J. Biol. Chem. 261, 1002–1008.
Walker, G., Dijck, P., 2006. Physiological and molecular responses of yeasts to the environment. In: Querol, A., Fleet, G.H. (Eds.), Yeasts in Food and Beverages. Springer-Verlag, Berlin Heidelberg, pp. 111–112.
Xia, J., Jones, A.D., Lau, M.W., Yuan, Y.J., Dale, B.E., Balan, V., 2011. Comparative lipidomic profiling of xylose-metabolizing S. cerevisiae and its parental strain in different media reveals correlations between membrane lipids and fermentation capacity. Biotechnol. Bioeng. 108, 12–21. https://doi.org/10.1002/bit.22910.
Yazawa, H., Iwahashi, H., Kamisaka, Y., Kimura, K., Uemura, H., 2009. Production of polyunsaturated fatty acids in yeast *Saccharomyces cerevisiae* and its relation to alkaline pH tolerance. Yeast 26, 167–184. https://doi.org/10.1002/yea.1659.
You, K.M., Rosenfield, C., Knipple, D.C., 2003. Ethanol tolerance in the yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content. Appl. Environ. Microbiol. 69, 1499. https://doi.org/10.1128/AEM.69.3.1499.