Vicente *et al. AMB Express* (2025) 15:90
https://doi.org/10.1186/s13568-025-01896-4

AMB Express

RESEARCH Open Access

Ochratoxin A reduction in wine fermentation: evaluating the potential of *Lachancea thermotolerans*

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Abstract
Ochratoxin A is a mycotoxin commonly found in wine, primarily produced by fungal species from the *Aspergillus* and *Penicillium* genera. Due to its nephrotoxic, neurotoxic, immunotoxic, and carcinogenic properties, ochratoxin A contamination in wine is a significant concern for public health. This study investigates the potential of *Lachancea thermotolerans* in reducing ochratoxin A levels during wine fermentation, evaluating its fermentative performance and impact on key enological parameters. Fermentation trials with 32 *L. thermotolerans* strains demonstrated considerable variability in fermentation kinetics, ethanol production, and sugar consumption. The yeast exhibited strain-dependent variability in the production of organic acids, including succinic and lactic acid, leading to significant differences in total acidity and pH. Additionally, *L. thermotolerans* produced glycerol levels comparable to or exceeding those of *Saccharomyces cerevisiae*. The ability of *L. thermotolerans* to reduce ochratoxin A was highly strain-dependent, with reductions ranging widely. The most effective strains achieved ochratoxin A reductions exceeding those previously reported for *S. cerevisiae*. However, an inverse correlation was observed between ochratoxin A reduction and polyphenol retention, suggesting that strains with high ochratoxin A adsorption may also bind anthocyanins and polyphenols, affecting wine color and structure. These findings highlight *L. thermotolerans* as a promising non-*Saccharomyces* yeast for mitigating ochratoxin A contamination in wine while contributing positively to acidity modulation and sensory attributes. The study underscores the importance of strain selection to balance ochratoxin A detoxification with desirable enological properties, particularly in regions where contamination poses a significant challenge to wine safety and quality.

Keywords *Lachancea thermotolerans*, Ochratoxin A, Lactic acid, Wine, Acidity

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Introduction

Ochratoxin A (OTA) is a mycotoxin frequently found in wine, primarily produced by *Aspergillus* and *Penicillium* species. It is recognized for its nephrotoxic, neurotoxic, immunotoxic, mutagenic, and teratogenic effects, which raise significant concerns about its impact on human health (Ding et al. 2023). The International Agency for Research on Cancer (IARC) has classified OTA as a probable human carcinogen (Group 2A) (Benito 2019). Even at low concentrations, OTA poses risks to vulnerable populations such as children, pregnant women, and individuals with renal disorders. Regulatory limits for OTA in wine have been established in many regions, with the European Union setting a critical threshold of 2 µg/kg (Benito 2019).

The presence of Ochratoxin A (OTA) in wine is typically associated with fungal contamination of grapes, primarily by *Aspergillus carbonarius*, under conditions of high humidity and poor vineyard management (Gambuti et al. 2005). However, other species of both *Aspergillus* and *Penicillium* also contribute to OTA contamination in grapes. Species such as *Aspergillus niger* and other members of the *A. niger* aggregate group (*A. welwitschiae*, *A. tubingensis*), *Aspergillus steynii*, *A. westerdijkiae*, and *A. ochraceus* from the Circumdati section, as well as *Penicillium nordicum* and *Penicillium verrucosum*, have all been identified as OTA producers in grapevine environments (Gil-Serna et al. 2018). While *A. carbonarius* is the most prevalent and potent producer of OTA in grapes, these other fungal species also play a significant role in contaminating wine, with their prevalence influenced by geographical and environmental conditions such as temperature and humidity.

The presence of Ochratoxin A (OTA) in wine varies across different countries and wine types, with a particular concern for those samples exceeding the legal limit of 2 µg/L established by the European Union. Studies show that while many samples of wine are contaminated with OTA, only a small proportion exceed the legal threshold (Gil-Serna et al. 2018). For instance, in Argentina, 9% of red wine samples tested positive for OTA, with a maximum level of 4.82 µg/L, but only 4% of the samples exceeded the 2 µg/L limit. Similarly, in Australia, 14% of red wines and 16% of white wines contained OTA, with maximum levels of 0.62 µg/L, but none surpassed the legal limit. In Greece, 68% of red wine samples, 53% of white wines, and 83% of dessert wines showed OTA contamination, with the highest level recorded at 2.82 µg/L. However, no samples exceeded the 2 µg/L limit. In Italy, 93% of red wine samples were positive for OTA, with levels reaching up to 7.63 µg/L, but only 2% exceeded the legal threshold. Spain had 19% of red wine samples and 8% of white wines contaminated with OTA, with maximum concentrations of 4.24 µg/L and 0.18 µg/L, respectively, and only a small proportion surpassing the legal limit.

One study investigated the role of eight different yeast strains from eight different species in reducing OTA levels in both white and red wines (Cecchini et al. 2006). The initial concentration of OTA in the musts was approximately 2 mg/L. The yeast strains studied included *S. cerevisiae, S. cerevisiae bayanus, S. bayanus, K. apiculata, T. delbrueckii, Schizosaccharomyces pombe, C. pulcherrima*, and *Saccharomycodes ludwigii*. The results revealed a significant reduction in OTA content, with white wines showing a decrease of 46.83–52.16% of the initial OTA concentration, and red wines showing a more pronounced reduction, ranging from 53.21 to 70.13%. Notably, *S. cerevisiae, Saccharomyces bayanus* var. *uvarum*, and *S. pombe* were particularly effective in reducing OTA levels, especially in red wines. The reduction was attributed to an adsorption process rather than degradation, as OTA appeared to bind to the yeast cells and yeast lees, highlighting the potential of yeast fermentation as an effective strategy for minimizing OTA contamination in wine. Another study (Meca et al. 2010) examined the reduction of Ochratoxin A (OTA) during the fermentation of Italian red wine Moscato, using 16 strains of *S. cerevisiae*. The initial OTA concentration in the must was 10 ng/g. The results showed that OTA absorption by the yeast strains ranged from a minimum of 32.6% to a maximum of 50.4%, with an average reduction of 42.6% by the end of fermentation. This indicates that the fermentation process significantly reduced OTA levels, demonstrating the potential of yeast strains to decrease OTA contamination in wine.

Lachancea thermotolerans (Benito 2018; Vicente et al. 2021) has attracted attention in winemaking due to its beneficial properties, especially its ability to produce lactic acid during fermentation, which helps to increase the acidity of wine (Ciani et al. 2006; Kapsopoulou et al. 2007; Jolly et al. 2014; Porter et al. 2019; Vilela 2019). This yeast is particularly useful in warmer wine regions, where it helps balance low acidity and high alcohol levels. In addition to lactic acid production, *L. thermotolerans* enhances wine quality by improving color stability, generating polysaccharides, and boosting aromatic profiles through ester formation. It also has potential in lowering ethanol, making it a versatile tool for winemakers.

This study is the first to investigate the absorption of Ochratoxin A (OTA) by *L. thermotolerans* during fermentation. It explores the interaction between these yeast strains and OTA, providing new insights into its potential to reduce mycotoxin contamination in musts. The use of *L. thermotolerans* yeast during alcoholic fermentation has shown promise in significantly reducing OTA levels in wine, offering a potential solution to mitigate the health risks posed by this mycotoxin.

Materials and methods

Microbiological material

Thirty-two strains of *L. thermotolerans* yeast were utilized in this study as selected strains (Table S1). The control strain used was *S. cerevisiae* AWRI-796 (Maurivin, Australia). All *L. thermotolerans* strains employed in this study have previously been identified as different strains according to their genotype determined by the micro satellite typing protocol for *L. thermotolerans*, amplifying six different microsatellites by multiplexed PCR, and resolving them by agarose electrophoresis (Vicente et al. 2023).

Vinification

Vinifications were performed using commercial *Vitis vinifera* L. *Tempranillo* grape juice, marketed as CarrefourBio (Carrefour Spain, Madrid, Spain), with a pH of 3.65, a malic acid content of 3.66 g/L, and lactic and acetic acid levels below 0.1 g/L. The grape juice was enriched to 200 g/L with an equimolar mixture of glucose and fructose (Fisher Scientific, Pittsburgh, USA). The initial nitrogen concentrations were 147 mg/L for primary amino nitrogen and 31 mg/L for ammonia nitrogen. The initial concentration of ochratoxin A was adjusted to 10 µg/L using Ochratoxin A from *Petromyces albertensis* (HPLC grade, Sigma-Aldrich, St. Louis, Missouri, USA).

All preparations were carried out under strict aseptic conditions within a Telstar Mini-H laminar flow hood (Telstar S.A., Madrid, Spain).

The fermentations were carried out in 150 mL sterile polypropylene bottle bottles (dhmaterial médico, Barcelona, Spain) filled up to 120 mL under rigorous aseptic conditions within a Telstar Mini-H laminar flow hood (Telstar S.A., Madrid, Spain). For the fermentations, conducted in triplicate, the final inoculation concentration was 2×10^6 cells/mL. Each fermentation vessel was equipped with a partially open polypropylene cap enabling CO_2 release while averting microbial contamination. These fermentations were replicated three times at 25 °C using a Zanotti Ecology climate-controlled chamber (Zanotti, Pieve di Soligo, Italy).

Fermentative kinetics of alcoholic fermentations were monitored by measuring weight loss every 72 h, and fermentations were considered complete when the weight loss was less than 0.01% per day. After fermentation, all wines were centrifuged (7000 rpm for 5 min) and stored at 4 °C until further analysis.

Basic oenological parameters determinations

A Miuramicro Autoanalyzer and its enzymatic kits (TDI, Barcelona, Spain) were used to perform determinations of L-malic acid, L-lactic acid and total acidity. The pH for final wine was determined with a Crison pH Meter Basic 20 (Crison, Barcelona, Spain). The determination of acetic acid, ethanol, glucose + fructose, succinic acid, glycerol, color intensity and total polyphenol index in the resulting wines was carried out using an FTIR autoanalyzer Bacchus 3 MultiSpec (TDI, Spain).

Determination of Ochratoxin A

Ochratoxin A (OTA) measurement was performed at the Laboratorio Arbitral Agroalimentario (Madrid, Spain), an accredited laboratory operating under UNE-EN ISO/IEC 17025 and following official food safety procedures. OTA concentration was determined by High Performance Liquid Chromatography (HPLC) with fluorescence detection, a reliable and widely accepted technique for mycotoxin analysis. A reverse phase C18 column INERTSIL ODS-3 V, 5 µm, 4.6 × 250 mm (GL Sciences, Tokyo, Japan) was used at 40 °C in an Agilent 1100 Series HPLC system coupled with a fluorescence detector (Agilent 1260) set at excitation and emission wavelengths of 330 and 470 nm, respectively. The mobile phase consisted of 4 mM monopotassium phosphate at pH 2.5 and methanol (30:70) at a flow rate of 1 mL min^{-1}. OTA was eluted and quantified by comparison with a calibration curve generated from OTA standards (Sigma-Aldrich, Steinheim, Germany). Sample preparation was carried out using OCHRAPREP immunoaffinity columns (R-Biopharm Rhone Ltd.). The limit of detection (LOD) was 0.015 µg/L, and the limit of quantification (LOQ) was 0.05 µg/L. These sensitivity parameters comply with the maximum levels established for OTA in wine by the European Union under Regulation (EU) No 915/2023 and its subsequent amendments. The applied method is validated and accredited for complex matrices such as wine.

Statistical analysis

All statistical analyses were conducted using R software version 4.4.0 (R Development Core Team, 2013). Analysis of variance (ANOVA) and Tukey *post-hoc* tests were utilized to compare the different groups and values.

Results

Fermentation kinetics

The fermentation kinetics observed in this study (Fig. 1) revealed notable variability among different *L. thermotolerans* strains. Most strains exhibited active fermentation within the first 15–20 days, followed by a stabilization phase where weight loss slowed, indicating a decrease in CO_2 production and sugar consumption. However, certain strains, such as LT011, LT055, and LT089, demonstrated prolonged fermentation with lower overall weight loss, suggesting reduced fermentative efficiency.

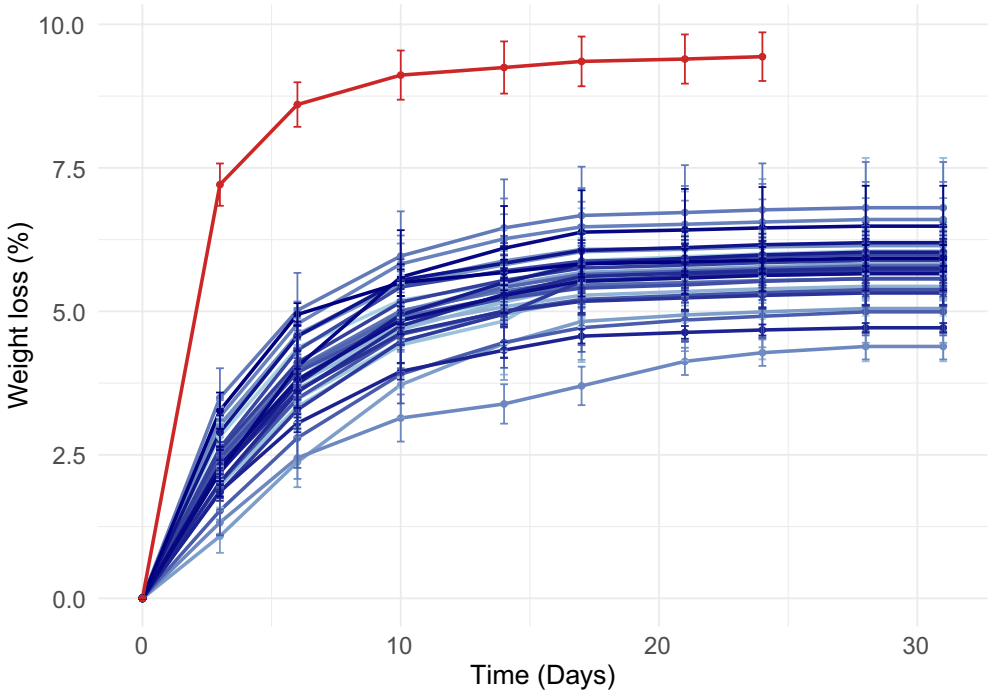

Fig. 1 Fermentation kinetics of different variants, measured gravimetrically by total weight loss, during pure fermentation in Tempranillo grape juice. *L. thermotolerans* strains are represented in different shades of blue, the control strain, *S. cerevisiae* AWRI-796, is shown in red

Final ethanol concentrations

The ethanol production observed among *L. thermotolerans* strains showed significant variability, ranging from 6.42 ± 0.03% (LT089) to 9.36 ± 0.2% (LT090) (Fig. 2; Table S2). In contrast, *S. cerevisiae* (AWRI796) reached 11.49 ± 0.06%, confirming that all *L. thermotolerans* strains produced lower ethanol concentrations.

Final glucose + fructose concentrations

The glucose and fructose consumption among *L. thermotolerans* strains showed notable variability, ranging from 29.85 ± 3.84 g/L (LT090) to 80.55 ± 0.59 g/L (LT089) (Table S2), with significant differences between strains. In contrast, *S. cerevisiae* (AWRI796) almost completely depleted these sugars, leaving only 0.87 ± 0.3 g/L, highlighting its greater fermentative power under the same conditions. Strains such as LT089 (80.55 g/L), LT035 (65.97 g/L), and LT224 (68.79 g/L) exhibited the highest residual sugar levels, indicating lower fermentative activity. Conversely, strains such as LT090 (29.85 g/L), LT091 (30.37 g/L), and LT101 (34.25 g/L) displayed the lowest residual sugar values among *L. thermotolerans* strains, suggesting a more efficient fermentative performance within the typical range for this species.

Glycerol

The glycerol production among *L. thermotolerans* strains exhibited significant variability, ranging from 2.44 ± 2.44 g/L (LT309) to 5.66 ± 0.01 g/L (LT324) (Table S2). In comparison, *S. cerevisiae* (AWRI796) produced 4.86 ± 0.15 g/L, placing it within the mid-range of values observed for *L. thermotolerans*. Strains such as LT324 (5.66 g/L), LT011 (5.53 g/L), and LT288 (5.53 g/L) exhibited the highest glycerol concentrations, whereas strains like LT309 (2.44 g/L) and LT087 (3.3 g/L) produced the lowest amounts, highlighting strain-dependent variability in this metabolic trait.

Lactic acid

The lactic acid production among *L. thermotolerans* strains varied considerably, ranging from 0.00 ± 0.00 g/L (LT091, AWRI796) to 5.82 ± 0.47 g/L (LT086) (Fig. 2; Table S2). In contrast, *S. cerevisiae* (AWRI796) did not produce detectable levels of lactic acid. Strains such as LT086 (5.82 g/L), LT133 (2.49 g/L), and LT101 (2.21 g/L) exhibited the highest lactic acid concentrations, while strains such as LT091 (0.00 g/L) and LT011 (0.03 g/L) produced little or no lactic acid.

Total acidity

The total acidity among *L. thermotolerans* strains showed significant variation, ranging from 5.40 ± 0.00 g/L (LT091) to 10.23 ± 0.39 g/L (LT086) (Table S2). In contrast, *S. cerevisiae* (AWRI796) exhibited the lowest total acidity, with 5.39 ± 0.02 g/L. Strains such as LT086 (10.23 g/L), LT133 (7.46 g/L), and LT101 (7.24 g/L) exhibited the highest total acidity values, reinforcing the role of *L. thermotolerans* in increasing wine acidity. Conversely, strains such

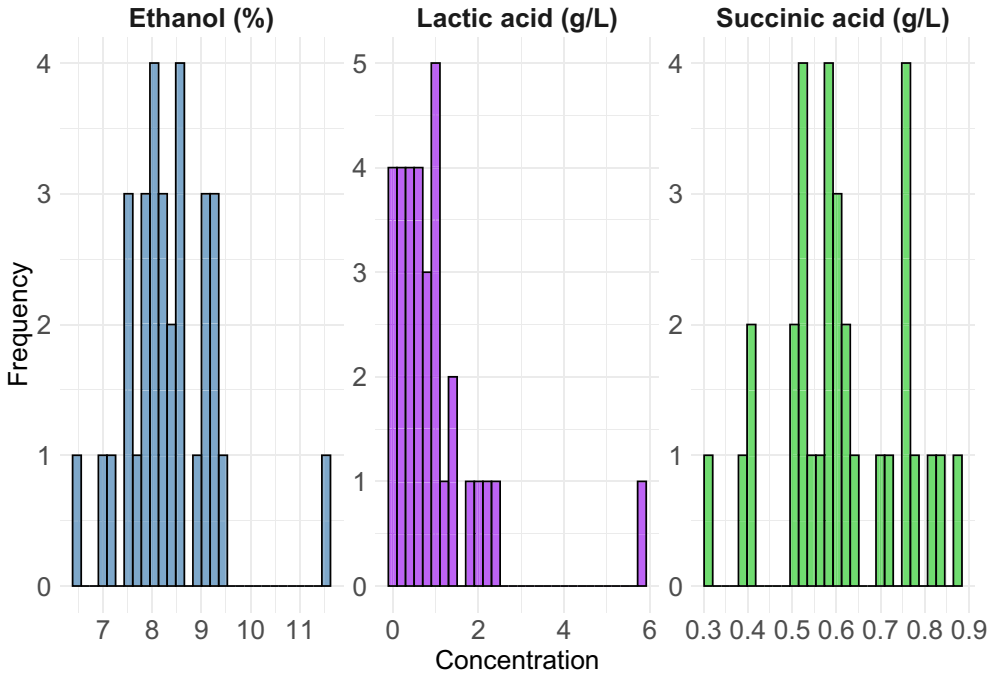

Fig. 2 Histogram for the mean values of ethanol (% v/v), lactic acid (g/L) and succinic acid (g/L) for the 32 studied *L. thermotolerans* strains after alcoholic fermentation

as LT091 (5.40 g/L) and LT011 (5.42 g/L) showed acidity levels similar to *S. cerevisiae*, indicating that not all *L. thermotolerans* strains contribute equally to acidity enhancement.

pH

The final pH values among *L. thermotolerans* strains showed significant variability, ranging from 3.17 ± 0.04 (LT086) to 3.65 ± 0.00 (LT091, LT011, LT018) (Table S2). In contrast, *S. cerevisiae* (AWRI796) exhibited the highest pH value of 3.68 ± 0.02. Strains such as LT086 (3.17) and LT133 (3.44) exhibited the lowest pH values, reinforcing the role of *L. thermotolerans* in acidification. Conversely, strains like LT091 (3.65) and LT011 (3.65) showed pH values close to those of *S. cerevisiae*, indicating strain-dependent differences in acidification ability. The pH reduction observed in *L. thermotolerans* fermentations ranged from 0.03 to 0.51 units compared to *S. cerevisiae*.

Malic acid

The final malic acid concentration among *L. thermotolerans* strains exhibited significant variability, ranging from 2.26 ± 0.03 g/L (LT118, 38.25% reduction) to 3.60 ± 1.66 g/L (LT305, 1.64% reduction) (Table S2). In contrast, *S. cerevisiae* (AWRI796) showed a malic acid concentration of 2.75 ± 0.07 g/L, corresponding to a 24.86% reduction, positioning it within the mid-range of values observed for *L. thermotolerans*. These results confirm that *L. thermotolerans* generally maintains malic acid at levels similar to *S. cerevisiae*. Strains such as LT305 (3.60 g/L, 1.64% reduction) and LT236 (2.94 g/L, 19.67% reduction) exhibited the highest malic acid concentrations, suggesting a lower consumption of this organic acid during fermentation. Conversely, strains like LT118 (2.26 g/L, 38.25% reduction) and LT089 (2.27 g/L, 37.98% reduction) showed the lowest levels, indicating potential differences in metabolic pathways related to malic acid retention.

Succinic acid

The production of succinic acid among *L. thermotolerans* strains varied significantly, ranging from 0.40 ± 0.05 g/L (LT145) to 0.88 ± 0.03 g/L (LT035) (Fig. 2; Table S2). In contrast, *S. cerevisiae* (SCAWRI796) produced the lowest amount, with 0.32 ± 0.03 g/L. Strains such as LT035 (0.88 g/L), LT236 (0.84 g/L), and LT157 (0.82 g/L) exhibited the highest succinic acid levels, whereas strains like LT145 (0.40 g/L), LT088 (0.41 g/L), and LT086 (0.41 g/L) produced the lowest amounts, closer to the levels observed in *S. cerevisiae*.

Acetic acid

The final acetic acid concentrations among *L. thermotolerans* strains exhibited considerable variability, ranging from 0.00 ± 0.00 g/L (LT018, LT038, LT089, LT133, LT139, LT157, LT162, LT211) to 0.08 ± 0.03 g/L (LT101) (Table S2). In contrast, *S. cerevisiae* (AWRI796) produced the highest acetic acid concentration, reaching 0.30 ± 0.01 g/L. Strains such as LT101 (0.08 g/L) and LT074 (0.07 g/L) exhibited the highest acetic acid

levels among *L. thermotolerans* isolates, yet these values remained significantly lower than those observed in *S. cerevisiae*. Conversely, strains like LT018, LT038, LT089, and several others (0.00 g/L) did not produce detectable levels of acetic acid, reinforcing the idea that *L. thermotolerans* exhibits strain-dependent variability in acetic acid metabolism.

Color intensity

The color intensity among *L. thermotolerans* strains exhibited notable variability (Fig. 3; Table S2), ranging from 1.67 ± 0.03 (LT324) to 2.98 ± 0.10 (LT133). In contrast, *S. cerevisiae* (AWRI796) showed a color intensity of 2.11 ± 0.24, placing it within the mid-range of values observed for *L. thermotolerans*. Strains such as LT133 (2.98) and LT038 (2.77) exhibited the highest color intensity values, suggesting their potential role in enhancing wine color. Conversely, strains such as LT324 (1.67) and LT091 (1.69) displayed the lowest color intensity values.

Total polyphenol index

The total polyphenol index (IPT) among *L. thermotolerans* strains exhibited significant variability (Fig. 4), ranging from 16.65 ± 0.46 (LT055) to 22.02 ± 0.13 (LT086). In contrast, *S. cerevisiae* (AWRI796) showed an IPT of 19.31 ± 0.73, placing it within the mid-range of values observed for *L. thermotolerans*. Strains such as LT086 (22.02) and LT211 (21.83) exhibited the highest IPT values, suggesting their potential role in improving polyphenol retention or extraction during fermentation. Conversely, strains such as LT055 (16.65) and LT324 (16.70) showed the lowest IPT values.

Tonality

The tonality values among *L. thermotolerans* strains exhibited moderate variability, ranging from 0.89 ± 0.03 (LT055) to 1.29 ± 0.01 (LT088) (Table S2). In contrast, *S. cerevisiae* (AWRI796) showed a tonality of 1.16 ± 0.02, placing it within the mid-range of values observed for *L. thermotolerans*. Strains such as LT088 (1.29) and LT035 (1.26) exhibited the highest tonality values, whereas LT055 (0.89) and LT324 (0.99) displayed the lowest tonality values.

Ochratoxin A reduction

The reduction of ochratoxin A (OTA) among *L. thermotolerans* strains exhibited significant variability, ranging from 54.01 ± 4.07% (LT086) to 91.36 ± 0.32% (LT324), with an average reduction of 62% (Fig. 5; Table S2). In contrast, *S. cerevisiae* (AWRI796) showed an OTA reduction of 75.85 ± 0.89%, positioning it within the mid-range of values observed for *L. thermotolerans*. Strains such as LT324 (91.36%) and LT055 (87.27%) exhibited the highest OTA reduction rates. Conversely, strains like LT086 (54.01%) and LT089 (59.31%) showed the lowest reduction rates, highlighting the strain-dependent variability in OTA detoxification mechanisms.

Discussion

Fermentation kinetics

The differences could be attributed to variations in carbohydrate metabolism or ethanol tolerance, as previously reported in studies on *L. thermotolerans* (Hranilovic et al. 2021; Vicente et al. 2024a). Compared to *S. cerevisiae* (AWRI796), *L. thermotolerans* strains exhibited lower

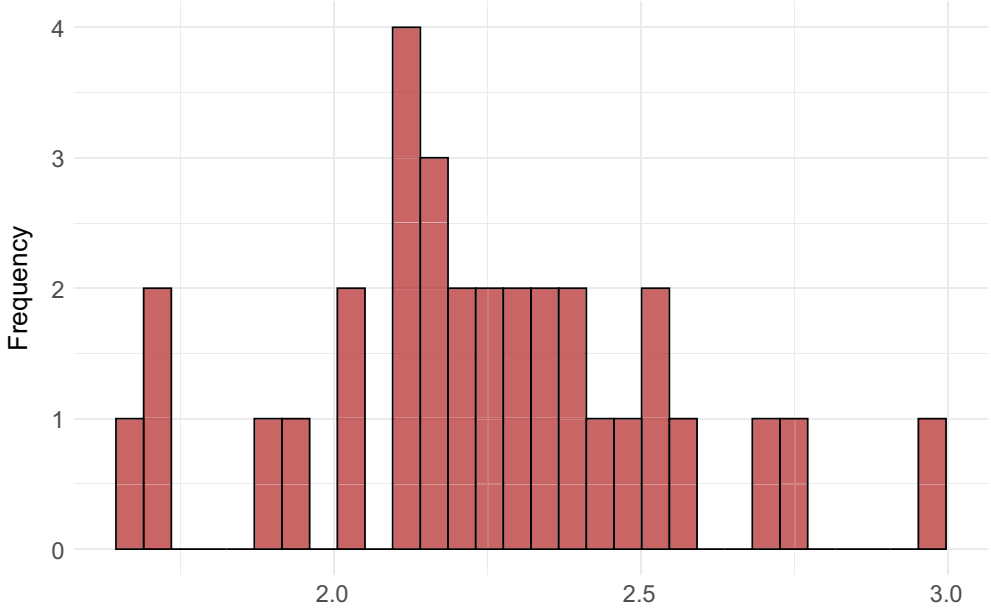

Fig. 3 Histogram for the mean values of colour intensity for the 32 studied *L. thermotolerans* strains after alcoholic fermentation

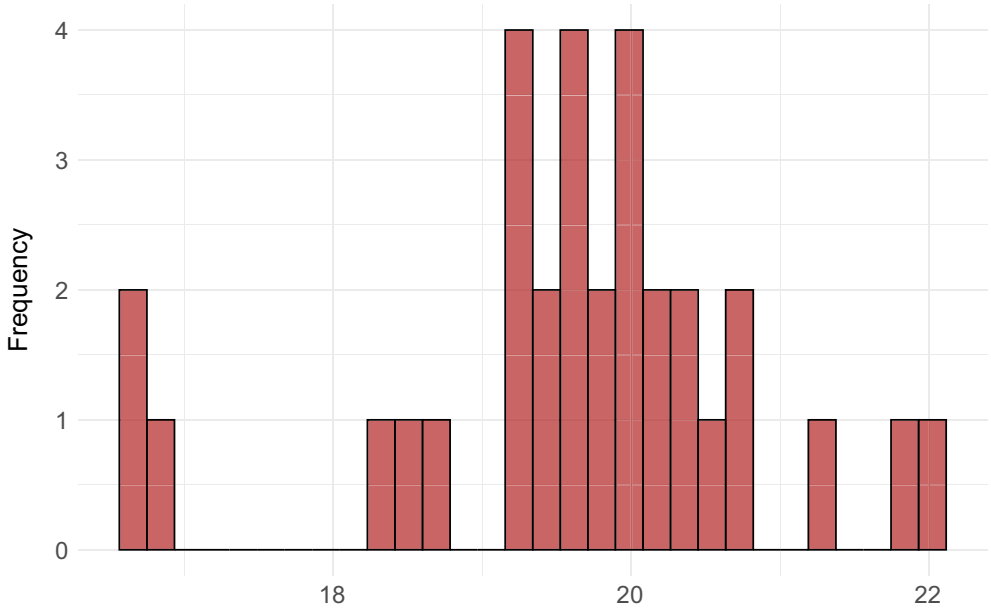

Fig. 4 Histogram for the mean values of total polyphenol index for the 32 studied *L. thermotolerans* strains after alcoholic fermentation

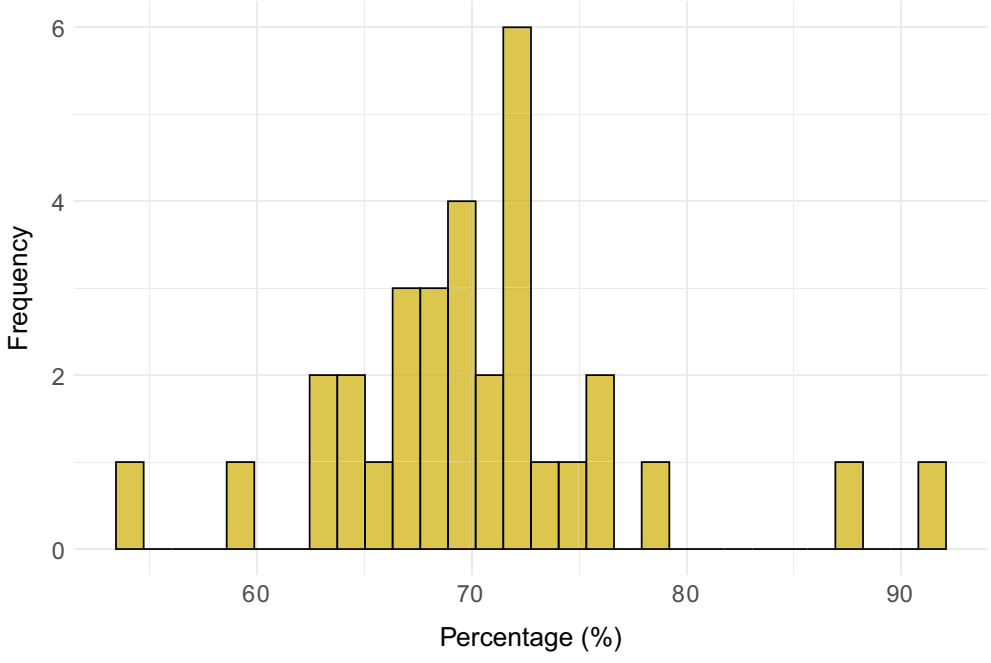

Fig. 5 Histogram for the mean values of ochratoxin A reduction (%) for the 32 studied *L. thermotolerans* strains after alcoholic fermentation

total weight loss, aligning with prior research indicating their moderate fermentative power, which typically results in ethanol levels ranging from 6 to 9% (v/v), significantly lower than the 11–13% (v/v) observed in *S. cerevisiae* fermentations (Benito 2018; Vicente et al. 2022). These findings reinforce the necessity of co-inoculating *L. thermotolerans* with stronger fermentative yeasts to ensure complete fermentation. Furthermore, the fermentation kinetics patterns identified in this study provide valuable insights for selecting specific *L. thermotolerans* strains with tailored enological properties.

Ethanol

This variation is consistent with previous studies that emphasize the influence of genetic factors on the fermentative performance of *L. thermotolerans* (Vicente et al. 2021). The observed ethanol concentrations, ranging between 6 and 10% (v/v), align with values previously

reported for this species and depend on strain variability and fermentation conditions (Vicente et al. 2021; Benito 2018). The lower ethanol production is associated with a reduced fermentative capacity and a metabolic shift toward lactic acid synthesis and the formation of other secondary metabolites (Benito 2018; Hranilovic et al. 2021).

The lower fermentative capacity of *L. thermotolerans* is widely recognized as the main reason for its application in mixed fermentations with *S. cerevisiae* or *S. pombe* (Benito 2020), ensuring the complete fermentation of sugars. This strategy allows the production of wines with reduced alcohol content and enhanced acidity, which is particularly advantageous in warm viticultural regions where grape over-ripening increases sugar concentration in the must (Vicente et al. 2021).

Glucose + fructose

These findings are consistent with previous studies that emphasize the lower sugar consumption of *L. thermotolerans* (Benito 2018; Vicente et al. 2021). The high residual sugar levels observed in certain strains align with earlier research showing that most *L. thermotolerans* strains leave significantly higher sugar concentrations unfermented, making them unsuitable for use as sole fermenting yeasts in winemaking (Vicente et al. 2021). On the other hand, strains with lower residual sugar values demonstrated a more efficient fermentative performance, although still consuming less sugar than *S. cerevisiae*. This reinforces the idea that *L. thermotolerans* is best suited for mixed fermentations rather than being used as a single starter culture, allowing it to contribute to wine complexity while ensuring complete sugar fermentation (Hranilovic et al. 2021).

Glycerol

These results confirm that *L. thermotolerans* generally produces glycerol at levels comparable to or slightly higher than *S. cerevisiae*, consistent with previous research indicating that some *non-Saccharomyces* yeasts may contribute to increased glycerol content in wine (Benito 2018; Vicente et al. 2021). The ability of *L. thermotolerans* to produce significant amounts of glycerol is particularly relevant for enhancing wine texture and mouthfeel, as glycerol increases viscosity and smoothness, contributing to the sensory perception of the final product.

The variability in glycerol production among strains reinforces the importance of strain selection in enological applications. Strains such as LT324, which exhibited the highest glycerol levels, could be particularly beneficial in winemaking processes where improved mouthfeel and viscosity are desirable. Conversely, strains like LT309 and LT087, which produced lower glycerol concentrations, may be less suited for applications requiring enhanced texture. These findings further validate that *L. thermotolerans* can generate significant glycerol concentrations, sometimes surpassing *S. cerevisiae*, though differences among strains must be considered for optimal selection in wine production (Hranilovic et al. 2021).

Lactic acid

The substantial variation observed among *L. thermotolerans* strains aligns with previous findings indicating that lactic acid production is highly strain-dependent and influenced by genetic factors and fermentation conditions (Hranilovic et al. 2021; Vicente et al. 2025). The ability of *L. thermotolerans* to produce lactic acid plays a significant role in increasing wine acidity, which is particularly valuable in winemaking, especially in warm viticultural regions where grape over-ripening leads to reduced natural acidity and higher pH values (Vicente et al. 2021). The strains with the highest lactic acid concentrations (e.g., LT086, LT133, LT101) confirm the potential of the species for acidity enhancement. Conversely, strains with little or no lactic acid production (e.g., LT091, LT011) indicate broad metabolic variability within the species. This metabolic trait is essential for selecting *L. thermotolerans* strains for enological applications, particularly in cases where a controlled increase in acidity is desired to improve wine freshness, microbial stability, and overall balance (Benito 2018; Vicente et al. 2021).

Total acidity

The ability of *L. thermotolerans* to enhance total acidity is largely attributed to its production of lactic acid, which is absent in *S. cerevisiae*-dominant fermentations (Benito 2018). The high total acidity levels observed in certain *L. thermotolerans* strains align with previous studies reporting strain-dependent variability in organic acid metabolism. The variation in total acidity observed among strains suggests that the selection of *L. thermotolerans* isolates should be tailored depending on enological goals, particularly in warm viticultural regions where acidity preservation is crucial for maintaining wine freshness and balance. The strains exhibiting the highest acidity levels could be beneficial for increasing total acidity in wines lacking natural acidity, whereas lower-acidity strains may be preferable for balanced fermentations when used in combination with *S. cerevisiae*.

pH

The acidification effect observed in *L. thermotolerans* strains is particularly relevant in winemaking, as a lower pH enhances microbial stability, improves color stability in red wines, and contributes to overall wine freshness. These findings are consistent with previous studies that highlight the ability of *L. thermotolerans* to lower pH due

to its lactic acid production (Benito 2018). However, the variability in pH reduction across strains suggests that different *L. thermotolerans* isolates may exert varying degrees of impact on acidity management. While some strains effectively reduce pH, others exhibit a more limited acidifying effect, making strain selection crucial for enological applications. The pH reductions observed in this study (0.03–0.51 units) align with prior reports indicating pH decreases of 0.1–0.4 units when using *L. thermotolerans* under similar enological conditions (Benito 2018; Vicente et al. 2021). These results reinforce the potential of *L. thermotolerans* for winemaking in warm viticultural regions, where acidification strategies are essential to balance wine freshness and stability.

Malic acid

The results confirm that *L. thermotolerans* does not totally degrade malic acid, aligning with previous research that differentiates it from other *non-Saccharomyces* yeasts such as *Schizosaccharomyces pombe*, which completely degrades malic acid during fermentation (Benito 2018; Vicente et al. 2024b). The variability in malic acid retention observed in this study suggests that different *L. thermotolerans* strains have distinct metabolic behaviors, which could impact their enological applications.

Previous studies recommend using *L. thermotolerans* strains with high malic acid degradation for red wine production, as their ability to consume malic acid could facilitate and accelerate the subsequent malolactic fermentation process (Vicente et al. 2024b). In contrast, strains that exhibit low or no malic acid degradation are preferred for white wines made from grape juices with naturally low acidity. These strains help preserve malic acid, which is essential for maintaining freshness and stability in wines where acidity correction is necessary. The strain-dependent differences observed in this study reinforce the importance of selecting *L. thermotolerans* strains based on their metabolic profiles to achieve desired acidity levels in different winemaking contexts.

Succinic acid

Strains exhibiting the highest succinic acid levels reinforce the idea that specific *L. thermotolerans* isolates can significantly contribute to the acidic balance and sensory profile of wines, particularly in applications where increased acidity and mineral perception are desirable (Vicente et al. 2021, 2024a). Unlike previous studies that have only reported succinic acid production for a single strain or a limited number of *L. thermotolerans* isolates, this study examines 32 different strains, providing a broader and more comprehensive analysis of succinic acid variability within the species. The results confirm that *L. thermotolerans* consistently produces more succinic acid than *S. cerevisiae*, yet with notable variation depending on the strain studied. This variability highlights the importance of strain selection in enological applications, as different isolates can have distinct effects on wine acidity, sensory attributes, and mineral character (Benito 2018; Vicente et al. 2021).

Acetic acid

The results confirm that *L. thermotolerans* generally produces lower acetic acid levels than *S. cerevisiae*, which aligns with previous findings indicating that *L. thermotolerans* generates lower volatile acidity (Vilela 2018), making it a suitable candidate for winemaking applications where reduced acetic acid formation is desired (Hranilovic et al. 2021). High acetic acid concentrations can negatively impact wine quality, contributing to off-flavors and spoilage risks, and thus, the lower levels found in *L. thermotolerans* fermentations represent an enological advantage (Vicente et al. 2021).

The strain-dependent variability in acetic acid production observed in this study highlights the importance of selecting specific *L. thermotolerans* isolates based on their metabolic profiles. Strains such as LT101 and LT074, which exhibited slightly higher acetic acid concentrations, still remained well below the levels found in *S. cerevisiae*, reinforcing the potential of *L. thermotolerans* to improve wine balance while avoiding excessive volatile acidity. Conversely, the complete absence of detectable acetic acid in strains such as LT018, LT038, and LT089 suggests that certain *L. thermotolerans* strains may be particularly well suited for applications where minimal volatile acidity is critical. The variability observed among strains underscores the importance of carefully selecting *L. thermotolerans* strains to achieve optimal fermentation performance without compromising wine stability.

Color intensity

Previous studies indicate that *L. thermotolerans* can enhance color intensity by reducing pH, which shifts anthocyanin molecules toward their red-colored flavylium cation form, intensifying the visual perception of wine color (Benito 2018; Vicente et al. 2021). However, the results also suggest that certain *L. thermotolerans* strains may absorb anthocyanins during fermentation, leading to a strain-dependent effect on color retention. Strains such as LT133 and LT038, which exhibited the highest color intensity, likely contributed to color enhancement via acidification and improved anthocyanin stability. Conversely, strains like LT324 and LT091, which showed the lowest color intensity, may have adsorbed anthocyanins onto their cell walls, resulting in reduced final wine color.

This strain-dependent effect on anthocyanin retention aligns with previous findings that suggest *L. thermotolerans* can either stabilize or deplete anthocyanins, depending on the specific strain and fermentation conditions (Vicente et al. 2021). These findings highlight the importance of strain selection when using *L. thermotolerans* in winemaking, particularly in red wine production, where color stability is a crucial quality attribute.

Total polyphenol index

These results confirm that *L. thermotolerans* can influence polyphenol retention in wine, either maintaining or slightly modifying it depending on the strain. This aligns with previous research indicating that *L. thermotolerans* affects polyphenolic composition through its impact on pH, organic acid production, and interactions with phenolic compounds (Benito 2018; Vicente et al. 2021).

Strains such as LT086 and LT211, which exhibited the highest IPT values, may contribute to improved polyphenol retention by lowering pH, which enhances the solubility and stability of polyphenolic compounds, particularly anthocyanins in red wines (Vicente et al. 2021). In contrast, strains like LT055 and LT324, which showed the lowest IPT values, may have promoted polyphenol adsorption onto yeast cell walls or degradation during fermentation. This strain-dependent variability underscores the importance of selecting appropriate *L. thermotolerans* strains in enological applications, particularly in red winemaking, where polyphenol preservation is crucial for color stability, astringency, and antioxidant properties (Benito 2018; Vicente et al. 2021).

These findings highlight the need for further studies on the mechanisms through which different *L. thermotolerans* strains influence polyphenol retention and how these variations impact wine sensory attributes and aging potential.

Tonality

Previous studies indicate that *L. thermotolerans* can influence wine tonality through its effect on pH reduction and organic acid production, which can stabilize anthocyanins and modify the spectral properties of wine pigments (Benito 2018; Vicente et al. 2021).

Strains such as LT088 and LT035, which exhibited the highest tonality values, may contribute to a shift toward orange or brown hues, potentially due to increased anthocyanin polymerization or oxidative reactions during fermentation. This trend suggests that some *L. thermotolerans* strains might promote oxidative reactions that alter anthocyanin structures.

Conversely, strains like LT055 and LT324, which displayed the lowest tonality values, could be associated with a greater retention of red hues, possibly due to lower oxidation rates or mechanisms stabilizing anthocyanins. These findings align with previous research suggesting that *L. thermotolerans* can either enhance or slightly alter wine color depending on strain variability and fermentation conditions (Vicente et al. 2021).

Understanding these strain-dependent differences is essential for winemakers aiming to optimize color stability, particularly in red wines where tonality plays a key role in visual appeal and perceived quality.

Ochratoxin A reduction

The most substantial reductions in the OTA content are generally observed in red wines, where factors such as higher polyphenol content and longer maceration times may enhance yeast-mediated OTA adsorption (Cecchini et al. 2006). In this context, our findings regarding the inverse relationship between OTA reduction and polyphenol retention provide a useful framework for designing tailored fermentation strategies aimed at mitigating OTA while minimizing the loss of phenolic quality.

Previous studies have reported significant OTA reductions across different yeast species, with values ranging from 46 to 70%, depending on the type of wine (Cecchini et al. 2006). Reductions reported for individual yeast species in red wine were: *S. cerevisiae* (53.21%), *S. bayanus var. uvarum* (55.91%), *S. bayanus* (56.91%), *Kloeckera apiculata* (62.52%), *Torulaspora delbrueckii* (65.71%), *S. pombe* (70.13%), *Metschnikowia pulcherrima* (61.98%), and *S. ludwigii* (60.46%). Additionally, a study on the fermentation of Italian red Moscato wine using 16 strains of *S. cerevisiae* found OTA reductions ranging from 32.6 to 50.4%, with an average reduction of 42.6% (Meca et al. 2010).

The results of this study confirm that *L. thermotolerans* can be as effective as, or even surpass, *S. cerevisiae* in OTA reduction, reinforcing its potential as a tool for improving wine safety and quality. The variability observed among strains highlights the importance of strain selection in enological applications (Fig. 5), particularly in regions where OTA contamination poses a significant risk to grape production and wine quality.

The results suggest an inverse relationship between OTA reduction and the retention of color and polyphenols among *L. thermotolerans* strains. Strains such as LT324 (91.36%) and LT055 (87.27%) achieved the highest OTA reduction but showed the lowest color intensity and total polyphenol index (TPI), whereas LT086 (54.01%) and LT089 (59.31%) exhibited lower OTA reduction and higher phenolic retention. This trend indicates that strains with strong OTA adsorption capacity may also bind anthocyanins and polyphenolic compounds to their cell walls, reducing their retention in the final product. To mitigate this trade-off, strategies such as early yeast removal (e.g., racking shortly after OTA adsorption) could help limit polyphenol loss. Additionally,

co-inoculation with other non-Saccharomyces yeasts like *Schizosaccharomyces pombe* has been shown to enhance color stability through increased production of stable anthocyanins (e.g., vitisins) and to contribute to OTA detoxification without significantly impacting phenolic content (Benito 2020). These findings highlight the relevance of both strain selection and fermentation design in optimizing wine safety and quality.

Additionally, strains LT324 and LT055 produced moderate levels of lactic acid (1.04 g/L and 0.71 g/L), which were significantly lower than those of the highest-producing strains, reaching up to 5.82 g/L.

Overall, the results of this study highlight the remarkable ability of *L. thermotolerans* to reduce Ochratoxin A (OTA) concentration in wine fermentations, with reductions ranging from 54.01 to 91.36%, depending on the strain. These findings confirm the potential of *L. thermotolerans* as a valuable tool for improving wine safety, particularly in regions where OTA contamination poses a significant risk. Additionally, an inverse relationship was observed between OTA reduction and the retention of polyphenols and color intensity in wine. Strains with higher OTA adsorption capacity tended to exhibit lower color intensity and total polyphenol index values, suggesting that strong OTA-binding strains may also interact with anthocyanins and polyphenolic compounds. This effect underlines the importance of strain selection in winemaking, as different isolates may influence wine composition in distinct ways. These findings emphasize the need for careful selection of *L. thermotolerans* strains for enological applications.

Abbreviations

S. cerevisiae	*Saccharomyces cerevisiae*
L. thermotolerans	*Lachancea thermotolerans*
OTA	*Ocrathoxin A*

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13568-025-01896-4.

Supplementary Material 1.

Acknowledgements

Funding was provided by the Spanish Ministry of Science and Innovation, and the State Investigation Agency under the framework of Project VinSegCalClim (PID2020-119008RB-I00/AEI/10.13039/501100011033). Javier Vicente conducted this research under a fellowship from Complutense University of Madrid (CT58/21-CT59/21).

Author contributions

Javier Vicente, Wendu Tesfaye, Fernando Calderón, and Santiago Benito carried out the fermentation work, participated in the experimental design, and performed the basic enological parameter analyses. Daniel Vidal and Fernando García conducted the ochratoxin A analyses. Santiago Benito and Javier Vicente coordinated the writing of the manuscript. All authors reviewed the final manuscript.

Funding

Not applicable (written in Acknowledgements).

Data availability

Data is provided within the manuscript or supplementary information files

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 1 April 2025 / Accepted: 24 May 2025
Published online: 11 June 2025

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