

Article

Enhancing the Production of Sour Beers by Adding Blueberries and Fermenting with *Lachancea* and *Metschnikowia*

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Abstract

The increasing demand for alcohol-free craft beers with functional properties and distinctive sensory attributes has motivated the brewing industry to investigate alternative production strategies, such as the application of non-*Saccharomyces* yeasts, to obtain sour beers while reducing production time and associated costs. This study explores the combined use of *Lachancea thermotolerans* L31 and *Metschnikowia pulcherrima* M29 in the production of beers brewed with blueberries or enriched with grape anthocyanin concentrate. Physicochemical parameters such as pH, color, bitterness, total polyphenols, antioxidant capacity, and anthocyanin and volatile profiles were evaluated, and a sensory analysis was performed. The results showed that both the addition of blueberries and that of anthocyanin concentrate and fermentation with *Lachancea* and *Metschnikowia* significantly influenced the chemical and sensory properties of the beer. Mainly, pH values decreased from 4.35 to 3.50 and from 3.69 to 3.26, while antioxidant activity increased from 3 to 10 times, depending on the type of yeast and the addition of fruit. Alcohol content remained constant at approximately 5.0% v/v. This strategy allows for the production of beer with a distinctive profile and functional benefits, representing a step forward in craft beer development and opening new avenues for research and innovation in the sector.

Keywords: antioxidant activity; non-*Saccharomyces*; pH; ABTS⁺; HPLC-DAD; yeast acidification; craft beer; fruit beer; co-inoculation; low-alcohol beer



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1. Introduction

The earliest evidence of beer dates back to 2800 BC [1], although its origins are believed to lie in the Egyptian and Babylonian civilizations, around 8000 years ago [2]. Its initial popularity stemmed from its nutritional value and low susceptibility to contamination [3]. Over time, beer has evolved to meet society's needs, diversifying into countless styles. Nowadays, beer is considered a cultural element and is the most consumed alcoholic beverage worldwide [4,5], with global consumption of approximately 1.88 billion hectoliters in 2023 [6].

Currently, the brewing industry is experiencing a boom in craft beer production, pursuing new flavors and sensory experiences [7,8]. These goals have driven the recent rise in popularity of sour beer, characterized by a high concentration of organic acids and a low pH compared to traditional beers [1]. These beers are traditionally produced through

spontaneous fermentation involving yeasts and bacteria (either acetic acid bacteria or lactic acid bacteria) [9], requiring long production times (1 to 3 years) and having high production costs [1]. Consequently, the brewing industry is exploring alternative methods, replacing bacterial acidification with yeast-driven approaches that use non-*Saccharomyces* species [10] known for their high acid production [11,12]. Among these, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, and *Torulaspora delbrueckii* are the most promising choices [13].

Nowadays, people are seeking healthier lifestyles that involve consuming functional foods [10]. A common approach is enriching these with anthocyanins, natural water-soluble pigments responsible for the red and purple [14] colors in vegetables, flowers, and fruits [15]. Anthocyanins have strong antioxidant capacity and provide benefits such as anti-tumorigenic, anti-diabetic, anti-inflammatory, and anti-angiogenic activities [15]. Their use in the food industry is regulated by the *Codex Alimentarius* [16]. They constitute a dietary source of antioxidants. In the brewing industry, adding anthocyanins or anthocyanin-rich fruits increases both the total anthocyanin levels and the antioxidant activity of beers [17–21].

At the same time, a healthy lifestyle implies a reduction in alcohol intake. Because of this, the craft beer industry has lately experienced an increase in the consumption of low-alcohol and 0.0% beers. In the European Union, the production of low-alcohol beers rose by 13.5% from 2022 to 2023 due to higher demand, while the production of alcoholic beers fell by 5% [22]. Currently, there are two main methods to obtain low-alcohol beers. The first strategy involves physical removal of ethanol from fermented beer [10], which entails expensive equipment and significant energy demand [23]. On the other hand, biological methods focus on the use of alternative yeasts unable to ferment maltose, thus exhibiting low fermentative capacity and low ethanol production [24]. Examples of these yeasts are *L. thermotolerans* and *M. pulcherrima*, as previously noted.

Lachancea thermotolerans is a non-*Saccharomyces* yeast, found in grape juice [25] and recently used in the wine industry to produce low-pH wines with innovative sensory profiles [26]. It is a heterofermentative yeast, capable of converting sugars into ethanol and lactic acid [13], producing between 1 and 9 g/L depending on the strain [27,28]. In beer wort, it ferments glucose, fructose, sucrose, and maltose, but not maltotriose [29,30]. This yeast is able to grow as long as bitterness remains below 90 IBUs and alcohol content does not exceed 10% *v/v* ethanol [31,32]. Its high volatile compound production contributes to floral and fruity aromas in the final product [13,28,33–35]. *Metschnikowia pulcherrima*, also found in grape juice, and used in the wine industry [36], does not produce lactic acid. It only produces 0.5–1% *v/v* ethanol in wort [10,37], and is sensitive to ethanol concentrations above 4% *v/v* [38,39]. Due to its high proteolytic activity, it is used in co-inoculations to enhance yeast growth and produce fruity aroma compounds [34,37,40]. The low fermentative activity and high production of volatile acids make *L. thermotolerans* and *M. pulcherrima* interesting choices for crafting low-alcohol sour craft beers [41,42].

The main objective of this study is to replace bacterial acidification with yeast-driven acidification using the non-*Saccharomyces* yeasts *L. thermotolerans* and *M. pulcherrima* to produce sour beers enriched with natural anthocyanins.

2. Materials and Methods

2.1. Malt

Two different types of malt were used for wort production: dehydrated and milled pale malt Château Pilsen 2RS from the 2023 harvest (Castle Maltin, Beloeil, Belgium) and dehydrated roasted Carafa Type I malt (Weyermann, Bamberg, Germany). The latter was manually ground with a manual disc mill.

2.2. Hops

Lemondrop hop with 7.1% α -acids was used in pellet form for wort production.

2.3. Yeast Strains and Culture Media

Three different yeast strains were used for the primary fermentation of the wort: *Saccharomyces cerevisiae* 7VA (enotecUPM, ETSIAAB, UPM, Madrid, Spain), Lallemand's commercial strain *Lachancea thermotolerans* L31 (Blizz™, Lallemand Iberia, Spain, <https://www.lallemandwine.com/es/spain/productos/levaduras-enologicas/blizz/> (accessed on 10 October 2025)), and *Metschnikowia pulcherrima* M29 (enotecUPM, ETSIAAB, UPM, Madrid, Spain). The yeasts were isolated in Ribera de Duero, Valladolid, Spain. They were stored in the freezer in tubes containing YPD medium (1% yeast extract, 2% peptone, 2% glucose, 1.5% agar) by the Microbiology Department (ETSIAAB, UPM, Madrid, Spain).

Prior to inoculation, the yeasts were refreshed in Petri dishes with YPD medium (1% yeast extract, 2% peptone, 2% glucose, 0.8% agar) and later grown in sterilized YPD liquid medium (1% yeast extract, 2% peptone, 2% glucose) at 25 °C and 240 rpm. After 48 h, a subsequent step was performed, and pure cultures of yeast were inoculated at 2% into fresh sterilized YPD liquid medium for another 24 h in the same conditions.

For the implantation test, two media were used. YPD agar medium was used to evaluate the growth of *L. thermotolerans* L31 and *M. pulcherrima* M29 in pure culture, while chromogenic agar (45.9%) was used as a differential medium to evaluate the growth of both yeasts in mixed culture. The chromogenic agar used was *Candida Chromogenic Agar* by Condalab (Madrid, Spain).

2.4. Grape Anthocyanin Concentrate

Liquid grape anthocyanin concentrate was obtained from Secna (Benifaió, Valencia, Spain) and sterilized at 121 °C for 15 min.

2.5. Blueberries

Bags of frozen blueberries were acquired from Crop's (Ooigem, Belgium). On the day fermentation began, 200 g of blueberries was weighed and crushed in a food processor (Vorwerk, Wuppertal, Germany) for 5 min at maximum speed. The resulting blueberry puree was transferred to sterilized glass jars and stored in the fridge at 4 °C for an hour before use.

2.6. Wort Preparation

A Grainfather (Auckland, New Zealand) 40 L capacity mash tun was used for the mashing process. For producing the wort, 18 L of tap water was conditioned with 5.85 g of calcium sulfate dihydrate ($\text{CaSO}_4 \cdot \text{H}_2\text{O}$) to a final concentration of 90 mg/L of calcium. Once adjusted, the water was heated to 72 °C and added to the mash tun, along with 6 kg of milled pale Château Pilsen 2RS malt and 37.5 g of roasted Carafa Type I malt. The mash tun temperature was set at 68 °C for 1 h.

The malt was sparged in the mash tun by lifting the lauter tun and adding 16.5 L of water previously heated to 77 °C and conditioned with $\text{CaSO}_4 \cdot \text{H}_2\text{O}$ in the same conditions as above. Water was added in three rounds of 6, 6, and 4.5 L, respectively, making circular movements and letting it drain in between additions. After washing, spent grain was removed and the pH of the wort was adjusted to 5.27 with 4 mL of phosphoric acid before the boiling step. The wort was brought to a boil and maintained at that temperature for 90 min. After the first 30 min, 30 g of Lemondrop hops was added. Boiling continued for an additional 60 min.

Immediately after boiling, the wort was whirlpooled for a minute and was left to cool for 30 min. A volume of 600 mL of wort was pumped into 18 bottles, each of 1 L capacity, previously sterilized at 121 °C for 15 min. The bottles were capped and stored in the fridge for 24 h at 4 °C until the inoculation of the wort to avoid contamination.

2.7. Implantation Test

Before inoculation of the wort, an implantation test was performed to study the growth kinetics of *L. thermotolerans* L31 and *M. pulcherrima* M29 in the wort. During the experiment, 6 different conditions were evaluated separately and in triplicate, varying the proportion of yeast and the type of culture: 1:1, 1:5, 1:10 (L31:M29), sequential inoculation (M29 on day 0 followed by L31 on day 2), 1:0 (L31 in pure culture, and finally 0:1 (M29 in pure culture).

Different conditions were evaluated in 50 mL sterile flasks, pouring in 30 mL of previously sterilized wort (121 °C, 15 min) and inoculating the yeast at 2% *v/v* of the final volume in all conditions. For the conditions 1:5 and 1:10, M29 yeast culture was concentrated 5× and 10× by centrifugation at 503× *g* for 5 min at −4 °C, and re-suspended in sterile YPD liquid medium.

Flasks were lidded with septum lids and pierced with needles to allow the escape of CO₂. The needles had been previously soaked in 96% ethanol for 5 min.

Once inoculated, the flasks were stored in an oven at 25 °C and in darkness for 7 days. On days 0, 2, 5, and 7, cultures were plated on Petri dishes for a CFU count in triplicate. Conditions 1:0 and 0:1 (pure cultures of L31 and M29, respectively) were plated in YPD agar medium, while conditions 1:1, 1:5, 1:10, and sequential were evaluated in chromogenic agar.

2.8. Fermentation and Storage

Six different fermentation conditions were evaluated throughout the experiment, each in triplicate.

Out of 18 bottles, 6 contained no additive. Another 6 were supplemented with 200 g of blueberry purée, and the last 6 bottles contained 250 µL of liquid anthocyanin concentrate.

The wort was inoculated at 2% of the final volume in sterile conditions. Inoculum was produced as explained in Section 2.3 and the cell count of the inoculum was 1×10^6 CFU/mL. Nine bottles were inoculated with pure culture of *S. cerevisiae* 7VA, three containing fruit, three containing anthocyanin concentrate, and three without additives. Another nine bottles were inoculated with a co-culture of *L. thermotolerans* L31 and *M. pulcherrima* M29 at a 1:10 ratio. Table 1 summarizes the codes and conditions evaluated during the experiment.

Table 1. Primary fermentation conditions.

Code	Replicates	Yeast	Additive
7.VA	3	<i>S. cerevisiae</i> 7VA	No
7.VA.F	3	<i>S. cerevisiae</i> 7VA	Blueberry puree
7.VA.A	3	<i>S. cerevisiae</i> 7VA	Anthocyanin concentrate
LM	3	<i>L. thermotolerans</i> L31 + <i>M. pulcherrima</i> M29	No
LM.F	3	<i>L. thermotolerans</i> L31 + <i>M. pulcherrima</i> M29	Blueberry puree
LM.A	3	<i>L. thermotolerans</i> L31 + <i>M. pulcherrima</i> M29	Anthocyanin concentrate

Each bottle was fitted with a sterile cap and a fermentation valve with 2 mL of glycerol, allowing fermentation CO₂ to escape and avoiding microbial contamination.

Fermenters were left at room temperature (20 °C) in darkness for the corresponding time, until the specific gravity (SG) of the beers reached 1015 kg/m³ and remained constant

for 3 days. To monitor the fermentation process, pH, density, and glucose levels were measured every 3–5 days. To obtain fermentation samples, 50 mL of wort was poured into a 100 mL beaker in sterile conditions.

The specific gravity of beers 7.VA.A and LM.A stalled at high SG values on day 10, and therefore required re-inoculation with *S. cerevisiae* 7VA and *L. thermotolerans* L31 + *M. pulcherrima* M29 (1:10), respectively, following the same procedure as the initial inoculation.

Once fermentation was complete, bottles were stored in a refrigerated room (4 °C) in darkness for 7 days. Then, the beers were transferred to clean sterile glass bottles and stored again in the same conditions until bottling, to remove solids and yeast residues.

2.9. Bottling and Secondary Fermentation

The beers were bottled in 250 mL amber bottles previously sterilized at 121 °C for 15 min. To promote CO₂ production and head formation, a secondary fermentation was carried out in the bottle.

A stock solution of 400 g/L of table sugar (Hacendado, Valencia, Spain) was prepared, sterilized, and added to the beer to a final concentration of 5 g/L. Then, 200 mL of beer and 0.4 g/L of active dry yeast *S. cerevisiae* QA23™ (Lalvin QA23™, Lallemmand Iberia, Barcelona, Spain, <https://www.lallemmandwine.com/es/spain/productos/levaduras-enologicas/lalvin-qa23> (accessed on 2 August 2025)) were added to the bottles in that order.

Finally, the bottles were capped with crown caps, using a manual capper, and stored at room temperature for 15 days for secondary fermentation. Afterward, the beers were stored at 4 °C in darkness for 10 days.

2.10. Instrumental Analysis

2.10.1. Density

Density was measured at 20 °C with a 50 mL graduated cylinder and a 1000–1050 kg/m³ range density meter.

2.10.2. pH

pH was analyzed at 20 °C with a pre-calibrated portable pH meter from Metria (Barcelona, Spain), M22 model.

2.10.3. Glucose Levels

Residual glucose in all samples was measured with the Food Quality Enology enzyme kit (Biosystems, Barcelona, Spain) by measuring the absorbance at 500 nm with a UV–vis spectrophotometer (Selecta, Madrid, Spain).

2.10.4. Color

Beer color was analyzed following the ABSC protocol for color evaluation (no. 10) [43], by measuring the absorbance at 700 nm with a UV–vis spectrophotometer (Selecta, Spain). Samples were previously filtered with an MCE membrane syringe filter with a pore size of 0.45 µm. Color was expressed in EBC units.

2.10.5. Bitterness

Beer bitterness was evaluated according to the corresponding ABSC protocol (no. 23A) [44] and expressed in IBU. Each 10 mL of each sample was treated with 1 mL of 3 M HCl, 20 mL of isooctane, and 50 µL of octane, then vortexed for 15 min and finally centrifuged at 3578× *g* for another 15 min. The organic phase was recovered, and its absorbance was measured at 275 nm with a quartz cuvette.

2.10.6. Alcoholic Content

The alcoholic strength of the samples was determined using the Barus ebulliometer method, based on the difference in boiling temperatures between water and ethanol at 1 atm. The equipment consisted of a lower boiler, an upper cooling system, precision thermometers, and a calibrated dual-entry scale. Because atmospheric pressure in Madrid differs from 1 atm, the method was previously calibrated using 10 mL of distilled water to establish the boiling reference. Subsequently, 50 mL of each sample was analyzed by recording the stabilized boiling temperature, which was then used to determine the alcohol content. Between samples, the boiler was rinsed and the cooling system refilled with tap water at room temperature.

2.10.7. Determination of Volatile Compounds by GC-FID

Volatile compounds were identified by gas chromatography (GC) with an Agilent Technologies 6850 gas chromatograph (GC System Network, Santa Clara, CA, USA) coupled to a flame ionization detector (FID) (Hewlett-Packard, Palo Alto, CA, USA). A BD-624 column (60 m × 0.25 mm × 1.4 µm) was used. Before injection of the samples, they were filtered through an MCE syringe filter with a 0.45 µm pore size. A volume of 1 mL of each sample was poured into 1.5 mL capacity chromatographic vials and spiked with 100 µL of 4-methyl-2-pentanol (50 mg/L, Fluka Chemie, GmbH, Buchs, Switzerland) as an internal standard. The method was calibrated with calibration curves prepared in hydroalcoholic solutions containing 13% *v/v* ethanol, using different concentrations, in the range of 1–500 mg/L, of each of the following compounds: acetaldehyde, methanol, 1-propanol, diacetyl, ethyl acetate, 2-butanol, isobutyl alcohol, 1-butanol, acetoin, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutyl acetate, ethyl butyrate, ethyl lactate, 2,3-butanediol, isoamyl acetate, hexanol, 2-phenylethanol, and 2-phenylethyl acetate. $R^2 > 0.999$ for all compounds except for 2,3-butanediol ($R^2 = 0.991$). Limit of detection (LOD): 0.1 mg/L.

For better resolution of the peaks, a temperature gradient was used. The oven temperature was initially set to 40 °C for 5 min, then increased by 10 °C per minute until reaching 250 °C. The analysis was carried out in splitless mode. The injector and detector temperatures were 200 °C and 300 °C, respectively. The injection volume was 1 µL and hydrogen was used as the carrier gas. This method corresponded to the OIV protocol of volatile compounds (Resolution OIV-OENO 553-2016, <https://www.oiv.int/public/medias/4968/oiv-oeno-553-2016-es.pdf>, accessed on 9 January 2026). Samples were analyzed in triplicate in the following order: 7.VA, 7.VA.F, 7.VA.A, LM, LM.F, LM.A. A water blank was injected first to condition the column, and the first sample was injected twice. Water cycles were performed every 5 samples to prevent instrumental contamination.

The chromatograms were interpreted through the integration of different peaks and comparison with the available bibliography.

2.10.8. Anthocyanin Profile by HPLC

Anthocyanin profiles were determined according to the method previously described in Bartolomé et al. (2025) [7].

An Agilent Technologies (Palo Alto, CA, USA) 1260 HPLC system equipped with a diode-array detector (DAD) was used for identifying anthocyanins in the samples. The column was a Poroshell 120 C18 reverse-phase column (Phenomenex, Torrance, CA, USA), with dimensions of 50 mm × 4.6 mm and particle size of 2.7 µm. Before injection, 1 mL of each sample was filtered through 0.45 µm MCE syringe filters into 1.5 mL chromatographic vials. The mobile phase consisted of water/formic acid (95:5 *v/v*) as solvent A; and methanol/formic acid (95:5 *v/v*) as solvent B. The gradient was 0–2 min, 25% B; 2–10 min, linear increase to 50% B; 10–11 min, 50% B; 11–12 min, linear decrease to 2% B; 12–17 min,

re-equilibration. The system pressure was set to 200 bar throughout the run, and the flow rate was set at 1 mL/min. The injection volume was 50 μ L. The monitoring wavelength was set at 525 nm for the detection of red pigments. Chromatograms were interpreted by comparison with the published literature, and anthocyanins were quantified using a calibration curve of malvidin-3-O-glucoside as the external standard ($R^2 = 0.9999$, LOD = 0.1 mg/L).

2.10.9. Total Polyphenol Index (TPI)

The TPI of the beer was determined by UV spectrophotometry. Samples were diluted 1:50 in distilled water and vortexed for 10 s. Absorbance at 280 nm was then measured with a UV-vis SELECTA spectrophotometer (Barcelona, Spain) using quartz cuvettes [45,46].

2.10.10. Determination of Antioxidant Activity

The antioxidant activity of the beers was measured according to the ABTS protocol described by Re et al. (1999) [47]. The day before analysis, a 7 mM ABTS solution was prepared in distilled water. Potassium persulfate was added to a concentration of 2.45 mM and kept in darkness for 12–16 h to obtain the ABTS^{•+} radical. On the day of analysis, a 2.5 mM Trolox calibration curve (Sigma Aldrich, Merck, St. Louis, MO, USA) was prepared in 95% ethanol. ABTS^{•+} was diluted in 95% ethanol until reaching $A_{734\text{nm}} = 0.7 \pm 0.02$. Then, 1 mL of ABTS^{•+} ($A_{734\text{nm}} \approx 0.70$) and 10 μ L of Trolox or sample were mixed and incubated at 20 °C for 4 min in darkness. Absorbance at 734 nm was measured and used to calculate the percentage inhibition and, consequently, the antioxidant activity in Trolox equivalents. Samples were analyzed in triplicate.

2.11. Sensory Analysis

Sensory analysis was performed by a panel of 8 trained judges from the Department of Chemistry and Food Technology of ETSIAAB (UPM), including men and women, aged 20–50 years. Each panelist tasted 6 beers and rated 20 different attributes (including visual, olfactory, and taste attributes) on a scale from 1 (low perception) to 5 (high perception) using a tasting sheet. The tasting test was carried out in a sensory evaluation room with white light and ventilation, at 21 °C, and a sample preparation room. Samples were coded with three-digit random numbers to avoid biased scores and served in wine glasses over a white tablecloth.

The sensory assessment was performed in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (UPM Ethics Committee) of the Universidad Politécnica de Madrid (AIDLAPPMLB-AMB-HUMANOS-20221026 on 14 November 2022).

2.12. Statistical Analysis

Data analysis was performed with Microsoft Excel 365 (v2504) (Microsoft, Washington, DC, USA) and expressed as mean $\pm$ SD for triplicate samples. A two-factor ANOVA with LSD post hoc test was carried out with Statgraphics Centurion v19.6.04 software (Statgraphics Technologies Inc., The Plains, VA, USA). The factors studied were yeast species (7.VA, LM) and type of additive (no additive, fruit, anthocyanins). The significance level was set at p -value < 0.05. The XLSTAT (2025.1.1 ver.) Excel add-in (Addinsoft, Paris, France) was used to perform a PCA of the sensory analysis data and volatile compound profiles.

3. Results

3.1. Implantation Test

An implantation test was performed prior to the beer production, to determine the growth capacity of the yeasts in wort. The assay was conducted in duplicate. Figure 1 shows the growth kinetics of the yeasts in wort.

Pure cultures of *L. thermotolerans* and *M. pulcherrima* (Figure 1A,B) were both inoculated at approximately log 6 CFU/mL and both showed similar growth kinetics as pure cultures in wort, reaching a maximum population of around log 7.5 CFU/mL on day 5.

When co-inoculating L31 and M29, L31 appeared to display a dominance effect over M29, as shown in Figure 1C,D,F. In the 1:1, 1:5, and sequential inoculation conditions, a marked decline in the M29 population was observed from day 5 onward, associated with an increase in the L31 population, which remained stable at approximately 7 log CFU/mL throughout the experiment. The 1:10 condition (Figure 1E) showed a similar dominance pattern of L31 over the M29 population; however, M29 viability on day 5 was approximately 50% higher than in the other conditions, suggesting improved survival under this ratio. For this reason, a 1:10 inoculation ratio (L31:M29) was selected for the primary fermentation of the beer.

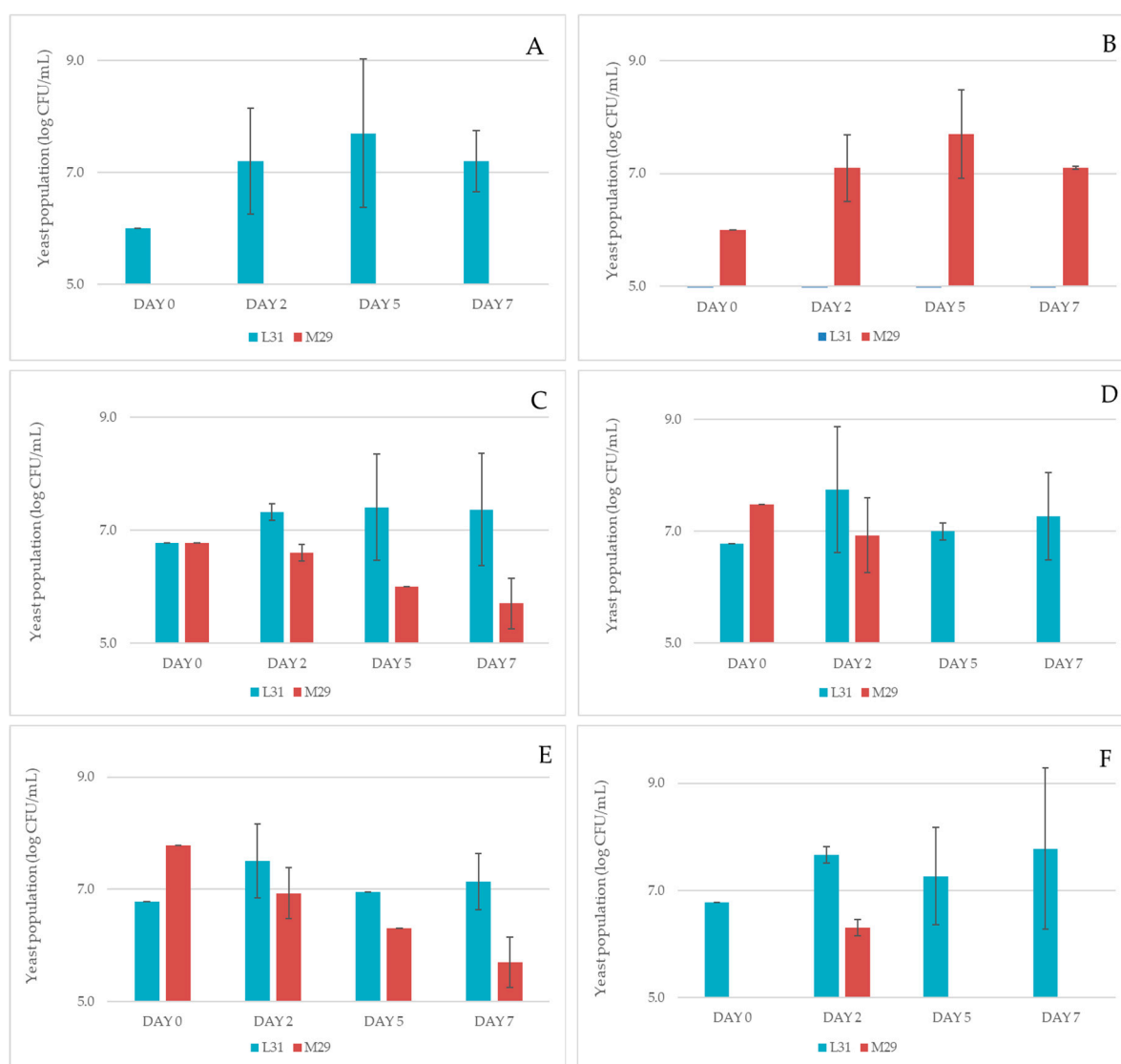


Figure 1. Evolution of the yeast population in wort during the implantation test. (A) Pure culture of *L. thermotolerans* L31; (B) pure culture of *M. pulcherrima* M29; (C) co-inoculation 1:1; (D) co-inoculation 1:5; (E) co-inoculation 1:10; (F) sequential inoculation.

3.2. Fermentation

The initial values of density and pH before wort inoculation were 1051 kg/m³ and 5.22, respectively. The progression of these parameters during fermentation is shown in Figure 2. The glucose consumption can be found in Figure S1 in Supplementary Materials.

The density of the wort (Figure 2A) decreased progressively during fermentation. The beers with fruit, 7.VA.F and LM.F, reached the desired value (1015 kg/m³) in 5 and 10 days, respectively. Therefore, they showed the highest fermentation rate. Next, the beers without additives (7.VA and LM) ended fermentation on day 10 and around day 20, respectively. This means that beers co-inoculated with L31-M29 ferment more slowly than beers inoculated with *Saccharomyces cerevisiae*. Finally, the density of beers with grape anthocyanin concentrate (7.VA.A and LM.A) stalled at high values on day 10. Thus, they had to be re-inoculated with their corresponding yeasts. Beer 7.VA.A reached the end of fermentation on day 26, while beer LM.A reached the desired density on day 30.

Regarding pH (Figure 2B), beers with fruit showed lower pH values, followed by beers fermented with *L. thermotolerans* and *M. pulcherrima*. On the other hand, beers with anthocyanin concentrate added showed similar pH values to beer brewed with *S. cerevisiae*. Addition of anthocyanin concentrate raised the final pH of LM.A beers, inhibiting beer acidification.

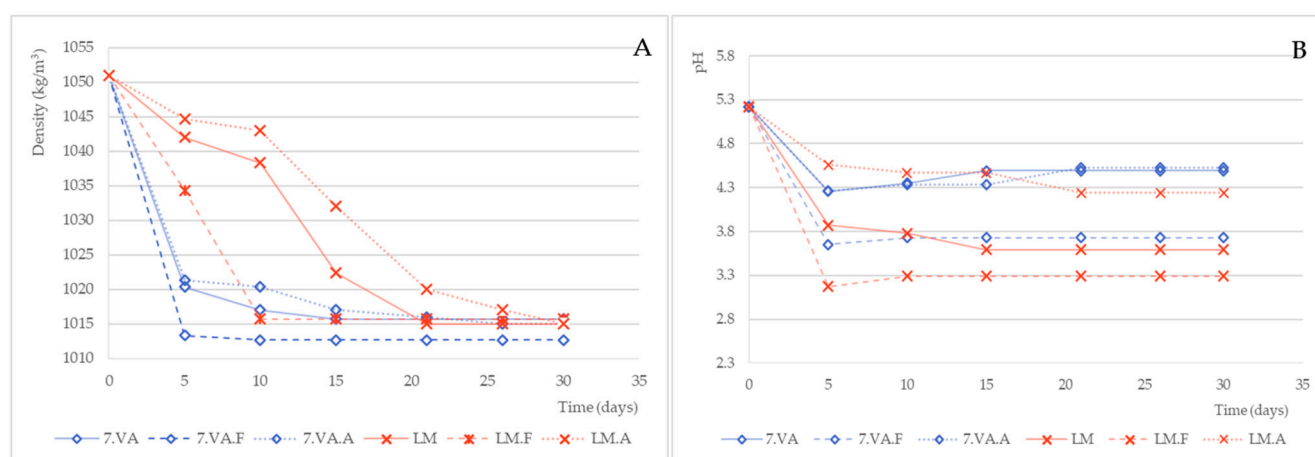


Figure 2. Evolution of (A) density (kg/m³) and (B) pH during fermentation of the wort.

3.3. Physicochemical Parameters in Final Beer

After secondary fermentation, pH, density, glucose levels, color, bitterness, and alcoholic content were analyzed. The results are summarized in Table 2.

Table 2. Physicochemical parameters in final beer.

	7.VA	7.VA.F	7.VA.A	LM	LM.F	LM.A
pH	4.35 ± 0.006 ^a	3.69 ± 0.009 ^b	4.32 ± 0.008 ^c	3.50 ± 0.017 ^d	3.26 ± 0.002 ^e	4.28 ± 0.027 ^c
Original gravity	1.053 ± 0.00 ^a	1.053 ± 0.00 ^a	1.053 ± 0.00 ^a	1.053 ± 0.00 ^a	1.053 ± 0.00 ^a	1.053 ± 0.00 ^a
Density (kg/m ³)	1015 ± 0.16 ^a	1012 ± 0.17 ^b	1016 ± 0.16 ^{a,c}	1016 ± 0.17 ^c	1015 ± 0.10 ^d	1017 ± 0.16 ^e
Attenuation	0.641 ± 0.015 ^a	0.723 ± 0.008 ^b	0.679 ± 0.006 ^{a,b}	0.539 ± 0.062 ^c	0.666 ± 0.017 ^{a,b}	0.641 ± 0.009 ^{a,b}
Glucose (g/L)	0.060 ± 0.017 ^{a,b}	0.040 ± 0.005 ^a	0.090 ± 0.026 ^b	0.019 ± 0.003 ^a	0.034 ± 0.002 ^a	0.046 ± 0.012 ^a
Color (EBC)	12.88 ± 0.77 ^a	22.75 ± 0.34 ^b	15.56 ± 0.25 ^{c,d}	14.64 ± 0.19 ^c	23.24 ± 0.49 ^b	17.08 ± 0.74 ^d
Bitterness (IBUs)	23.92 ± 0.58 ^a	11.93 ± 0.05 ^b	23.42 ± 0.75 ^a	22.32 ± 0.28 ^a	9.67 ± 0.96 ^b	16.23 ± 0.54 ^c
Alcohol (%v/v)	4.97 ± 0.05 ^a	5.10 ± 0.00 ^{a,b}	5.03 ± 0.03 ^{a,b}	4.70 ± 0.13 ^{a,b}	4.98 ± 0.13 ^{a,b}	5.05 ± 0.06 ^b

Data are mean ± standard deviation ($n = 3$). Data with different letters within each column are significantly different (p -value < 0.05). 7.VA: *S. cerevisiae* without additive; 7.VA.F: *S. cerevisiae* with fruit; 7.VA.A: *S. cerevisiae* with grape anthocyanin concentrate; LM: *Lachancea-Metschnikowia* without additive; LM.F: *Lachancea-Metschnikowia* with fruit; LM.A: *Lachancea-Metschnikowia* with grape anthocyanin concentrate.

Glucose levels decreased in all the beers as fermentation progressed. Whereas beers with blueberries showed the highest residual glucose levels, beers brewed with *S. cerevisiae* generally had twice the glucose levels of beers fermented with *L. thermotolerans* and *M. pulcherrima*. In all cases, glucose levels remained low, meaning almost all the glucose in the wort was consumed.

Beer color (expressed as EBC units) is strongly associated with the type of additive and not influenced by the type of yeast used for fermentation. The beers with blueberries (7.VA.F and LM.F) had red–maroon hues, similar to garnet wines. They were the darkest ones and therefore had the highest EBC values. The beers with anthocyanin concentrate had intermediate EBC values, corresponding to dark-copper beers, like brown ale beers. The beers with no additives were the lightest and most golden beers, with lowest EBC values.

The fruit beers were significantly less bitter than the others, regardless of the type of yeast used for fermentation. Therefore, bitterness of beer is affected by the addition of fruit, but not by the grape anthocyanins or by the yeast species used.

Finally, all the beers showed similar alcohol contents, around 5% *v/v*. No significant differences were observed in alcoholic content for any beer.

3.4. Antioxidant Activity, Total Polyphenol Index (TPI), and Anthocyanin Concentration

Antioxidant activity (ABTS), TPI, and anthocyanin concentration were measured to determine the antioxidant capacity of the beers. The data are summarized in Table 3.

Table 3. Total Polyphenol Index (TPI), antioxidant activity (Trolox equivalents/L), and total anthocyanin content in final beers.

	TPI	ABTS ⁺ (mmol TE/L)	Anthocyanins (mg/L)
7.VA	23.97 ± 1.02 ^{a,b}	0.14 ± 0.04 ^a	n.d.
7.VA.F	24.13 ± 0.31 ^{a,b}	1.03 ± 0.05 ^b	41.23 ± 3.30 ^a
7.VA.A	23.77 ± 1.75 ^{a,b}	0.37 ± 0.05 ^c	0.37 ± 0.13 ^b
LM	21.27 ± 0.19 ^a	0.46 ± 0.07 ^d	n.d.
LM.F	27.62 ± 0.55 ^c	1.29 ± 0.02 ^e	33.37 ± 0.49 ^c
LM.A	25.33 ± 0.73 ^{b,c}	0.69 ± 0.03 ^c	3.107 ± 1.26 ^b

Data are mean ± standard deviation (*n* = 3). Data with different letters within each column are significantly different (*p*-value < 0.05). 7.VA: *S. cerevisiae* without additive; 7.VA.F: *S. cerevisiae* with fruit; 7.VA.A: *S. cerevisiae* with grape anthocyanin concentrate; LM: *Lachancea-Metschnikowia* without additive; LM.F: *Lachancea-Metschnikowia* with fruit; LM.A: *Lachancea-Metschnikowia* with grape anthocyanin concentrate.

As can be seen, antioxidant capacity and TPI are directly related and depend on the type of additive used. This antioxidant activity is due to the presence of melanoidins and polyphenols, directly extracted from the malt and hops during the boiling process, and the blueberries added before fermentation.

The addition of fruit increases the antioxidant activity almost tenfold in beer 7.VA.F (from 0.14 to 1.03 TE/L) and threefold in beer LM.F (from 0.46 to 1.29 TE/L), as well as the TPI. The beers with blueberries showed the highest antioxidant activity, followed by the beers with grape anthocyanin content, which also showed higher antioxidant activity and TPI than the beers with no additives. Therefore, the type of additive in the beer strongly influenced these parameters, whereas the yeast species had no effect.

3.5. Anthocyanin Profile by HPLC

The concentration of anthocyanins depended on the type of additive used, as expected (Table 3). The beers with fruit showed the highest anthocyanin levels (both above 30 mg/L). Anthocyanin levels in the beers with grape anthocyanin concentrate were low (0.37 mg/L for 7.VA.A and 3.11 mg/L for LM.A), while no anthocyanins above the limit of detection (LOD = 0.1 mg/L) were found in the beers with no additives, according to Table 3. The type of additive directly affects the anthocyanin content, while the type of yeast does not. Thus,

higher anthocyanin content resulted in higher antioxidant activity and a redder appearance, since anthocyanins are naturally red pigments.

Figure 3 shows the chromatograms obtained from the samples. The chromatograms are consistent with the data shown in Table 3. Since no anthocyanins were detected in beers without additives, these chromatograms are not included.

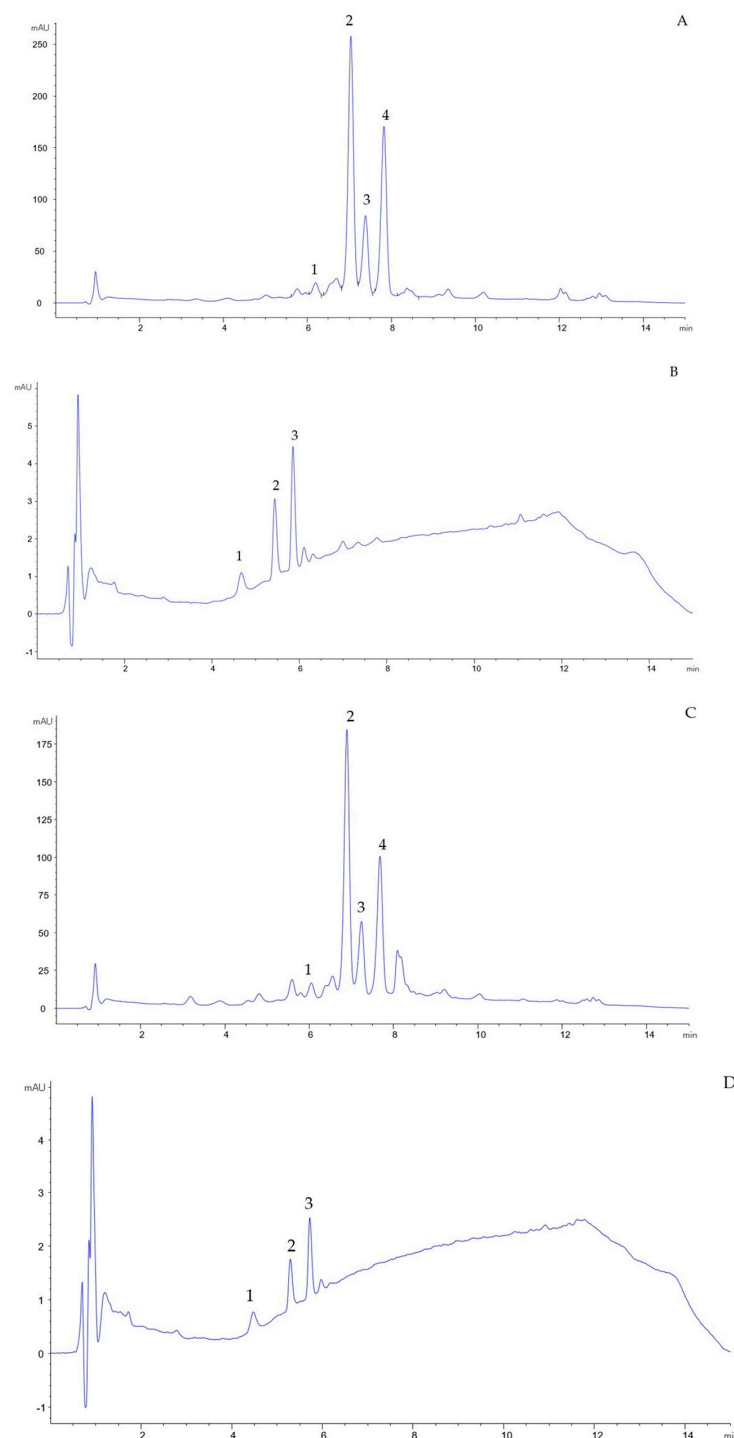


Figure 3. Anthocyanin profiles of the final beers. (A) 7.VA.F (*S. cerevisiae* with blueberries); (B) 7.VA.A (*S. cerevisiae* with grape anthocyanin concentrate); (C) LM.F (*Lachancea-Metschnikowia* with blueberries); (D) LM.A (*Lachancea-Metschnikowia* with grape anthocyanin content). Peaks identified in (A,C): (1) malvidin-3-glucoside, (2) delphinidin-3-glucoside, (3) malvidin-3-arabinoside, and (4) peonidin-3-galactoside. Peaks identified in (B,D): (1) cyanidin-3-glucoside, (2) petunidin-3-glucoside, and (3) malvidin-3-glucoside.

Figure 3A,C correspond to beers with blueberries and display similar anthocyanin profiles, with peak intensities of approximately 200 mAU. This agrees with the anthocyanin content data, where beers with fruit show the highest anthocyanin levels. Identified peaks are included in the caption of the figure.

In Figure 3B,D (beers with grape anthocyanin concentrate), three main peaks were identified. They are identified in the figure caption.

3.6. Volatile Compounds by GC-FID

The data corresponding to the concentrations of volatile compounds in the beers are summarized in Table 4. The perception thresholds, retention times, and olfactory descriptors can be found in Table S1 in the Supplementary Materials.

Table 4. Concentration of volatile compounds (mg/L) in final beers.

Compound	7.VA	7.VA.F	7.VA.A	LM	LM.F	LM.A
Alcohols						
Methanol	11.80 ± 0.08 ^a	67.90 ± 18.98 ^c	19.69 ± 4.94 ^a	30.27 ± 13.31 ^b	62.53 ± 2.36 ^{b,c}	6.44 ± 2.67 ^a
1-propanol	20.61 ± 1.11 ^{a,c}	28.51 ± 1.76 ^b	21.88 ± 1.33 ^c	15.50 ± 1.31 ^a	36.36 ± 1.30 ^d	22.08 ± 2.15 ^c
2-butanol	3.41 ± 1.04 ^{a,b}	6.07 ± 0.91 ^{a,b}	6.61 ± 0.50 ^b	5.87 ± 1.17 ^{a,b}	6.06 ± 0.64 ^{a,b}	2.97 ± 0.80 ^a
Isobutanol	n.d.	n.d.	n.d.	24.68 ± 0.98 ^{a,b}	29.35 ± 4.40 ^c	20.94 ± 2.25 ^{b,c}
1-butanol	8.57 ± 1.53 ^a	9.92 ± 2.20 ^a	7.65 ± 0.53 ^a	7.74 ± 0.72 ^a	8.23 ± 3.51 ^a	5.57 ± 0.32 ^a
3-methyl-1-butanol	5.49 ± 1.01 ^{a,b}	4.06 ± 0.26 ^a	4.84 ± 0.34 ^a	3.97 ± 0.12 ^a	7.33 ± 0.98 ^b	4.61 ± 0.29 ^a
2-methyl-butanol	45.36 ± 2.99 ^{a,b}	79.85 ± 1.78 ^b	45.09 ± 3.09 ^a	58.71 ± 3.20 ^{a,c}	60.17 ± 3.29 ^c	46.89 ± 7.20 ^{a,c}
2,3-butanediol	167.40 ± 9.84 ^a	176.13 ± 9.92 ^a	151.66 ± 1.78 ^a	176.37 ± 9.23 ^a	179.22 ± 6.05 ^a	158.14 ± 6.07 ^a
Hexanol	4.30 ± 0.21 ^a	5.30 ± 0.62 ^a	4.34 ± 0.28 ^a	4.31 ± 0.25 ^a	10.80 ± 5.32 ^a	4.26 ± 0.26 ^a
2-phenylethyl alcohol	n.d.	n.d.	n.d.	26.75 ± 11.18 ^a	36.81 ± 11.07 ^a	32.07 ± 1.91 ^a
Esters						
Ethyl acetate	19.84 ± 2.39 ^a	37.74 ± 1.93 ^b	22.36 ± 2.54 ^a	24.68 ± 0.98 ^a	29.35 ± 4.40 ^{a,b}	20.94 ± 2.25 ^a
Isobutyl acetate	27.96 ± 4.36 ^a	32.39 ± 3.70 ^a	22.41 ± 2.16 ^a	19.50 ± 1.40 ^a	30.44 ± 5.52 ^a	25.60 ± 1.74 ^a
Ethyl butyrate	7.88 ± 0.65 ^{a,b}	11.40 ± 1.91 ^a	9.31 ± 1.03 ^b	7.59 ± 1.07 ^{a,b}	6.64 ± 0.86 ^b	5.39 ± 0.18 ^{a,b}
Ethyl lactate	17.45 ± 3.64 ^a	15.37 ± 3.84 ^a	26.65 ± 2.52 ^{a,b}	34.01 ± 1.92 ^{a,b}	49.76 ± 14.59 ^b	9.83 ± 1.29 ^a
Isoamyl acetate	5.60 ± 0.61 ^{a,b}	4.40 ± 2.03 ^a	8.91 ± 0.34 ^{b,c}	12.19 ± 1.55 ^c	9.88 ± 0.87 ^{b,c}	5.63 ± 0.19 ^{a,b}
2-phenylethyl acetate	5.57 ± 0.24 ^a	5.66 ± 0.22 ^a	5.74 ± 0.41 ^a	5.94 ± 0.23 ^a	7.15 ± 0.87 ^a	7.41 ± 1.01 ^a
Carbonyl compounds						
Acetaldehyde	11.09 ± 2.93 ^a	28.70 ± 2.56 ^{a,b}	34.63 ± 9.19 ^{a,b}	51.69 ± 9.09 ^b	43.85 ± 7.83 ^b	11.86 ± 2.71 ^a
Diacetyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetoin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Data are mean ± standard deviation ($n = 3$). Data with different letters within each column are significantly different (p -value < 0.05). 7.VA: *S. cerevisiae* without additive; 7.VA.F: *S. cerevisiae* with fruit; 7.VA.A: *S. cerevisiae* with grape anthocyanin concentrate; LM: *Lachancea-Metschnikowia* without additive; LM.F: *Lachancea-Metschnikowia* with fruit; LM.A: *Lachancea-Metschnikowia* with grape anthocyanin concentrate. n.d. means “non-determined”.

Regarding alcohols, methanol, 1-propanol and 2-methyl-1-butanol were above their perception thresholds in all samples, and increased significantly in the fruit-added beers. These compounds are related to odors such as solvent, alcoholic, wine-like, and banana notes. The hexanol and 2,3-butanediol levels were also above threshold in all samples, but no significant differences were observed within groups. In contrast, 2-butanol, 1-butanol, isobutanol, and 3-metil-1-butanol did not exceed their perception thresholds. 2-phenylethyl alcohol only went over the threshold in sample LM.F.

When analyzing esters, it can be noted that isobutyl acetate, ethyl butyrate, isoamyl acetate, and 2-phenylethyl acetate levels were above their perception thresholds in all the samples, providing fruity odors such as banana and pear. Neither the type of yeast nor

type of additive affected the levels of these compounds. Ethyl acetate and ethyl lactate levels were both below their perception thresholds.

Regarding carbonyl compounds, diacetyl and acetoin, responsible for buttery and woody odors, respectively, were both below their perception thresholds. However, acetaldehyde concentration exceeded the perception threshold in all samples, except 7.VA and LM.A. Acetaldehyde is associated with green-leaf aromas and is considered a flaw at high concentrations.

3.7. Sensory Analysis

The results obtained from the sensory analysis are summarized in Figure 4 as spider charts.

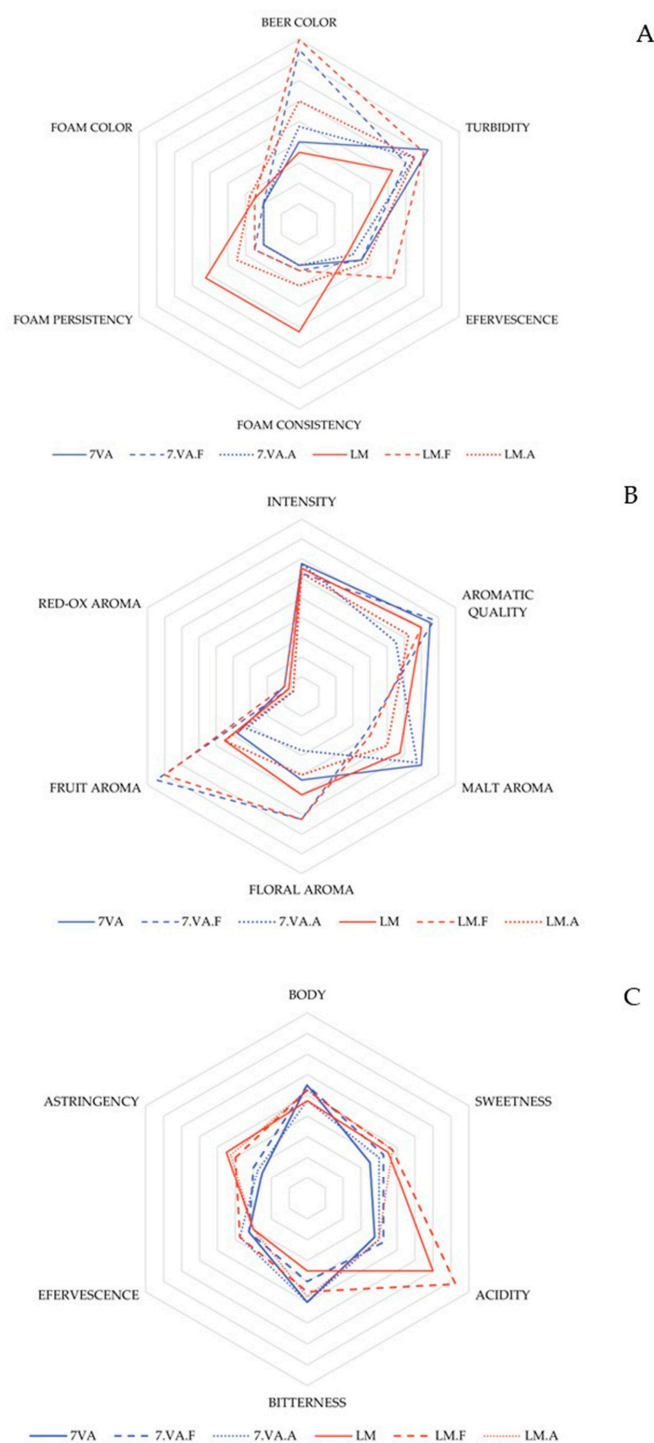


Figure 4. Results of sensory analysis of final beers. (A) Visual attributes; (B) olfactory attributes; (C) gustatory attributes.

Concerning the data from visual attributes, the beers with fruit (7.VA.F and LM.F) obtained high scores in the “beer color” attribute, meaning they were perceived as dark red beers, followed by the beers with anthocyanin concentrate (7.VA.A and LM.A), which were copper-colored, and finally the beers without additives (7.VA and LM), perceived as the lightest and most golden beers. These data agree with the EBC values of the beers. The addition of fruit significantly affected the color. No significant differences were observed in turbidity or effervescence.

Regarding olfactory attributes, the addition of fruit and the type of yeast significantly affected the fruitiness of the beer. Therefore, the beers with blueberries and beers brewed with *Lachancea-Metschnikowia* were perceived as fruitier and more floral. On the other hand, no differences in aromatic quality or aromatic intensity were found. The beers brewed with *S. cerevisiae* were rated highest in the “malt aroma” attribute.

In terms of gustatory attributes, all the beers were perceived as light-bodied, with low effervescence and similar bitterness levels. The beers with fruit and beers brewed with *Lachancea-Metschnikowia* were significantly more acidic, due to the low pH of blueberries and the production of lactic acid from *L. thermotolerans*, respectively.

3.8. PCA for GC-FID and Sensory Analysis

Principal component analysis (PCA) was performed using the GC-FID and sensory analysis data to better interpret the results. Figures 5 and 6 illustrate the results.

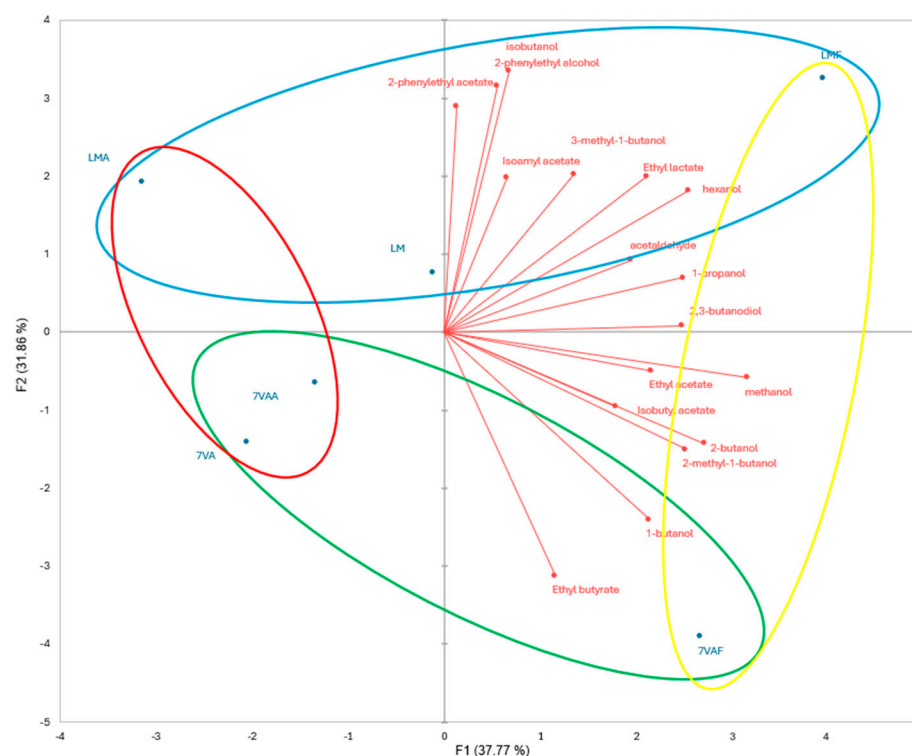


Figure 5. PCA for results obtained from GC-FID analysis. Blue: *Lachancea-Metschnikowia* beers; green: *S. cerevisiae* beers; yellow: beers with blueberries; red: beers with grape anthocyanin concentrate.

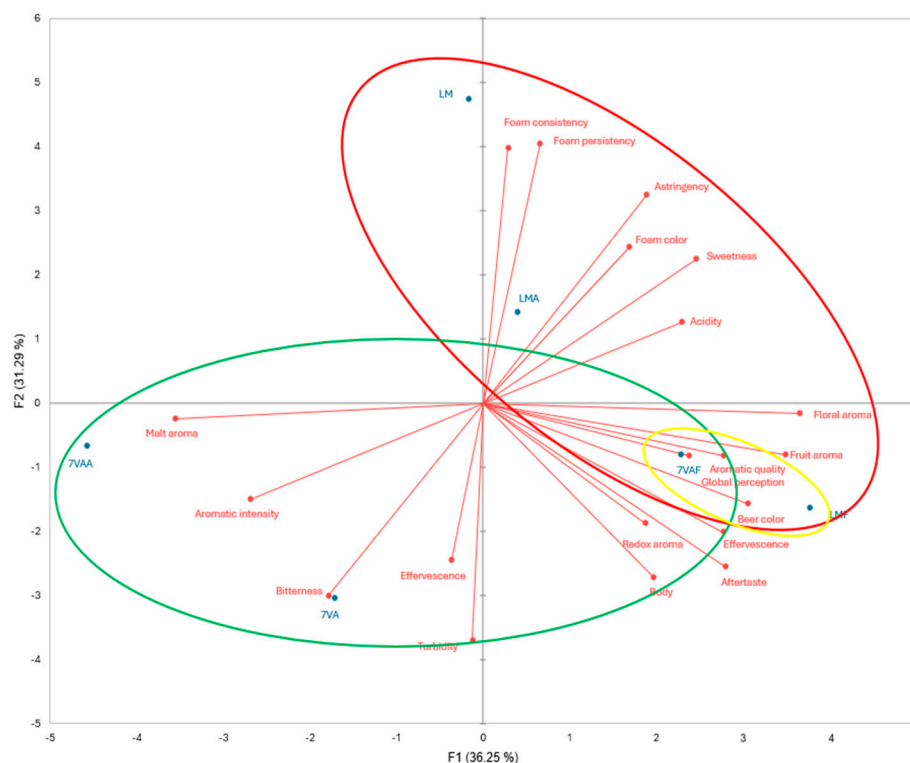


Figure 6. PCA for results obtained from sensory analysis. Red: *Lachancea-Metschnikowia* beers; green: *S. cerevisiae* beers; yellow: beers with fruit.

The PCA for GC-FID (Figure 5) explains 69.39% of the data variability. The beers are clearly separated into four groups. *Lachancea-Metschnikowia* beers (blue) are associated with higher levels of 2-phenylethyl acetate, 2-phenylethyl alcohol, isoamyl acetate, and isobutanol, and therefore with aromas such as fruity, banana, rose, and apple notes. The *S. cerevisiae* beers (green) are associated with production of ethyl butyrate and 1-butanol. The third group corresponds to the beers with blueberries (yellow), related to higher levels of methanol, 2-butanol, and 2-methyl-1-butanol. 7.VA.F is closely linked to production of 1-butanol and ethyl butyrate, thus these beers were perceived as fruity and tropical. Finally, the beers with anthocyanin concentrate (red) are characterized by low aroma production and a poorly defined volatile profile.

The PCA for sensory analysis (Figure 6) explains 67.54% of the data variability. In this case, the beers are grouped into three groups. The beers brewed with *Lachancea-Metschnikowia* (red) are grouped together and they are associated with higher acidity, astringency, and less sweetness. They are also less hazy, less bitter, and less effervescent. The *S. cerevisiae* beers are perceived as hazier, more bitter, and more effervescent. Finally, the beers with blueberries are gathered in the third group (yellow) and show higher aromatic quality, darker color, and a floral–fruity aromatic profile. They are also rated with the highest “global perception” score.

4. Discussion

When co-inoculating L31 and M29 in beer wort, L31 showed better growth than M29, since the M29 population decreased significantly during the first days of fermentation. Some studies have shown that *L. thermotolerans* can metabolize glucose, fructose, sucrose, and maltose similarly to *S. cerevisiae* [26,29], while it is unable to metabolize maltotriose. Meanwhile, *M. pulcherrima* can assimilate glucose and fructose, but not maltose or maltotriose [37], meaning that *L. thermotolerans* has access to a greater pool of assimilable

nutrients than *M. pulcherrima* in wort. Thus, it is not surprising that the *L. thermotolerans* population easily outcompetes *M. pulcherrima* and leads to its decline in beer wort. This phenomenon also occurs in wine; *Lachancea* has been shown to be a better competitor than *Metschnikowia* [48].

The addition of blueberries had a significant impact on yeast fermentative capacity. This might be due to an increase in the concentration of fermentable sugars derived from blueberries. *S. cerevisiae* showed higher fermentative power than *L. thermotolerans* and *M. pulcherrima*, which agrees with other studies on the matter, in both beers and wine [30,38,49]. Indeed, it is estimated that whereas *S. cerevisiae* needs 16 g of sugar to produce 1% v/v of ethanol, *L. thermotolerans* requires 21.5 g to produce the same amount, due to its inability to ferment maltose. Beers with anthocyanin concentrate exhibited the lowest fermentation rate and had to be re-inoculated on day 10. This was linked to a high sulfite concentration in the wort, resulting from the addition of grape anthocyanins. According to Nally et al. (2018) [50], many yeast genera, such as *L. thermotolerans* and *M. pulcherrima*, are highly sensitive to sulfites, and their fermentative power decreases above concentrations of 40 and 60 mg/L, respectively [38].

When comparing all the beers, those with blueberries stood out as the most acidic. During fermentation, pH decreases because of the production of organic acids derived from the metabolism of yeasts [51]. Since the addition of berries increased the glucose levels in the initial wort, the metabolism of yeasts may have been enhanced, producing more organic acids in these beers. Another hypothesis is that blueberries are fruits grown in acidic soils, so they usually have low pH, which can affect the pH in the final beers. Similarly, beers brewed with *Lachancea-Metschnikowia* show lower pH than beers brewed with *S. cerevisiae*, due to the capacity of *L. thermotolerans* to produce lactic acid [26,49,52].

The beers with fruit showed the highest residual glucose levels among all the beers, since the addition of fruits leads to an increase in glucose levels in the initial wort. On the other hand, the higher concentrations of glucose in the beers brewed with *S. cerevisiae* can be explained by the fact that this yeast can utilize a wider range of sugars in the wort than *L. thermotolerans* and *M. pulcherrima* [13], which therefore relied primarily on glucose as their carbon source. This may explain why glucose levels were lower in the LM beers. The results obtained here are lower than those reported in other studies, such as Postigo et al. (2023) [49] or Zdaniewicz et al. (2020) [52], but similar to those reported by Pirrone et al. (2025) [29] or Postigo et al. (2022) [37] in their studies on *L. thermotolerans* and *M. pulcherrima*. This could mean that fermentative capacity is strongly strain-dependent.

Beer bitterness appeared to be affected by both the type of additive and the type of yeast used. The presence of blueberries resulted in low bitterness (both IBU and perceived scores), agreeing with Rinaldi et al. (2022) [53] and Kawa-Rygielska et al. (2019) [54]. These studies reported that beers with gooseberries and cherries showed a decrease in bitterness, although based on Rinaldi's research, the effect was not dose-dependent. In addition, the yeast used for fermentation can also modify the α -isoacid level in beers, since these molecules can bind to yeast cell walls, causing their precipitation and lowering the IBU values. This phenomenon is strongly strain-dependent, and many studies have shown that the use of *L. thermotolerans* reduces bitterness [49,55], while others have reported an increase in bitterness when using *L. thermotolerans* and *M. pulcherrima* in beer production [37,56].

The beers with blueberries acquired a reddish color, and showed EBC values similar to those of commercial beers with raspberries, blackberries, or cherries [57]. Some studies on this subject have shown that the addition of fruits such as Kamchatka berries [58] increases the EBC value of beers to a similar extent to the results obtained here. The beers with grape anthocyanin concentrate were darker than beers without additives, and lighter than those with blueberries. This difference might be explained by the fact that anthocyanins are

pH-dependent pigments that require low pH to remain stable [16]. Since pH in the beers with anthocyanins was higher, the grape anthocyanins may have degraded.

The addition of fruit and grape anthocyanin concentrate to the beers increased the anthocyanin levels, resulting in higher TPI and increased antioxidant activity. This effect was significantly stronger when adding fruits, which agrees with other research [58], as a consequence of the transfer of polyphenols and flavonoids from the fruit to the beer. This increase in antioxidant activity is more remarkable in certain anthocyanin-rich fruits such as cherry, raspberry, and grapes [15]. It also depends on the amount, physical form, and timing of fruit addition [59,60]. Grape anthocyanin concentrate was added in the same proportion and at the same time, so the samples would be expected to show similar anthocyanin levels. The anthocyanin stability might have been affected by either the pH of the beers [61,62], or by storage time, which is considered one of the main causes of anthocyanin stability loss [63]. Generally, the beers with blueberries showed more complex anthocyanin profiles, with mainly malvidin, delphinidin, petunidin, and their derivatives, since these are the most common anthocyanins in blueberries [64,65]. The anthocyanin profiles of beers supplemented with anthocyanin concentrate was simpler and had mainly malvidin and petunidin, which are the predominant anthocyanins in red grapes [16]. Cyanidin is also found in grapes, although in a lower proportion [66]. Overall, anthocyanin composition and profile depend closely on the variety of fruit and agricultural practices [67].

It is also important to mention that the addition of blueberries does not only influence the sugar content of the wort. Many other nutrients, such as amino acids and vitamins, may also increase in beer due to the addition of fruit. Baigts-Allende et al. (2021) compared twenty-six fruit beers by analyzing the amino acid and phenolic profiles of samples, finding significant differences among beers [57]. Although most studies focus on variations in phenolic and anthocyanin profiles [59,68], the presence of other fruit-derived bioactive compounds, such as amino acids and vitamins, may represent an important field of research for the development of new functional beverages.

The aromatic profiles of the beers did not vary substantially among samples. The results generally agreed with those reported by Peces-Pérez et al. (2022) [69] and Kobayashi et al. (2008) [70]. No effect of yeast type was observed on the aromatic profiles of the beers except for isobutanol, whose concentration increased when using *Lachancea-Metschnikowia*. In other studies, levels of isobutanol remained constant regardless of the yeast used [49,55]. The fact that volatile compound profiles did not change when using *S. cerevisiae* or *Lachancea-Metschnikowia* contrasts with Zdaniewicz et al. (2024) [71] and Peces-Pérez et al. (2022) [69] but agrees with Bartolomé et al. (2025) [7]. Again, aromatic profiles may vary depending on the strain used. The addition of fruit caused major variations in compounds such as methanol, 1-propanol, and 2-methyl-1-butanol, with the beers exhibiting higher levels of these compounds than those reported in other studies [30,35,69,72], but again, similar to those in Kobayashi et al. (2008) [70]. Methanol is produced during fermentation because of the degradation of pectin. Since beers are usually not brewed with fruits, they do not present high levels of ethanol. When fruit is added, the pectin content of the wort increases, causing an increase in methanol levels. The presence of pectin in blueberries may explain the rise in methanol levels in beers [73–75]. 2-methyl-1-butanol is produced from pyruvate derived from glycolysis. Therefore, addition of fruit leads to an increase in 2-methyl-1-butanol levels, because of higher glucose levels in the wort [70]. Finally, acetaldehyde levels were relatively high in all the samples. Acetaldehyde is an intermediate in the ethanolic fermentation pathway and a direct precursor of ethanol. High concentrations of this compound are related to incomplete fermentation processes and are considered a

flaw in beer [69]. Conversely, low levels of diacetyl and acetoin indicate complete beer maturation and are associated with a desirable sensory profile [71,76].

Although the use of fruit yielded the most promising results for sour beer production, the use of *L. thermotolerans* and *M. pulcherrima* as a co-inoculum in primary fermentation also had notable effects on the final beers. Primary fermentation of LM beers completed fermentation within 20 days, reaching a final density of 1016 kg/m³. LM beers, brewed with non-*Saccharomyces* yeasts, were significantly more acidic than *S. cerevisiae* beers, with pH values of 3.50 and 4.35, respectively. The acidification of beer when using non-*Saccharomyces* yeasts is a fact proven in many other studies [7,31,49], and is attributed to lactic acid production [26]. These beers also showed increased antioxidant activity (a threefold increase, from 0.14 mmol TE/L in LM beers to 0.46 mmol TE/L in 7.VA beers) but there were no differences in anthocyanin content, since no anthocyanins were detected in any sample. This agrees with the results obtained in Bartolomé et al. (2025) [7], who also observed a moderate rise in antioxidant activity in beers brewed with *L. thermotolerans*. According to Wu et al. (2024) [77], the use of non-*Saccharomyces* yeasts in beer brewing may result in increased antioxidant activity due to the production of antioxidant metabolites such as melatonin. Beers brewed with *Lachancea-Metschnikowia* exhibited a fruitier aroma, associated with the enhanced production of esters such as isoamyl acetate, as well as acetaldehyde (responsible for banana and green apple odors, respectively). These results agree with the data from the sensory analysis, since LM beers were perceived as more acidic and less sweet than 7VA beers. They were also perceived as fruitier and less malt-aromatic than *S. cerevisiae* beers, which correlates with the data from the volatile compound profiles. Considering this, the exclusive use of non-*Saccharomyces* yeasts should be considered a viable alternative for sour beer production.

5. Conclusions

To sum up, the use of non-*Saccharomyces* yeasts (*L. thermotolerans* and *M. pulcherrima*) allowed the substitution of bacterial acidification in sour beers due to the high production of lactic acid and volatile acids, shortening production time and resulting in beers with a sensory profile similar to those of sour beers. Furthermore, the addition of blueberries significantly increased the total anthocyanin content and therefore the antioxidant activity of the beer, representing an example of a functional beverage. Future research could focus on the use of different yeast strains as co-inocula to produce low-alcohol beers, reducing production costs and providing greater microbiological stability throughout the process. Not only does the use of non-*Saccharomyces* yeasts emerge as an innovative option, but the use of natural anthocyanins and fruits as additives to enhance the sensory profile of beers through sustainable production strategies also represents a broad research field yet to be fully explored in the brewing industry.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation12020091/s1>, Figure S1: Glucose intake kinetics in beer primary fermentation; Table S1: Characteristics and aromatic descriptors for volatiles analyzed by GC-FID in beer [69].

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (UPM Ethics Committee) of Universidad Politécnica de Madrid (AIDLAPPMLB-AMB-HUMANOS-20221026 on 14 November 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Verbal informed consent was obtained from the participants. The rationale for utilizing verbal consent is that the research presented no greater than minimal risk to participants.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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