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Whisky innovation using Patagonian yeasts to diversify flavour and aroma

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Abstract

Why was the work done: Whisky fermentations are typically performed using specific strains of *Saccharomyces cerevisiae*, which produce a wash with high ethanol yields but a limited diversity of aromas and flavours. Innovation is an important theme for alcoholic beverages and incorporating non-conventional yeasts in their production can enhance sensory complexity and enable regional differentiation. This study investigated the potential of native yeasts from Argentine Patagonia to produce whiskies with unique flavour profiles.

How was the work done: Eight native Patagonian yeasts (*Saccharomyces uvarum, Saccharomyces eubayanus, Hanseniaspora smithiae, Lachancea nothofagi, Lachancea cidri*) were tested in pure and mixed fermentations using distillery wort. A commercial strain of *Saccharomyces cerevisiae* (Safspirit M1™) was included for comparison. Fermentation kinetics, sugar consumption, and ethanol production were analysed, while sensory evaluation and chemical analyses of volatile compounds were performed on the first distillate or 'low wines' (20-23% ABV).

What are the main findings: Native yeasts produced lower ethanol yields than the commercial strain but resulted in low wines with enhanced complexity of flavour and aroma. Mixed fermentations increased ester and phenolic notes. *S. eubayanus* and *S. uvarum* were identified as promising yeasts for their ability to generate distinctive flavour compounds while maintaining good fermentation performance. Low wines made with native yeasts were preferred by a sensory panel over the control made with a commercial yeast.

Why is the work important: This research highlights the potential of Patagonian yeasts as a tool for whisky innovation, enabling flavour diversity. It also contributes to industry efforts to explore microbial biodiversity as a route to enhancing product differentiation and local whisky production.

Keywords

whisky, flavour, non-conventional yeasts, mixed fermentations, beverage innovation

Introduction

Whisky is a distilled alcoholic beverage produced from the fermentation of malted grains (barley, maize, rye, wheat), with the 'wash' distilled and matured by storage in wooden barrels. Its organoleptic properties are influenced by the choice of raw materials, the barrels used for ageing, and the fermentation and distillation processes, together with the yeast and bacterial microbiota (Russell and Stewart 2014). The introduction of pure yeast cultures in fermented beverage production has facilitated the standardisation and optimisation of processes and products. However, this approach has also led to a reduction in sensory complexity (Domizio et al. 2007; Cubillos et al. 2019). In whisky production, the yeast most used is *Saccharomyces cerevisiae*, with several commercial yeasts ('M-type') hybrids of *S. cerevisiae* and the amylolytic *S. cerevisiae* var. *diastaticus* (Daute et al. 2024). This property results in higher attenuation and ethanol yields with consistent levels of desirable congeners (Watson 1981; Campbell 2003; Russell and Stewart 2014; Waymark and Hill 2021). Most yeast research in the whisky industry has focused on improving and optimising fermentation efficiency and ethanol production, rather than their role in the organoleptic profile. This reflects the emphasis on consistency and yield, prioritising robust fermentation performance over flavour complexity. As a result, differences in whisky profiles reflect the malting, distillation, and the maturation processes rather than choice of yeast (Wanikawa 2020; Esteban-Decloux et al. 2023; Picard et al. 2023). Despite this, yeast plays a crucial role in the production of fermented beverages, as it significantly influences the final flavour and aroma through the production of various congeners (Walker et al. 2019; Kelly et al. 2023). For this reason, many alcoholic producers are focusing on selecting non-conventional yeasts to create diversity and foster innovation. Although commercial and efficient *S. cerevisiae* yeast strains continue to dominate whisky production, the use of novel yeasts is a growing trend (Walker and Hill 2016; Burini et al. 2021; Daute 2022).

Yeasts of the order Saccharomycetales are facultative anaerobes that ferment sugars to ethanol and carbon dioxide as the main fermentation products (Suh et al. 2006; Walker and Stewart 2016), along with a diverse mix of compounds including esters and higher alcohols (Olaniran et al. 2017), phenols (Mukai et al. 2010), and sulphides (Moreira et al. 2008). The production (and level) of these compounds depends on the yeast species, and the specific yeasts used (Stewart 2017). Non-conventional yeasts (other than the traditional domesticated yeasts) often exhibit lower fermentative performance compared to commercial strains, as they are less efficient at consuming the available sugars, and are less tolerant to ethanol stress. However, they can contribute novel flavours and aromas, making them key players in product innovation (Padilla et al. 2016; Burini et al. 2021). These yeasts may be used in isolation or in mixed fermentations (co-culture systems) (Benito et al. 2016). Despite their potential to introduce diversity to alcoholic beverages, their application in whisky production remains largely unexplored (Waymark and Hill 2021; Daute et al. 2024).

Non-conventional yeasts with the potential for innovation are diverse (Gschaedler 2017; Burini et al. 2021). A notable example is *Saccharomyces eubayanus*, a wild yeast isolated for the first time in Patagonia (Libkind et al. 2011), and used commercially by Heineken for the first 100% Patagonian beer (Burini et al. 2021). Another native yeast - S*accharomyces uvarum* - is used to produce wine and cider (Masneuf-Pomarède et al. 2010; Flores et al. 2019). Similarly, yeasts belonging to the *Hanseniaspora* and *Lachancea* genera contribute distinctive aroma and flavour compounds in wine, cider and beer (Moreira et al. 2008; Benito et al. 2016; Domizio et al. 2016; Morata et al. 2018; Canonico et al. 2019).

The aim of this study was to evaluate the potential of non-conventional yeasts isolated from Patagonia (Argentina) to produce whiskies (as low wines) with novel flavour profiles and regional distinction. Fermentations were peformed with *S. eubayanus, S. uvarum, Hanseniaspora* spp., and *Lachancea* spp., both individually and in combination with a commercial strains of *S. cerevisiae*. The washes were distilled and the 'low wines' (20-23% alcohol by volume/ABV) evaluated.

Materials and methods

Yeasts

Eight yeasts isolated from the natural environment of Argentine Patagonia (Culture Collection of IPATEC, Río Negro, Argentina) were assessed: *Saccharomyces uvarum* CRUB 209 (SU 209), *Saccharomyces uvarum* CRUB 222 (SU 222), *Saccharomyces eubayanus* CRUB 1568T (SE 1568), *Saccharomyces eubayanus* CRUB 1963 (SE 1963), *Lachancea nothofagi* CRUB 197 (LN 197), *Lachancea cidri* CRUB 568 (LC 568), *Hanseniaspora smithiae* CRUB 1583 (HS 1583) and *Hanseniaspora smithiae* CRUB 1602 (HS 1602). The *S. cerevisiae* var. *diastaticus*, POF^+, STA1 gene commercial strain Safspirit M1™ (Fermentis) was used as a control and with co-inoculation in the mixed fermentations.

Fermentation

Fermentations were performed in 1 L flasks containing 600 mL of all-malt distillery wort (Madoc - Single Malt Whisky, Patagonia, Argentina), with a density of 14.2°Bx (Alla France refractometer, 0-32 ± 0.2°Bx) and a pH of 5.70 (Sartorius PR15 pH meter). The wort was used according to distillery conditions, without sterilisation or oxygenation. Pre-cultures (inoculum) of each strain were propagated aerobically in malt extract medium (8°Bx, supplemented with 0.25% w/v yeast extract and 0.3% w/v casein peptone) at 20°C with orbital shaking (140 rpm). When the pre-cultures reached the exponential growth phase, the fermentations were inoculated at a cell density of 0.5 x 10^6 viable cells/mL/°Bx and fitted with airlocks containing sterile water. Cell number and viability were determined using alkaline methylene violet staining with a Neubauer chamber and microscope (Olympus CX22LED). Fermentation kinetics were monitored by weight loss every 24 h (AND EK 1200A ± 0.1 g) until reaching a constant weight. At the end of fermentation, the supernatant was obtained by centrifugation of the wash (5000 x g, 10 min, 4°C), filtered (0.22 µm) and stored at 4°C. Fermentations were in duplicate and with controlled temperature ramps to replicate the fermentation conditions at the distillery: 20°C for 19 h, 25°C for 4 h, 30°C for 48 h and 25°C until weight loss was complete.

In addition, the sugar consumption and sensory analysis of all yeasts was performed using an all-malt wort (control wort) produced from Pilsen malt (Robobrew All Grain Brewing System) using a small-scale congress mash protocol (Evans et al. 2011), sterilised by autoclaving (121°C for 15 minutes). On cooling, zinc was added (0.3 mg/L as $ZnSO_4 . 7H_2O$) together with 15 mg/L oxygen (Hach - HQ30D). The initial density of the wort was 15°Bx with a pH of 5.5. Fermentation performance and sensory analysis of the different yeasts is reported in Supplementary Information (Figures S1-3).

Microbiological characterisation

Selective isolation of microorganisms present at the end of fermentation (wash) was performed using pour plates with selective culture media. Acetic acid bacteria and/or *Escherichia coli* were evaluated in Wallerstein's differential medium (WLD), non-*Saccharomyces* yeasts in Lin's Cupric Sulphate Medium (LCSM), and *Lactobacillus* and *Pediococcus* species in Hsu's LP medium. Plates were examined using a stereoscopic microscope to quantify the colony forming units (CFU/mL).

Mixed fermentations with native yeasts and a commercial yeast

S. eubayanus CRUB 1963, *S. uvarum* CRUB 209 and commercial strain Safspirit M1™, were used in co-cultures in sequential inoculation. Fermentations were performed in duplicate in an all-malt distillery wort with a density of 15°Bx, and without oxygenation or sterilisation. Flasks containing 1.5 L of wort were inoculated with pre-cultures from each native yeast (as previously described) at a cell density of 0.5 x 10^6 viable cells/mL/°Bx, and incubated at 20°C for 23 h, followed by 25°C for 25 h. After 48 h, the M1 strain was inoculated in each flask at the same cell density (mixed fermentations SE 1963 + M1 and SU 209 + M1), and incubated at 30°C for another 48 h (fermentations were stopped at 96 h in accordance with production times). Fermentation was monitored daily by density measurements with a refractometer. The control fermentations involved the commercial M1 yeast which was inoculated on both occassions (M1 + M1).

The washes obtained from the mixed fermentations were individually distilled in a 3.5 L copper artisanal alembic still obtaining a low wine between 20-23% ABV. The first 50 mL fraction - the 'head' of the distillation, containing elevated levels of methanol - was discarded, and the next 600 mL - the 'low wines' - collected for analysis.

Analysis

Sugar consumption (glucose, maltose and maltotriose) and ethanol production were analysed by HPLC-Waters 600E (Nguyen et al. 2008), using a Rezex™ ROA-Organic Acid H+ (8%) column (300 × 7.8 mm) heated at 60°C and equilibrated with 0.005N H_2SO_4. Elution was performed isocratically (100% 0.005N H_2SO_4) at a flow rate of 0.6 mL/min. Identification and quantification were carried out with calibration curves of the standards D-glucose 99.8% (Merck), maltose 98% (Sigma), maltotriose 95% (Sigma) and absolute ethanol 99% (Sintorgan). Ethanol content was also measured with an Alcohol and Extract Meter (Alex 500, Anton Paar).

Phenolic compounds 4-vinylguaiacol (4-VG), 4-ethylguaiacol (4-EG), 4-vinylphenol (4-VP) and 4-ethylphenol (4-EP) were quantified according to Saez et al (2011) using a Phenomenex Luna™ 5 µm C18(2) 100 Å column (250 × 4.6 mm), a gradient elution of acetonitrile and water with formic acid (0.12 g/L, pH 3.5) and a flow rate of 0.9 mL/min. Spectra were acquired at wavelengths beween 220–700 nm. The standards, 4-vinylphenol (10.1% w/w in propylene glycol; Sigma), 4-ethylphenol (99% w/w; Sigma), 4-vinylguaiacol (99.7% w/w; Sigma) and 4-ethylguaiacol (98.1% w/v; Sigma) were resuspended in a beer matrix, and used for identification and quantification (Burini et al. 2022). The measurement of methanol and ethyl esters (hexanoate, lactate, octanoate, decanoate, butyrate, dodecanoate, isobutyrate and acetate) were determined by quantitative analysis using gas chromatographic flame ionisation (GC-FID) detection according to the AOAC, 16th edition, method 972.11; 26.1.36 and outsourced to CLONAR (Mar del Plata, Argentina).

Sensory analysis

A descriptive sensory analysis of washes and low wines was performed by a trained sensory panel at IPATEC. The eight panellists (five women and three men, ages 25-45) were trained in descriptive analysis, terminology and sensory descriptors (ASBC, Sensory Analysis-4; ASBC, Sensory Analysis-10). The sensory attributes for aroma were: malty, estery, phenolic and sulphury, and for flavour were: malty, estery, bitter, sweet, sour acidic, alcoholic and phenolic (ASBC-Sensory Analysis–12; Wanikawa 2020). Each attribute was scored on a six-point intensity scale, where '0' = 'not present' and '5' = 'very strong', with the average value determined. The washes (4.9-8.9% ABV) and the low wines 20-23% ABV) were assessed without dilution. Samples were attemperated to 12°C for full perception of aroma and flavour (ASBC, Sensory Analysis–10); served in transparent 40 mL polycarbonate plastic cups, covered with aluminium foil and coded with random three-digit numbers. Panellists received detailed information regarding the products to be assessed, their role in the study, potential risks (e.g., allergenic ingredients), data usage, and confidentiality. Only those who provided informed consent, with the freedom to withdraw at any time, participated in the evaluation. Individuals under 21 years old, pregnant women, or those with conditions that preclude alcohol consumption were not included in the study (IFST, 2020).

Statistical analysis

Statistical analyses were performed using non-parametric Kruskal-Wallis test (Shapiro-Wilk test, p-value < 0.05; dplyr and car packages, R Core Team, 2022). For sensory analyses, a two-way ANOVA was applied, verifying normality and homoscedasticity (SigmaPlot 11).

Results

Performance of native yeasts in fermentations of distillery wort

Fermentation kinetics, measured as carbon dioxide (CO_2) production over time, for the eight native yeasts and the commercial strain Safspirit M1™ in distillery wort are shown in Figure 1.

All strains reached the stationary phase in approximately 48 hours. However, the Patagonian yeasts exhibited secondary fermentative activity, at around 56 hours. This was not observed in experiments conducted in the controls with sterile all-malt wort (Figure S1).

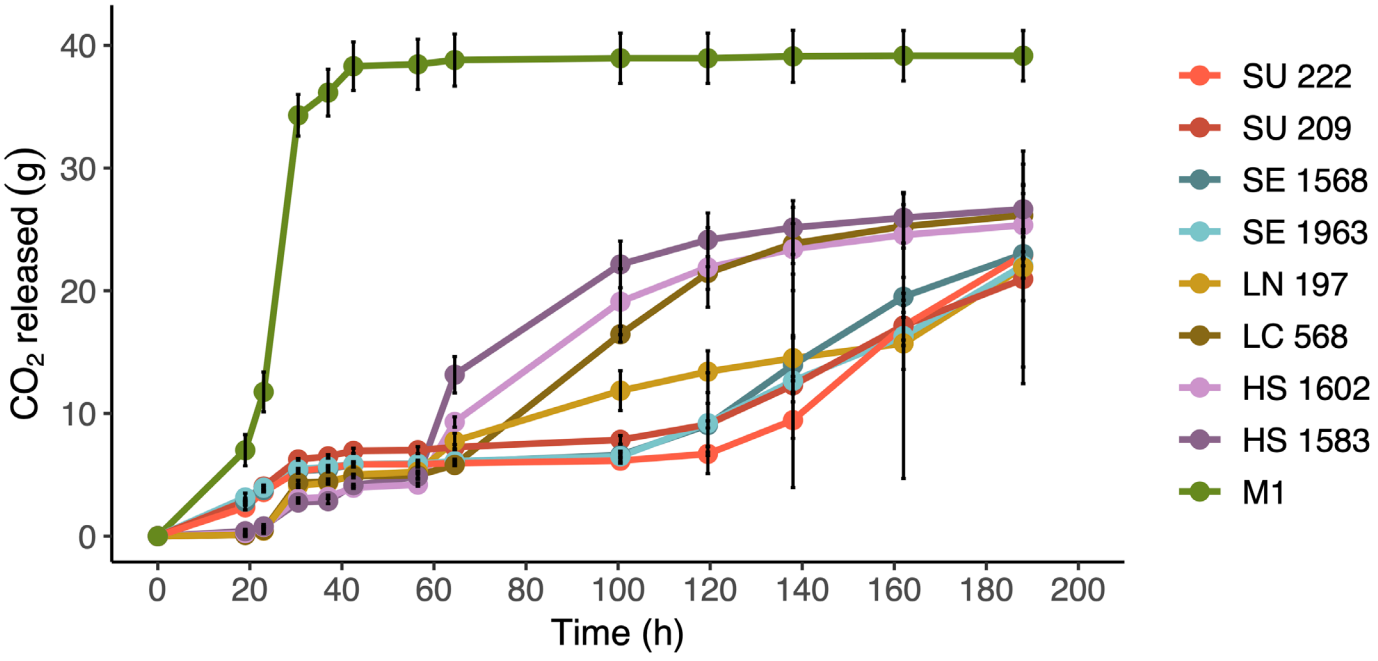
The sugar profile of the distillery wort consisted of 122 g/L maltose, 27 g/L maltotriose, and 29 g/L glucose (reported as percentages in Figure 2). The commercial strain Safspirit M1™ exhibited the highest sugar consumption in the wort (Figure 2), utilising all of the glucose, 98.1 ± 0.02% of the maltose, and 89.4 ± 0.02% of the maltotriose. Dextrins declined by 68% (cf the unfermented wort) and ethanol production (8.9 ± 0.5% ABV, Figure 2) was greatest with this commercial yeast. For the wild yeasts, sugar consumption was in all cases lower than with Safspirit M1™ (Figure 2). Glucose consumption varied between 94.2 - 95.7%, maltose consumption was higher in the fermentations with *H. smithiae* CRUB 1583 (87 ± 4.9%) and maltotriose consumption was higher for *S. eubayanus* CRUB 1568T (40.1 ± 2.9%). The decline in dextrins ranged between 23 - 68%, and ethanol formation ranged from 4.9 - 7.4% ABV, with the lowest for *L. nothofagi* CRUB 197 and the highest for *H. smithiae* CRUB 1583 (Figure 2). In the control fermentations with sterile wort (Figure S1), the commercial strain Safspirit M1™ exhibited the greatest consumption of sugars and highest ethanol production compared to the native yeasts. It is noteworthy that in this control wort medium, all yeasts exhibited lower attenuation and ethanol production compared to fermentations in distillery wort. Further, in the control wort none of the native yeasts consumed maltotriose (Figure S2, SI).

Microbiological contamination of the fermentations

Contaminant microorganisms were found in all fermentations with distillery wort, with growth on WLD and HLP media, suggesting the presence of aerobic and anaerobic bacteria, (presumably *Pediococcus* and *Lactobacillus* species). In all cases, WLD medium showed counts exceeding 500 CFU/mL. In contrast, all samples tested negative for the presence of non-*Saccharomyces* yeasts in Lin's Cupric Sulphate Medium (LCSM).

Figure 1.

Fermentation kinetics at laboratory scale in distillery wort of the native yeasts and control strain M1, as carbon dioxide production (g) over time (h). SU 209: *S. uvarum* CRUB 209, SU 222: *S. uvarum* CRUB 222, SE 1568: *S. eubayanus* CRUB 1568T, SE 1963: *S. eubayanus* CRUB 1963, LN 197: *Lachancea nothofagi* CRUB 197, LC 568: *Lachancea cidri* CRUB 568, HS 1602: *Hanseniaspora smithiae* CRUB 1602 and HS 1583: *Hanseniaspora smithiae* CRUB 1583, M1: Safspirit M1™.



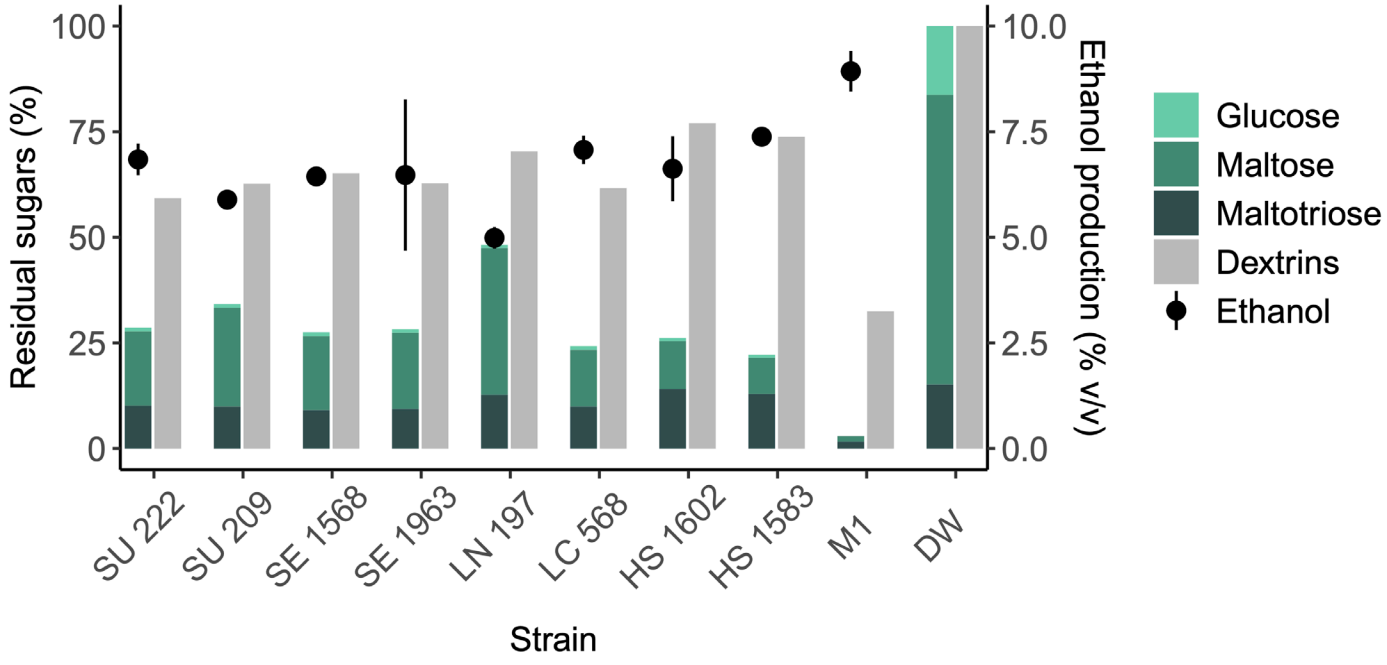
Sensory profile of native yeasts fermentation of distillery wort

The descriptive sensory analysis conducted by the sensory panel is summarised in radar charts (Figure 3). The flavour, 'sour acidic' was the descriptor with the greatest intensity for all washes. Significant differences were detected between the commercial M1 strain (with lower acidic intensity) and the yeasts *S. eubayanus* and *S. uvarum* ($p < 0.05$); this was consistent with the final pH values of 3.7 for the commercial M1 strain and 3.2 for the *Saccharomyces* native yeasts. The washes obtained with the M1 yeast were significantly more alcoholic ($p < 0.05$) than those produced with the wild yeasts except for *S. eubayanus* and *H. smithiae* which were not significant. The reference M1 strain was also more bitter than native yeasts ($p < 0.05$), with the exception of *S. eubayanus* and *L. cidri*. For flavour, ester levels ranged from 1.1 to 1.4 in the washes and were similar for all yeasts (with no significant differences). While phenols varied between 0.4-1.4, differences ($p < 0.05$) were only observed in intensity between *S. uvarum* and three yeasts (*L. nothofagi*, *L. cidri*, and *H. smithiae* CRUB 1602).

For the different native yeasts, the aroma profile from fermentations with *S. uvarum* and *S. eubayanus* had the highest intensity of phenols (2.3-3.0), with a significant difference ($p < 0.05$) to *Lachancea* and *Hanseniaspora* (range of 0.6-1.2). *S. eubayanus* presented the highest levels of esters (2.9 ± 0.9), although no significant differences were found between the yeasts. For the control strain M1, phenols were at an intermediate level (intensity of 2.1), showing significant differences with *L. cidri* 568 and *H. smithiae* 1602 ($p < 0.05$). It is noteworthy that the panel also perceived phenols from this commercial strain in fermentations with the control sterile wort (Supplementary Information, Figure S3), . Overall, with *S. uvarum,* phenols were predominant, esters and phenolic aromas were in balance with *S. eubayanus*, with esters predominate with the *Hanseniaspora* strains and esters and sulphury aromas predominate with *Lachancea* yeast.

Figure 2.

Residual sugars (% relative to the unfermented wort), residual dextrins (% relative to the unfermented wort) and ethanol produced (% v/v) in laboratory scale fermentations in distillery wort (DW) for the native yeasts and the commercial strain M1. In the DW bars, the total dextrins in the unfermented wort are represented in grey as 100%, and in different shades of green the percentages of glucose, maltose and maltotriose that represent 100% of the fermentable sugars in the wort are shown.



Mixed fermentations with native yeasts and Safspirit M1™ in distillery wort

S. eubayanus CRUB 1963 and *S. uvarum* CRUB 209 were selected for co-inoculation with Safspirit M1™ strain in mixed fermentations. Their fermentation kinetics are illustrated in Figure 4. These yeasts were chosen for their acceptable sugar consumption, short fermentation times, and for their distinctive aroma and flavour profiles. In mixed fermentations, the native yeasts reached stationary phase in 48 hours, but fermentative activity increased after inoculation of the M1 strain (marked with a vertical dashed line, Figure 4). In the case of the control mixed fermentation (M1 + M1), the curve was continuous reaching stationary phase at 96 hours.

Sugar consumption in the mixed fermentations

At the endpoint (96 hours), fermentations with the native mixed yeasts had a higher residual sugar content and lower ethanol production compared to the control (Figure 5). The control M1 yeast completely consumed glucose, 97.0 ± 0.2% of maltose and 84.0 ± 1.7% of maltotriose. Whereas the mixed fermentations had a similar sugar consumption which was lower than for the control

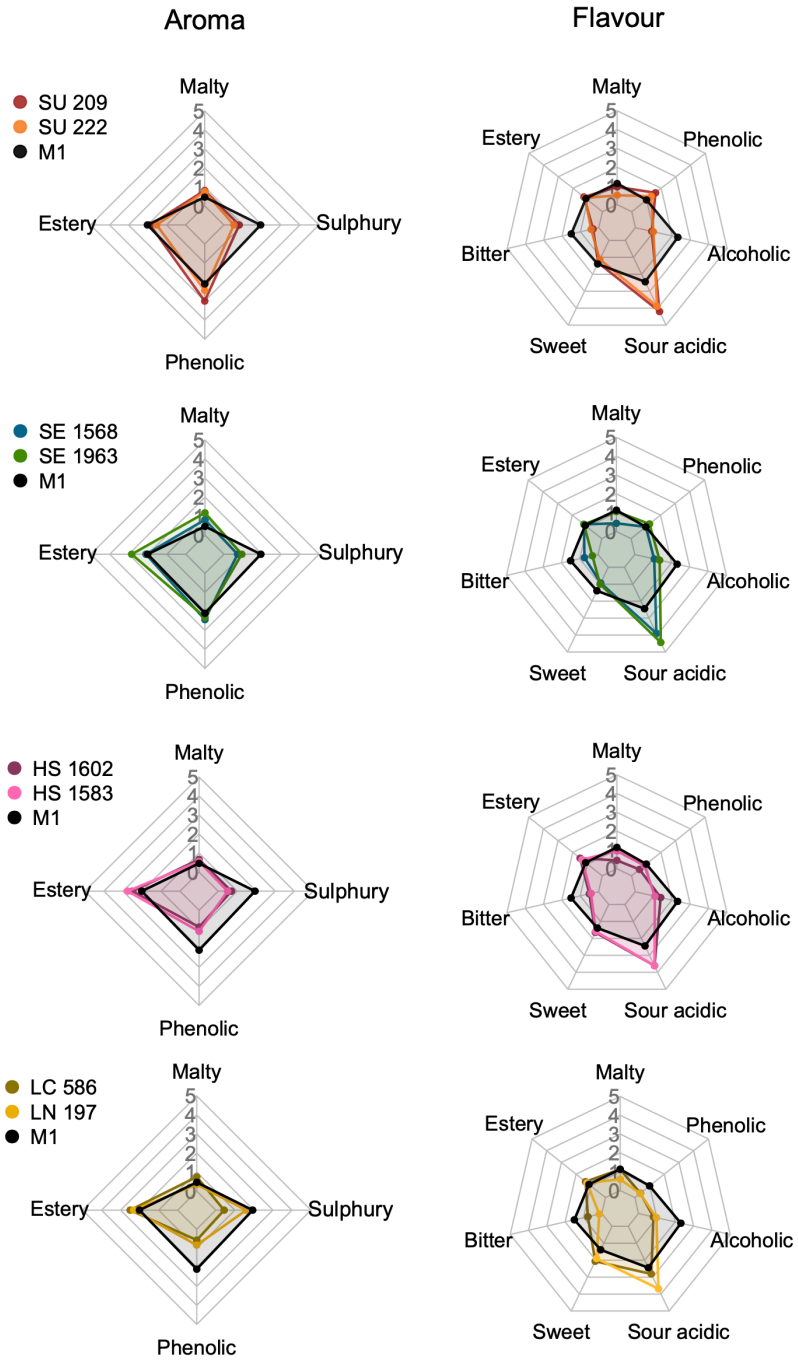


Figure 3.

Aroma (left) and flavour (right) sensory profile of laboratory scale washes by the native yeasts (separated by genus). The M1 control strain is represented in all the radar charts in black.

fermentation utilising 87.3-100% glucose, 96.1-96.5% maltose and 45.0-46.4% maltotriose. The relative level of dextrin declined to between 55.4-62.2%. (Figure 5). Ethanol production was 6.8 ± 0.2% ABV for *S. uvarum* CRUB 209 + M1, 6.6 ± 0.2% ABV for *S. eubayanus* CRUB 1963 + M1 compared to 8.6 ± 0.9% ABV for the control (M1). Accordingly, the ethanol yield was 78.8% for the mixed fermentation with *S. uvarum*, 76.1% for the mixed fermentation with *S. eubayanus*, compared to 100% with the control fermentation.

Sensory evaluation of the mixed washes

The sensory profile of the mixed fermentation washes as radar charts is shown in Figure 6. In the mixed washes with both native yeasts, the predominant flavour was sour acidic (4.0-4.2), which were higher than the control M1 + M1 wash (1.8 ± 1.3; $p < 0.05$). With aroma, fruity esters (described as apple and banana) predominated (2.9 ± 1.1 and 2.7 ± 1.3, respectively), with *S. uvarum* 209 + M1 and the control strain (M1 + M1). But no significant

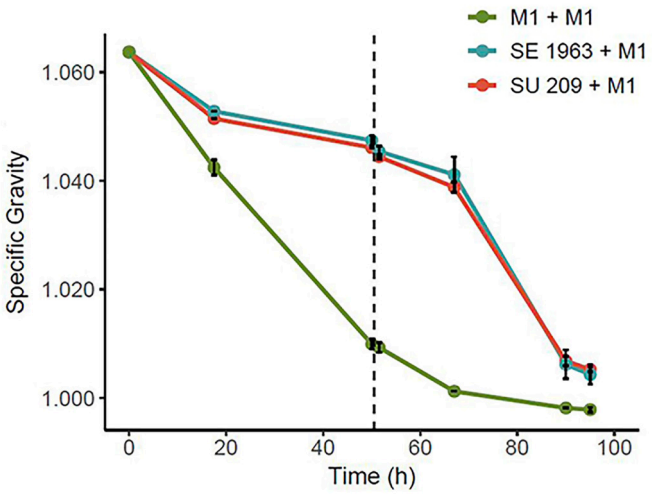


Figure 4.

Fermentation kinetics at laboratory scale in distillery wort of the mixed fermentations (washes), as specific gravity (g/mL) against time (h). The point of innoculation of strain M1 is indicated by the vertical dashed line. SE 1963 + M1 (*S. eubayanus* CRUB 1963 + M1 control strain); SU 209 + M1 (*S. uvarum* CRUB 209 + M1 control strain). M1 + M1 (control fermentation inoculated on both occasions with the commercial M1 strain).

Figure 5.

Residual sugars (% relative to the unfermented wort), residual dextrins (% relative to the unfermented wort) and ethanol produced (% v/v) in laboratory scale fermentations of distillery wort (DW) for the mixed washes. In the DW bars, the total dextrins in the unfermented wort are represented in grey as 100%, and in different shades of green the percentages of glucose, maltose and maltotriose that represent 100% of the fermentable sugars in the wort are shown.

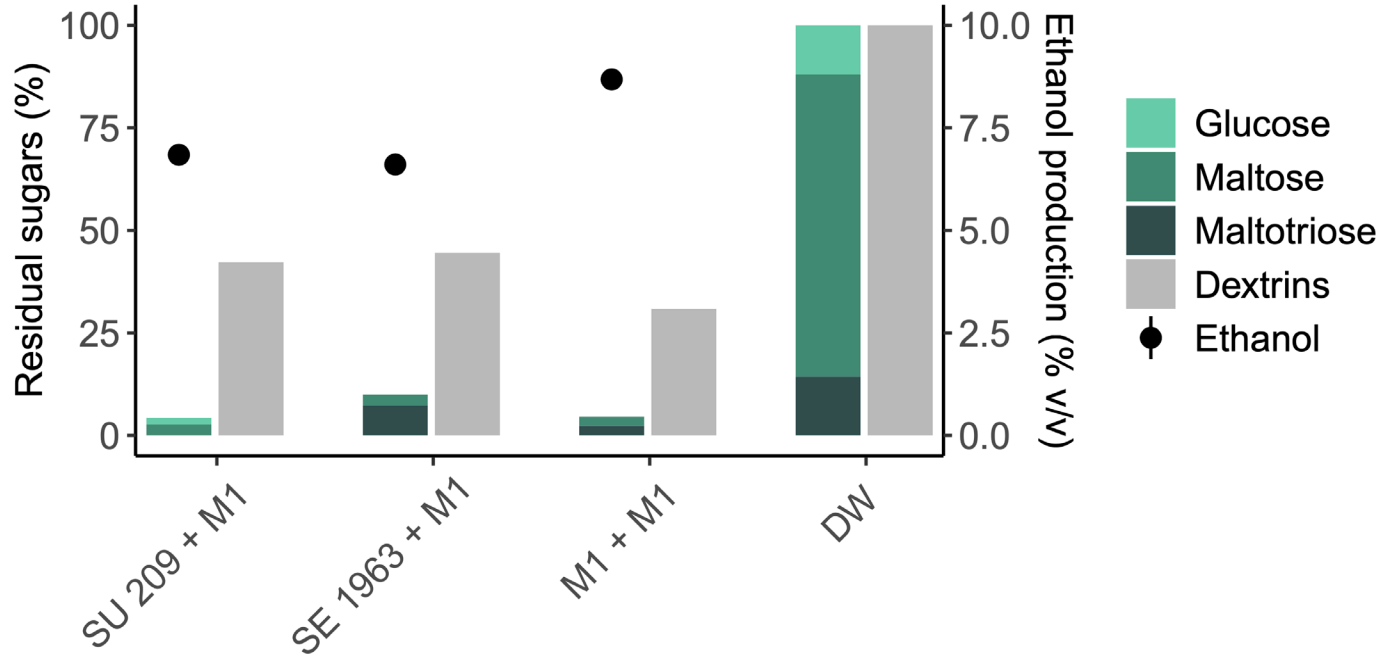


Table 1.

Volatile phenol and ester concentrations (mg/L) in distilled low wines from mixed (SU + M1 and SE + M1) and control (M1 + M1) washes. Compounds with concentrations above their respective sensory thresholds (indicated in the second column, mg/L) are in bold.

[a] 4-vinylphenol (odour threshold for beer, Wackerbauer et al. 1982), 4-vinylguaiacol (Jalo 2022), 4-ethylphenol (Jalo 2022), 4-ethylguaiacol (Poisson and Schieberle 2008), ethyl acetate (Salo et al. 1972), ethyl isobutyrate (Gao et al. 2014), ethyl butyrate (Poisson and Schieberle 2008) and - from Waymark and Hill (2021) - ethyl dodecanoate, ethyl decanoate, ethyl octanoate, ethyl lactate and ethyl hexanoate.

Volatile Compound	Threshold[a]	SU + M1	SE + M1	M1 + M1
4-vinylphenol	0.2	**0.3**	**0.7**	**1.9**
4-vinylguaiacol	1.4	**8.2**	**8.2**	**10.0**
4-ethylphenol	2.7	1.6	1.7	0
4-ethylguaiacol	0.007	**0.6**	**0.3**	0
Ethyl acetate	17	**89**	**115**	**95**
Ethyl isobutyrate	0.06	0	**0.1**	**0.2**
Ethyl butyrate	0.01	**0.9**	**0.6**	**1.1**
Ethyl dodecanoate	0.6	**0.8**	**0.9**	**0.9**
Ethyl decanoate	1.1	**1.5**	**1.5**	**2**
Ethyl octanoate	0.2	**2.6**	**2.5**	**2.4**
Ethyl lactate	14	9.5	12.2	9
Ethyl hexanoate	0.08	**10.2**	**8.2**	**9.9**

differences were observed with *S. eubayanus* + M1 (1.1 ± 1.2). A higher intensity of phenols ($p < 0.05$) was found in *S. eubayanus* 1963 + M1 (0.9 ± 0.2) compared to *S. uvarum* + M1, where no phenols were perceived. With *S. eubayanus* + M1, the profile of phenols, esters and sulphides exhibited similar intensities.

Analysis of low wines from mixed washes with native yeasts and Safspirit M1™

Levels of volatile phenols (4-vinylguaiacol, 4-vinylphenol, 4-ethylguaiacol and 4-ethylphenol) and esters (ethyl hexanoate: ethyl lactate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl butyrate, ethyl isobutyrate, ethyl acetate) in the distilled low wines together with their perception threshold in whisky are reported in Table 1.

The type and concentration of phenols (mg/L) in the low wines from both mixed washes with the wild yeasts was similar, but distinct to that from the M1 control wash. In the low wines of *S. uvarum* CRUB 209 + M1 and *S. eubayanus* CRUB 1963 + M1, the four phenols were detected, although 4-vinylphenol (4-VP) and 4-ethylphenol (4-EP) were at levels below their threshold. The clove like 4-vinylguaiacol (4-VG) predominated, exceeding the perception threshold more than fivefold; for 4-ethylguaiacol (4-EG) (spicy, smoky, clove-like) the levels exceeded the perception threshold but to a lesser degree, with a higher value for *S. uvarum* CRUB 209 + M1. For the M1 control strain, 4-VG was the predominant phenol (seven fold greater than the threshold); but - unlike the mixed washes with native yeasts - 4-VP (medicinal, band aid, smoky) exceeded the threshold, whereas 4-EG and 4-EP were not detected.

The profile of esters was similar for the low wines from the mixed fermentations of the three yeasts. Values exceeded the perception threshold for ethyl acetate (solvent, rose-like), ethyl butyrate (pineapple-like, berry), ethyl octanoate (sour apple, fruity, winey, waxy) and ethyl hexanoate (apple-like, aniseed, pineapple) and, to a lesser extent, (but also above threshold) of ethyl dodecanoate (floral, soapy, fruity) and ethyl decanoate (apple, fruity, sweet). Ethyl isobutyrate (fruity, butterscotch) was not detected in the distillates of *S.uvarum* but was prominent with the commercial M1 yeast.

Sensory evaluation of mixed low wines

Figure 7 shows the sensory evaluation of the aroma and flavour of distilled low wines from the co-culture fermentations and that with the control M1 strain. All distillates were similar - with a

predominant alcoholic character, a malty aroma and flavour and absence of sulphury notes. The low wines from the native yeasts were described as having a more ester-rich profile, a sweeter flavour, and a lower perception of phenolic notes compared to the control distillate from the commercial yeast.

Figure 6.

Aroma (left) and flavour (right) sensory profile in washes from mixed (*S. uvarum* 209 + M1 and *S. eubayanus* 1963 + M1) and control (M1 + M1) laboratory scale fermentations of distillery wort.

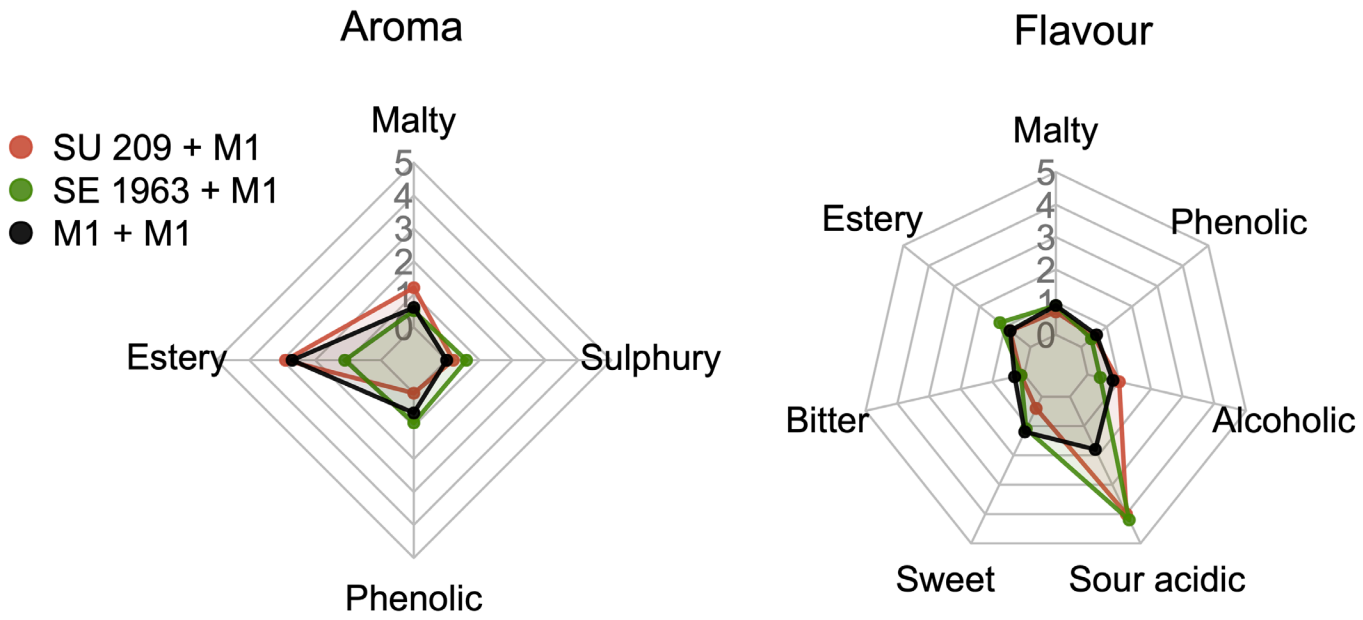
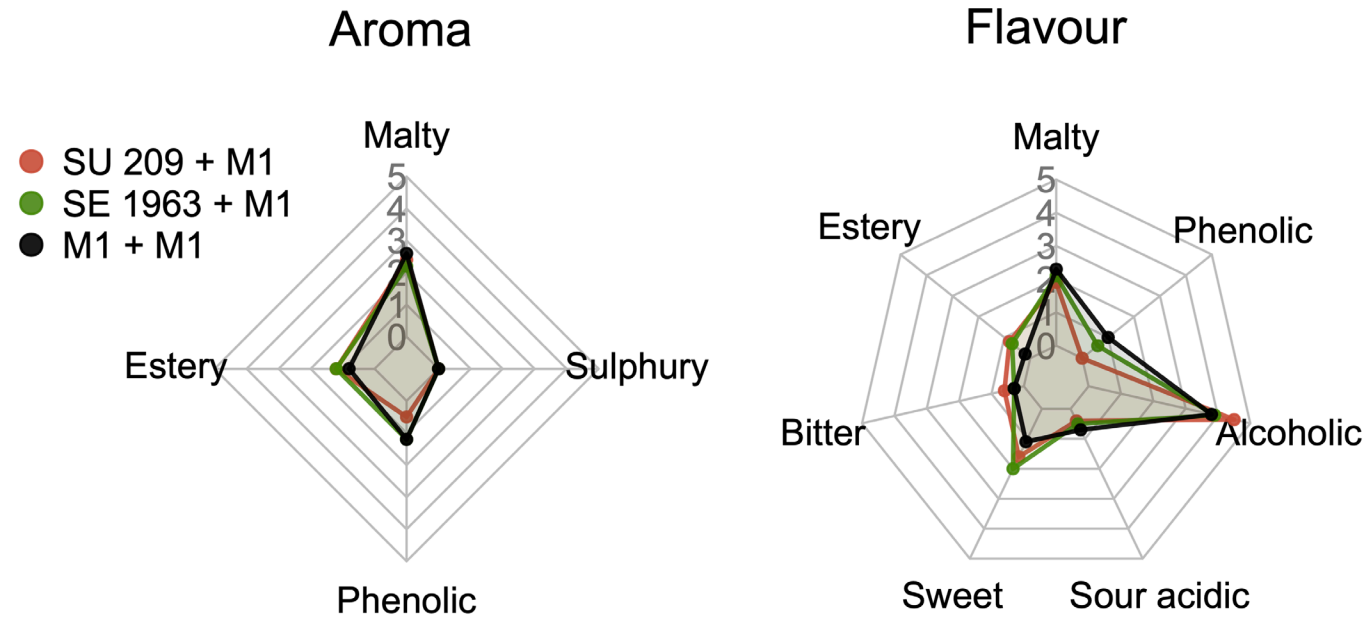


Figure 7.

Aroma (left) and flavour (right) sensory profile in washes from mixed (*S. uvarum* 209 + M1 and *S. eubayanus* 1963 + M1) and control (M1 + M1) laboratory scale fermentations of distillery wort.



Discussion

In this work, we show that fermentation with novel 'wild' yeasts provides an opportunity in the flavour development of whisky. While fermentation is typically performed by a limited number of *S. cerevisiae* strains, wild yeast can provide a viable strategy for new yeasts for whisky production. Here, for the first time, the fermentation performance and organoleptic profile for different yeasts isolated from Patagonia (Argentina) was assessed. These yeasts - *Saccharomyces uvarum, Saccharomyces eubayanus, Lachancea nothofagi, Lachancea cidri and Hanseniaspora smithiae* – offer the potential to produce regionally distinctive whiskies. The fermentative and organoleptic character of washes from distillery and sterile, all-malt wort revealed differences between the native yeasts and the commercial strain (M1) in terms of ethanol yield, together with aroma and flavour profiles. In agreement with (Daute 2022), the commercial M1 yeast exhibited superior fermentative performance, reflecting adaptation to ensure rapid and complete fermentation of malt wort sugars with high levels of ethanol production (Walker et al. 2019). The domestication of yeasts under these conditions has led to efficient maltose utilisation and the evolution of specific transporters that facilitate maltotriose assimilation (Gallone et al. 2016).

In contrast, non-conventional wild yeasts have evolved in natural environments distinct from anthropogenic fermentation conditions. *Lachancea* spp. and *Saccharomyces eubayanus* were isolated from the bark of *Nothofagus* (southern beech) trees, while *Hanseniaspora smithiae* and *Saccharomyces uvarum* yeasts were from the stromata of *Cyttaria hariotii* and *Cyttaria johowii*, fungal parasites of *Nothofagus* trees. These substrates lack the sugars maltose and maltotriose, explaining the reduced fermentation performance (attenuation and ethanol yield) of the native yeasts and reflecting their limited exposure/adaptation to the major sugars in malt wort. Accordingly, the use of these wild yeasts as the sole microorganisms in the fermentation of barley/malt wort, presents limitations to production of beverages that require high fermentation efficiency and alcohol yield. However, the aromatic contributions of these yeasts may add value in fermentations in tandem with other microorganisms.

A key difference in the production of whisky and beer is that in whisky the malt wort is not boiled, enabling limit dextrinase to degrade dextrins to fermentable sugars (Walker et al. 2012) with contaminating microorganisms able to proliferate in fermenter. Microbiological analysis with WLD and HLP media showed the presence of aerobic and anaerobic lactic acid bacteria in the distillery wort, which are found in late-stage whisky fermentations (van Beek and Priest 2002; Reid et al. 2020). The presence of bacteria influenced fermentation kinetics and ethanol yield in the washes obtained with the native yeasts, evidenced by the emergence of a secondary fermentation phase after 56 hours, which was not observed in the sterile control wort (Supplementary information Figure S1). This is likely a result of bacteria competing for the increased sugar availability due to the lower fermentation capacity of the wild yeasts. A similar secondary fermentation pattern and reduced ethanol yield have been reported for other non-conventional yeasts, which was attributed to the growth of contaminating microorganisms (Daute 2021). In contrast, no secondary fermentation was observed in fermentations with the commercial M1 yeast, which would out-compete contaminating bacteria and limit their growth by rapidly utilising sugars and nutrients and/or producing inhibitory levels of ethanol (Narendranath et al. 1997). Furthermore, the higher ethanol yield observed in distillery wort fermentations compared to sterile control wort fermentations is likely a result of enzymic (limit dextrinase) and microbial degradation of dextrins and complex sugars, increasing the availability of simple sugars for yeast metabolism (Narendranath et al. 1997; Park et al. 2014; Daute 2021). The increased activity of lactic acid bacteria (LAB) in native yeast fermentations also affected sensory characteristics, resulting in higher perceived acidity. The final pH for these fermentations was 3.2-3.7 below that of whisky (4.0–4.5) (Aylott 2014; Walker et al. 2019), indicating that the fermentation parameters promoted LAB growth beyond those normally observed in whisky fermentation, which is consistent with the long fermentation periods and the proliferation of these bacteria.

The sensory evaluation of all washes exhibited a phenolic character in both aroma and flavour, suggesting the yeasts have a phenolic off-flavour (POF^+) phenotype. The production of volatile

phenols is linked to the presence of PAD1, FDC1, and/or VPR genes (Vanbeneden et al. 2008), which remain functional in wild (non-domesticated) yeasts (Gallone et al. 2016). Previous studies have reported volatile phenol production in all-malt wort for *S. eubayanus* and noted intraspecific variability (Urbina et al. 2020; Burini et al. 2022). Similarly studies with *Lachancea* and *Hanseniaspora* species in brewery wort have highlighted variability in volatile phenol production (Methner et al. 2019; Toh et al. 2020; Rodríguez Madrera et al. 2021; Postigo et al. 2023; Aguiar-Cervera et al. 2024). Whilst there are no reports of *S. uvarum* fermentations in all-malt wort, studies with cider fermentations have detected volatile phenol production at levels below the sensory threshold (Flores et al. 2019). Phenols were also detected for the commercial M1 strain. The SafSpirit™ M-1 strain is a hybrid between *S. cerevisiae* and *S. cerevisiae* var. *diastaticus*, which would explain the POF^+ phenotype (Krogerus and Gibson 2020). In addition, there are reports that bacteria of the *Lactobacillus* and *Pediococcus* genus exhibit POF^+ phenotype (van Beek and Priest, 2000), suggesting that they could also contribute to the phenolic profile.

The production of fruity esters by *Lachancea* and *Hanseniaspora* yeasts were found in barley worts (Canonico et al. 2019; Bellut et al. 2020; Rodríguez Madrera et al. 2021; Aguiar-Cervera et al. 2024). Similarly, with *S. eubayanus*, there are reports of moderate concentrations of isoamyl acetate (banana and pear aroma) and ethyl acetate (fruity aroma at low concentrations and solvent-like aroma at high concentrations) (Krogerus et al. 2015; Mertens et al. 2015; Burini et al. 2022; Kelly et al. 2023). The bacterial microflora in whisky wort also impacts on the sensory profile by adding acidity and fruity aromas (Reid et al. 2020; Wanikawa 2020; Daute 2022). The increase in lactic and acetic acids in the fermenting wort promotes fruity and floral notes through the production of ethyl lactate and ethyl acetate (van Beek and Priest 2000), which are detected in distillates (Reid et al. 2020).

These results suggest that the dynamics of sugar consumption by the native yeasts with their lower fermentative efficiency, together with their complex interactions with other microorganisms, could be complemented by a second fermentation using commercial strains used in whisky fermentation.

Moreover, wild yeasts provide different profiles of aroma and flavour compounds to that of the commercial strain. Notably, *S. eubayanus* and *S. uvarum* contribute to the phenolic and fruity profiles and accordingly were selected for mixed fermentations.

To evaluate the feasibility of producing whiskies with novel sensory profiles using native yeasts while maintaining ethanol yield, sequential mixed fermentations were conducted with the selected yeasts in co-cultures with the M1 strain. The combination of non-*Saccharomyces* yeasts with a commercial *Saccharomyces* yeast to enhance ethanol yield and flavour complexity is used in brewing, winemaking and distilled beverages (Duarte et al. 2013; Nuñez-Guerrero et al. 2016). In this study, native yeasts were initially inoculated alone due to their lower fermentation kinetics, allowing them to dominate the early fermentation phase, the period of highest aroma and flavour metabolite production (White and Zainasheff 2010). The commercial strain was then introduced to ensure sugar consumption and increase ethanol yield. Although this strategy facilitated full sugar consumption in the wort and yielded higher ethanol levels compared to wild yeasts alone, it did not achieve the level of ethanol found with the M1 strain alone. The lower ethanol yield observed in mixed washes, relative to the (M1 + M1) control, could be attributed to the inefficient conversion of sugars to ethanol by wild yeasts, nutrient depletion caused by the initial inoculated native yeasts which may inhibit M1 growth (Fleet 1998; Taillandier et al. 2014), and/or potential antagonistic interactions between yeasts (Fleet 2003; Albergaria et al. 2010; Branco et al. 2014). However, the resulting fermentation exhibited greater aromatic and flavour complexity, underscoring the role of the initial fermentation with wild yeasts in enhancing the depth and richness of the final product.

For the three distilled low wines, all esters - except ethyl lactate - exceeded their sensory perception thresholds. Esters are among the most significant congeners and have been reported in both low wines and whiskies (Kelly et al. 2023; Poisson and Schieberle 2008; Waymark and Hill 2021; Daute 2022). While ester concentrations were comparable between the mixed and control fermentations, sensory analysis revealed that low wines from

native yeasts were perceived as sweeter and richer in esters. Although quantification of volatile phenol showed high concentrations exceeding perception thresholds, these compounds were not perceived with high intensity by the sensory panel. The higher ethanol ABV in low wines possibly influenced the sensory perception by modulating the thresholds of aroma compounds, resulting in varying intensity levels in the final distillate. Further, the composition of the whisky matrix also contributes to differences in perception of fruity ester (Ickes and Cadwallader 2017; Karlsson and Friedman 2017; Daute 2022; MacGarry 2023). While sensory analyses of malt whisky is usually performed on 'new-make spirit' (ca. 65% ABV after the second distillation), in this study we chose to evaluate the 'low wines' from the first distillate as an approximation of fermentation derived aromas. It is important to note, that low wines retain the metabolic signature of the yeast consortium prior to second distillation and maturation in cask, allowing a clearer attribution of observed differences in microbial activity. While some volatile compounds produced by the yeast may carry over into the final spirit, the medium chain ethyl esters (C6-C12) and the vinyl/ethyl phenols exhibit vapour–liquid partition coefficients that suggest they would be largely retained in the new-make spirit.

Finally, this study confirms that wild yeasts can be used alongside commercial yeasts but also shows that the resulting low wines display novel flavour profiles that could positively influence the development of innovative whiskies of the future.

Conclusions

This the first report of the fermentation dynamics of non-conventional yeasts isolated from natural Patagonian environments for whisky production. While native yeasts have lower efficiency in sugar consumption compared to the commercial M1 strain, mixed fermentations with the industrial strain - alongside interactions with the native microbial flora in the distillery wort - enhanced the organoleptic complexity of the distilled low wines. Moreover, they provide a distinctive contribution to the development of unique products, helping to expand existing markets and create new ones. Further, using autochthonous yeasts imparts a distinctive sensory signature in the distillate.

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Author contributions

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Julieta A. Burini: writing (original draft), methodology, investigation, visualisation, conceptualisation.
Clara Bruzone: writing (review and editing), investigation.
Diego Libkind: writing (review and editing), funding acquisition, conceptualisation.
Martín Moliné: writing (review and editing), methodology, conceptualisation, supervision.

Conflict of interest

The authors declare there are no conflicts of interest.

References

Aguiar-Cervera J, Visinoni F, Zhang P, Hollywood K, Vrhovsek U, Severn O, Delneri D. 2024. Effect of *Hanseniaspora vineae* and *Saccharomyces cerevisiae* co-fermentations on aroma compound production in beer. *Food Microbiol* 104585. https://doi.org/10.1016/j.fm.2024.104585

Albergaria H, Francisco D, Gori K, Arneborg N, Gírio F. 2010. *Saccharomyces cerevisiae* CCMI 885 secretes peptides that inhibit the growth of some non-*Saccharomyces* wine-related strains. *Appl Microbiol Biotechnol* 86:965-972. https://doi.org/10.1007/s00253-009-2409-6

American Society of Brewing Chemists. Methods of Analysis, 8th ed. Selection and training of assessors. The Society, St. Paul, MN, 1984, rev. 2009.

American Society of Brewing Chemists. Methods of Analysis, 8th ed. Descriptive analysis. The Society, St. Paul, MN, 1983, rev. 2009.

American Society of Brewing Chemists. Methods of Analysis, 8th ed. Flavor terminology and reference standards. The Society, St. Paul, MN, 1986.

Aylott R. 2014. Whisky analysis, p 123-146. *In* Russell I, Stewart GG (eds), *Whisky: Technology, Production and Marketing*, Elsevier, UK.

Bellut K, Krogerus K, Arendt EK. 2020. *Lachancea fermentati* strains isolated from kombucha: fundamental insights, and practical application in low alcohol beer brewing. *Front Microbiol* 11:764. https://doi.org/10.3389/fmicb.2020.00764

Benito Á, Calderón F, Benito S. 2016. Combined use of *S. pombe* and *L. thermotolerans* in winemaking. Beneficial effects determined through the study of wines' analytical characteristics. *Molecules* 21:1744. https://doi.org/10.3390/molecules21121744

Branco P, Francisco D, Chambon C, Hébraud M, Arneborg N, Almeida MG, Caldeira J, Albergaria H. 2014. Identification of novel GAPDH-derived antimicrobial peptides secreted by *Saccharomyces cerevisiae* and involved in wine microbial interactions. *Appl Microbiol Biotechnol* 98:843-853. https://doi.org/10.1007/s00253-013-5411-y

Burini JA, Eizaguirre JI, Loviso C, Libkind D. 2021. Non-conventional yeasts as tools for innovation and differentiation in brewing. *Rev Argent Microbiol* 53:359-377. https://doi.org/10.1016/j.ram.2021.01.003

Burini JA, Eizaguirre JI, Loviso C, Libkind D. 2022. Selection of *Saccharomyces eubayanus* strains from Patagonia (Argentina) with brewing potential and performance in the craft beer industry. *Eur Food Res Technol* 1-13. https://doi.org/10.1007/s00217-021-03897-6

Campbell I. 2003. Wild yeasts in brewing and distilling, p 247–266. *In* Priest FG, Campbell I (eds), *Brewing Microbiology*, Springer US, Boston, MA.

Canonico L, Galli E, Ciani E, Comitini F, Ciani M. 2019. Exploitation of three non-conventional yeast species in the brewing process. *Microorganisms* 7:11. https://doi.org/10.3390/microorganisms7010011

Cubillos FA, Gibson B, Grijalva-Vallejos N, Krogerus K, Nikulin J. 2019. Bioprospecting for brewers: Exploiting natural diversity for naturally diverse beers. *Yeast* 36:383–398. https://doi.org/10.1002/yea.3380

Daute M, Jack F, Harrison B, Walker G. 2021. Experimental whisky fermentations: Influence of wort pretreatments. *Foods* 10:2755. https://doi.org/10.3390/foods10112755

Daute M. 2022. Exploiting yeast diversity in whisky fermentations for biocatalysis of desirable flavour compounds. PhD thesis, Abertay University. https://rke.abertay.ac.uk/ws/portalfiles/portal/35753380/Daute_ExploitingYeastDiversity_PhD_Thesis_2022.pdf

Daute M, Jack F, Walker G. 2024. The potential for Scotch malt whisky flavour diversification by yeast. *FEMS Yeast Res* foae017. https://doi.org/10.1093/femsyr/foae017

Domizio P, Lencioni L, Ciani M, Di Blasi S, Pontremolesi C, Sabatelli MP. 2007. Spontaneous and inoculated yeast populations dynamics and their effect on organoleptic characters of Vinsanto wine under different process conditions. *Int J Food Microbiol* 115:281-289. https://doi.org/10.1016/j.ijfoodmicro.2006.10.052

Domizio P, House JF, Joseph CML, Bisson LF, Bamforth CW. 2016. *Lachancea thermotolerans* as an alternative yeast for the production of beer. *J Inst Brew* 122:599-604. https://doi.org/10.1002/jib.362

Duarte WF, Amorim JC, Schwan RF. 2013. The effects of co-culturing non-*Saccharomyces* yeasts with *S. cerevisiae* on the sugar cane spirit (cachaça) fermentation process. *Antonie Van Leeuwenhoek* 103:175–94. https://doi.org/10.1007/s10482-012-9798-8

Esteban-Decloux M, N'Guessan T, Grangeon H. 2023. Behaviour of volatile compounds during batch multi-stage distillation of whisky: experimental and simulation data. *J Inst Brew* 129:192–208. https://doi.org/10.58430/jib.v129i3.28

Evans DE, Goldsmith M, Dambergs R, Nischwitz R. 2011. A comprehensive revaluation of small-scale congress mash protocol parameters for determining extract and fermentability. *J Am Soc Brew Chem* 69:13–27. https://doi.org/10.1094/ASBCJ-2011-0111-01

Fleet GH. 1998. The microbiology of alcoholic beverages, p 217-262. *In* Wood BJB (ed), *Microbiology of Fermented Foods*, Springer, US.

Fleet GH. 2003. Yeast interactions and wine flavour. *Int J Food Microbiol* 86:11-22. https://doi.org/10.1016/S0168-1605(03)00245-9

Flores MG, Rodríguez ME, Origone AC, Oteiza JM, Querol A, Lopes CA. 2019. *Saccharomyces uvarum* isolated from Patagonian ciders shows excellent fermentative performance for low temperature cidermaking. *Food Res Int* 126:108656. https://doi.org/10.1016/j.foodres.2019.108656

Gallone B, Steensels J, Prahl T, Soriaga L, Saels V, Herrera-Malaver B, Merlevede A, Roncoroni M, Voordeckers K, Miraglia L, Teiling C, Steffy B, Taylor M, Schwartz A, Richardson T, White C, Baele G, Maere S, Verstrepen KJ. 2016. Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. *Cell* 166:1397-1410.e16. https://doi.org/10.1016/j.cell.2016.08.020

Gao W, Fan W, Xu Y. 2014. Characterization of the key odorants in light aroma type chinese liquor by gas chromatography-olfactometry, quantitative measurements, aroma recombination, and omission studies. *J Agric Food Chem* 62:5796-804. https://doi.org/10.1021/jf501214c

Gschaedler A. 2017. Contribution of non-conventional yeasts in alcoholic beverages. *Curr Opin Food Sci* 13:73-77. https://doi.org/10.1016/j.cofs.2017.02.004

Ickes CM, Cadwallader KR. 2017. Effects of ethanol on flavor perception in alcoholic beverages. *Chemosens Percept* 10:119-134. https://doi.org/10.1007/s12078-017-9238-2

IFST. 2020. IFST guidelines for ethical and professional practices for the sensory analysis of foods. Institute of Food Science and Technology.

Jalo V. 2022. Analysis of volatile aroma-active compounds from a headspace of a novel whiskey glass. Master thesis. University of Turku.

Karlsson BC, Friedman R. 2017. Dilution of whisky–the molecular perspective. *Sci Rep* 7:6489. https://doi.org/10.1038/s41598-017-06423-5

Kelly J, O'Connor C, Kilcawley KN. 2023. Sources of volatile aromatic congeners in whiskey. *Beverages* 9:64. https://doi.org/10.3390/beverages9030064

Krogerus K, Magalhães F, Vidgren V, Gibson B. 2015. New lager yeast strains generated by interspecific hybridization. *J Ind Microbiol Biotechnol* 42:769-778. https://doi.org/10.1007/s10295-015-1597-6

Krogerus K, Gibson B. 2020. A re-evaluation of diastatic *Saccharomyces cerevisiae* strains and their role in brewing. *Appl Microbiol Biotechnol* 104:3745-3756. https://doi.org/10.1007/s00253-020-10531-0

Libkind D, Hittinger CT, Valerio E, Goncalves C, Dover J, Johnston M, Goncalves P, Sampaio JP. 2011. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *PNAS* 108:14539–14544. https://doi.org/10.1073/pnas.1105430108

MacGarry S. 2023. The effects of ethanol on the flavour of whisky. PhD thesis. Edinburgh Napier University. https://doi.org/10.17869/enu.2023.3175295

Masneuf-Pomarède I, Bely M, Marullo P, Lonvaud-Funel A, Dubourdieu D. 2010. Reassessment of phenotypic traits for *Saccharomyces bayanus* var. *uvarum* wine yeast strains. *Int J Food Microbiol* 139:79-86. https://doi.org/10.1016/j.ijfoodmicro.2010.01.038

Mertens S, Steensels J, Saels V, De Rouck G, Aerts G, Verstrepen KJ. 2015. A large set of newly created interspecific *Saccharomyces* hybrids increases aromatic diversity in lager beers. *Appl Environ Microbiol* 81:8202-8214. https://doi.org/10.1128/AEM.02464-15

Methner Y, Hutzler M, Matoulková D, Jacob F, Michel M. 2019. Screening for the brewing ability of different non-*Saccharomyces* yeasts. *Fermentation* 5:101. https://doi.org/10.3390/fermentation5040101

Morata A, Loira I, Tesfaye W, Bañuelos MA, González C, Suárez Lepe JA. 2018. *Lachancea thermotolerans* applications in wine technology. *Fermentation* 4:53. https://doi.org/10.3390/fermentation4030053

Moreira N, Mendes F, Guedes de Pinho P, Hogg T, Vasconcelos I. 2008. Heavy sulphur compounds, higher alcohols and esters production profile of *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii* grown as pure and mixed cultures in grape must. *Int J Food Microbiol* 124:231–238. https://doi.org/10.1016/j.ijfoodmicro.2008.03.025

Mukai N, Masaki K, Fujii T, Kawamukai M, Iefuji H. 2010. PAD1 and FDC1 are essential for the decarboxylation of phenylacrylic acids in *Saccharomyces cerevisiae*. *J Biosci Bioeng* 109:564–569. https://doi.org/10.1016/j.jbiosc.2009.11.011

Narendranath NV, Hynes SH, Thomas KC, Ingledew WM. 1997. Effects of lactobacilli on yeast-catalyzed ethanol fermentations. *Appl Environ Microbiol* 63:4158-4163. https://doi.org/10.1128/AEM.63.11.4158-4163.1997

Nguyen N, McGinley M, Mott J. 2008. Faster real-time response to bacterial infection of bioethanol fermentation using a short Rezex™ ROA column. *Appl Noteb* 1:1–4.

Nuñez-Guerrero ME, Páez-Lerma JB, Rutiaga-Quiñones OM, González-Herrera SM, Soto-Cruz NO. 2016. Performance of mixtures of *Saccharomyces* and non-*Saccharomyces* native yeasts during alcoholic fermentation of Agave duranguensis juice. *Food Microbiol* 54:91-97. https://doi.org/10.1016/j.fm.2015.10.011

Olaniran AO, Hiralal L, Mokoena MP, Pillay B. 2017. Flavour-active volatile compounds in beer: production, regulation and control. *J Inst Brew* 123:13–23. https://doi.org/10.1002/jib.389

Padilla B, Gil JV, Manzanares P. 2016. Past and future of non-*Saccharomyces* yeasts: From spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front Microbiol* 7:411. https://doi.org/10.3389/fmicb.2016.00411

Park JY, Lee JY, Choi SH, Ko HM, Kim IC, Lee HB, Bai S. 2014. Construction of dextrin and isomaltose-assimilating brewer's yeasts for production of low-carbohydrate beer. *Biotechnol Lett* 36:1693-1699. https://doi.org/10.1007/s10529-014-1530-5

Picard M, Oulieu C, Nonier MF, Vivas N, Vivas N. 2023. The role of oak wood in the mint and floral notes of whisky: identification of common terpenoids by aromatic fractionation. *J Inst Brew* 129:62–79. https://doi.org/10.58430/jib.v129i1.8

Poisson L, Schieberle P. 2008. Characterization of the key aroma compounds in an American Bourbon whisky by quantitative measurements, aroma recombination, and omission studies. *J Agric Food Chem* 56:5820-5826. https://doi.org/10.1021/jf800383v

Postigo V, Esteban S, Arroyo T. 2023. *Lachancea thermotolerans*, an innovative alternative for sour beer production. *Beverages* 9:20. https://doi.org/10.3390/beverages9010020

R Core Team. 2022. R: The R Project for Statistical Computing. *https://www.r-project.org*

Reid SJ, Speers RA, Willoughby N, Lumsden WB, Maskell DL. 2020. Pre-fermentation of malt whisky wort using *Lactobacillus plantarum* and its influence on new-make spirit character. *Food Chem* 320: 126605. https://doi.org/10.1016/j.foodchem.2020.126605

Rodríguez Madrera R, Pando Bedriñana R, Suárez Valles B. 2021. Evaluation of indigenous non-*Saccharomyces* cider yeasts for use in brewing. *Eur Food Res Technol* 247:819-828. https://doi.org/10.1007/s00217-020-03665-y

Russell I, Stewart GG. 2014. Distilling yeast and fermentation, p 123-146. *In* Russell I, Stewart GG (eds), *Whisky: Technology, Production and Marketing*, Elsevier, UK.

Saez JS, Lopes CA, Kirs VE, Sangorrín M. 2011. Production of volatile phenols by *Pichia manshurica* and *Pichia membranifaciens* isolated from spoiled wines and cellar environment in Patagonia. *Food Microbiol* 28:503–509. https://doi.org/10.1016/j.fm.2010.10.019

Salo P, Nykänen l, Suomalainen H. 1972. Odor thresholds and relative intensities of volatile aroma components in an artificial beverage imitating whisky. *J Food Sci* 37:394-398. https://doi.org/10.1111/j.1365-2621.1972.tb02647.x

Stewart GG. 2017. The production of secondary metabolites with flavour potential during brewing and distilling wort fermentations. *Fermentation* 3:63. https://doi.org/10.3390/fermentation3040063

Suh SO, Blackwell M, Kurtzman CP, Lachance MA. 2006. Phylogenetics of Saccharomycetales, the ascomycete yeasts. *Mycologia* 98:1006-1017. https://doi.org/10.1080/15572536.2006.11832629

Taillandier P, Lai QP, Julien-Ortiz A, Brandam C. 2014. Interactions between *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in wine fermentation: Influence of inoculation and nitrogen content. *World J Microbiol Biotechnol* 30:1959-1967. https://doi.org/10.1007/s11274-014-1618-z

Toh DWK, Chua JY, Lu Y, Liu SQ. 2020. Evaluation of the potential of commercial non-*Saccharomyces* yeast strains of *Torulaspora delbrueckii* and *Lachancea thermotolerans* in beer fermentation. *IJFST* 55:2049-2059. https://doi.org/10.1111/ijfs.14399

Urbina K, Villarreal P, Nespolo RF, Salazar R, Santander R, Cubillos FA. 2020. Volatile compound screening using HS-SPME-GC/MS on *Saccharomyces eubayanus* strains under low-temperature Pilsner wort fermentation. *Microorganisms* 8: 755. https://doi.org/10.3390/microorganisms8050755

van Beek S, Priest FG. 2000. Decarboxylation of substituted cinnamic acids by lactic acid bacteria isolated during malt whisky fermentation. *Appl Environ Microbiol* 66:5322-5328. https://doi.org/10.1128/AEM.66.12.5322-5328.2000

van Beek S, Priest FG. 2002. Evolution of the lactic acid bacterial community during malt whisky fermentation: a polyphasic study. *Appl Environ Microbiol* 68:297-305. https://doi.org/10.1128/AEM.68.1.297-305.2002

Vanbeneden N, Gils F, Delvaux F, Delvaux FR. 2008. Formation of 4-vinyl and 4-ethyl derivatives from hydroxycinnamic acids: Occurrence of volatile phenolic flavour compounds in beer and distribution of Pad1-activity among brewing yeasts. *Food Chem* 107:221-230. https://doi.org/10.1016/j.foodchem.2007.08.008

Wackerbauer K, Kramer P, Siepert J. 1982. Phenolische aromastoffe in bier. *Brauwelt* 15:618-626

Walker JW, Bringhurst TA, Broadhead AL, Brosnan JM, Pearson SY. 2001. The survival of limit dextrinase during fermentation in the production of Scotch whisky. *J Inst Brew* 107:99-106. https://doi.org/10.1002/j.2050-0416.2001.tb00082.x

Walker GM, Hill AE. 2016. *Saccharomyces cerevisiae* in the production of whisk(e)y. Beverages 2:38. https://doi.org/10.3390/beverages2040038

Walker GM, Stewart GG. 2016. *Saccharomyces cerevisiae* in the production of fermented beverages. *Beverages* 2:30. https://doi.org/10.3390/beverages2040030

Walker GM, Lappe-Oliveras P, Moreno-Terrazas CR, Kirchmayr M, Arellano-Plaza M, Gschaedler-Mathis AC. 2019. Yeasts associated with the production of distilled alcoholic beverages, p 477-512. *In* Romano P, Ciani M, Fleet GH (eds), *Yeasts in the Production of Wine*, Springer, NY, USA.

Wanikawa A. 2020. Flavors in malt whisky: a review. *J Am Soc Brew Chem* 78:260-278. https://doi.org/10.1080/03610470.2020.1795795

Watson DC. 1981. The development of specialised yeast strains for use in Scotch malt whisky fermentations. *Adv Biotechnol* 57-62. https://doi.org/10.1016/b978-0-08-025382-4.50014-9

Waymark C, Hill AE. 2021. The influence of yeast strain on whisky new make spirit aroma. *Fermentation* 7:311. https://doi.org/10.3390/fermentation7040311