



Impact of *Schizosaccharomyces* species on apple juice fermentation: High glycerol production, malic acid degradation, and species-specific aroma profiles

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ABSTRACT

The increasing significance of non-*Saccharomyces* strains in fermented beverages comes from their ability to modulate sensory properties and produce diverse metabolites. This study evaluated the oenological potential and fermentation capacity of five *Schizosaccharomyces* species (*S. pombe* var. *pombe*, *S. pombe* var. *malidevorans*, *S. japonicus*, *S. octosporus*, and *S. cryophilus*). While all strains assimilate and ferment glucose, fructose, and sucrose, they show no utilization of sweeteners such as xylitol, erythritol, or stevia. Notably, the strains exhibited tolerance to 15–30% glucose, and most of them could grow at 15 °C. Fermentation trials revealed that all species successfully produced 4–6% (v/v) ethanol in apple juice, characterized by high glycerol production and efficient malic acid degradation, while maintaining acetic acid levels comparable to the *Saccharomyces bayanus* control. Volatile organic compound (VOC) analysis identified ester-rich aroma profiles—featuring fruity, banana-like, and pineapple-like notes—comprising both common and species-specific components. These findings demonstrate that *Schizosaccharomyces* species are viable candidates for cider production, offering a unique combination of deacidification, glycerol enrichment, and novel aromatic complexity, particularly for use in mixed-culture fermentation strategies.

1. Introduction

There is a growing demand for producing a wide variety of wine styles and other fermented beverages with enhanced sensory complexity and improved technological performance. To meet this need, the use of non-*Saccharomyces* yeast species provides an appealing alternative. Although these species generally cannot produce high levels of ethanol independently, they can significantly influence beverage quality through their metabolic diversity (Belda et al., 2017; Vejarano, 2020). Non-*Saccharomyces* yeasts are known to modify aroma profiles, color stability, acidity, and glycerol content by producing enzymes and secondary metabolites such as higher alcohols, esters, organic acids, and

polysaccharides (Benito et al., 2016a; Padilla et al., 2016; Ellis et al., 2022). Recent studies further emphasize their role in mixed fermentations and their potential to modulate ethanol yield and sensory complexity (Ciani et al., 2010; Morata et al., 2020).

Among these alternative yeasts, the fission yeast *Schizosaccharomyces pombe*—a widely used model organism in genetic and molecular biology research (Acs-Szabo et al., 2024)—has attracted considerable interest due to its valuable oenological properties (Loira et al., 2018). Several studies have demonstrated that *S. pombe* can reduce malic acid content via malo-ethanolic fermentation, thereby lowering total acidity and improving microbiological stability (Gallander, 1977; Taillandier et al., 1988; Dharmadhikari and Wilker, 1998; Benito et al., 2013). In addition,

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this species is capable of releasing significant amounts of polysaccharides, particularly mannoproteins, which contribute positively to aroma perception, mouthfeel, and physicochemical stability (Domizio et al., 2017; Loira et al., 2018). *S. pombe* strains are also characterized by high pyruvic acid production, promoting the formation of vitisin A and enhancing color stability, especially in red wine vinification (Benito et al., 2013, 2014; Morata et al., 2016; Ivit et al., 2018; Loira et al., 2018). Due to its high sugar tolerance (Kurtzman et al., 2011), this species is also well-suited to produce sweet wines. Furthermore, it generates substantial amounts of glycerol (Benito et al., 2014), a desirable trait for improving wine mouthfeel. Importantly, *S. pombe* typically does not produce biogenic amines, which are associated with potential health risks (Kovacova-Hanuszkova et al., 2015; Loira et al., 2018; Wang et al., 2021). Owing to these advantages, numerous studies advocate its application in co-fermentation systems (Benito et al., 2013, 2019; Yu et al., 2022), secondary fermentation (Ivit et al., 2018), and red wine production (Benito et al., 2012).

Beyond *S. pombe*, the genus *Schizosaccharomyces* comprises closely related species—including *S. japonicus*, *S. cryophilus*, and *S. octosporus*—whose biotechnological potential remains largely unexplored. Genomic analyses have revealed substantial evolutionary divergence within the genus, suggesting possible metabolic variability and strain-dependent technological properties (Jefferies et al., 2015). However, comparative studies evaluating their fermentation performance in fruit-based substrates are scarce.

Cider, a fermented beverage produced from apple juice, has recently experienced significant growth in global popularity, including in regions where it was previously uncommon (Becot et al., 2016; Ostrom et al., 2022). Its refreshing sensory profile and bioactive compound content contribute to consumer appeal. Moreover, the rising demand for diverse, premium-quality products has fuelled the expansion of the global cider market (Becot et al., 2016; Ostrom et al., 2022). The final quality of cider is influenced by multiple factors, including fruit composition, fermentation parameters, and—most notably—the microorganisms involved in the fermentation process (Valles et al., 2007; Lorenzini et al., 2019; Calugar et al., 2021). Although *S. cerevisiae* is the industry standard, reliance on a single yeast species may result in a less distinctive aromatic profile. Consequently, identifying novel yeast species suitable for mixed or alternative fermentations has become an important objective for beverage innovation. Recent investigations suggest that non-*Saccharomyces* yeasts can significantly influence ester production, acidity balance, and mouthfeel in apple-based fermentations relevant to cider production (Valles et al., 2007).

Despite the well-documented oenological properties of *S. pombe*, little information is available regarding the fermentation characteristics of other *Schizosaccharomyces* species in apple-based substrates. To our knowledge, systematic comparative studies assessing their fermentation kinetics and technological traits in apple juice under controlled laboratory conditions are currently lacking. Therefore, in this study, we evaluated the fundamental fermentation properties and kinetics of *S. japonicus*, *S. malidevorans*, *S. cryophilus*, and *S. octosporus* in pasteurized apple juice under laboratory-scale conditions, as a model system relevant to cider production. A laboratory strain of *S. pombe* and a commercial *Saccharomyces bayanus* strain were included as reference controls.

2. Materials and methods

2.1. Strains

The strains used in this study are listed in Table 1. Wild-type *Schizosaccharomyces* species and an *S. japonicus* mutant strain (334), which was isolated in our laboratory and had strong flocculation capacity (Acs-Szabo et al., 2023), were tested. A commercially available *S. bayanus* strain (Mangrove Jack's Cider Yeast - M02 Cider; https://help.mangrovejacks.com/hc/en-us/article_attachments/13551379984785) was used

Table 1
Strains used in this study.

Species	Collection number	Description
<i>Schizosaccharomyces pombe</i> var. <i>pombe</i>	0-1, ATCC 24843	Wild type, 1972 h ⁻
<i>Schizosaccharomyces japonicus</i>	7-286, NCYC 2354	Wild type, h ⁹⁰
<i>Schizosaccharomyces pombe</i> var. <i>malidevorans</i>	9-1, CCY44-6-1	Wild type
<i>Schizosaccharomyces octosporus</i>	6-7, CCY44-2-2	Wild type
<i>Schizosaccharomyces cryophilus</i>	6-21, CBS11777	Wild type
<i>Schizosaccharomyces japonicus</i> mutant strain	334	<i>lig4Δ::KanMX6</i> , h ⁹⁰ high flocculation capacity
<i>Saccharomyces bayanus</i>	10-1668	A commercial <i>S. bayanus</i> strain, Mangrove Jack's Cider Yeast - M02 Cider

as a control. The strains were maintained at −80 °C in cryopreservation.

2.2. Assimilation test

Since fruit juices (e.g., grape juice or apple juice) contain a unique mixture of natural sugars—primarily glucose, fructose, and sucrose—we wanted to investigate whether our yeast strains can utilize these sugars as a carbon source and grow in their presence. Therefore, the yeast cells were grown on YPA (1% yeast extract, 1% peptone, 2% glucose, 2% agar; VWR, Radnor, Pennsylvania, US; incubated at 25 °C for 1 day), and cell suspensions were prepared in Milli-Q (MQ) water at a density of 1×10^6 cells/mL. Next, a 10 µL aliquot of each suspension was spotted onto the surface of standard YPA and modified YPA media. The latter of which contained 2% fructose or sucrose in place of glucose. In addition, we prepared a YPA medium in which we used 2% sweetener (xylitol, erythritol, or stevia) instead of glucose to investigate whether the strains could utilize these flavour-enhancing additives. The agar plates were incubated at 15 °C and 25 °C, and the growth of the cells was monitored after 1 and 2 weeks. All tests were performed three times.

2.3. Fermentation test

To evaluate the fermentation capacity of the yeast strains, cultures were grown on YPA at 25 °C for 24 h, after which cell suspensions were prepared in MQ water (1×10^6 cells/mL). A 100 µL aliquot of each suspension was inoculated into 4 mL of YPL medium (YPA without agar) and modified YPL (containing 2% fructose or sucrose). To determine if alternative sweeteners could induce fermentation, media containing 2% xylitol, erythritol, or stevia were also tested. All media were autoclaved with inverted Durham tubes before inoculation. The cultures were incubated at both 15 °C (cool fermentation is frequently preferred for ciders) and 25 °C. Gas production (indicated by visible bubbles in the Durham tubes) was monitored daily for 12 days. All experiments were performed in triplicate.

2.4. Acid production

In previous studies, high acetic acid production was observed in certain *S. pombe* strains (Benito et al., 2012), which is an undesirable trait. Therefore, we also examined the acid production of our strains on a medium containing CaCO₃ (0.5 g yeast extract, 5 g glucose, 0.5 g CaCO₃, 2 g agar/100 mL) (Csoma et al., 2023). Yeast cells were cultured in YPL at 25 °C for 1 day. After centrifugation, cell suspensions were prepared (MQ water; 1×10^6 cells/mL). Ten µL of cell suspension was dropped onto the surface of the agar plates, which were incubated at 25 °C for 14 days. Acid production was indicated by a clear zone appearing around the cells. All tests were performed three times.

2.5. pH tolerance

The pH of apple juices and ciders typically ranges between 3.0 and 4.0 (Wei et al., 2020), which is a critical parameter for cider producers. To determine the pH tolerance of our yeast strains, we cultured them in YPL at 25 °C for 1 day. After centrifugation, cell suspensions were prepared with MQ water. Ten microliters of cell suspensions (4×10^6 cells/mL) and their 10-, 100-, and 1000-fold dilutions were spotted onto YPA plates, which we buffered to different pH values using boric acid (3.5–6.5). The agar plates were incubated at 15 °C for 17 days and 25 °C for 5 days, and the growth of cells was investigated. All tests were performed three times.

2.6. Sugar tolerance

While the total sugar concentration of typical apple juices is generally around 120 g/L (Li et al., 2020), some varieties or other fruit juices exhibit significantly higher levels (Wei et al., 2020; Csoma et al., 2021). These elevated sugar concentrations, combined with low pH, create a hostile environment for fermentation. Consequently, the osmotolerance of yeasts involved in fermentation is an important feature. To evaluate this trait of our *Schizosaccharomyces* strains, we monitored their cell growth in YPA medium containing 2%, 15%, 20%, or 30% glucose, adjusted to pH 3.5 and 5.0. The strains were cultured in YPL at 25 °C for 1 day, after which cell suspensions were prepared with MQ water (4×10^6 cells/mL and their 10-, 100-, and 1000-fold dilutions) and 10 µL were spotted onto agar plates, which were incubated at 15 °C and 25 °C. Growth of the cells was monitored after 5 days. All tests were performed three times.

2.7. Alcohol tolerance

Yeasts consume the natural sugars present in fruit juice, producing ethanol as a byproduct; consequently, their alcohol tolerance is a critical trait. Low tolerance can lead to premature cell death or the production of undesirable compounds. Therefore, we aimed to characterize this trait in our specific yeast strains. Since apple ciders generally have an ethanol content of 4–9% (Wei et al., 2020), we prepared modified YPA media containing 6%, 8%, 10%, and 12% (v/v) ethanol. Cell suspensions were prepared from 1-day-old YPL cultures (incubated at 25 °C) using ultrapure (MQ) water. These cell suspensions (4×10^6 cells/mL), along with their 10-, 100-, and 1000-fold serial dilutions, were spotted onto the ethanol-containing agar surfaces (10 µL). The plates were incubated at 25 °C, and cell growth was assessed after five days. All assays were performed in triplicate to ensure reproducibility.

2.8. Sulphur tolerance

Potassium metabisulfite is frequently used in winemaking and cidermaking, acting as an antioxidant and a potent antimicrobial agent. To evaluate sulphur tolerance of the yeast strains, cell suspensions were prepared with MQ water from 1-day-old YPL cultures (incubated at 25 °C) and 10 µL of cell suspensions (4×10^6 cells/mL), and their dilutions (10×, 100×, 1000×) were dropped onto the surface of YPA agar plates supplemented with 100, 200, 300, and 400 mg/L $K_2S_2O_5$ (Csoma et al., 2021). The Petri dishes were incubated at 25 °C, and the growth of the cells was investigated after 5 days. All tests were performed three times.

2.9. Enzyme production

Enzyme production by fermenting yeasts is important for enhancing the aromatic profile of ciders. Therefore, various agar plates were prepared to evaluate enzyme production. To test esterase activity, we prepared a medium containing 1 g peptone, 0.5 g NaCl, 0.4 g $CaCl_2 \times 2H_2O$, 1 g Tween 80 (added after sterilization), and 3 g agar/100 mL (Carrasco

et al., 2012).

Medium containing 1 g yeast extract, 2 g peptone, 0.5 g cellobiose, and 2 g agar/100 mL was used for testing the β -glucosidase activity, while medium with 2 g pectin, 1 g yeast extract, 0.2 g KH_2SO_4 , 0.05 g $CaCl_2$, 1 g NH_4SO_4 , 0.8 g $MgSO_4 \times 7H_2O$, 0.05 g $MnSO_4$, 3 g agar/100 mL was used for evaluation of pectinase activity (Villena et al., 2005; Merín et al., 2015).

10 µL of cell suspensions (4×10^6 cells/mL) and their dilutions (10×, 100×, 1000×) were spotted onto the surface of the agar plates, which were incubated at 25 °C. All tests were performed three times.

2.10. Biogenic amine production

Since biogenic amines are generally considered harmful byproducts, we wanted to assess their formation. To evaluate histamine production, we used a medium containing 3 g yeast extract, 1 g glucose, 2 g histidine, 0.15 g bromocresol purple, and 2 g agar/100 mL, pH 5.2 (set with boric acid). Tyramine production was tested on the agar plates containing 3 g yeast extract, 1 g glucose, 2 g tyrosine, 0.15 g bromocresol purple, and 2 g agar/100 mL, pH 5.2. Putrescine production was investigated on agar plates containing 3 g yeast extract, 1 g glucose, 2 g ornithine, 0.15 g bromocresol purple, and 2 g agar/100 mL, pH 5.2 (Barbosa et al., 2018).

2.11. Fermentation of apple juice

The wild-type yeast strains were grown on YPA containing 10% glucose (pH 5.0) at 25 °C for 3 days. Cell suspensions were prepared with MQ water, and 4×10^6 cells/mL were inoculated into pasteurized Golden Delicious apple juice (300 mL juice in a 500 mL Erlenmeyer flask). The Erlenmeyer flasks were incubated at 22 °C, without shaking, for 31 days. The weight loss (caused by CO_2 production) was determined each day; the fermentative power was expressed as g of CO_2 /100 mL of apple juice. Weight loss was determined from the average of three replicates. Parameters of the Apple juice (Golden) were Brix 13.2%; expected alcohol 6.9 v/v%; pH 3.24 (measured with a pH meter); relative density 1.056 (14% sugar). The experiment was also repeated with Jonagold (JG) (Brix 12.8%; expected alcohol 6.8%; pH 3.8; relative density 1.052), Jonathan (JT) (Brix 10%; expected alcohol 5.2%; pH 3.2; relative density 1.040), and a new apple variety (P) juice (Brix 12.6%; expected alcohol 6.7%; pH 3.15; relative density 1.051). Fig. 1 shows the experimental flow chart, which was generated using Graphviz (Ellson et al., 2002) via the Graphviz Online interface (<https://dreampuf.github.io/GraphvizOnline/>).

Apple juice pasteurization was performed by a commercial contractor. The juice was heated to 80 °C in large steel tanks and subsequently transferred into sterile, 5-liter aluminium foil bags. These bags were stored in insulated containers, allowing them to cool to ambient temperature over 16 h.

2.12. Analytical measurement

The chemical compositions of the fermented apple juice samples were determined using a Bruker Alpha II FT-IR spectrophotometer equipped with a diamond ATR (attenuated total reflectance) cell and OPUS 7.8 software. Measurements were performed at room temperature without chemical pretreatment; the samples were treated in an ultrasonic water bath only before injection for degassing. A small amount of the sample was placed directly on the ATR crystal. A fresh background spectrum was obtained before each measurement, and the signal-to-noise ratio was improved by averaging multiple scans. The raw spectra were subjected to baseline correction, ATR compensation, and normalization to ensure comparability between samples. According to the manufacturer's validation data for the Bruker Alpha II food analysis calibrations, typical RMSEP values fall within the 0.05–0.15% (v/v) range for ethanol and 0.1–0.3 g/L for organic acids, indicating the expected analytical uncertainty of the FTIR-based quantification.

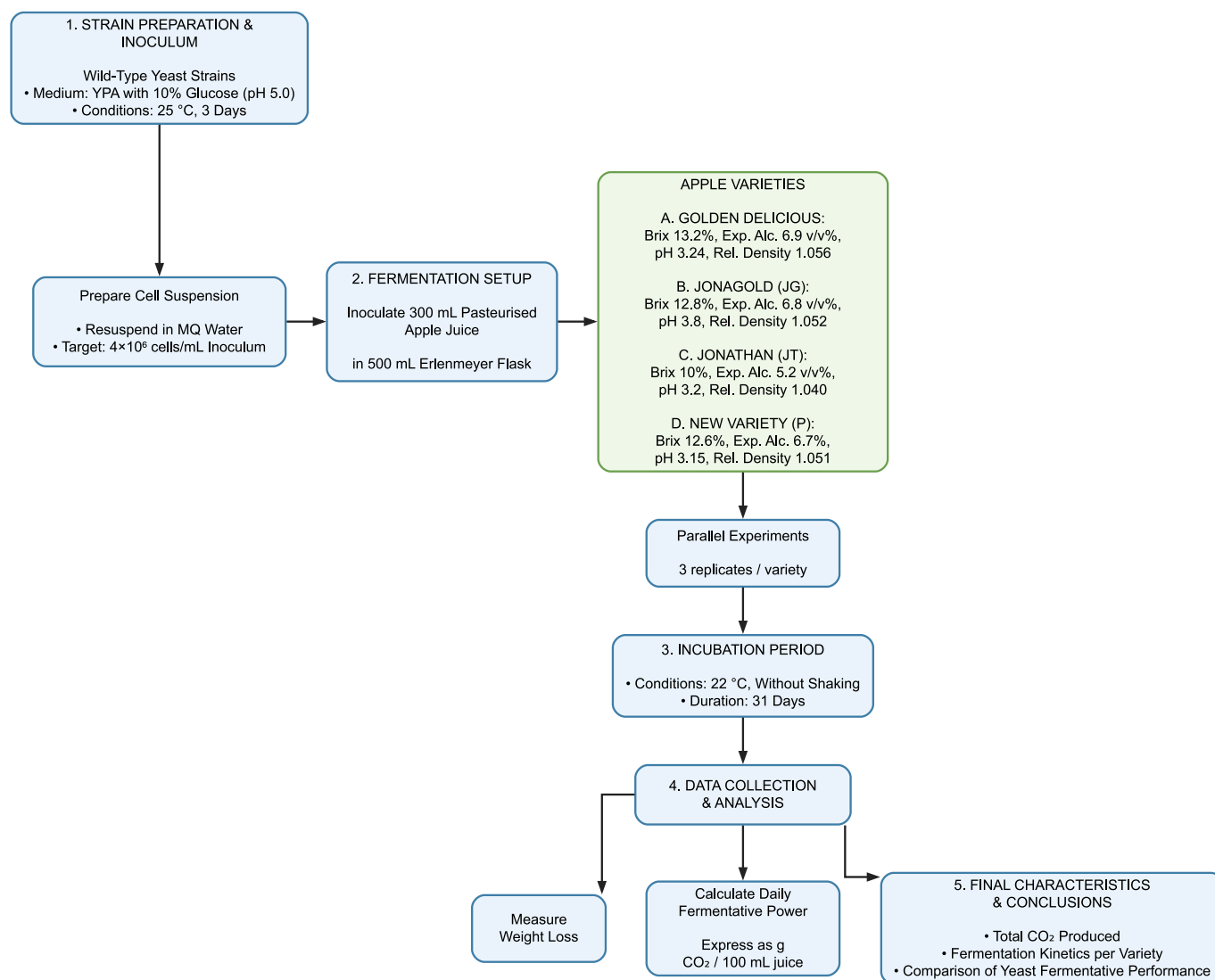


Fig. 1. Experimental fermentation flow chart for multiple apple varieties.

During the quantitative analysis, we determined the alcohol content (v/v%), reducing sugars, sucrose, the glucose/fructose ratio, relative density, glycerol content, and individual organic acids (acetic acid, citric acid, malic acid, tartaric acid, lactic acid). In addition, we measured titratable acidity, volatile acidity, and pH. Quantification was performed using multivariate calibration models, which were validated based on certified reference analytical results. Calibration and validation were performed in accordance with standard FT-IR food analysis protocols, ensuring the accuracy and reproducibility of the method

2.13. VOCs analysis

The profile of VOCs was determined by a Bruker Scion 456 gas chromatograph. It was equipped with a Bruker SHS-40 Headspace Sampler (Bruker Corporation, Billerica, MA, US), a Bruker SQ mass spectrometer, and a Br-5 capillary column (30 m 0.25 mm i.d. 1.0 m film thickness). Helium was used as a carrier gas. The measurements were prepared and performed as described in Rakonczás et al. (2023). Mass spectrometric data were used for the identification of the VOCs (National Institute of Standards and Technology (NIST), version 2005, mass spectral library). Semi-quantitative analysis was performed to identify VOCs. CAS Numbers (Chemical Abstracts Service) of the identified components were obtained from the <https://commonchemistry.cas.org>

/database. The odour descriptions were obtained from the databases listed in the footnote to Table S3.

2.14. Statistical analyses

The normal distribution of the data was verified using the Shapiro-Wilk test. Since most of the datasets were not normally distributed, the Kruskal-Wallis test was used for multiple comparisons, followed by Dunn's post hoc test for pairwise comparisons. Holm-Bonferroni corrections were applied to minimize the probability of Type I errors. Generally, *p*-values below 0.05 were considered statistically significant; however, the Holm-Bonferroni adjustments modified the level of significance accordingly. All statistical analyses were performed using the Past software v5.2.1 (Hammer et al., 2001).

Principal component analysis (PCA) was performed to identify the main variables that distinguish the different yeast strains during the fermentation trials. Since the variables were measured in different units, the PCA was based on a correlation matrix. The PCA plot was generated using Past software v5.2.1 (Hammer et al., 2001).

3. Results

3.1. Assimilation and fermentation of sugars and sweeteners in fission yeast species

The assimilation and fermentation capacities of the strains were evaluated using fundamental sugars (glucose, fructose, and sucrose) and polyol sweeteners (xylitol, erythritol, and stevia). While the growth and fermentative capacity of the *Schizosaccharomyces* species were lower than those of the control strain *S. bayanus* on media containing 2% sugar, all strains successfully utilized and fermented the tested sugars at 25 °C (Table 2). At the reduced temperature of 15 °C, glucose fermentation was maintained in all strains except for *S. cryophilus* and *S. octosporus*. Notably, only *S. malidevorans* retained the ability to ferment fructose and sucrose at this lower temperature. None of the fission yeast strains exhibited the capacity to assimilate or ferment any of the tested sweeteners, regardless of the incubation temperature (Table 2).

3.2. Stress tolerance of fission yeast species

Further experiments demonstrated that all fission yeast species exhibited tolerance to high sugar concentrations, with growth on media containing 15–30% glucose comparable to that of the control strain (Table 3). Notably, *S. cryophilus* displayed sensitivity at pH 3.5; however, this effect was mitigated by increasing glucose levels. While all strains tolerated ethanol concentrations of 6% and 8%, their growth rates were pH dependent. Both *S. pombe* and *S. malidevorans* retained the ability to grow in 10% ethanol, albeit less vigorously than in the control. Regarding sulphur tolerance, *S. pombe* and *S. malidevorans* again emerged as the most resilient species, exhibiting growth patterns like those of the control strain (Table 3).

3.3. pH tolerance, enzyme, acid, and biogenic amine production capacity

Building on previous findings regarding pH sensitivity, cell growth was evaluated on YPA media across a range of pH values. At 25 °C,

S. pombe and *S. malidevorans* exhibited growth levels nearly equivalent to the control strain, *S. bayanus*, throughout the pH 3.5–6.5 range. In contrast, other fission yeast species demonstrated greater susceptibility to pH changes (Fig. S1A). *S. cryophilus* proved particularly sensitive to pH 6.5 and to levels below pH 5 (Fig. S1A). Upon reducing the incubation temperature to 15 °C, pH tolerance was further compromised—most notably in *S. octosporus*—whereas *S. malidevorans* retained its proliferative capacity under these conditions (Fig. S1B).

The enzymatic profiles, biogenic amines, and acid production of the strains were also evaluated. As detailed in Table S1, glycosidase and pectinase production were restricted to *S. pombe*, *S. malidevorans*, and *S. octosporus*, while all strains produced acid on CaCO₃-containing medium (Fig. S2). Production of biogenic amines (histamine, tyramine, and putrescine) was not detected on Petri dishes (data not shown).

3.4. Fermentation of apple juice

Following the initial assessment of the yeast strains' basic capabilities, their fermentation capacity in Golden Delicious apple juice was investigated. In this stage of the study, fermentation kinetics were the primary focus, and CO₂ evolution was used as a reproducible indicator of metabolic performance under cider-relevant conditions. Because CO₂ production reflects fermentative activity rather than direct microbial growth and shows clear correspondence with the technological parameters. These results demonstrated that all fission yeast species were able to ferment the juice; however, their fermentation kinetics were slightly slower than those of the control strain (Fig. 2). Despite this, the weight loss after 31 days of fermentation for *S. pombe* and *S. malidevorans* was comparable to that observed for *S. bayanus* (Table S2). Alcohol production by the fission yeasts ranged from 4.4% to 6.5%, with *S. pombe* and *S. malidevorans* exceeding 6%, similar to *S. bayanus* (Fig. 3, Table S3). This was further confirmed by principal component analysis (PCA), which highlighted that *S. pombe* and *S. malidevorans* also specialize in deacidification (forming a pH-proximal cluster) (Fig. 4). Notably, all fission yeasts exhibited more robust malic acid degradation and produced similar or higher levels of glycerol than the control (Fig. 3, Table S3). Malic acid, tartaric acid, glycerol, and lactic acid correlate

Table 2
Assimilation and fermentation of sugars and sweeteners.

Strains/sugar	Glucose	Fructose	Sucrose	Xylitol	Erythritol	Stevia
25 °C assimilation						
<i>S. pombe</i>	++	++	++	–	–	–
<i>S. malidevorans</i>	++	++	++	–	–	–
<i>S. octosporus</i>	+	+	+	–	–	–
<i>S. cryophilus</i>	WD	WD	WD	–	–	–
<i>S. japonicus</i>	+	+	+	–	–	–
<i>S. japonicus</i> mutant strain	+	+	+	–	–	–
<i>S. bayanus</i>	+++	+++	+++	W	W	W
25 °C fermentation						
<i>S. pombe</i>	+	+	+	–	–	–
<i>S. malidevorans</i>	++	++	++	–	–	–
<i>S. octosporus</i>	+	+	+	–	–	–
<i>S. cryophilus</i>	+ D	+ D	–	–	–	–
<i>S. japonicus</i>	++	+++	++	–	–	–
<i>S. japonicus</i> mutant strain	+	+	+++	–	–	–
<i>S. bayanus</i>	+++	++	+++	–	–	–
15 °C fermentation						
<i>S. pombe</i>	+	–	–	–	–	–
<i>S. malidevorans</i>	++	++	+	–	–	–
<i>S. octosporus</i>	–	–	–	–	–	–
<i>S. cryophilus</i>	–	–	–	–	–	–
<i>S. japonicus</i>	++	–	++	–	–	–
<i>S. japonicus</i> mutant strain	++	–	+	–	–	–
<i>S. bayanus</i>	+++	++	+++	–	–	–

The number of + correlates with the strength of growth. –: no growth; W: weak growth; D: delayed growth.

Table 3
Sugar, ethanol, and sulphur tolerance.

Strains/glucose conc.	2 w/w%		15 w/w%		20 w/w%		30 w/w%	
	pH 5.0	pH 3.5	pH 5.0	pH 3.5	pH 5.0	pH 3.5	pH 5.0	pH 3.5
25 °C								
<i>S. pombe</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>S. malidevorans</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>S. octosporus</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>S. cryophilus</i>	+++	–	+++	+	+++	++	+++	++
<i>S. japonicus</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>S. japonicus</i> mutant strain	+++	+++	+++	+++	+++	++	+++	+
<i>S. bayanus</i>	+++	+++	+++	+++	+++	+++	+++	+++
15 °C								
<i>S. pombe</i>	+++	+++	+++	+++	+++	+++	+++	++
<i>S. malidevorans</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>S. octosporus</i>	+++	+	+++	+++	+++	+++	+++	+++
<i>S. cryophilus</i>	++	–	+++	+	+++	+	+++	++
<i>S. japonicus</i>	+++	+++	+++	+++	+++	+++	++	+
<i>S. japonicus</i> mutant strain	+++	+++	+++	++	+++	++	++	–
<i>S. bayanus</i>	+++	+++	+++	+++	+++	+++	+++	+++
Ethanol conc. 25 °C								
	6 v/v%		8 v/v%		10 v/v%		12 v/v%	
	pH 5.0	pH 3.5	pH 5.0	pH 3.5	pH 5.0	pH 3.5	pH 5.0	pH 3.5
<i>S. pombe</i>	+++	+++	+++	+	+	+	–	–
<i>S. malidevorans</i>	+++	+++	+++	++	++	++	–	–
<i>S. octosporus</i>	+++	++	+	–	–	–	–	–
<i>S. cryophilus</i>	+++	–	++	–	+	–	–	–
<i>S. japonicus</i>	+++	++	+	–	–	–	–	–
<i>S. japonicus</i> mutant strain	+++	++	+	–	–	–	–	–
<i>S. bayanus</i>	+++	+++	+++	+++	+++	+++	+	+
K ₂ S ₂ O ₅ conc. 25 °C								
	100 mg/L		200 mg/L		300 mg/L		400 mg/L	
	pH 5.0	pH 3.5	pH 5.0	pH 3.5	pH 5.0	pH 3.5	pH 5.0	pH 3.5
<i>S. pombe</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>S. malidevorans</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>S. octosporus</i>	+++	+++	+++	++	+++	–	++	–
<i>S. cryophilus</i>	+++	–	+++	–	+++	–	++	–
<i>S. japonicus</i>	+++	+++	+++	+	+++	–	++	–
<i>S. japonicus</i> mutant strain	+++	++	+++	–	+++	–	++	–
<i>S. bayanus</i>	+++	+++	+++	+++	+++	+++	+++	+++

The number of + indicates the strength of growth; –: no growth; w: weak growth; conc.: concentration.

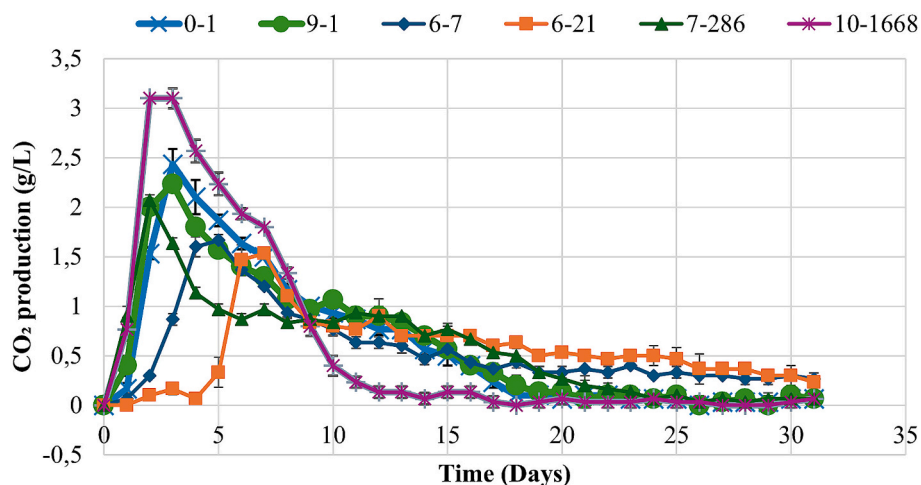


Fig. 2. Evaluation of the fermentation power of *Schizosaccharomyces* species in apple juice (Golden). 0-1 *S. pombe*, 9-1 *S. malidevorans*, 6-7 *S. octosporus*, 6-21 *S. cryophilus*, 7-286 *S. japonicus*, 10-1668 *S. bayanus*.

with higher organic acid and glycerol concentrations (Fig. 4). Furthermore, their acetic acid production was not substantially higher

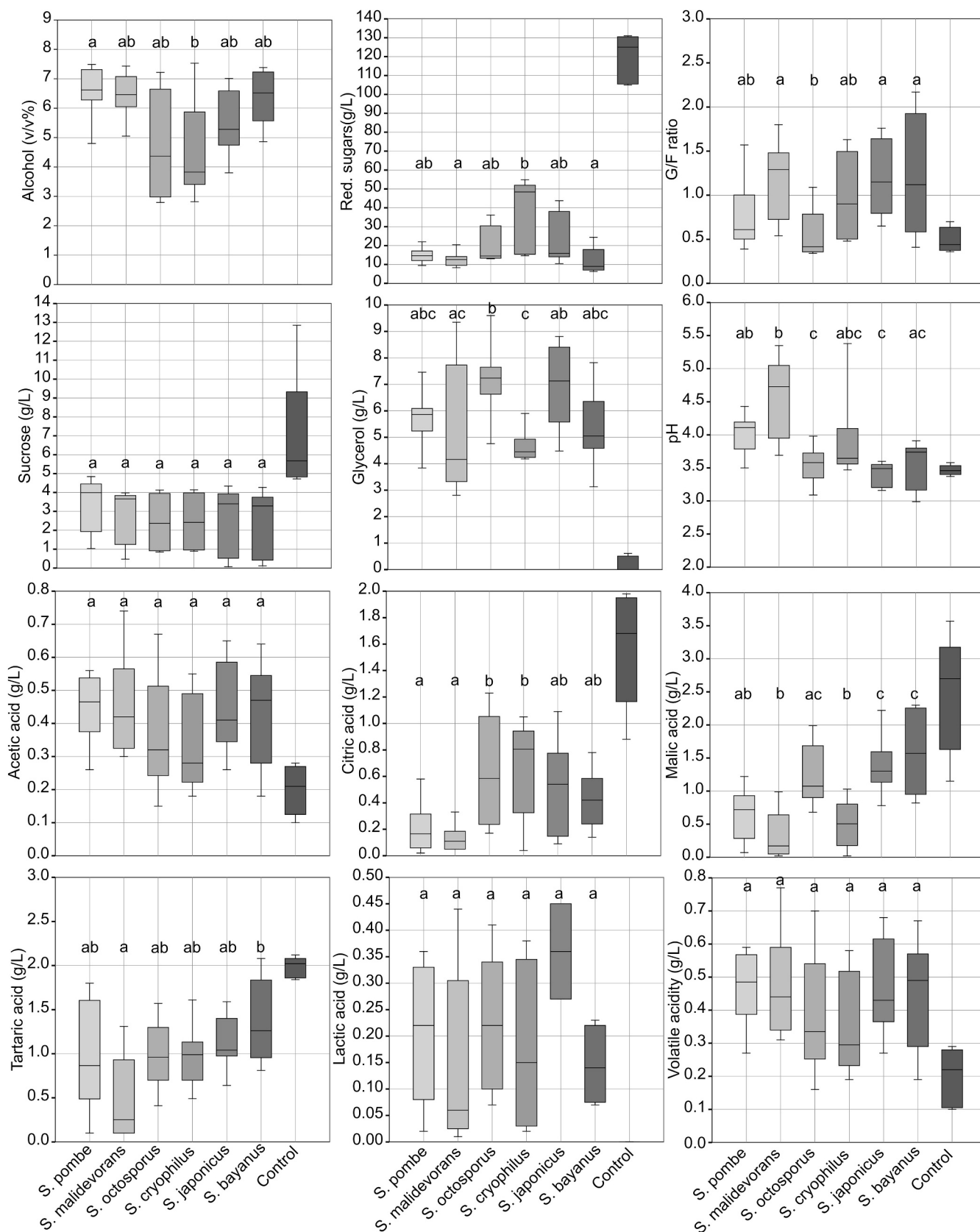


Fig. 3. Analytical data of fermented apple juice (Golden).

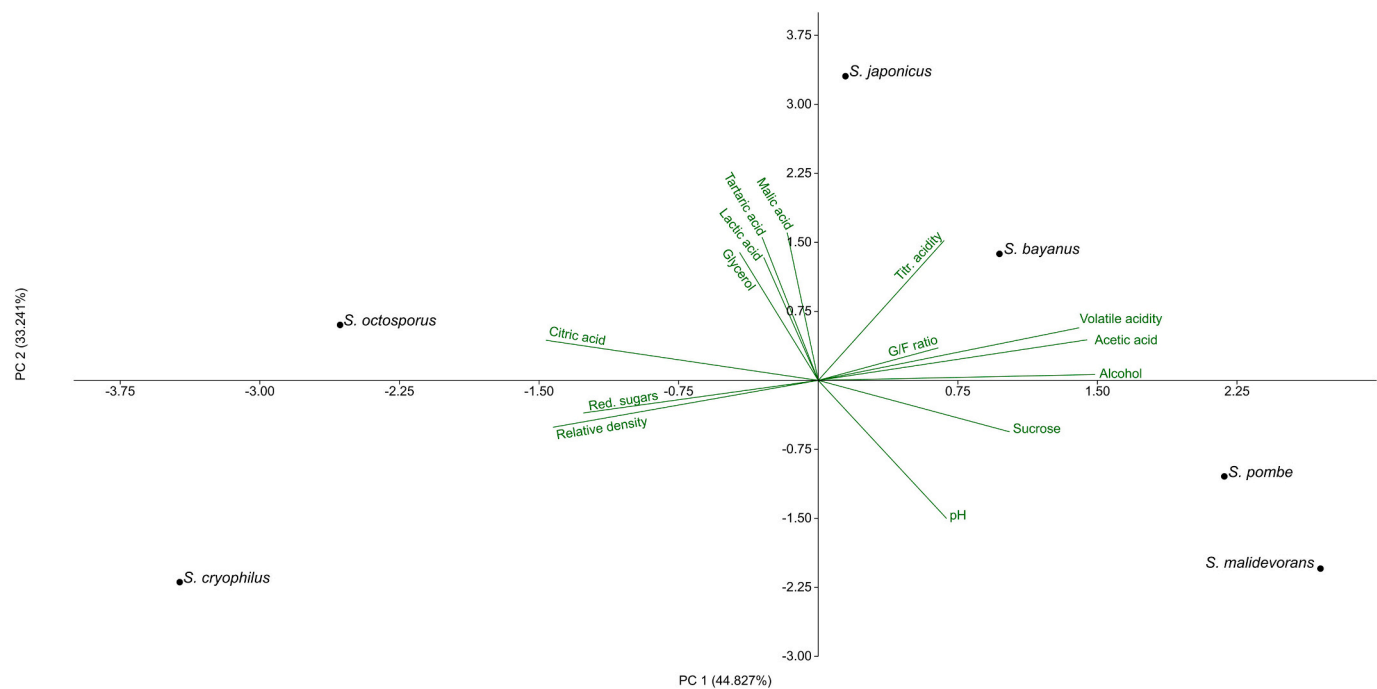


Fig. 4. PCA of the analytical data obtained in fermented apple juice (Golden).

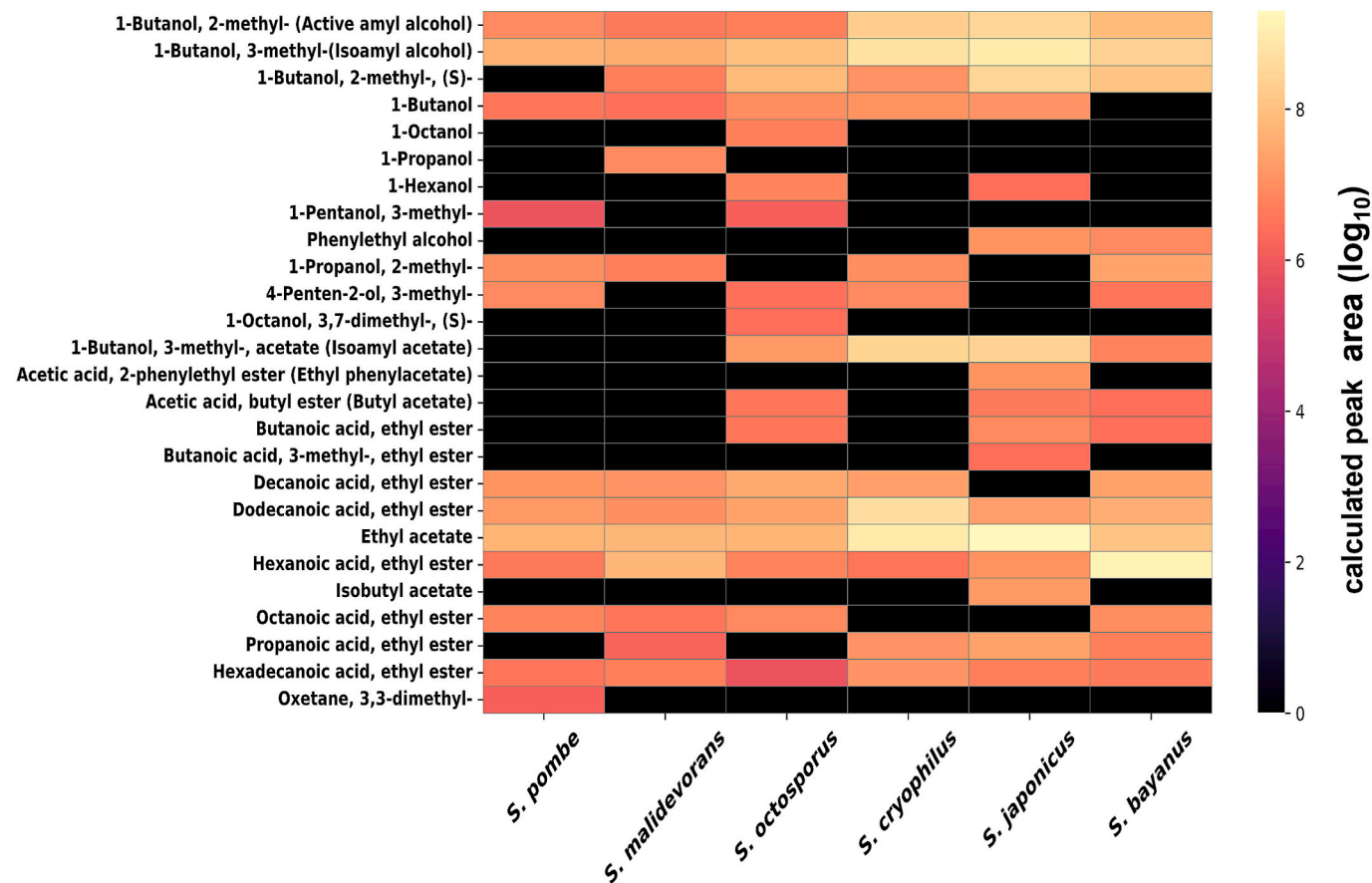


Fig. 5. The heat map shows the VOC profiles of yeast species.

than that of *S. bayanus* (Fig. 3, Table S3). This PCA biplot also demonstrates that the metabolic profiles of *Schizosaccharomyces* species exhibit remarkable intra-genus diversity. Furthermore, it highlights that

S. japonicus possesses a distinct metabolic phenotype characterized by elevated glycerol and lactic acid synthesis, whereas *S. cryophilus* appears physiologically less adapted to these specific fermentation conditions, as

Table 4

Analytical data of fermented apple juices Jonagold (JG), Jonathan (JT), and a new apple variety (P).

Parameters/ strains	<i>S. malidevorans</i>			<i>S. japonicus</i>		
	JG	JT	P	JG	JT	P
Alcohol (v/v %)	4.78 ± 0.72	3.95 ± 0.35	6.23 ± 1.39	5.16 ± 1.03	2.80 ± 1.62	5.85 ± 1.14
Red. sugars (g/L)	39.57 ± 2.69	44.60 ± 3.23	37.96 ± 2.32	44.51 ± 2.24	83.40 ± 30.35	43.70 ± 2.14
G/F ratio	0.72 ± 0.06	0.55 ± 0.03	0.87 ± 0.11	0.62 ± 0.05	0.46 ± 0.04	0.65 ± 0.00
Sucrose (g/L)	1.08 ± 0.10	0.89 ± 0.07	0.22 ± 0.07	1.83 ± 0.04	7.49 ± 4.31	1.64 ± 0.33
Relative density	1.0022 ± 0.0017	1.0047 ± 0.0023	1.0014 ± 0.0015	1.0053 ± 0.0011	1.0247 ± 0.0148	1.0057 ± 0.0011
Glycerol (g/L)	4.16 ± 0.03	4.14 ± 0.04	3.39 ± 0.22	5.59 ± 0.23	3.16 ± 1.32	4.41 ± 0.27
Acetic acid (g/L)	0.62 ± 0.08	0.46 ± 0.03	0.49 ± 0.07	0.65 ± 0.09	0.46 ± 0.08	0.65 ± 0.09
Citric acid (g/L)	0.02 ± 0.01	0.12 ± 0.18	0.02 ± 0.00	0.07 ± 0.07	0.86 ± 0.55	0.05 ± 0.05
Malic acid (g/L)	0.20 ± 0.13	0.15 ± 0.09	0.37 ± 0.26	1.19 ± 0.24	1.73 ± 0.18	2.26 ± 0.39
Tartaric acid (g/L)	0.51 ± 0.42	0.72 ± 0.02	0.33 ± 0.40	0.66 ± 0.09	0.93 ± 0.53	0.60 ± 0.19
Lactic acid (g/L)	0.49 ± 0.20	0.36 ± 0.01	0.69 ± 0.28	0.48 ± 0.16	0.36 ± 0.16	0.46 ± 0.32
Tit. acidity (g/L)	2.59 ± 1.38	3.43 ± 0.35	2.28 ± 0.24	4.00 ± 0.26	5.36 ± 0.33	3.47 ± 0.23
Volatile acidity (g/L)	0.65 ± 0.09	0.48 ± 0.03	0.51 ± 0.07	0.67 ± 0.10	0.46 ± 0.07	0.68 ± 0.10
pH	4.25 ± 0.66	3.85 ± 0.30	4.14 ± 0.24	3.51 ± 0.04	3.40 ± 0.08	3.87 ± 0.09

Parameters/ strains	<i>S. bayanus</i>			UAJ		
	JG	JT	P	JG	JT	P
Alcohol (v/v %)	4.13 ± 1.72	4.82 ± 0.16	4.49 ± 2.02	nd	nd	0.08 ± 0.01
Red. sugars (g/L)	79.49 ± 26.24	35.21 ± 1.84	78.88 ± 29.65	130.4 ± 0.19	128.4 ± 0.20	131.6 ± 0.15
G/F ratio	0.48 ± 0.03	0.81 ± 0.01	0.45 ± 0.04	0.46 ± 0.05	0.41 ± 0.08	0.4 ± 0.09
Sucrose (g/L)	4.88 ± 1.21	0.36 ± 0.27	7.95 ± 5.27	8.44 ± 0.25	12.6 ± 0.10	17.89 ± 0.14
Relative density	1.0226 ± 0.0134	1.0005 ± 0.0005	1.0243 ± 0.0158	1.0492 ± 0.06	1.0456 ± 0.03	1.0519 ± 0.10
Glycerol (g/L)	3.02 ± 0.41	4.03 ± 0.06	2.58 ± 0.82	1.58 ± 0.11	nd	1.83 ± 0.23
Acetic acid (g/L)	0.56 ± 0.04	0.56 ± 0.05	0.58 ± 0.02	0.37 ± 0.09	0.29 ± 0.05	0.51 ± 0.03
Citric acid (g/L)	0.36 ± 0.25	0.23 ± 0.11	0.47 ± 0.48	1.31 ± 0.04	1.61 ± 0.02	1.71 ± 0.01
Malic acid (g/L)	3.16 ± 0.64	1.53 ± 0.83	3.69 ± 0.28	3.96 ± 0.03	1.66 ± 0.02	3.61 ± 0.04
Tartaric acid (g/L)	1.43 ± 0.19	0.93 ± 0.29	1.44 ± 0.40	2.08 ± 0.01	1.68 ± 0.04	2.01 ± 0.02
Lactic acid (g/L)	0.10 ± 0.13	0.22 ± 0.13	0.14 ± 0.00	nd	0.17 ± 0.02	nd
Tit. acidity (g/L)	4.92 ± 1.22	3.84 ± 0.25	6.74 ± 1.16	6.62 ± 0.08	5.29 ± 0.05	7.89 ± 0.06
Volatile acidity (g/L)	0.59 ± 0.04	0.58 ± 0.06	0.60 ± 0.02	0.39 ± 0.03	0.31 ± 0.02	0.53 ± 0.02
pH	3.51 ± 0.20	3.58 ± 0.07	3.23 ± 0.11	3.26 ± 0.0,09	3.36 ± 0.11	3.15 ± 0.10

UAJ: unfermented apple juice; nd: not detected. Results represent the mean ± SD for three replicates.

indicated by its clustering with residual sugars (Fig. 4).

To investigate the influence of apple juice variety on these parameters, fermentations were repeated using Jonagold (JG), Jonathan (JT), and a new apple variety (P) juices with the most effective strains (*S. malidevorans* and *S. japonicus*; *S. malidevorans* showed the highest ethanol concentration, while *S. japonicus* was good at glycerol production) (*S. bayanus* was used as a control). The results indicate that the analytical characteristics of the fermented apple juice are significantly affected by the type of apple juice used (Table 4). Nevertheless, *S. malidevorans* consistently demonstrated robust malic acid degradation across all juice varieties (Table S3, Table 4). Similarly, *S. japonicus* maintained high glycerol production levels in almost every tested juice (Table S3, Table 4). Ethanol yields were between 2.8 and 6.2 v/v%, depending on apple variety. Fission yeasts showed generally higher ethanol levels in JG and P juices than *S. bayanus* (Table S3, Table 4).

3.5. Analysis of VOCs

Beyond the analytical data presented in Table S3 and Table 4, volatile organic compounds (VOCs) were also analysed. Semi-quantitative analysis revealed that the fission yeasts exhibited distinct aroma profiles, consisting of both shared and species-specific components (Fig. 5, Table S4). Their VOC production was primarily dominated by alcohols and esters (Fig. 5, Table S4). The most prevalent esters were characterized by sweet, floral, and green notes, as well as fruity aromas reminiscent of banana, pineapple, apple, and apricot. Notably, the production of compounds such as ethyl acetate and ethyl hexanoate (hexanoic acid, ethyl ester) was enhanced compared to the control strain (Table S4). As for alcohols, it is noteworthy that the fission yeast strains produced significantly higher amounts of fusel alcohols compared to the control strain. Specifically, higher levels of isoamyl alcohol were observed (with *S. octosporus* and *S. japonicus* showing the highest yields), along with 1-butanol (most notably in *S. octosporus*) and 2-methyl-1-propanol (primarily produced by the *S. pombe* and *S. cryophilus* strains) (Table S4). Furthermore, the synthesis of 1-octanol by *S. octosporus* and 1-hexanol by both *S. octosporus* and *S. japonicus* may impart a fresher, more herbaceous character to the final product.

4. Discussion

The consumption of fermented fruit juices is on the rise, driven by their health-promoting properties and the trend of increasingly hot summers. Furthermore, there is a growing consumer demand for a wider variety of beverage styles and types. Utilizing yeast strains with diverse metabolic capabilities, e.g., non-*Saccharomyces* species, is one way to address these needs (Benito et al., 2019; Calugar et al., 2021; Yu et al., 2022); however, this approach requires a comprehensive understanding of the specific characteristics of various yeast species and strains. In this context, comparative evaluation at the genus level is essential to distinguish conserved metabolic traits from strain-specific effects.

Building on the known oenological benefits of *S. pombe* (Benito et al., 2013, 2014; Domizio et al., 2017; Loira et al., 2018), this research explores the potential of several related fission yeast species. We evaluated *S. japonicus*, *S. octosporus*, *S. cryophilus*, and the *S. pombe* variant *S. malidevorans*, alongside a highly flocculent mutant *S. japonicus* strain (Acs-Szabo et al., 2023) (Table 1). The primary goal was to assess their fundamental properties and suitability for apple juice fermentation. To provide a baseline for comparison, a laboratory *S. pombe* strain and a commercial *S. bayanus* strain were used as controls. By extending the investigation beyond *S. pombe*, the present study enables a broader assessment of the biotechnological potential of the *Schizosaccharomyces* genus.

All strains demonstrated the ability to assimilate and ferment glucose, fructose, and sucrose, both confirming and extending the previous findings (Benito et al., 2016a; Brysch-Herzberg et al., 2019). In contrast, none of the tested strains were able to utilize the sweeteners

xylitol, erythritol, or stevia (Table 2). This aligns with the data reported by Choi et al. (2023), who also noted that *S. japonicus* is unable to utilize xylitol. This lack of fermentation may be advantageous in products where sweeteners are added to enhance flavour without the risk of unintended secondary fermentation. From a technological perspective, this metabolic limitation may contribute to product stability in sweetened beverages.

Growth at lower temperatures was also evaluated, with all strains exhibiting growth at 15 °C (Fig. S1). This contrasts with the findings of Helston et al. (2010), who reported poor growth at 18 °C for these fission yeast species. Such discrepancies may be attributable to the significant influence of medium composition and pH on yeast growth. Our results indicate that fission yeasts are more sensitive to pH fluctuations than *S. bayanus* (Fig. S1); specifically, *S. cryophilus* did not tolerate pH values below 5. A similar phenotype was observed in *S. octosporus* at 15 (Fig. S1). Given that apple juice typically has a pH between 3 and 4, this sensitivity could limit industrial applicability. However, all species successfully fermented apple juice at pH 3–4 (Fig. 2), suggesting that the natural juice matrix mitigates the stress effects observed in defined media. This observation highlights the importance of evaluating yeast performance in real substrate systems rather than relying exclusively on synthetic laboratory media. In addition, we noticed an improved pH tolerance of *S. cryophilus* at elevated glucose concentrations (Table 3), which may be linked to glucose-induced intracellular alkalization (Haworth and Fliegel, 1993). This emphasizes the interplay between nutrient availability and stress adaptation. Although all *Schizosaccharomyces* species, including *S. cryophilus*, were able to ferment apple juices at pH values 3–4, initially, the fission yeasts exhibited slower fermentation kinetics compared to the *S. bayanus* control (Fig. 2), consistent with previous findings for *S. japonicus* in various apple juices (Leali et al., 2024). *S. pombe* and *S. malidevorans* achieved comparable total weight loss by the end of fermentation (Table S2), demonstrating robust fermentative capacity. It was confirmed by PCA (Fig. 4). Furthermore, *S. pombe* and *S. malidevorans* exhibited the highest ethanol tolerance, withstanding concentrations of 8% and 10%. In contrast, the other species only tolerated up to 6% ethanol, a tolerance that was notably pH-dependent (Table 3). These results align with previous data from grape must fermentations, where selected *S. pombe* strains also tolerated 10% ethanol (Benito et al., 2016b). Given that the ethanol levels generated during apple juice fermentation typically range between 4% and 6% (Table S3 and Table 4)—and cider alcohol content generally spans 1.2% to 8.5% (Lea and Piggott, 2003)—ethanol toxicity should not pose a barrier to the application of fission yeasts in cider production. Instead, fermentation kinetics, stress resilience, and metabolic efficiency are likely more decisive determinants of industrial suitability.

Another favourable characteristic of these fission yeast species was their strong glycerol production, which was either comparable to or superior to that of the *S. bayanus* control (Table S3 and Table 4). These results mirror findings from previous studies using other *S. pombe* and *S. japonicus* strains in the fermentation of synthetic and commercial grape juices (Domizio et al., 2017; Benito et al., 2014; Romani et al., 2018). In our study, glycerol production by *S. japonicus* was remarkably high across almost all apple juice varieties tested (Table S3, Fig. 4, Table 4). These levels exceeded those observed in other *Saccharomyces* species, such as *Hanseniaspora vineae* and *Pichia kluyveri* (Wei et al., 2020) or *Saccharomyces uvarum* (González et al., 2019); however, the fermentation time varied. Elevated glycerol synthesis is often associated with redox balancing during fermentation and may positively influence mouthfeel and sensory perception. Particularly high glycerol production by *S. japonicus* suggests species-specific differences in redox metabolism and carbon flux distribution.

Similarly, significant malic acid degradation was observed in all species (Table S3 and Table 4), which, based on PCA, correlates with, e. g., higher organic acid and glycerol production (Fig. 4). While malic acid degradation has been previously documented for *S. pombe* and *S. japonicus* (Taillandier et al., 1988; Benito et al., 2012; Domizio et al.,

2018; Leali et al., 2024), the consistent performance across all tested species suggests that efficient malo-ethanolic fermentation may represent a conserved genus-level metabolic characteristic. This capability offers a practical alternative to bacterial malolactic fermentation, potentially reducing microbiological risks and simplifying acidity management in acidic fruit substrates. Our results from apple juice fermentation confirm these findings, with malic acid degradation being comparable or superior to the control (Tables S3 and S4), particularly in the case of *S. pombe* and *S. malidevorans* (Fig. 4). Since all tested species performed well in both glycerol production and malic acid degradation, these appear to be genus-specific characteristics. Furthermore, high sugar tolerance was also consistent across all strains, with successful growth at 15% and 30% glucose (Table 3). Consequently, these strains may be suitable not only for cider production but also for fermenting high-sugar fruit juices. Furthermore, our results demonstrated that the examined species did not produce harmful biogenic amines, and their sulphite tolerance was comparable to or lower than that of the control strain (Table 3), suggesting that eliminating the yeasts from the final product would be straightforward. While previous studies have highlighted high acetic acid production in *S. pombe* (Benito et al., 2014, 2019), others have noted that *S. pombe* can reduce acetic acid levels under specific conditions (Song et al., 2019). Given that our findings showed acetic acid production was not much higher than that of the *S. bayanus* control in Golden, JT, and P juices (Tables S3 and S4), this metabolic trait in *Schizosaccharomyces* species is likely highly dependent on the type of fruit juice or specific fermentation parameters. This context-dependent metabolic behaviour underscores the necessity of the strain- and matrix-specific evaluation before industrial implementation. The growth curves also supported the species-specific fermentation kinetics observed: *S. pombe* showed faster growth and correspondingly stronger fermentation kinetics, whereas *S. japonicus* exhibited slower growth but produced a more ester-rich and fruity aroma profile.

The VOCs of cider produced from Golden Delicious apple juice were also analysed. Our findings indicate that the VOC profiles generated by fission yeasts were distinct, consisting primarily of alcohols and esters (Fig. 5, Table S4). Esters (which are produced through the reaction of alcohols and fatty acids or acetyl-CoA) with sweet, banana-like, fruity, and floral notes were most abundantly detected in the samples. Ethyl acetate and ethyl hexanoate were enhanced compared to the control strain (Table S4). This aligns with findings by He et al. (2021), who characterized ciders fermented with *S. pombe* as being ‘more floral,’ ‘fruitier,’ and ‘sweeter’ than those fermented with *S. cerevisiae*. Beyond these general patterns, several VOC groups showed species-specific tendencies that aligned with the fermentation behaviour observed in this study. The higher alcohols produced by *S. pombe* and *S. malidevorans* were consistent with their more intensive sugar metabolism, whereas the ester-rich profile of *S. japonicus* corresponded to its slower but more aroma-oriented fermentation. These relationships provide physiological context for the VOC differences, although they remain correlative and do not constitute direct mechanistic evidence. The predominance of esters may reflect species-specific acetyltransferase activity and differential regulation of aroma precursor metabolism.

Additionally, the fission yeast strains produced higher amounts of isoamyl alcohol and other fusel alcohols compared to the control *S. bayanus* strain (Table S4). While high concentrations of fusel alcohols can lead to off flavours, at lower levels they impart characteristic aromas such as marzipan and banana to the fermented juice. These compounds are derived from amino acid catabolism (Ehrlich pathway) (Hazelwood et al., 2008) and are often synthesized in response to nitrogen limitation. We emphasize that these metabolic explanations remain hypothetical, as no direct evidence for pathway activation or enzyme-level regulation was obtained in this study. Given that Boudreau et al. (2017) highlighted that apple juices are frequently deficient in yeast assimilable nitrogen (YAN), it is possible that nitrogen deficiency contributed to the production of these aroma components. It is interesting to note that we have observed on several occasions that *S. japonicus* produced fruity

aromas even on nitrogen-rich media (the aforementioned YPA). In addition, other interesting compounds have been produced. The synthesis of 1-octanol by *S. octosporus* and of 1-hexanol by both *S. octosporus* and *S. japonicus* can impart a fresher, spicier character to the final product (Table S4).

Comparative analysis also revealed that these fission yeast species produced similar types and species-specific volatiles (Fig. 5, Table S4). These data further highlight that despite the high conservation of gene pools and gene orders among related fission yeast species (Rhind et al., 2011; Acs-Szabo et al., 2021), their physiological capabilities can differ significantly. Overall, thanks to its extremely fruity flavour profile and its banana and rose aromas, *S. japonicus* may be ideal for producing ciders with intense aromas. Its related species may be better suited for making more traditional beverages, which, however, can produce new aromatic components during mixed fermentation compared to *S. bayanus* cider yeast.

In summary, the examined *Schizosaccharomyces* species successfully fermented apple juice, produced moderate ethanol levels (4–6%), demonstrated efficient glycerol production and malic acid degradation. They also maintained acceptable acetic acid levels and produced many interesting species-specific volatiles. However, it should be noted that this study was conducted under laboratory-scale conditions. Further validation under industrial processing conditions will be necessary to confirm scalability and commercial applicability. This should include sensory evaluation and long-term stability assessment.

5. Conclusion

The fission yeast species studied exhibited complementary metabolic profiles. *S. japonicus* displayed superior glycerol production and a distinct fruity aroma, whereas *S. pombe* and *S. malidevorans* showed robust ethanol tolerance and fermentation kinetics. Additionally, all species demonstrated high sugar tolerance. These shared and divergent traits suggest that controlled mixed-culture fermentations could allow the precise modulation of acidity, mouthfeel, and aromatic complexity. A key limitation of this work is the absence of yeast-assimilable nitrogen (YAN) measurements, which restricts the physiological interpretation of the fermentation behaviour and should be addressed in future studies. Taken together, these genus-level metabolic characteristics position *Schizosaccharomyces* species as promising candidates for innovation in apple-based beverage production.

CRedit authorship contribution statement

Szonja Takács: Methodology. **Anna Bodnár:** Methodology. **László Attila Papp:** Methodology. **Lajos Acs-Szabo:** Methodology. **Dávid Domonkos:** Funding acquisition. **Hajnalka Csoma:** Writing – review & editing, Conceptualization. **Ida Miklós:** Writing – original draft, Conceptualization.

Consent for publication

All authors have read and agreed to the version of the manuscript.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijfoodmicro.2026.111991>.

Data availability

No data was used for the research described in the article.

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Further reading

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