





## Article

# Alternative Yeast Strains in Beer Production: Impacts on Quality and Nutritional Value

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## Abstract

Discovering new yeast species can be crucial for creating new types of beers. In this study, we investigated three new yeast species, *Saccharomyces bayanus*, *Schizosaccharomyces japonicus* and *Schizosaccharomyces pombe* var. *malidevorans*, which have not been previously used in the brewing industry. Colour, total acidity, bitterness, aroma profile, total phenolic, flavonoid, mineral content and organoleptic characteristics of beers fermented by these strains were analysed to discover their applicability in the brewing industry. They did not significantly affect the nutritional value and colour of the beers, but showed increased acidity compared to the control *Saccharomyces cerevisiae*. GC-MS (Gas Chromatography-Mass Spectrometry) analysis revealed 33 aroma compounds, some of which were identical and some unique. *S. cerevisiae* and *S. bayanus* produced a similar number (19–20) of aroma compounds, while *S. japonicus* produced the fewest, including some undesirable compounds. Isobutyl alcohol, isoamyl alcohol, acetol, dimethylpyrazine, acetic acid, 4-cyclopentene-1,3-dione, butyrolactone, 2-furanmethanol, phenylethyl alcohol, maltol and pyranone that provide desired aromas in beers could be found in every sample. The new yeasts significantly increased polyphenols and decreased flavonoid content. Based on the results above and the taste scores, the strains *S. bayanus* and *S. pombe* var. *malidevorans* may be suitable for brewing, while *S. japonicus* is less or only suitable for combined fermentation.

**Keywords:** beer; yeast strains; quality; nutritional value; organoleptic characteristics



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## 1. Introduction

Raw materials of the brewing industry determine the quality of beer. As one of the primary ingredients, malt gives most of beer's mineral content, which is influenced by the region, species and vintage [1]. The degree of roasting has a great influence on its colour, aroma and taste; furthermore, the species of grain might affect foam stability too. Dark and caramel malts contain high amounts of antioxidants, formed during Maillard reaction and caramelization. These compounds might increase the shelf life of beer [2]. The most important parameter of brewing water is hardness, which is given by the dissolved salts [3]. Therefore, the quality of brewing water also has a great influence on the mineral content of beer, indirectly affecting its foam stability and bitterness [1]. Hops provide the specific

bitterness of beer due to their alpha and beta acid content, which varies between 4–19% based on the type [4], but they also contain volatile compounds, e.g., terpene hydrocarbons, esters, carbonyls and alcohols [5]. Since it is also a great source of antioxidants, it can be considered a natural preservative [6].

Out of the processing aids, yeasts are the most important, which convert fermentable carbohydrates to ethanol and carbon dioxide. In addition, numerous metabolites are produced by the yeast cells during fermentation, such as different esters, ethers, aldehydes and ketones, which also contribute to the aroma profile of the beverage [7]. Traditional lager yeasts are *Saccharomyces pastorianus* strains, while *S. cerevisiae* yeasts are applied for ale beer production [8]. Certain yeast strains have different properties during fermentation. Their optimum temperature varies, as does their pressure and ethanol tolerance. The proportion of their metabolites also varies, which is also influenced by the fermentation parameters, so that certain types of beer have different organoleptic characteristics [9].

As there is a growing demand among young people for different types of fermented beverages, including craft beers, this study aimed to produce ale beers by using yeast strains that are less common in the food industry and to investigate their properties that are important from the brewing industry's point of view. The fermentation properties of different yeast strains were investigated, whereupon the yeasts *S. bayanus*, *S. japonicus* and *S. pombe var. malidevorans* were selected, and compared with *S. cerevisiae* used as a control. The selected *Schizosaccharomyces* strains belong to fission yeasts, which have not been applied for brewing previously. Total phenolic content (TPC), flavonoid content, mineral content, colour, acidity, bitterness and aroma profile of the products were determined; furthermore, a sensory analysis was also performed to discover the applicability of the above-mentioned yeasts in the brewing industry.

*S. cerevisiae* is as old as mankind. It was already used by the Egyptians to produce fermented products. Nowadays, it is used to produce beer, wine and bread, but it is also employed as a dietary supplement [10]. *S. bayanus* is a distant relative of *S. cerevisiae*. Although they belong to the same genus, there are many differences between the two species. While we already know the importance of *S. cerevisiae* in beer production, *S. bayanus* plays more of a role in winemaking and apple cider production [11]. *S. bayanus* is suitable for growth at low temperatures and fermentation at higher sugar concentrations. Besides the previously mentioned cryo-tolerance, its fermentation profile in grape must differs from that of *S. cerevisiae*. Such differences are lower acetic acid and ethanol production. On the other hand, in the case of *S. bayanus*, a larger amount of glycerol and succinic acid production was observed, accompanied by the synthesis of malic acid, instead of its degradation [12]. In contrast, *S. japonicus* and *S. pombe* belong to the fission yeasts, which divide by fission instead of budding. Although the two *Schizosaccharomyces* species are closely related and descend from a common ancestor [13], they have both common and different features. *S. japonicus* shows dimorphism, i.e., it can grow in unicellular (yeast) and filamentous (hyphae) forms, depending on the environmental conditions [14].

Because of this capacity, *S. japonicus* is regarded as an early separated and most divergent fission yeast species, which is also an attractive model organism for molecular biologists. In recent years, its applicability in the production of cider or makgeolli has begun to be investigated, and it has been proven to be suitable for making cider or as an alternative starter culture for fermenting makgeolli [15,16]. It can also release larger amounts of cell wall-derived polysaccharides [17], which play a fundamental role in the technological properties and organoleptic characteristics of wines [18,19]. *S. pombe*, another model organism that grows only in yeast form, has been extensively studied for oenological traits. Due to its unique ability to degrade acids, such as malic acid, this microorganism could be suitable to solve some problems of modern winemaking, such as the improvement

of food quality or food safety [20,21]. A high release of polysaccharides is also characteristic of this species, as well as a high pH and temperature resistance [22]. Moreover, *S. pombe* produces less urea but higher levels of pyruvic acid and glycerol [23].

Although it was originally isolated from millet beer in East Africa [24], its broader use in brewing remains limited. While fission yeasts such as *S. japonicus* and *S. pombe* have been studied in winemaking and cider fermentation, their potential in beer brewing remains largely unexplored. Recent studies, however, have demonstrated that *S. pombe* in mixed-culture fermentation achieves higher attenuation, efficient nitrogen utilization, and increased ester and higher-alcohol production [25], while *S. japonicus* strains show promising fermentation traits and strong malic acid reduction capacity [15,26]. Reviews also highlight the increasing role of non-*Saccharomyces* yeasts, including fission yeasts, in creating innovative beer styles with distinctive sensory properties [27,28]. We have therefore set ourselves the goal of investigating *S. japonicus* and *S. pombe* var. *malidevorans* for beer fermentation. This study addresses this gap by evaluating their fermentation performance, metabolite production, and impact on key beer quality parameters—including phenolic and flavonoid content, mineral composition, acidity, bitterness, aroma profile, and sensory characteristics—in comparison with the conventional brewing yeast *S. cerevisiae*.

## 2. Materials and Methods

### Preparation of the applied yeasts

Yeasts were available in the collection of the Department of Genetics and Applied Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, where they were stored at  $-70\text{ }^{\circ}\text{C}$ . Table 1 indicates the origin and registration number of the yeast.

**Table 1.** The applied yeast strains.

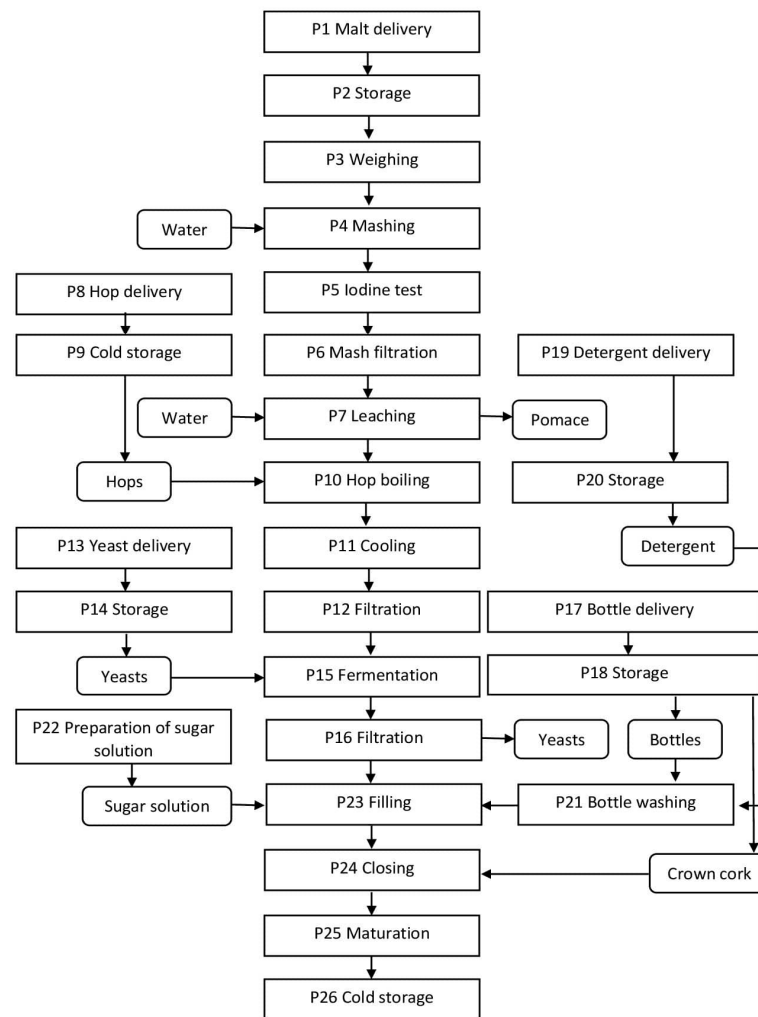
Registration Number	Strain	Origin
10-1676	<i>Saccharomyces cerevisiae</i>	Fermentis SafAle “T-58”
10-1688	<i>Saccharomyces bayanus</i>	Mangrove Jack’s M02
7-286	<i>Schizosaccharomyces japonicus</i>	CCY 44-5-1
9-1	<i>Schizosaccharomyces pombe</i> var. <i>malidevorans</i>	CCY 44-6-1

Yeasts were cultured on YPA (pH 6) or in YPL (pH 6) media. YPA medium contained yeast extract (1 g), peptone (1 g), glucose (10 g) and agar (3 g), while YPL contained yeast extract (1 g), peptone (1 g) and glucose (10 g) (VWR International LTD., Radnor, PA, USA). pH was adjusted with tartaric acid. Cells, grown on YPA, were inoculated into 300 mL of YPL media.

These cultures were incubated at  $20\text{ }^{\circ}\text{C}$  for 5 days by continuous shaking, thus the cells were growing instead of fermenting. The cells were counted in a Bürker chamber on the day of brewing. The necessary amount of cells was centrifuged (Beckman Coulter centrifuge, Beckman Coulter GmbH, Aachen, Germany) (4000 rpm, 15 min,  $4\text{ }^{\circ}\text{C}$ ). The supernatant was discarded, and the pellet was suspended in 100 mL of distilled water per tube, which was then stored in the refrigerator until the inoculation of the wort. The starting cell concentration of the wort was  $9 \times 10^6$  cells/mL.

### Brewing

The flowchart of the brewing process is illustrated in Figure 1. Every brewing was carried out in triplicate.



**Figure 1.** Flowchart of the brewing process.

Malts, hops and *S. cerevisiae* (Fermentis Division of S.I. Lesaffre, Marcq-en-Baroeul, France) and *S. bayanus* (Bevie Handcraft NZ Limited, Auckland, New Zealand) yeasts were purchased in a local brewing specialty shop. Hops were stored in a refrigerator until the brewing process. Four batches of pale ale beer were produced by the following recipe:

Malts: pale ale malt (4.0 kg), wheat malt (0.5 kg), caramel malt (0.2 kg); Surrogates: -; Mashing water: 15 L; Mashing: protease step—50 °C, 20 min, amylase step—67 °C, 75 min; Leaching water: 11 L; Hop boiling: 60 min; Hop addition: Citra hop pellets (alpha-acid: 13%): 40 g, 0 min, Citra hop pellets (alpha-acid: 13%): 10 g, 50 min; Yeast strains: *S. cerevisiae*/*S. bayanus*/*S. japonicus*/*S. pombe var. malidevorans*; Fermentation: 20 °C, 14 days approx.

The exact time of fermentation was determined by the efficiency of fermentation. Products were bottled on the day when gravity reached 1.006. No priming sugar was added before bottling. Products were stored at room temperature (20 °C) for 2 weeks before their analysis.

## Analytical methods

### Sample preparation

Samples were degassed by an ultrasonic water bath (Bandelin Sonorex Digital DT 255H, BANDELIN Electronic GmbH & Co., Berlin, Germany), and then filtered through folded filter paper (grade: 292, Munktell Ahlstrom, Helsinki, Finland). This sample preparation method was required for every method below, except for the sensory analysis.

### Determination of total phenolic content (TPC)

The principle of the method is that phosphotungstic and phosphomolybdic acids found in Folin–Ciocalteu reagent oxidize phenolic compounds, resulting in a blue-coloured solution. Colour intensity is proportional to the concentration of phenolic compounds, therefore the absorbance of the mixtures is measured by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, Altrincham, England) at a wavelength of 760 nm, against the mixture of methanol and distilled water (MeOH:DW, 80:20). After diluting (1:10) the samples with the mixture of methanol and distilled water (80:20; MeOH:DW further), those were filtered through folded filter paper (Munktell Ahlstrom, grade: 292, Helsinki, Finland). 0.5 mL of this filtrate was taken to a test tube, then 2.5 mL of Folin–Ciocalteu reagent (0.2 N) was added. After a 5 min rest, 2 mL of Na<sub>2</sub>CO<sub>3</sub> solution (75 g/L) was added, and samples were rested in the dark at room temperature for 2 h before measuring their absorbance.

To prepare the calibration solutions, a gallic acid stock solution is used. Applied chemicals: 3,4,5-trihydroxybenzoic acid (Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany), sodium carbonate (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), methanol (Scharlab S.L., Barcelona, Spain), Folin–Ciocalteu reagent (VWR International S.A.S., Rosny-sous-Bois, France). Results were expressed in mg GAE/100 mL (mg gallic acid equivalent/100 mL) [29].

### Determination of flavonoid content

The determination of flavonoid content was also carried out by a spectrophotometric method. Absorbance of the rose-coloured complex created during the analysis was measured at a wavelength of 510 nm by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, Altrincham, England) against a blank solution. After diluting (1:10) the samples with MeOH:DW, those were filtered through folded filter paper (Munktell Ahlstrom, grade: 292, Helsinki, Finland). 1.0 mL of this filtrate was added to 4 mL of MeOH:DW. Then 0.3 mL of NaNO<sub>2</sub> solution (10% m/m) was added, followed by 0.3 mL of AlCl<sub>3</sub> solution (10% m/m). At last, 2 mL of NaOH solution (1 M) was added to the tubes before voluming them up to 10 mL with MeOH:DW. Absorbance of the samples could be measured immediately. To prepare the calibration solutions, a catechin stock solution was used. Applied chemicals: catechin (Cayman Chemical Company, Ann Arbor, MI, USA), aluminium chloride (Scharlab S.L., Barcelona, Spain), sodium nitrite (Scharlau Chemie S.A., Sentmenat, Spain), sodium hydroxide (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), methanol (Scharlab S.L., Barcelona, Spain). Results were expressed in mg CE/100 mL (mg catechin equivalent/100 mL) [30].

### Determination of element content

An open-system digestion was carried out as sample preparation. 15 mL sample was measured into a digestion tube, then 10 mL HNO<sub>3</sub> (69%, VWR International LTD., Radnor, PA, USA) was added and the samples were rested overnight. Pre-digestion of the samples was carried out at 60 °C for 30 min. Then 3 mL H<sub>2</sub>O<sub>2</sub> (30%, VWR International LTD., Radnor, PA, USA) was added prior to the main digestion, which took 90 min at 120 °C. After cooling down, samples were diluted to 50 mL with ultrapure distilled water (Millipore S.A.S., Molsheim, France). and filtered through qualitative filter paper (grade: 388, Sartorius Stedim Biotech S.A., Gottingen, Germany) [31]. The concentration of minerals was determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer; Thermo Scientific iCAP 6300, Cambridge, UK) with the following operation parameters: Power rate—1350 W; nebulizer gas flow rate—1 dm<sup>3</sup>/min; cooling gas flow rate—12 dm<sup>3</sup>/min; auxiliary gas flow rate—1 dm<sup>3</sup>/min; sample input speed—1 cm<sup>3</sup>/min; stabilisation time—3 s. The emission wavelengths (nm) were as follows: Na—589.5; K—766.4; Ca—317.9; Mg—279.5; P—185.9; S—182.0. Results were expressed in mg/L.

### Determination of beer's colour

To determine the colour of beers, the degassed samples were poured into cuvettes and their absorbance was measured by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, Altrincham, England) at a wavelength of 430 nm, against distilled water. Results were expressed in EBC (European Brewing Convention) ( $EBC = A_{430} \times 19.7$ ) [32].

### Determination of total acidity

The samples were diluted (one decimal) with boiled distilled water, then titrated with 0.1 M sodium hydroxide (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) in the presence of phenolphthalein indicator (Scharlab S.L., Barcelona, Spain). Results were expressed in %lactic acid [33].

### Determination of bitterness

Bitter acids were extracted from the samples by an organic solvent in an acidic medium. After the extraction and the separation of the two phases, absorbance of the solvent phase was measured by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, Altrincham, England) at a wavelength of 275 nm, against the solvent. Applied chemicals: 2,2,4-trimethyl pentane (VWR International S.A.S., Rosny-sous-Bois, France), hydrochloric acid (VWR International S.A.S., Rosny-sous-Bois, France). Results were expressed in mg/L (IBU) [34].

### Analysis of aroma profile

To profile the aroma compounds of the last batches, a Bruker Scion 456 gas chromatograph (SCION Instruments, Goes, The Netherlands) was applied, equipped with a Bruker SHS 40 headspace sampler, coupled to a Bruker SQ mass spectrometer (SCION Instruments, Goes, The Netherlands), and fitted with a BR-5 capillary column (30 m  $\times$  0.25 mm i.d.; 1.0  $\mu$ m film thickness). The carrier gas was helium, flow rate was 1 mL/min in constant flow mode. A 5000  $\mu$ L sample was incubated in the headspace vials (60  $^{\circ}$ C, 20 min) in the automatic sampler with no agitation. A 1000  $\mu$ L headspace sample was injected into the column. The transfer line was maintained at 230  $^{\circ}$ C, while the injector temperature was 250  $^{\circ}$ C (20:1 split ratio). The oven was set to an initial temperature of 40  $^{\circ}$ C for 2 min, then increased to 280  $^{\circ}$ C with an intensity of 10  $^{\circ}$ C/min, held for 3 min. The mass spectrometer was operated in electron impact ionisation mode (70 eV). Source temperature was 180  $^{\circ}$ C, scanning rate was 1 scan/s, and mass spectra were recorded in full scan mode. Identification of the volatile compounds was based on the data obtained from the National Institute of Standards and Technology (NIST) mass spectral library [35].

### Sensory Analysis

The products of the third brewing were submitted to a sensory analysis by 25 volunteers. Based on the Regulation of the Regional and Institutional Ethical Committee, University of Debrecen, ethical permission was not required due to the presence of no additional substances besides the normally applied raw materials of beer. The Regulation is in compliance with Act no. CLIV on public health (1997), Regulation no. 23/2002 (V.9.) on medical research including human testing, and the Ovideo Convention. The evaluation was carried out by the application of the following form indicated in Table 2.

**Table 2.** Evaluation form of sensory analysis.

Evaluation Form				
Gender (underline)				
Female		Male		
Age (underline)				
18–25 years	26–35 years	36–45 years	46–55 years	56–65 years
Sensory evaluation of the samples				
Evaluation of <b>colour</b> -1: dislike extremely; 5: like extremely				
1	2	3	4	5
Evaluation of <b>foam</b> -1: dislike extremely; 5: like extremely				
1	2	3	4	5
Evaluation of <b>aroma</b> -1: dislike extremely; 5: like extremely				
1	2	3	4	5
Evaluation of <b>flavour</b> -1: dislike extremely; 5: like extremely				
1	2	3	4	5
<b>Characteristics</b> that come to mind after tasting the sample (please underline at least 3 of these)				
refreshing	bread flavour (yeast)	citrus aroma	cheesy	
hazy	fruity	clear	honey	
foot smell	bitter	sweet	sour	
acidic	light	corn	musty	
grass taste	metal flavour	alcoholic	buttery	
caramel aroma	spicy	medicinal	sulphurous	

### Statistical Analysis

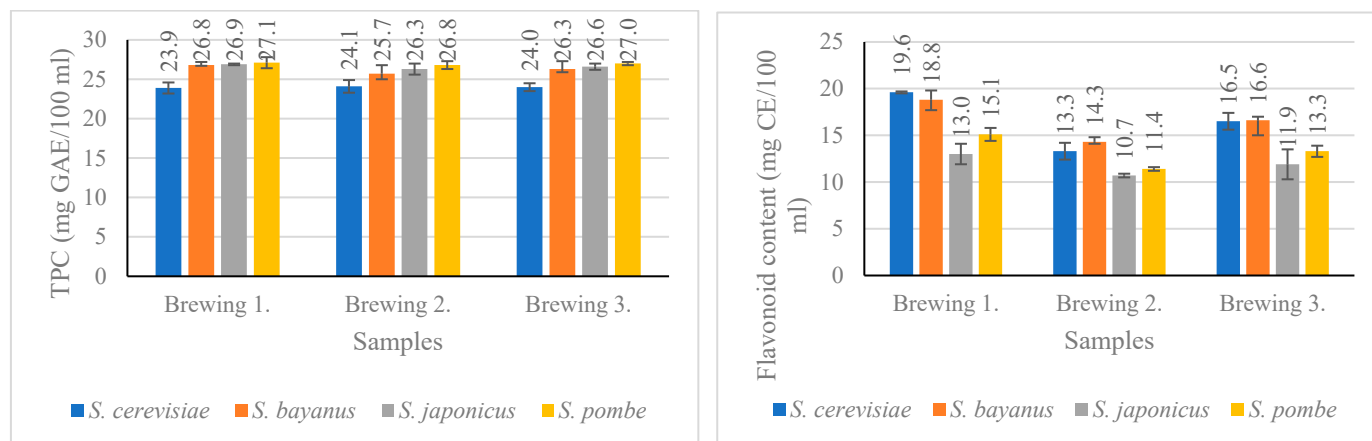
Analytical measurements were carried out in triplicate. For the statistical analysis, SPSS (version 23, SPSS Inc., Chicago, IL, USA) was used. After the descriptive analysis, the normality of the data was tested, which justified the absence of any outliers. To identify statistically verified differences between the mean results of the three brewings from the aspect of yeast strains, One-Way ANOVA was performed. Before the analysis, the test of homogeneity was also carried out at the level of 5% significance. Based on its results (homogeneous variables only), a Tukey test was performed.

## 3. Results

### 3.1. Nutritional Value

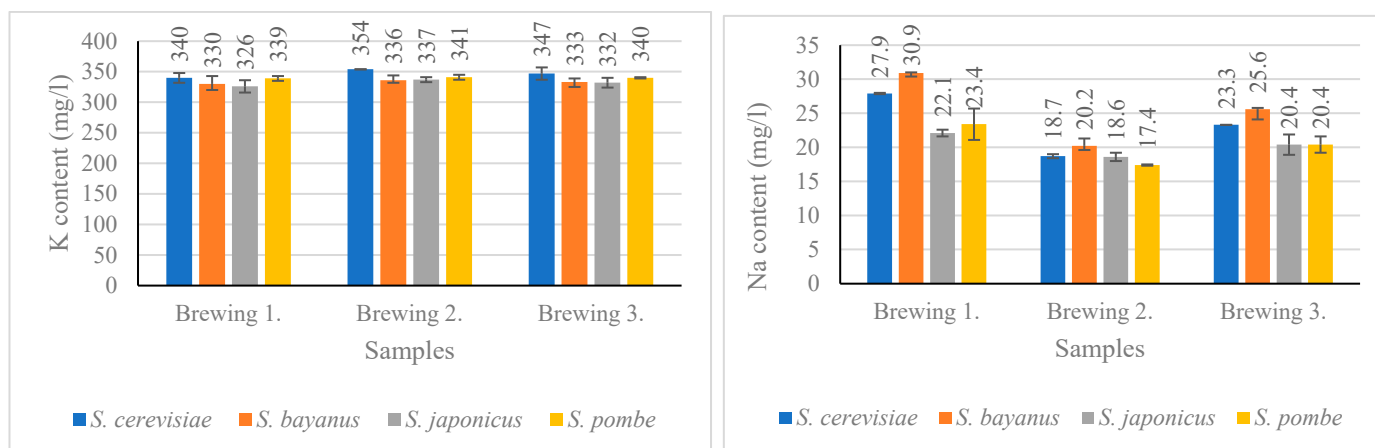
Total phenolic content and flavonoid content of the samples are indicated in Figure 2, showing similar tendencies between the three brewings. Phenolic content of the samples was increased by every yeast strain applied compared to *S. cerevisiae*, which had a TPC of 23.9–24.1 mg GAE/100 mL, and the difference was significant in every case ( $p < 0.0005$ ). In contrast, samples fermented by *S. bayanus* showed 25.7–26.8; and samples fermented by *S. japonicus* showed 26.3–26.9 mg GAE/100 mL. The highest polyphenol content was measured in the samples of *S. pombe* (26.8–27.1 mg GAE/100 mL). As seen in the figure, TPC of the samples fermented by alternative yeast strains had similar polyphenol content; however, the application of *S. pombe* caused the highest increase in this parameter in every case. The difference between the TPC of alternative yeasts' products was not significant. On the other hand, the application of the two *Schizosaccharomyces* strains caused a decrease

in the beverages' flavonoid content by 20–35%. The use of *S. bayanus* did not change the flavonoid content of the sample in either of the cases, the difference in the concentrations measured in those and the control beers was about 5%. Regarding the mean flavonoid content of the samples, no significant difference could be observed. Polyphenol and flavonoid content of the samples are in compliance with the study of Yao et al. (2022), which presented a polyphenol content of 241 mg GAE/L and a flavonoid content of 193 mg RE/L in the case of their pale ale beer fermented by *S. cerevisiae* [36]. On the other hand, Nardini and Garaguso (2020) reported a polyphenol content of conventional ale beers ranging between 383 and 482 mg GAE/L; however, their flavonoid content was much lower than our samples, 51.9–73.2 mg CE/L [37].



**Figure 2.** Total phenolic content (TPC) and flavonoid content of the samples.

Potassium and sodium content of the samples are presented in Figure 3. As seen in the figure, the highest potassium concentrations (340–357 mg/L) were measured in the beers fermented by *S. cerevisiae*; however, the application of *S. pombe* resulted in similar potassium content; therefore, this was the only case when no significant difference could be observed between the mean K concentrations ( $p = 0.334$ ). *S. bayanus* and *S. japonicus* decreased the samples' K content, but only by 3–5%, which was also significant compared to the control sample ( $p_1 = 0.028$ ;  $p_2 = 0.017$ ). The highest Na concentrations were measured in beers fermented by *S. bayanus*; however, the Na content of the control samples was lower by 7–10% only. The application of *Schizosaccharomyces* strains decreased the Na content of the samples, which might be considered beneficial. These differences were not statistically verified in either of the cases.



**Figure 3.** Potassium and sodium content of the samples.

Figure 4 indicates the calcium and magnesium content of the beverages. As seen, the lowest calcium concentrations were produced by the control samples, while the application of *S. japonicus* increased its concentration by 20% approximately, and the difference between the mean Ca content of the above-mentioned samples was also statistically verified ( $p = 0.041$ ). Other yeast strains also caused a slight increase in calcium content, but the difference could not be verified. On the other hand, the magnesium content of the samples decreased using alternative yeast strains in every case, mostly in case of *S. bayanus*, except for the first brewing, when the sample mentioned showed similar (46.9–46.2 mg/L) magnesium content. Favorably, the decrease in magnesium content was not significant in general.

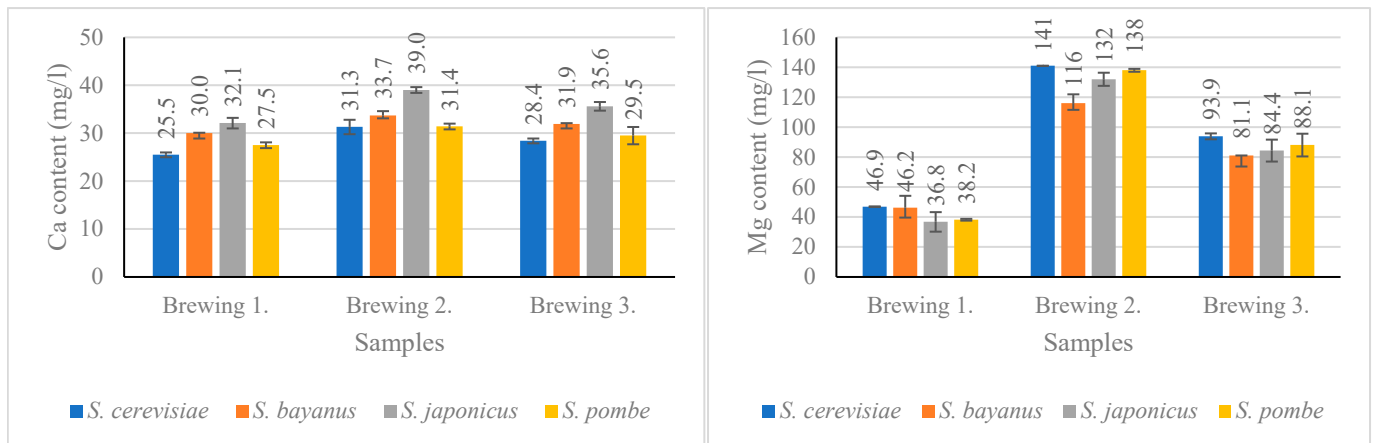


Figure 4. Calcium and magnesium content of the samples.

The phosphorus and sulphur content of the samples is shown in Figure 5. P was the second most abundant element among the analysed minerals. The concentrations of P were similar in the case of *S. cerevisiae* and *S. bayanus*, while *S. japonicus* decreased its concentration by 4–8%, and *S. pombe* increased its concentration by 5–9%. Observing the measured sulphur content, the results are not as consistent as previously. Sample fermented by *S. bayanus* showed an outstanding S content in case of the first brewing, while it also had the lowest S concentration in case of the second brewing. Similar S content was measured from every sample after the third brewing; however, the two *Schizosaccharomyces* strains' S concentration was similar in every case. There was no significant difference between the mean phosphorus and sulphur contents in any case.

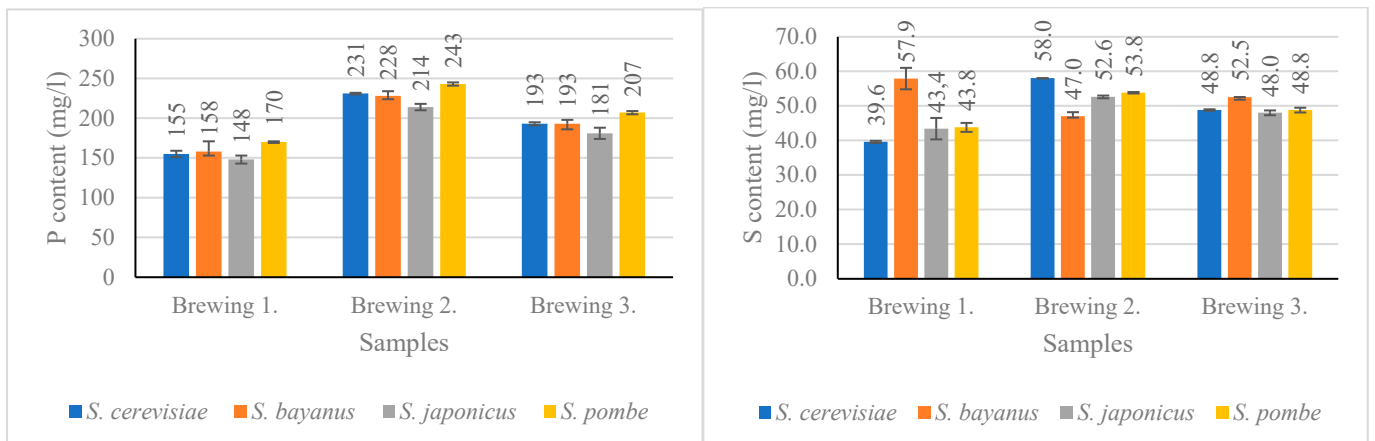


Figure 5. Phosphorus and sulphur content of the samples.

Macro element composition of the produced samples complies with the data previously reported regarding beer composition. Based on the study of Gama et al. (2017), K content of 30 commercial pale and dark beers ranged between 183 and 418 mg/L, and they reported the following ranges for the elements analysed: Ca—9.82–96.0 mg/L; P—37.4–150 mg/L; S—10.3–50.7 mg/L [38]. Pires et al. (2019) analysed 7 beers (pilsner, ale and dark) by microwave-induced plasma optical emission spectrometry, and reported a K content between 248 and 548 mg/L, and the ranges for other elements were the following: Na—51.0–171 mg/L; Ca—12.0–112 mg/L; Mg—24.0–79.0 mg/L [39]. Compared to these studies, we have measured similar K, Ca, Mg, S, Cu and Mn content. The Na content of our samples was remarkably lower than that reported by Pires et al. (2019) [39], which is favourable, and we have measured slightly higher P content than Gama et al. (2017) [38].

### 3.2. Beer Quality Parameters

Mean results of colour, total acidity and bitterness of the samples are summarized in Table 3. The colour of each sample complied with the requirements of Directive no. 2-702, Codex Alimentarius Hungaricus, as their colour intensity was lower than 20 EBC [40]. The highest colour was measured in the beers fermented with *S. bayanus*, which differed significantly from the other samples. The lowest values were reported by *S. japonicus*, although they did not differ significantly from the control sample ( $p = 0.065$ ). The acidity of the samples varied between 0.0500 and 0.0711% lactic acid. The highest acidity was measured in all cases in the beverages fermented with *S. japonicus*, but only differed significantly compared to the control sample ( $p = 0.031$ ). In contrast, the mean acidity of the control sample was significantly lower than the acidity of all other beers. Table 2 also shows extremely small differences between the samples in terms of bitterness, which is mainly related to the recipe—each batch was produced with the same hopping; therefore, no significant difference in bitterness could be detected.

**Table 3.** Colour, total acidity and bitterness of the samples.

Sample	Quality Parameter		
	Colour (EBC)	Total Acidity (%Lactic Acid)	Bitterness (IBU)
<i>S. cerevisiae</i>	14.0 ± 0.5 <sup>ab</sup>	0.0503 ± 0.0003 <sup>a</sup>	84.8 ± 1.0 <sup>a</sup>
<i>S. bayanus</i>	17.1 ± 0.3 <sup>c</sup>	0.0606 ± 0.0041 <sup>b</sup>	84.7 ± 0.9 <sup>a</sup>
<i>S. japonicus</i>	12.6 ± 0.7 <sup>a</sup>	0.0670 ± 0.0041 <sup>b</sup>	81.9 ± 2.3 <sup>a</sup>
<i>S. pombe</i>	14.7 ± 0.7 <sup>b</sup>	0.0627 ± 0.0041 <sup>b</sup>	84.3 ± 1.5 <sup>a</sup>

Lowercase letters indicate the significant differences at a significance level of 5%.

### 3.3. Organoleptic Characteristics

Organoleptic characteristics were investigated only in the case of the last brewing to gather information on the effect of different yeast strains on consumers' preferences.

The presence of 33 aroma compounds was identified by GC-MS; the results of the qualitative analysis are summarized in Table 4, and the proportions (area%) of the compounds are illustrated in Figure 6. While beverages fermented by *S. cerevisiae* and *S. bayanus* contained a similar number (19–20) of aroma compounds, the one fermented by *S. pombe* contained 26, and the one prepared by *S. japonicus* contained only 16. Isobutyl alcohol, isoamyl alcohol, acetol, dimethylpyrazine, acetic acid, 4-cyclopentene-1,3-dione, butyrolactone, 2-furanmethanol, phenylethyl alcohol, maltol and pyranone could be found in every sample; however, different species produced them in different proportions. These compounds provide aroma, which is extremely desired in the case of beer, such as sweet, balmy, alcoholic, smoky, bitter and floral aromas; however, unfortunately, sour acetic

acid was also present in every sample, which might be the result of the slow growth of yeasts, ending up in the presence of acetic acid bacteria [36]. Since the sample fermented by *S. pombe* contained the highest variety of volatiles, several of these substances were present exclusively in this sample, such as methylpyrazine, 2(5H)-furanone, 2-acetylfuran, propanoic acid, 1,3-butanediol, solerone and 2-hydroxy- $\gamma$ -butyrolactone. Most of these compounds are pleasant, with the exception of propanoic acid. On the other hand, the absence of furfural and methionol is also favorable. In the case of *S. japonicus*, many volatiles could not be detected. The lack of furfural, formic acid, propanoic acid, caproic acid and n-octanoic acid has a positive effect on beer aroma; however, it still contains acetic acid, isobutyric acid and methionol (exclusively), which might cause sour and cauliflower aroma. 2-methoxy-4-vinylphenol could also be detected only in this beverage, which might cause a clove aroma. Furfural could only be detected in beverages fermented by *Saccharomyces* strains; however, this compound causes rancid and dry aroma. On the other hand, there was no volatile compound that was present in both beers produced with *Schizosaccharomyces* strains but missing from the other samples. 5-hydroxy-4-pentanolide was present only in the case of *S. cerevisiae*, while phenylethyl acetate was only detected in the beer fermented by *S. bayanus*, which are both pleasant aroma compounds.

**Table 4.** Identified aroma compounds of the samples.

Compound	Effect	Presence			
		<i>S. cerevisiae</i>	<i>S. bayanus</i>	<i>S. japonicus</i>	<i>S. pombe</i>
1-propanol	Mild, alcoholic	+	+	+	
Isobutyl alcohol	Sweet, musty	+	+	+	+
Isoamyl alcohol	Balmy, alcoholic	+	+	+	+
Methylpyrazine	Roasted, peanut				+
Acetole	Sweet, caramel	+	+	+	+
Dimethylpyrazine	Roasted, peanut	+	+	+	+
Acetic acid	Sour	+	+	+	+
Furfural	Rancid, dry	+	+		
2(5H)-furanone	Curry, walnut, sweet				+
Pyrrole	Sweet, peanut	+	+		+
2-acetylfuran	Smoky, caramel				+
Formic acid	Strong, acidic	+	+		+
Propanoic acid	Hot, unpleasant				+
Isobutyric acid	Sour		+	+	+
4-cyclopentene-1,3-dione	Bitter, smoky	+	+	+	+
Butyrolactone	Bitter	+	+	+	+
2-furanmethanol	Roasted, smoky	+	+	+	+
Phenylethyl acetate	Rose, honey		+		
1,3-butanediol	Fruity				+
Caproic acid	Fatty, cheesy		+		+

Table 4. Cont.

Compound	Effect	Presence			
		<i>S. cerevisiae</i>	<i>S. bayanus</i>	<i>S. japonicus</i>	<i>S. pombe</i>
Methionol	Cauliflower			+	
Phenylethyl alcohol	Floral, rose	+	+	+	+
Maltol	Sweet, caramel	+	+	+	+
2-pyrrolidine	Tart, sour	+		+	+
n-octanoic acid	Rancid	+	+		+
2-methoxy-4-vinylphenol	Clove			+	
Solerone	Caramel, roasted				+
2-hydroxy-gamma-butyrolactone	Mildly sweet				+
Pyranone	Caramel, roasted	+	+	+	+
3-pyridinol-2-methyl	Tarty	+	+		+
5-hydroxy-4-pentanolide	Caramel, sweet	+			
4-hydroxymethyl-gamma-butyrolactone	Mildly buttery			+	
5-hydroxymethylfurfural	Sweet	+	+		+

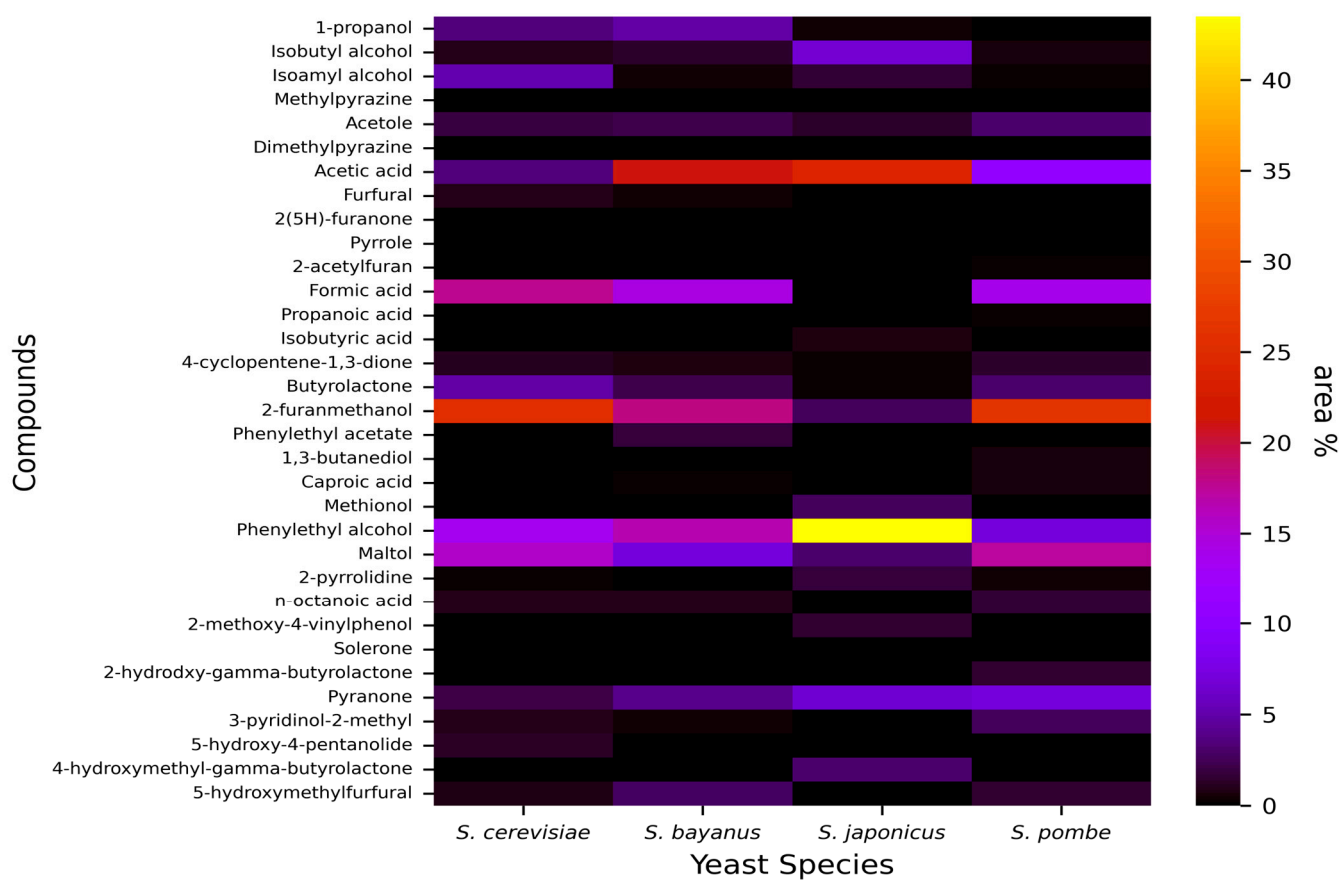
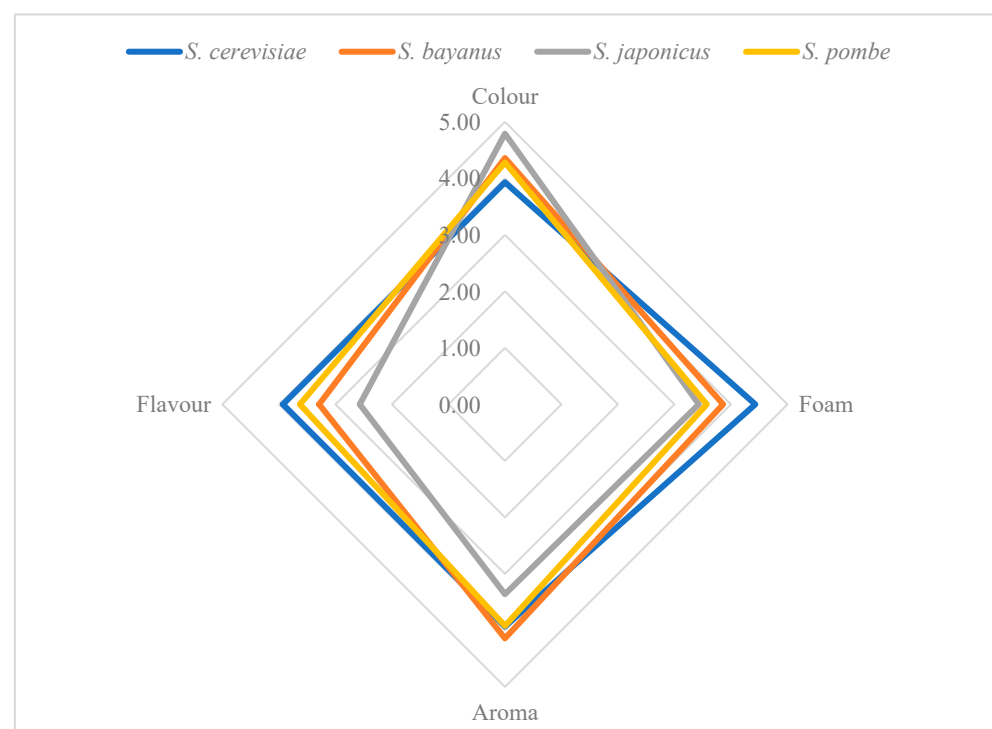


Figure 6. Proportions of the identified aroma compounds.

In general, the sample prepared by *S. pombe* showed the most favorable balance between pleasant and unpleasant aroma compounds, the two samples of *Saccharomyces* strains showed the most similarities, and the application of *S. japonicus* resulted in the lowest number of volatiles; however, the number of unpleasant compounds was also lower than in the rest of the samples.

Figure 7 indicates the results of the sensory analysis. The lowest score regarding its colour was received by the control sample (3.93). On the other hand, flavour (3.93) and foam (4.43) of this sample were the most desirable according to the volunteers. The application of *S. bayanus* resulted in the highest score in aroma (4.14), while the beverage fermented by *S. japonicus* received the highest score regarding its colour (4.79), although this sample received an extremely low flavour score (2.57) compared to the other beverages. Apart from the control sample, the application of *S. pombe* resulted in the most pleasant flavour (3.63) based on the sensory analysis.



**Figure 7.** Results of the sensory analysis.

The evaluators were also asked to emphasize the attributes that came to mind after tasting the samples. The most common attributes in the case of the control sample were refreshing, bitter and citric aroma, but a few evaluators also perceived a medicinal taste. Regarding the sample fermented by *S. bayanus*, several volunteers felt the flavour of honey, spices and caramel, and a few participants also marked “bread character”, which is not a surprise considering the characteristics of unfiltered beer. It was also interesting that only a few female evaluators mentioned medicinal and sulphurous flavours.

The low flavour score of the beverage fermented by *S. japonicus* was further explained by the description of the participants. Most of them felt a specific cheesy or corny flavour; however, its texture was described as positive and clear. “Light” attribute was also mentioned by numerous volunteers, presumably due to its slight foaming. In the case of *S. pombe*, the sample was mostly described by the following attributes: refreshing, citrus, bread aroma, acidic and sour.

## 4. Discussion

Here, we focused on investigating yeast strains not previously used in brewing. The budding yeast (*S. bayanus*) and fission yeasts (*S. japonicus* and *S. pombe*) were tested to explore whether they might be suitable for beer production.

Based on our results, the inoculation of the same wort with different yeast strains can cause significant differences in the examined parameters. We showed that the TPC values produced by the alternative yeast strains were higher, while their flavonoid production was slightly lower (between 10–18 mg CE/L) compared to the control *S. cerevisiae* (Figure 2). Similar effects have been described for *S. pombe* and *S. japonicus* in winemaking, where their fermentations led to increased polysaccharide and phenolic compound release [17–19]. Despite the differences, the average polyphenol and flavonoid content of our samples showed good agreement with Yao's results [36]. Regarding nutrient parameters, all strains caused similar K content (Figure 3), which fell within the range observed by Gama et al. (2017) [38] and resulted in higher Ca and lower Mg levels (Figure 4) than the control strain. However, the average Mg concentration of our samples was higher than in many classic beer varieties [41,42]. Further analysis revealed that *S. bayanus* increased, while the *Schizosaccharomyces* strains slightly decreased Na concentration (Figure 3), which was remarkably lower than reported by Pires et al. 2019 [39] and can be considered beneficial. We measured increased P content in beer fermented by *S. pombe*, and this species appeared to be the best in terms of S levels (Figure 5).

The identified aroma compounds also demonstrated that the tested yeast strains had a unique aroma profile, meaning they produced aroma compounds that were partly similar but partly different (Table 4 and Figure 6). The two samples of the *Saccharomyces* strain showed the greatest similarity and a higher number of compounds, while the fission yeasts were more distinct from each other, and *S. japonicus* yielded the fewest volatiles (Table 4 and Figure 6). Earlier research also reported that *S. pombe* and *S. japonicus* fermentations in wine and cider generate distinctive volatile profiles compared to *Saccharomyces* [15,16,22].

Certain compounds provide aromas that are extremely desired in the case of beers, such as sweet, balmy, alcoholic, smoky, bitter and floral aromas (Table 4 and Figure 6). Based on these results, these strains might be applicable for brewing. This was confirmed by the beer quality parameters, which also met the criteria regarding the specific beer type in every case. Colour intensity was increased mostly by *S. bayanus* (Table 3), which could be attractive to the consumers, based on the results of the sensory analysis (Figure 7). However, our experiments also highlighted some less favourable properties, such as the fact that *S. japonicus* can significantly increase the overall acidity of the beverage, which was described negatively by sensory evaluators (Figure 7). This agrees with recent findings on organic acid accumulation in *S. japonicus* [14,15,27]. In contrast, *S. pombe* is widely applied to reduce activity in wine due to its malic acid-degrading ability [20–22].

Although the control sample received the highest flavour scores, the application of *S. bayanus* and *S. pombe* var. *malidevorans* could also be adequate to create pleasing products for the consumers (Figure 7). Beverages fermented by these strains received higher scores for colour and aroma, furthermore, the evaluators described these samples by such positive attributes as honey, spicy, caramel, refreshing and citric. In contrast, overall, the application of *S. japonicus* was the least appealing for the consumers (Figure 7); however, this could also be improved by combined fermentation using multiple yeast strains, similar to winemaking [20]. In other words, the tested strains may be suitable for beer fermentation, but further studies are needed to determine how to maintain or possibly increase their positive and reduce their negative properties. *S. bayanus* and *S. pombe* var. *malidevorans* could be the most suitable strains. We believe that these data can contribute to the development of appropriate mixed starter cultures, thereby creating a new and more

complex aroma profile. A further possible way to improve quality of beer could be the application of different flavouring and colouring agents, such as fruits and their products, herbs, spices, vegetable juices or extracts.

Considering that all tested strains could be applied in the brewing industry based on analytical measurements, the results of the sensory analysis should be the most important factor during the evaluation of the yeasts. Although the control sample received the highest flavour scores, the application of *S. bayanus* and *S. pombe* var. *malidevorans* could also be suitable to create consumer-pleasing products. Beverages fermented by these strains received higher scores for colour and aroma; furthermore, the evaluators described these samples by such positive attributes as honey, spicy, caramel, refreshing and citric. In contrast, overall, the application of *S. japonicus* was the least appealing to consumers, overall, but this could also be improved by a combined fermentation with several yeast strains.

In order to improve the analysed parameters, colouring and flavouring substances could be tested with different plant-based products, and other types of beer should also be analysed in further studies, as the choice of raw materials could also have a considerable influence on the quality and nutritional parameters, as well as on the volatile substances. In other words, the tested strains may be suitable for beer fermentation, but further studies are needed to determine how to maintain or possibly increase their positive and reduce their negative properties. *S. bayanus* and *S. pombe* var. *malidevorans* appear to be the most promising strains. We believe that these data can contribute to the development of appropriate mixed starter cultures, thereby creating new and more complex beer styles.

## 5. Conclusions

During the investigation of *S. bayanus* budding yeast, *S. japonicus* and *S. pombe* fission yeasts, various findings were reached regarding their applicability in the brewing industry. Inoculating the same wort with varying strains of yeast may cause significant changes in specific parameters.

Fermentation with *S. bayanus* resulted in significantly higher TPC and quite similar flavonoid content compared to *S. cerevisiae*. Its element content was mostly identical, except for a slightly higher Na and Ca concentration. Regarding the beer quality parameters, this sample showed the highest colour intensity and 20% higher acidity than the control sample. Its aroma profile was similar too. Still, it contained more aroma compounds, such as phenylethyl acetate, which provided a rose and honey aroma, compared to the one fermented with *S. cerevisiae*. On the other hand, it also contained sour isobutyric acid. During the sensory assessment, this sample received the highest score for its aroma, and its colour and foam score could also be considered high.

*S. pombe* increased the polyphenol content of beer significantly, but unfortunately, it also decreased its flavonoid content by 10–20%. P and S content of this beer was like the control sample; a slight increase could be observed in its Ca content, and Na content of the beers fermented with *S. pombe* was lower than the control sample's in every case. Like *S. bayanus*, the application of *S. pombe* resulted in slightly higher colour intensity and significantly higher acidity. This sample was the richest in aroma compounds, containing desirable compounds exclusively, such as methylpyrazine (roasted, peanut), 2(5H)-furanone (curry, walnut, sweet), 2-acetylfuran (smoky, caramel), 1,3-butanediol (fruity), solerone (caramel, roasted), which the sensory evaluators also noticed, since this sample received a flavour score almost as high as the control sample. Furthermore, its aroma and colour were also as desirable as those of the sample fermented with *S. cerevisiae*.

Regarding the nutritional value, the other *Scizosaccharomyces* strain's samples showed similar results to the previously described samples fermented with *S. pombe*, except for the outstanding Ca content and slightly lower P content measured. The fermentation with this

yeast strain resulted in the lowest colour intensity and an exceptionally high total acidity; however, this character was not desirable according to the sensory assessment, since this sample received the lowest flavour scores, which is also confirmed by the low number of volatile compounds present in the sample.

Unfortunately, out of these compounds, several may cause undesirable flavors, such as sour and cauliflower-like notes. On the other hand, this sample received the highest scores regarding its colour.

Based on our results, the investigated strains could be applied for brewing, considering all analyzed parameters; however, combined fermentation is strongly recommended in the case of *S. japonicus* due to its low flavour and aroma scores. The addition of colouring and flavouring agents could also increase the popularity of these products, but testing the fermentation of other beer types could also provide more information on these yeast strains.

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## Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of Variance
CE	Catechin Equivalent
EBC	European Brewing Convention
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography-Mass Spectrometry
IBU	International Bitterness Unit
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
NIST	National Institute of Science and Technology
TPC	Total Phenolic Content
YPA	Yeast Peptone Agar
YPL	Yeast Peptone Liquid

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