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Assessment of growth and fermentation of some yeasts on soybean residue hydrolysate

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Abstract:

This study explored the growth and fermentation capabilities of four distinct yeast strains, including *Saccharomyces cerevisiae var. boulardii* CNCM I-745, *S. cerevisiae* 7012, *S. cerevisiae* 7028, and *S. cerevisiae var. diastaticus* BE-134, with the aim of identifying the most suitable strain for the production of a fermented beverage from soybean residue hydrolysate (SRH). The results were as follows: *S. cerevisiae* 7012 exhibited the most efficient fermentation, with residual sugar content of 2.16 g/100 ml, an ethanol concentration of 1.39% v/v, and a favourable aroma, receiving a sensory score of 4.3/5 points. Within this sensory profile, the aromatic compound 2-phenylethanol was found to be the predominant component. Additionally, *S. cerevisiae var. boulardii* CNCM I-745 demonstrated superior biomass production ability, achieving a cell density of 7.71 log CFU/ml after 48 hours of fermentation solution also showed the highest antioxidant activity, equivalent to 1.63 mg of ascorbic acid per 100 ml. Consequently, these two yeast strains were selected for combined use in fermentation to leverage the probiotic characteristics of *S. cerevisiae var. boulardii* CNCM I-745 and the flavouring capacity of *S. cerevisiae* 7012 in creating fermented beverages from SRH.

Keywords: **fermentation, *S. cerevisiae*, soybean residue, yeast.**

Classification number: **3.5**

1. Introduction

Soybean residue, a by-product of the soy milk and tofu industries, contains an array of valuable nutrients that can be exploited. Through enzymatic hydrolysis, the concentration of solutes in soybean residue can be significantly enhanced, rendering it appropriate for the growth and fermentation of microorganisms. Fermentation not only facilitates the digestion and absorption of nutrients but also augments the nutritional value of soybean residue. This process can eliminate undesired odours, augment the quantity of digestible fibre, and synthesise aromatic compounds that enrich the sensory appeal of the product. In fact, soybean residue has been successfully fermented to produce a diversity of products, including soy sauce, jam, animal feed, food additives, and beverages [1, 2].

One strategy for altering the odour of soybean residue is through bioconversion using yeast. Certain yeasts and soybean lipases can break down the lipids in soybean residue, generating free fatty acids that can be partially metabolised via β-oxidation to create methyl ketones, secondary alcohols, esters, alkanes, and lactones [3]. Unwanted aldehydes can be oxidised by aldehyde dehydrogenases to form fatty acids or reduced by yeast alcohol dehydrogenases to form alcohols. Yeast alcohol acyltransferase can catalyse the reaction between alcohols and yeast metabolic intermediates to form esters. Yeast proteinases and peptidases can further decompose the proteins in soybean residue, and the resultant free amino acids can be metabolised by yeasts via the Ehrlich pathway to generate higher alcohols and esters [4].

Solid-state fermentation (SSF) of yeast on soybean residue has been demonstrated to enhance its nutritional quality. Fermentation leads to a rise in protein, ash, total polyphenolics, and antioxidant activity, whilst simultaneously reducing the raw fibre content, carbohydrates, and lipids. Furthermore, SSF promotes the bioconversion of isoflavones β-D-glucoside to aglycone forms via the action of β-glucosidases [5, 6].

Yeast fermentation may be employed to produce bio-flavours from soybean residue or to create a food ingredient with improved sensory characteristics. Dairy yeasts, owing to their enhanced lipolytic capability, typically metabolise the fat fraction of soybean residue into methyl ketones. Conversely, soybean residue fermented by wine yeasts contains a higher concentration of esters than that fermented by dairy yeasts [7].

Yarrowia lipolytica yeast was discovered to biotransform soybean residue by catabolising aldehydes and their metabolites mainly to methyl ketones. This resulted in a reduction in grassy, off-odour and a slightly pungent, musty, and cheese-like odour in the fermented soybean residue. The fermentation process yielded volatile aromatics, such as 3-methylbutanal and 2-phenylethanol, from amino acids. The fermented soybean residue exhibited increased levels of umami-tasting substances, a cheese-like odour, improved digestibility, and enhanced antioxidant capacity [8].

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According to research, the synergy of carbohydrase from Celluclast® 1.5L and Viscozyme® L with protease from *Y. lipolytica* resulted in soybean residue with elevated antioxidant activity and higher concentrations of total amino acids and ferulic acid post-fermentation. The interaction between carbohydrase and *Y. lipolytica* disrupted the secondary cell wall of the soybean residue, which may elucidate these findings [9].

S. cerevisiae var. boulardii is acknowledged as a probiotic and a vital constituent in producing biologically active substances through fermentation. This yeast strain aids in the synthesis of active phytochemicals, such as isoflavones [10] and phenolics [11], thus increasing the antioxidant capacity of the end product. Additionally, its utilisation enhances the bioavailability of essential minerals and B vitamins, whilst reducing the concentration of antinutrients like phytates [12]. The extracellular fraction of *S. boulardii* culture is enriched with polyphenolic metabolites, including vanillic acid, cinnamic acid, phenyl ethyl alcohol (rose oil), erythromycin, amphetamine, and vitamin B6. *S. boulardii* exhibits growth patterns comparable to *S. cerevisiae* but possesses greater stress tolerance. Moreover, *S. boulardii* comparatively produces 6-10 times more antioxidant potential, and 20-70 times higher total phenolics and flavonoids in the extracellular fraction [13].

Recently, there has been burgeoning interest in employing yeast to biosynthesise natural aromatic compounds from by-products, and yeast fermentation is regarded as a promising technique. Numerous yeasts have the ability to produce 2-phenylethanol (2-PE) from L-phenylalanine. 2-PE has varied applications in several industries, particularly in food and beverage, as it enhances the organoleptic properties of the final product [14].

In this study, four yeasts were evaluated for their growth and fermentation ability on soybean residue hydrolysed by protease and carbohydrase complexes. The study aimed to select a suitable yeast for producing fermented beverages from SRH.

2. Subject and methodology

2.1. Subject

Four yeasts were employed in this study, including *S. cerevisiae var. boulardii* CNCM I-745 as a yeast used for probiotic production activated from Bioflora (Biocodex, France); *S. cerevisiae* 7012 and *S. cerevisiae* 7028 as yeasts used in ethanol production obtained from the collection of School of Biotechnology and Food Technology, Hanoi University of Science and Technology; and *S. cerevisiae var. diastaticus* SafAle™ BE-134 as a dry yeast used in beer production obtained from Fermentis, France. These four yeast strains, *S. cerevisiae var. boulardii* CNCM I-745, *S. cerevisiae* 7012, *S. cerevisiae* 7028, and *S. cerevisiae var. diastaticus* SafAle™ BE-134, were marked as I-745, 7012, 7028, and BE-134, respectively.

SRH used in the study was prepared as follows: each soybean residue sample from Vinasoy Bac Ninh factory was autoclaved and mixed with water to achieve 5% w/w. The slurry was adjusted to pH 7.0 and hydrolysed with Alcalase® 2.4 L for 1 hour; further adjusted to pH 4.5 with H3PO4 10%, and hydrolysed with Viscozyme® L and Pectinex® Ultra SP-L for 3 hours [15]. The treated SRH was then supplemented with 2% w/w sucrose, boiled in water for 15 minutes and cooled to approximately 30°C before being inoculated with yeasts in sterile conditions.

The starter culture medium is 10°Brix malt, a 10% w/v of yeast extract, autoclaved at 121°C for 15 minutes. To prepare the secondary culture medium, pretreated SRH was centrifuged to remove the undissolved part, and the supernatant was autoclaved at 121°C for 15 minutes. The determination of yeast cell number and mass is discussed in a subsequent section.

2.2. Research methodology

Evaluation of growth ability of four yeasts on SRH: These strains, grown on malt-extracted agar, were inoculated with 10 ml of starter culture medium at 30°C for 24 hours. Then, 10 ml of inoculations with the same initial cell density were subcultured in 90 ml of secondary culture medium at 30°C for 48 hours. The number of yeast cells was determined every 2 hours using a Neubauer counting chamber.

Evaluation of fermentation ability of four yeasts on SRH: Four yeasts from malt-extracted agar were cultured in glass tubes containing 10 ml of starter culture medium at 30°C for 24 hours. Grade 1 seed was drawn up 1 ml into 9 ml of secondary medium for further propagation at 30°C for 24 hours. Ten ml of the secondary medium from each strain was transferred to a fermentation vessel containing 90 ml of SRH, to form a density of 6.7 log CFU yeast/ml. The amount of CO_2 released was determined based on the decreasing weight of the fermenter every 2 hours for 48 hours. Analysis was conducted both before and after 48 hours of fermentation for all four yeasts, including parameters such as: pH, acidity, total sugar content, soluble protein and soluble protein in TCA, phenolic content, antioxidant activity, alcohol content, composition of volatiles, and sensory analysis of flavour.

2.3. Analysis methodology

Analysis of total sugar: The analytical solution was completely hydrolysed in 2 hours with 25% HCl, then neutralised to pH 7.0 with 10% NaOH, titrated to the specified volume and then centrifuged to collect the supernatant before analysis. The reducing sugar content in the solution was determined by the DNS method through absorbance at 540 nm of the final product with DNS reagent using a D-glucose standard curve [16].

Analysis of soluble protein and soluble protein in TCA: The analytical solution was neutralised to pH 7.0 and centrifuged to collect the supernatant. The content of soluble protein or soluble protein in 20% (w/w) trichloroacetic acid (TCA) was determined by the Lowry method. The amount of protein in the sample was determined by the absorbance at 750 nm of the supernatant with Folin reagent using the BSA standard curve [17, 18].

Analysis of phenolic: The slurry was centrifuged to collect the supernatant, after which it was precipitated with absolute ethanol at a ratio of 1:1 to remove proteins. The phenolic content in the supernatant was determined by the Folin-Ciocalteu method through absorbance at 760 nm of the final product with the Folin-Ciocalteu reagent, using the gallic acid standard curve [19].

Analysis of ethanol: The slurry was centrifuged to collect the supernatant. Ethanol content was determined by the high-performance liquid chromatography (HPLC) method, using the HPLC system of Agilent Technologies 1200 series (Germany) with a Biorad 87H analytical column, RID detector.

Analysis of antioxidant capacity: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) analysis was carried out to determine the changes in antioxidant activity before and after fermentation. The supernatant of the slurry was precipitated with absolute methanol at a ratio of 1:1 to remove proteins before analysis. The scavenging activity of the analysis sample could be assayed by measuring the decrease in absorbance at 517 nm of the stable free radical DPPH. The antioxidant content in the solution was expressed as mg ascorbic acid equivalent (mg AAE) per 100 ml [20, 21].

Analysis of volatiles: The slurry sample was extracted with ethyl acetate at a ratio of 1:1, then centrifuged to collect the supernatant. This supernatant was further filtered through a 0.2 µm membrane and analysed by GC-MS using a SCION 456-GC gas chromatograph (Netherlands). Based on the NIST 17 spectral library, containing information on retention time and mass spectrometry data, the presence of different volatiles in the analyte was determined. Semi-quantitative analysis was performed using peak areas on an equal amount of analytical samples [22]. The relative peak area (RPA) for each major group of volatiles was expressed as a percentage of the total peak areas of those compounds over the total peak area of all compounds.

Sensory evaluation: Comparison of the samples before and after fermentation was done by a scoring test according to Vietnamese Standard 3215-79 to determine the degree of aroma difference between samples. The sensory panel consisted of 10 members who had been trained. Each tester received one answer sheet and all samples to be evaluated. The tester would rate the aroma intensity of each product through a corresponding score on a 6-point scale with terms describing the intensity of that property already specified. The results were then statistically processed. Scores on the 6-point scale are: 0 - Odour of spoiled or odourless product; 1 - Strange odour; 2 - Less fragrant, beany odour; 3 - Weak aroma, very little beany odour; 4 - Light scent, relatively harmonious, slightly alcoholic smell; 5 - Mild aroma, smelling like fermented fruit, creating a feeling of liking.

Statistical analysis: The test of significance for experimental data was performed using one-way analysis of variance (One-Way ANOVA) and Tukey's test using SPSS® 22.0 software for Windows (SPSS Inc., Chicago, IL, USA) to evaluate any statistical differences between the treatments at $p \leq 0.05$.

3. Results

The growth and fermentation capabilities of four distinct yeast strains are shown in growth curves (Fig. 1), amount of CO_2 produced during 48 hours of fermentation (Fig. 2), composition of fermentation slurries (Table 1) and the changes in the major groups of volatiles in the slurries before and after fermentation (Table 2), as outlined below.

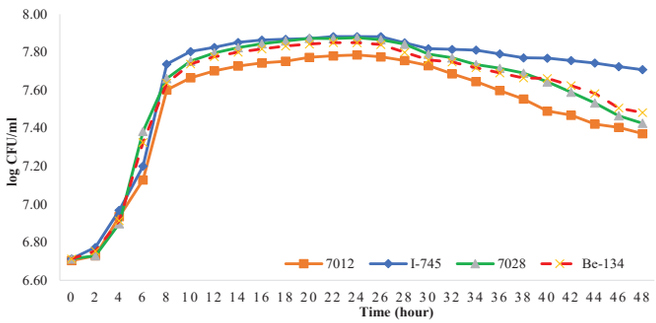

Fig. 1. Growth curves of the four yeast strains in pretreated SRH.

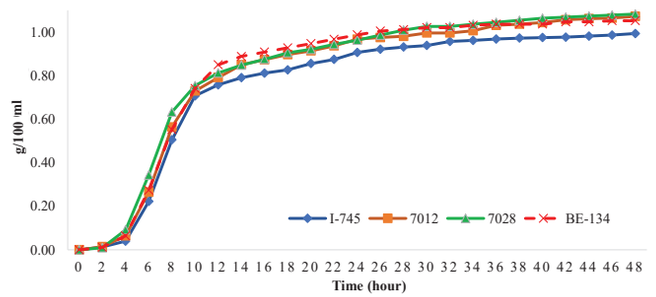

Fig. 2. Amount of CO_2 produced during 48 hours of fermentation by the four yeast strains.

Table 1. Composition of slurries before and after fermentation.

Analytical indicators	Before fermentation	After 48 hours of fermentation			
		I-745	*7012*	*7028*	*BE-134*
pH	$4.24^{a}\pm0.02$	$4.03^{b}\pm0.07$	$4.04^{b}\pm0.06$	$4.03^{b}\pm0.07$	$4.04^{b}\pm0.06$
Acidity (mg lactic/100 ml)	$385.9^{ab}\pm5.7$	$400.5^{a}\pm6.4$	$373.5^{b}\pm6.4$	$373.5^{b}\pm6.4$	$380.3^{b}\pm3.2$
°Brix	$5.25^{a}\pm0.07$	$3.15^{b}\pm0.07$	$3.00^{b}\pm0.14$	$3.00^{b}\pm0.14$	$3.05^{b}\pm0.07$
Total sugar (g/100 ml)	$4.70^{a}\pm0.04$	$2.29^{b}\pm0.04$	$2.16^{d}\pm0.02$	$2.20^{cd}\pm0.01$	$2.25^{bc}\pm0.01$
Ethanol (% v/v)	-	$1.23^{b}\pm0.07$	$1.39^{a}\pm0.03$	$1.25^{b}\pm0.07$	$1.36^{b}\pm0.02$
Soluble protein (mg/100 ml)	$617.7^{a}\pm11.8$	$451.3^{b}\pm16.6$	$468.3^{b}\pm20.7$	$465.0^{b}\pm23.0$	$459.3^{b}\pm21.5$
TCA-soluble protein (mg/100 ml)	$518.6^{a}\pm13.0$	$418.5^{b}\pm20.3$	$410.0^{b}\pm20.7$	$402.3^{b}\pm19.3$	$417.0^{b}\pm8.2$
Phenolic content (mg GAE/100 ml)	$31.10^{a}\pm2.31$	$30.08^{ab}\pm0.56$	$28.94^{b}\pm0.61$	$30.34^{ab}\pm0.87$	$31.60^{a}\pm1.83$
[@] mgAAE/100 ml	$0.63^{c}\pm0.04$	$1.63^{b}\pm0.02$	$1.50^{a}\pm0.02$	$1.50^{a}\pm0.04$	$1.51^{a}\pm0.04$
Sensory score	$1.20^{d}\pm0.42$	$2.90^{c}\pm0.57$	$4.30^{a}\pm0.48$	$2.70^{c}\pm0.48$	$3.60^{b}\pm0.52$

[a, b, c, d]: Statistical analysis using ANOVA at 95% confidence interval. Values with the same letter indicate no significant difference between the groups.
[@] Antioxidant capacity measured using DPPH scavenging assay. Antioxidant capacity is expressed as mg ascorbic acid equivalent (AAE)/100 ml.

Table 2. Some volatiles in the slurries before and after 48 hours of fermentation.

No	Content	Before fermentation		After 48 hours of fermentation							
				I-745		7012		7028		BE-134	
		S peak x 10^7	*RPA (%)*	*S peak x 10^7*	*RPA (%)*	*S peak x 10^7*	*RPA (%)*	*S peak x 10^7*	*RPA (%)*	*S peak x 10^7*	*RPA (%)*
1	2,4-Decadienal, (E,E)-	25.6	2.5	ND	ND	ND	ND	ND	ND	ND	ND
2	E-14-Hexadecenal	45.3	4.4	ND	ND	ND	ND	ND	ND	ND	ND
3	Oleic acid	96.2	9.4	80.7	4.4	21.1	1.3	44.5	1.2	67.4	3.5
4	Octadecanoic acid	79.7	7.8	ND	ND	ND	ND	ND	ND	ND	ND
5	2-Phenylethanol	7.53	0.7	502	27.3	648	39.0	564	15.3	614	31.6
6	Octanoic acid, ethyl ester	7.01	0.7	17.5	1.0	15.3	0.9	18.1	0.5	15.9	0.8
7	Decanoic acid, ethyl ester	ND	ND	10.5	0.6	12.5	0.9	12.5	0.3	14.6	0.8
8	Hexadecenoic acid, ethyl ester	ND	ND	68.8	3.7	52.7	3.2	64.2	1.7	63.1	3.3
9	Decanoic acid, 2-phenylethyl ester	ND	ND	9.58	0.5	6.02	0.4	12.6	0.3	12.3	0.6
10	Linoleic acid ethyl ester	ND	ND	137	7.4	63.0	3.8	93.3	2.5	117	6.0

ND: not detected.

4. Discussion

4.1. Evaluation of the growing ability of yeasts

The results in Fig. 1 demonstrate that, with the same initial cell density, all yeast strains grow well on the slurries. In this, the cell density increased from hour 2 to hour 10, then stabilised from hour 10 to hour 24 and began decreasing gradually from hour 26. All yeasts increased in density 10-12 times (from 6.7 to 7.8 log CFU/ml). I-745 is the yeast with the highest cell density in the fermentation broth. In the first 6 hours of culture, the density of this strain was equivalent to or even lower than the other 3 strains, but from the 8th hour, this strain increased in biomass very quickly and consistently reached the highest cell density until the end of fermentation. This result is consistent with the findings of Joaquín Mulero-Cerezo, et al. (2019) [23]. Thus, yeast strain I-745 is a probiotic that can grow well and maintain a large cell density in the SRH. High yeast growth is advantageous since our purpose aimed to create probiotic drinks from the SRH.

4.2. Evaluation of the fermentation ability of yeasts

The results in Fig. 2 show that, with the same cell density at hour 0, all yeasts have fermented well on the enzymatically treated slurries. Three yeast strains 7012, 7028 and BE-134 released almost the same amount of CO_2. I-745 has the highest cell density in the fermentation broth but the amount of CO_2 released by this yeast is the lowest.

The results in Table 1 demonstrate that although the pH of the post-fermentation slurries has not been reduced compared to that of the pre-fermentation, the acidity of the post-fermentation slurries has not changed substantially. The fermented slurry from I-745 has the highest fermentation acidity at 400.5 mg/100 ml.

After 48 hours of fermentation, all 4 yeast strains reduced the soluble dry matter content of the slurries from about 5 to 3°Brix. The total sugar content after fermentation differed; the remaining total sugar after fermentation by 7012 was the lowest, and was the highest remaining by I-745. The ethanol content generated by the four yeasts after 48 hours of fermentation fluctuated in the range of 1.23-1.39% v/v. In which 7012 produced the most, I-745 produced the least ethanol among the four strains. Due to the characteristics of a probiotic strain, *S. boulardii* CNCM I-745 tends to generate biomass mainly and does not favour the production of alcohol. Thus, 7012 was the best fermenting strain among the four yeast strains with the lowest residual sugar of 2.16 g/100 ml and the highest amount of ethanol generated at 1.39% v/v.

Soluble protein contents were significantly reduced after fermentation in all samples; this is due to the proteolytic activity of yeasts [24]. The contents of TCA-soluble protein also decreased after fermentation but still a certain amount of about >400 mg/100 ml (varied by each strain). This amount was quite close to the amount of soluble protein. Thus, it can be confirmed that soluble proteins are mainly short-chain peptides and amino acids. These differences were not significant among strains after 48 hour of fermentation.

The phenolic content of the samples did not increase after fermentation; even some samples decreased slightly compared to before fermentation. According to W.C. Vong, et al. (2019) [25], some phenolics metabolised during fermentation may be reduced or produced. Therefore, this amount changes without a specific rule and needs further research, but the reduction is insignificant, and after fermentation, there was still a certain amount of phenolics in the slurries.

The antioxidant capacity after fermentation of all four yeasts increased compared to the unfermented slurry. In the fermented slurries, there were certain amounts of short-chain peptides and certain contents of phenolics; these components contributed to the antioxidant capacity of the slurries. In addition, yeast cells themselves have also been recognised as a source of antioxidants.

Yeast has the ability to synthesise a number of biologically active compounds that can act as antioxidants such as glutathione [26], coenzyme Q or ubiquinone [27], carotenoids, etc. Some products metabolised by yeast after fermentation, some substances in cell components such as soluble proteins, sulphur-containing amino acids, β-glucan in yeast cell walls... also contribute to the ability to create this antioxidant ability [28]. The fermented slurries of I-745 exhibited the highest antioxidant activity. The antioxidant activity of *S. boulardii* higher than that of S. cerevisiae has also been reported by some authors [23, 29].

In terms of organoleptic quality, all yeasts showed a significant improvement in the aroma of the slurry compared to before fermentation, and the differences were statistically significant. The fermentation sample using 7012 was evaluated as the best aroma sample among the four yeasts with a sensory score of 4.3/5 points. The fermentation slurry has a light aroma, similar to that of fermented rice, relatively harmonious, with a slight alcohol smell.

4.3. Changing of volatile compounds after 48 hours fermentation of four yeasts

The results in Table 2 show that the hydrolysate slurry before fermentation still contains some undesirable odorous components, which are aldehydes that cause unpleasant odours such as 2,4-Decadienal, (E,E), E-14-Hexadecenal. But after fermentation, the aldehyde components were completely lost. This result is consistent with the report of W.C. Vong, et al. (2016) [8], in fresh soybean residue, volatile components are mainly aldehydes, these substances tend to decrease during heat treatment and fermentation.

The SRH also contained a large number of fatty acids such as oleic, octadecanoic, which were fatty acids available in the raw materials. Fatty acids tended to be completely or partially reduced in the post-fermentation slurry depending on the yeast strain, the most in the fermentation slurry of strain 7012 and the least in that of I-745.

The fermentation products by four yeasts contained several higher alcohols, typically 2-phenylethanol and esters. The results in Table 2 show that the composition of esters produced by the fermenting strains varied depending on the strain. The total amount was about 5.3-13.2% of the total volatile matter in the fermentation solution. I-745 was the most ester-producing strain of the four yeast strains.

2-phenylethanol was significantly high in the aroma spectrum of all strains, accounting for about 27.3-39.0% depending on the yeast strain, so this component greatly affected the sensory value of the fermentation slurries. Currently, this compound is considered to be very valuable and is being exploited through the biosynthetic pathway by yeast fermentation. Many yeasts, especially *S. cerevisiae*, are capable of producing 2-phenylethanol using L-Phe as a precursor [14]. The SRH slurry contained a lot of amino acid components, under the action of yeast, it produced volatile aromatic compounds in which 2-phenylethanol accounted for a very high proportion. The 7012 strains had the highest 2-phenylethanol content in the fermentation slurry. This result was consistent with the sensory evaluation results.

5. Conclusions

The experimental results above demonstrate that all four yeast strains thrived and fermented effectively on the SRH. Among them, *S. cerevisiae* 7012 was the best fermenting yeast, with residual sugar at only 2.16 g/100 ml, an ethanol content of 1.39% v/v, and the most appealing aroma, achieving a sensory score of 4.3/5 points. Within this aroma, the aromatic compound 2-phenylethanol accounted for the highest proportion. Conversely, the probiotic *S. boulardii* CNCM I-745 exhibited the most robust biomass production ability, achieving a cell density of 7.71 log CFU/ml after 48 hours, and the fermentation solution possessed the highest antioxidant activity, equivalent to 1.63 mg of ascorbic acid per 100 ml. Consequently, these two yeast strains were selected to be used in tandem for fermentation, capitalising on the probiotic properties of *S. boulardii* CNCM I-745 and the flavouring capacity of *S. cerevisiae* 7012 for fermented beverages derived from SRH.

CRediT author statement

Thi Van Anh Mai: Collected data and Wrote the paper; Kim Loan Nguyen and Dinh Trien Dang: Co-authors who collected a part of data; Thi Xuan Sam Nguyen and Thanh Hang Nguyen: Co-authors who provided research guidance.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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