# Comparison of the yeast *Saccharomyces cerevisiae var. boulardii* and top-fermenting brewing yeast strains during the fermentation of model nutrient media and beer wort

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Abstract. Recently, the yeast Saccharomyces cerevisiae var. boulardii have attracted the attention of Food Science researchers due to their unique properties, the main among which are probiotics. Thus, research is conducted on the use of this yeast as a starter culture in the technology of vogurt, fermented vegetables, fruit, vegetable juices, as well as beer. This paper is aimed at studying Saccharomyces cerevisiae var. boulardii 's fermentation performance compared to top-fermenting brewing yeast strains during fermentation of model nutrient media and beer wort. Fermentation activity of the studied strains was assessed based on the character of fermentation curves, as well as the values of the maximum substrate assimilation rate and apparent degree of fermentation. Moreover, during the study, beer was produced using the yeast Saccharomyces cerevisiae var. boulardii as a starter culture. According to the obtained results, it can be concluded that Saccharomyces cerevisiae var. boulardii have less fermentation activity compared to brewing strains. In turn, beer produced with Saccharomyces cerevisiae var. boulardii significantly differed in physicochemical, microbiological, and organoleptic parameters from the control sample obtained using the 047A brewing strain. Thus, it contained less ethanol and secondary metabolites; however, the concentration of living cells was significantly higher, which indicates a relatively high viability of the yeast Saccharomyces cerevisiae var. boulardii. From an organoleptic point of view, final beer has a positive sensory profile. The aroma of the product had a complex character: it included caramel, spicy, fruity and phenolic notes, as well as smoked and wine elements; while honey was the dominant note of the taste.

Key words: brewing, functional beer, fermented drinks, fermentation activity, probiotics, Saccharomyces cerevisiae var. boulardii, yeast.

# **INTRODUCTION**

The yeast *Saccharomyces cerevisiae var. boulardii* is currently the only registered eukaryotic probiotic microorganism recommended as a drug for both adults and children (Vandenplas et al., 2008; Łukaszewicz, 2012).

Initially, this yeast culture was identified as a separate species of the genus *Saccharomyces*. However, it was further shown that this yeast is a strain of the species *Saccharomyces cerevisiae* (Pais et al., 2020). It was found that *Saccharomyces cerevisiae* var. boulardii and *Saccharomyces cerevisiae* are more than 99% similar in terms of genome sequence considering Average Nucleotide Identity (ANI). At the same time, using the methods of molecular phylogenetics and typing, it was shown that *Saccharomyces cerevisiae* var. boulardii form a separate cluster within the species *Saccharomyces cerevisiae*, which is closer to wine yeast strains. Comparative genomic hybridization experiments also established that *Saccharomyces cerevisiae var. boulardii* and *Saccharomyces cerevisiae* are different strains of the same species but the loss of all intact Ty1/2 elements was reported only in *Saccharomyces cerevisiae var. boulardii*, which is one of the key differences from a genetic view point (Mitterdorfer et al., 2002; Khatri et al., 2017).

Despite the similarity from a genetic point of view, the yeast *Saccharomyces cerevisiae var. boulardii* significantly differ in physiological properties from other industrial and laboratory strains of *Saccharomyces cerevisiae* (see Table 1) (Pais et al., 2020). In particular, they are more resistant to both high temperature and acidity of the environment; unable to use galactose as a carbon source; and always diploid.

Physiological feature	Saccharomyces cerevisiae	Saccharomyces cerevisiae var. boulardii
Optimum growth temperature	30 °C	37 °C
Resistance to high temperature (52 °C)	45% viability	65% viability
Resistance to high acidity $(pH = 2 \text{ for } 1 \text{ hour})$	30% viability	75% viability
Assimilation of galactose	+	-
Ploidy	diploid and haploid	diploid

**Table 1.** Physiological features of Saccharomyces cerevisiae var. boulardii and Saccharomycescerevisiae (Pais et al., 2020)

The resistance of the probiotic strain to the high acidity of the medium can be explained by the cell wall characteristics. Hudson et al. (2016) showed that *Saccharomyces cerevisiae var. boulardii* have a thickened cell wall compared to laboratory strains of *Saccharomyces cerevisiae* (*BY4741 and W303*). When yeast culture was treated with the antifungal drug caspofungin, which inhibits  $\beta$ -1,3-D-glucan synthesis, and then placed in a nutrient medium with different acidity, a significant decrease in growth was observed at low pH values, indicating that the cell wall is responsible for the resistance of *Saccharomyces cerevisiae var. boulardii* to high acidity.

Another important property of *Saccharomyces cerevisiae var. boulardii* is that they are highly osmotolerant, which consists in maintaining the ability to ferment the substrate and multiply in environments with a high sugar concentration. Thus, Ávila-Reyes et al. (2016) reveled that even at sucrose concentrations more than 60%, this yeast strain showed little biomass growth and formed ethanol  $(1.5\% - v v^{-1})$ .

The listed properties of Saccharomyces cerevisiae var. boulardii, which can be called techno-functional, have attracted the attention of Food Science researchers. We can find some scientific data on the use of this yeast as a starter culture in the technology of yogurt (Lourens-Hattingh & Viljoen, 2001; Karaolis et al., 2013), fermented vegetables (Campbell et al., 2016), fruit and vegetable juices (Fratianni et al., 2014; Değirmencioğlu et al., 2016) as well as beer (Capece et al., 2018; Mulero-Cerezo et al., 2019; Pereira de Paula et al., 2021). In addition to the fact that the use of yeast as a starter culture allows imparting probiotic properties to food products this yeast strain improves its functionality and the functionality of accompanying bacteria in fermented food products. This effect is related to the capacity of Saccharomyces cerevisiae var. boulardii strains to stabilize pH and acidity of food matrices for prolonged periods of time. However, in some cases Saccharomyces cerevisiae var. boulardii may affect the sensorial characteristics of finished food products. Besides, it has an interesting beneficial effect on the nutritional value of foods since they synthesize folates and eliminate phytates and other antinutrients. In addition, Saccharomyces cerevisiae var. boulardii generates several metabolites or nutraceuticals known to exert positive health benefits (Lazo-Vélez et al., 2018).

The use of Saccharomyces cerevisiae var. boulardii in brewing is of a particular interest because strains of the species to which this culture belongs have been used for centuries as one of the indispensable raw materials in beer production. Although the use of *Saccharomyces cerevisiae var. boulardii* in brewing is considered by researchers as very promising; for the producer, in practical implementation, it seems extremely important to know how this yeast culture differs from traditional brewing strains in terms of fermentation performance. Thus, this research is aimed at studying *Saccharomyces cerevisiae var. boulardii* 's fermentation potential compared to top-fermenting brewing yeast strains during fermentation of model nutrient media and beer wort.

## **MATERIALS AND METHODS**

## **Objects of study**

Research objects were top-fermenting brewer's yeast strains (the culture bank of the BSG laboratory, Russia): 002A, 047A, 052A, and Saccharomyces cerevisiae var. boulardii (coded with S.c.b) isolated from Enterol® (Biocodex, France). Some characteristics of brewing yeast strains (002A, 047A, 052A) are presented in Table 2.

	Yeast strain			
Characteristics	002A	047A	052A	
Beer style	India pale ale	Wheat beer	Pale ale	
Optimal fermentation temperature, °C	18–20	20-22	18–24	
Flocculation	Medium	Low	Medium	

Table 2. The characteristics of the top-fermenting brewer's yeast strains

## Cultivation of yeast

Pure yeast cultures were stored on the YPD-agar (1% yeast extract, 2% peptone, 2% glucose, 2% agar) at 4 °C. The inoculum was grown in simple batch culture on YPD medium (1% yeast extract, 2% peptone, 2% glucose) by successive transfers with increasing amounts of nutrient medium. The multiplicity of increasing the volume of

medium in relation to the inoculum was 4:1. The flasks were filled with nutrient medium to 1/3 of the volume. The reseeding of the yeast culture was performed after the cells passed from the exponential growth stage to the stationary stage. Cultivation was proceeded at 28 °C.

# Study of the kinetics of the fermentation process on model nutrient media

The fermentation kinetics of *Saccharomyces cerevisiae 002A*, 047A, 052A strains, and *Saccharomyces cerevisiae var. boulardii* was studied on the following model media of different compositions:

- Medium 1: malt wort prepared from malt extract (Muntons 'Light', England)

- Medium 2: 1% yeast extract, 2% peptone, 9% glucose (YPD)

- Medium 3: 1% yeast extract, 2% peptone, 9% maltose (YPM).

The initial content of dry matter in nutrient media accounted for 12%, and pH was 5.5. The media were sterilized at 121 °C (1.1 atm) for 20 minutes. The fermentation process was performed for seven days in a simple batch culture in 100 mL Erlenmeyer flasks filled with 30 % sterile nutrient media at 20 °C. The pitching rate was  $12 \times 10^6$  cells mL<sup>-1</sup>. During the fermentation process, the concentration of the extract (g (100 g)<sup>-1</sup>) was determined by refractometer (PTR-46, Index Instruments Ltd, England). The value of pH was determined on titrator (848 Titrino plus, Metrohm AG, Switzerland).

Based on the obtained data, the rate of substrate assimilation ( $V_{assim}$ , Eq. 1) and apparent degree of fermentation (ADF, Eq. 2) were calculated.

$$V_{assim.} = \frac{\Delta S}{\Delta \tau}$$
(1)

where  $\Delta S$  – the change in substrate concentration (g (100 g)<sup>-1</sup>) over a period of time  $\Delta \tau$  (h).

$$ADF = \frac{OE - AE}{OE} \times 100\%$$
 (2)

where OE – original extract (% – m m<sup>-1</sup>); AE – apparent extract (% – m m<sup>-1</sup>) (Briggs, 2004).

# Processing of beer using *Saccharomyces cerevisiae var. boulardii* and top-fermenting brewer's yeast strains 047A

Industrial beer wort provided by OJSC Baltika (St. Petersburg, Russia) was used to produce beer with *Saccharomyces cerevisiae var. boulardii*. Table 3 shows the main physicochemical parameters of beer

wort used in the experiment.

To dete	rmine th	e com	pletion	
time of the	wort fee	rmenta	tion in	
connection	with	the	strain	
characteristics of the starter cultures,				
the real degree of fermentation (RDF)				

	•			
Indicator	Original wort extract	pН	Bitterness (IBU)	FAN (mg L <sup>-1</sup> )
	(0/1)		(IDO)	(mg L)

250

Table 3. Quality indicators of hopped wort

strain	maleutor	$(\% - m m^{-1})$	pm	(IBU)
cultures,	Value	11.85	5.15	20
n (RDF)				

was calculated for both strains according to the Eq. 3 (Briggs, 2004).

$$RDF = \frac{OE - RE}{OE} \times 100\%$$
(3)

where OE – original extract (% – m m<sup>-1</sup>); RE – real extract (% – m m<sup>-1</sup>).

The real degree of fermentation for S.c.b. was 62.8%, for 047A strain - 70.3%.

The fermentation process was performed in five-liter flasks, filled with 70% hopped wort (3.5 L). The concentration of yeast cells at the beginning of fermentation was  $12 \times 10^6$  cells mL<sup>-1</sup>. The process of obtaining beer included two stages: a) fermentation of the wort at the temperature of 20 °C until the RDF was reached; b) maturation of beer.

The maturation process included the bottling young beer after fermentation under aseptic conditions in 1.5-liter PET bottles. The degree of filling the bottles was 90%. Then, sterile glucose solution was added to each bottle at the rate of 0.5% carbohydrate content, and the bottles were closed with plastic plugs. For carbonation, young beer was kept in PET bottles for one day at  $20 \pm 2$  °C, cooled to the temperature of 5 °C, and then kept for seven days.

#### Analysis of young and final beer

In young and final beer, the real extract (RE,  $\% - m m^{-1}$ ), alcohol concentration ( $\% - v v^{-1}$ ), and the real degree of fermentation (RDF, %) were determined using an Alcolyzer Plus Beer analyzer (Anton Paar, Austria). The pH value was determined by an 848 Titrino plus titrator (Metrohm AG, Switzerland). The total concentration of yeast cells was determined using a Goryaev chamber while the concentration of living and dead cells was determined by staining with methylene blue.

The concentrations of secondary metabolites in beer samples were determined by gas chromatography using Hewlett-Packard - HP 6890 GC System with flame ionization detector (Agilent Technologies Inc., USA). Finally, the organoleptic indicators of beer (smell and taste) were assessed by certified tasters of OJSC Baltika (St. Petersburg, Russia).

# Statistical analysis

All experiments were performed in triplicate, the data were processed by the method of mathematical statistics with using MS Excel, finding the confidence interval and a probability of 0.95.

#### **RESULTS AND DISCUSSION**

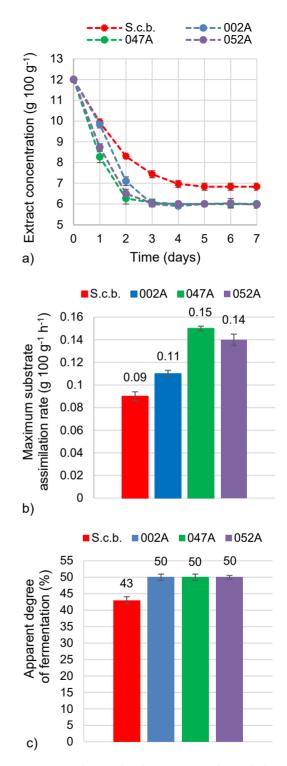
#### Study the kinetics of the fermentation process on model nutrient media

Fermentation activity is a key property of yeast, which partly determines its use in the fermented beverage industry, including brewing. For this reason, the first stage of the study was to compare the fermentation activity of *Saccharomyces cerevisiae var. boulardii* (coded with *S.c.b*) and top-fermenting brewing strains (002A, 047A, 052A) during fermentation of unhopped beer wort made from malt extract (medium 1). Fermentation activity of the studied strains was assessed based on the character of fermentation curves (Fig. 1, a), as well as the values of the maximum substrate assimilation rate (Fig. 1, b) and apparent degree of fermentation (Fig. 1, c).

According to the obtained data, it can be concluded that the yeast *Saccharomyces cerevisiae var. boulardii* have less fermentation activity compared to brewing strains. Firstly, this yeast culture can be characterized by a relatively low maximum substrate assimilation rate -  $0.090 \pm 0.005$  g (100 g)<sup>-1</sup> h<sup>-1</sup> (Fig. 1, b), which entails a longer fermentation process duration. Thus, there were required five days to complete fermentation for *Saccharomyces cerevisiae var. boulardii*, while for brewing strains this

period accounted for three days (Fig. 1, a). Secondly, the apparent degree of fermentation in the case of Saccharomyces cerevisiae var. boulardii was approximately 7% lower top-fermenting brewing than the strains (Fig. 1, c), which indicates the inability of this yeast culture to ferment all the beer wort sugars. The latter fact was also noted by other researchers; for example, according to Pereira de Paula et al. (2021) when producing wheat beer using Saccharomyces cerevisiae var. boulardii, the degree of fermentation was 6.82% less compared to the control strain Saccharomyces cerevisiae WB-06. Capece et al. (2018) noted not only a lower degree of attenuation of the yeast Saccharomyces cerevisiae var. boulardii compared to brewing strains (10.29-19.85% less), but also a decrease in this indicator when obtaining beer using the cofermentation process. Therefore, the inclusion of Saccharomyces cerevisiae var. boulardii in the composition of the mixed-starter culture in the ratio with Saccharomyces cerevisiae 1:1 led to the decrease in the degree of fermentation by 1.85% in average.

As it is known, the carbohydrate composition of beer wort is mainly represented by sugars fermentable by brewing strains such as glucose (10-15%), maltose (50-60%) and maltotriose (15-20%), as well as non-fermentable dextrins (20-30%) (Stewart, 2016). Apparently, as it was abovementioned, a lower degree of fermentation may be due to the inability of the yeast Saccharomyces cerevisiae var. boulardii to assimilate all wort sugars. In its turn, the relatively low rate of substrate assimilation during the fermentation of beer wort may be associated with a low assimilation rate of the quantitatively dominant sugar -

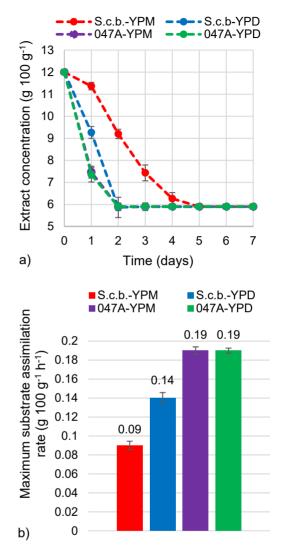


**Figure 1.** Change in the concentration of the extract (a); maximum substrate assimilation rate (b); apparent degree of fermentation (c).

maltose. To confirm these assumptions, fermentation with *Saccharomyces cerevisiae* var. boulardii was performed in model nutrient media (YPD and YPM), which differed

only in the type sugar, and brewing strain 047A was taken as a control.

Based on the presented data, it concluded can be that the Saccharomyces cerevisiae var boulardii is completely capable of fermenting maltose (Fig. 2, a), although the consumption rate of this disaccharide is significantly inferior to the rate of glucose fermentation (Fig. 2, b). In contrast, in the case of control (strain 047A), the rates of glucose and maltose consumption were approximately equal and significantly higher than those of Saccharomyces cerevisiae var. boulardii (Fig. 2, b), which is typical for brewing strains. In addition, a lag noticeable phase was on the fermentation curve (Fig. 2, a) during the fermentation of the medium with maltose (YPM). All this indicates a relatively low maltase activity of the yeast Saccharomyces cerevisiae var. *boulardii*. It is also worth noting that the values of the maximum rates of substrate assimilation during the fermentation of unhopped beer wort and the model medium with maltose are equal (Fig. 1, b and 2, b). This means that in the kinetics of beer wort fermentation Saccharomyces with cerevisiae var. boulardii, the process of maltose assimilation is decisive. Since both glucose and maltose are fermentable sugars by Saccharomyces cerevisiae var. boulardii, the reason



**Figure 2.** Change in the concentration of the extract (a); maximum substrate assimilation rate (b).

for the low apparent attenuation in beer wort fermentation seems to be related to the inability of this strain to consume maltotriose or to the extremely low rate of this process.

In addition, it is interesting to note that for *Saccharomyces cerevisiae var*. *boulardii*, the biomass yield on the maltose medium was higher than on the glucose one -  $9.15 \pm 0.18$  and  $6.98 \pm 0.30\%$ , respectively (data not presented). This observation suggests that the cultivation of this yeast strain on maltose as the main carbon source, despite the significant duration (five days), can be effective. However, we may assume that this process can be intensified by selecting cultivation modes due to varying such

factors as temperature, agitation, aeration, and initial sugar concentration. In addition, ultrasound is recognized as a promising tool for intensifying the enzymatic activity of microorganisms, which may be also applied to *Saccharomyces cerevisiae var. boulardii* (Suchkova et al., 2014).

# Processing of beers using *Saccharomyces cerevisiae var. boulardii* and top-fermenting brewer's yeast strains 047A, and their analysis

The final stage of the work was to produce beer from industrial hoped beer wort using the yeast *Saccharomyces cerevisiae var. boulardii* as a starter culture. For the purposes of obtaining beer with potential probiotic properties, the product has to be unfiltered and unpasteurized; in fact, wheat beer often possesses these criteria. Thus, strain 047A, which is intended for the production of wheat beer, was taken as a control. In addition to monitoring the main fermentation process by changes in real extract and ethanol concentrations (Fig. 3), the basic physicochemical and microbiological analysis of young and final beers was performed (Table 4) as well as determination of secondary metabolites concentrations (Table 5). Finally, an organoleptic analysis of the final products was made.

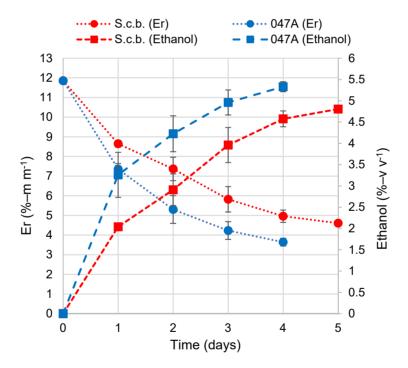


Figure 3. Change in the real extract and alcohol concentration during the main fermentation.

Fig. 3 shows the change in the real extract (Er) and alcohol concentration during the main fermentation process. From the presented data, we can conclude that the real degree of fermentation of the wort using 047A strain was achieved in four days, while for *Saccharomyces cerevisiae var. boulardii* five days were required. The difference in real degree of fermentation (RDF) between strains was 11.9%, which corresponds to the concentration range of maltotriose in beer wort. As expected, a beer sample made using

*Saccharomyces cerevisiae var. boulardii* was characterized by a high content of residual extract and, accordingly, a lower ethanol concentration (Table 4).

According to our research, in young beer, obtained using a probiotic strain (*S.c.b.*), the concentration of living cells was quite high and amounted to 8.29 million mL<sup>-1</sup> compared to the control, which was 2.10 million mL<sup>-1</sup> (Table 4). Although both the total concentration of cells and living cells decreased during the maturation process, the total concentration of cells and living cells was still quite high in the finished product compared to the control: 2.90 million mL<sup>-1</sup> and 1.17 million mL<sup>-1</sup>, respectively. Consequently, the yeast *Saccharomyces cerevisiae var. boulardii* demonstrate not only low flocculation activity, but also high viability. The relatively high viability of the probiotic strain is also noted by Mulero-Cerezo et al. (2019). Thus, a sample of craft beer obtained with *Saccharomyces cerevisiae var. boulardii*, after 45 days of storage, contained 8.3  $\pm$  1.4 $\times$ 10<sup>6</sup> CFU mL<sup>-1</sup>, while the control sample (beer produced using the *SF- 04* brewing strain) contained only 1.1  $\pm$  0.2 $\times$ 10<sup>5</sup> CFU mL<sup>-1</sup>.

Table 4. Results of physicochemical and microbiological analysis of young and final beers

Indicator	Young beer		Final beer	
Indicator	<i>S.c.b.</i>	047A	S.c.b.	047A
$Er(\% - m m^{-1})$	$4.61\pm0.07$	$3.64\pm0.09$	$4.46\pm0.12$	$3.52\pm0.13$
Ethanol ( $\% - v v^{-1}$ )	$4.81\pm0.13$	$5.33\pm0.21$	$5.56\pm0.04$	$5.85\pm0.18$
RDF (%)	$62.79\pm0.87$	$70.38 \pm 1.33$	$66.84\pm0.94$	$72.96 \pm 1.76$
pH	$4.21\pm0.17$	$4.06\pm0.08$	$4.26\pm0.11$	$4.10\pm0.07$
Cell concentration (million mL <sup>-1</sup> )	$9.67 \pm 1.45$	$2.96\pm0.44$	$2.90\pm0.43$	$0.90\pm0.14$
Concentration of living cells	$8.29 \pm 1.24$	$2.10\pm0.32$	$1.17\pm0.18$	$0.15\pm0.02$
(million mL <sup>-1</sup> )				

	Organoleptic	Young beer		Final beer	
Compound	threshold (mg L <sup>-1</sup> ) <sup>1</sup>	<i>S.c.b</i> .	047A	<i>S.c.b</i> .	047A
	Aldehydes				
Acetaldehyde	25.00	$10.02\pm1.50$	$16.61\pm2.50$	$29.73\pm4.46$	$19.13\pm2.87$
	Vicinal dike	tones			
Diacetyl	0.15	< 0.01	$1.12\pm0.17$	< 0.01	$0.56\pm0.08$
2,3-Pentanedione	1.00	< 0.01	$0.14\pm0.02$	< 0.01	< 0.01
	Higher alcol	nols			
n-Propanol	800.00	$16.65\pm2.50$	$27.73\pm4.16$	$20.82\pm3.12$	$39.14\pm5.87$
Isobutyl alcohol	200.00	$21.16\pm3.17$	$85.48 \pm 12.82$	$23.83\pm3.57$	$97.56 \pm 14.63$
Amyl alcohol	65.00	$68.13\pm10.22$	$100.01 \pm 15.00$	$74.16\pm11.12$	$109.60 \pm 16.44$
	Esters				
Ethyl acetate	21.00	$3.53\pm0.53$	$16.77\pm2.52$	$5.17\pm0.76$	$22.06\pm3.31$
Isoamyl acetate	1.40	< 0.01	$2.48\pm0.37$	< 0.01	$2.90\pm0.44$
Ethyl caproate	0.17	$0.13\pm0.02$	< 0.01	$0.15{\pm}~0.02$	< 0.01

**Table 5.** The content of secondary metabolites in young and final beers (mg L<sup>-1</sup>)

<sup>1</sup>Blanco et al. (2016).

Table 5 demonstrates that *Saccharomyces cerevisiae var. boulardii* produce fewer secondary metabolites than control. At the same time, in the final product, the concentration of only such components as acetaldehyde and amyl alcohol exceeded the

concentrations corresponding to the perception threshold. The increase in the concentrations of secondary metabolites after fermentation can be explained by secondary fermentation during beer carbonization using a primer. The particular attention should be paid to the concentration of diacetyl, which presence in the final product is considered to be one of the main beer defects. Both in young and final beers, the concentration of vicinal diketones did not exceed the perception threshold. This suggests that, when we use the yeast *Saccharomyces cerevisiae var. boulardii* in brewing, the probability of including a diacetyl rest in the main fermentation mode is insignificant.

When using new non-traditional cultures of yeast in brewing, the organoleptic properties of the final product are of particular interest. According to the data presented in Table 6, the use of the yeast *Saccharomyces cerevisiae var. boulardii* as a starter culture produces a beer with a positive sensory profile. The product aroma had a complex character: it included caramel, spicy, fruity and phenolic notes, as well as smoked and wine elements; while honey was the dominant note of the taste. It is worth mentioning that phenolic notes can be noticed in the composition of the sensory profile. This fact suggests that the yeast *Saccharomyces cerevisiae var. boulardii* is probably a positive for phenolic off-flavor yeast (POF+), as most wheat strains.

Indicator	Strain			
Indicator	<i>S.c.b.</i>	047A		
Aroma	Barely perceptible:	Barely perceptible:		
	alcohol, caramel, spicy	alcohol, wine, fruity, solvent-like		
	<u>Slight:</u>	<u>Slight:</u>		
	wine, fruity, smoked	green apple, phenolic, fatty, oily		
	Easily noticeable:	Easily noticeable:		
	phenolic	hoppy, nutty, herbal		
	Explicit:	Explicit:		
	hoppy	banana, pear		
Flavor	Easily noticeable:	Easily noticeable:		
	sour, hop bitterness with some coarse	sour, honeyed, with slight hop bitterness,		
	bitterness (residual), fullness of taste	watery		
	Explicit:	Explicit:		
	honey	coarse bitterness (residual)		

 Table 6. Organoleptic characteristics of final beer

According to the data obtained, it can be concluded that the use of the yeast *Saccharomyces cerevisiae var. boulardii* as a starter culture in beer technology is promising. The main advantage of its use is the possibility of obtaining beer with potential probiotic properties. In addition, it should be noted that the use of the yeast *Saccharomyces cerevisiae var. boulardii* in brewing allows us to obtain beer with a low RDF, which has a beneficial effect on such organoleptic properties of beer as body and drinkability, and this fact can also be used in the technology of non-alcoholic and low-alcohol beer. However, the fermentation activity of *Saccharomyces cerevisiae var. boulardii* is lower than brewing strains, which entails a longer fermentation process. To accelerate the fermentation process, further research is needed regarding its optimization, for example, by varying such parameters as pitching rate and temperature.

# CONCLUSIONS

In this work, the fermentation performance of the probiotic yeast culture *Saccharomyces cerevisiae var. boulardii* was studied compared to top-fermenting brewing yeast strains during fermentation of model nutrient media and beer wort. According to the obtained results, it can be concluded that *Saccharomyces cerevisiae var. boulardii* have less fermentation activity compared to brewing strains, which consists in a relatively low substrate assimilation rate and the value of the final degree of attenuation. It was determined that both glucose and maltose are fermentable sugars by the yeast *Saccharomyces cerevisiae var. boulardii*. Consequently, the low apparent attenuation in beer wort fermentation seems to be related to the inability of this strain to consume maltotriose or to the extremely low rate of this process. It was also noted that the biomass yield on the maltose medium was higher than on the glucose one.

Beer produced with *Saccharomyces cerevisiae var. boulardii* significantly differed in physicochemical, microbiological, and organoleptic parameters from the control sample obtained using the 047A brewing strain. It was primarily characterized by a high content of residual extract and, accordingly, a lower concentration of ethanol. This beer also contained significantly more live yeast cells, which indicates the relatively high viability of *Saccharomyces cerevisiae var. boulardii*. In its turn, the content of secondary metabolites was lower than in the control.

#### REFERENCES

- Ávila-Reyes, S.V., Camacho-Diaz, B.H., Acosta-Garcia, M.C., Jimenez-Aparicio, A.R. & Hernandez-Sanchez, H. 2016. Effect of salt and sugar osmotic stress on the viability and morphology of Saccharomyces boulardii. *International Journal of Environment*, *Agriculture and Biotechnology* 1, 593–602. doi: 10.22161/ijeab/1.3.43
- Blanco, C.A., Andrés-Iglesias, C. & Montero, O. 2016. Lowalcohol Beers: Flavor Compounds, Defects, and Improvement Strategies. *Critical Reviews in Food Science and Nutrition* 56(8), 1379–1388. doi: 10.1080/10408398.2012.733979
- Briggs, D.E., Boulton, C.A., Brookes, P.A. & Stevens, R. 2004. *Brewing Science and practice*, 863 pp.
- Campbell, C., Nanjundaswamy, A.K., Njiti, V., Xia, Q. & Chukwuma, F. 2016. Value-added probiotic development by high-solid fermentation of sweet potato with Saccharomyces boulardii. *Food Sci. Nutr* 5(3), 633–638. doi: 10.1002/fsn3.441
- Capece, A., Romaniello, R., Pietrafesa, A., Siesto, G., Pietrafesa, R., Zambuto, M. & Romano, P. 2018. Use of Saccharomyces cerevisiae var. boulardii in co-fermentations with S. cerevisiae for the production of craft beers with potential healthy value added. *International Journal* of Food Microbiology 284, 22–30. doi: 10.1016/j.ijfoodmicro.2018.06.028
- Değirmencioğlu, N., Gurbuz, O. & Şahan, Y. 2016. The Monitoring, Via an In vitro Digestion System, of the Bioactive Content of Vegetable Juice Fermented with Saccharomyces cerevisiae and Saccharomyces boulardii. J. Food Process Preserv 40, 798–811. doi: 10.1111/jfpp.12704
- Fratianni, F., Cardinale, F., Russo, I., Iuliano, C., Tremonte, P., Coppola, R. & Nazzaro, F. 2014. Ability of symbiotic encapsulated Saccharomyces cerevisiae boulardii to grow in berry juice and to survive under simulated gastrointestinal conditions. J. Microencapsul 31, 299–305. doi: 10.3109/02652048.2013.871361

- Hudson, L.E., McDermott, C.D., Stewart, T.P., Hudson, W.H., Rios, D., Fasken, M.B., Corbett, A.H. & Lamb, T.J. 2016. Characterization of the probiotic yeast Saccharomyces boulardii in the healthy mucosal immune system. *PLoS ONE* 11, 1–21. doi: 10.1371/journal.pone.0153351
- Karaolis, C., Botsaris, G., Pantelides, I. & Tsaltas, D. 2013. Potential application of Saccharomyces boulardii as a probiotic in goat's yogurt: survival and organoleptic effects. *International Journal of Food Science & Technology* 48(7), 1445–1452. doi: 10.1111/ijfs.12111
- Khatri, I., Tomar, R., Ganesan, K., Prasad, G.S. & Subramanian, S. 2017. Complete genome sequence and comparative genomics of the probiotic yeast Saccharomyces boulardii. *Sci. Rep.* **7**, 1–13. doi: 10.1111/ijfs.12111
- Lazo-Vélez, M.A., Serna-Saldívar, S.O., Rosales-Medina, M.F., Tinoco-Alvear, M. & Briones-García, M. 2018. Application of Saccharomyces cerevisiae var. boulardii in food processing: a review. J Appl Microbiol 125(4), 943–951. doi: 10.1111/jam.14037
- Lourens-Hattingh, A. & Viljoen B.C. 2001. Growth and survival of a probiotic yeast in dairy products. *Food Research International* **34**(9), 791–796.
- Łukaszewicz, M. 2012. Saccharomyces cerevisiae var. boulardii Probiotic Yeast. In Everlon Rigobelo (eds) *Probiotics*. Books on Demand, Rijeka, Croatia, 385–388 pp.
- Mitterdorfer, G., Mayer, H.K., Kneife, W. & Viernstein, H. 2002. Clustering of Saccharomyces boulardii strains within the species S. cerevisiae using molecular typing techniques. *Journal of Applied Microbiology* **93**, 521–530. doi: 10.1046/j.1365-2672.2002.01710.x
- Mulero-Cerezo, J., Briz-Redón, A. & Serrano-Aroca, A. 2019. Saccharomyces Cerevisiae Var. Boulardii: Valuable Probiotic Starter for Craft Beer Production. *Applied Sciences* **9**(16), 1–16. doi: 10.3390/app9163250
- Pais, P., Almeida, V., Yılmaz, M. & Teixeira, M.C. 2020. Saccharomyces boulardii: What Makes It Tick as Successful Probiotic? *Journal of Fungi* 6(2), 78. doi: 10.3390/jof6020078
- Pereira de Paula, B., de Souza Lago, H., Firmino, L., Fernandes Lemos Júnior, W.J., Ferreira Dutra Corrêa, M., Fioravante Guerra, A., Signori Pereira, K. & Zarur Coelho, M.A. 2021. Technological features of Saccharomyces cerevisiae var. boulardii for potential probiotic wheat beer development. *LWT - Food Science and Technology* 135, 1–8. doi: 10.1016/j.lwt.2020.110233
- Stewart, G.G. 2016. Saccharomyces species in the Production of Beer. *Beverages* 2(34), 1–18. doi:10.3390/beverages2040034
- Suchkova, E., Shershenkov, B. & Baranenko, D. 2014. Effect of ultrasonic treatment on metabolic activity of Propionibacterium shermanii, cultivated in nutrient medium based on milk whey. *Agronomy Research* 12(3), 813–820.
- Vandenplas, Y., Brunser, O. & Szajewska, H. 2008. Saccharomyces boulardii in childhood. *European Journal of Pediatrics* **168**(3), 253–265. doi: 10.1007/s00431-008-0879-7