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BIOCHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY AND LEAVENING POTENTIAL OF *SACCHAROMYCES CEREVISIAE* STRAINS ISOLATED FROM REGIONAL SOURDOUGHS

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Abstract: This study investigated the biotechnological potential of eleven *Saccharomyces cerevisiae* strains (1P–11P) isolated from artisanal and commercial sourdoughs originating from the Republic of Moldova, Romania, and Ukraine. The strains were cultivated under laboratory conditions on YPD medium, and several physiological, biochemical, and technological parameters were evaluated, including biomass production, protein and carbohydrate content, antioxidant activity, enzymatic activity, and dough leavening performance. The obtained results demonstrated significant differences among the strains ($p \leq 0.05$). Biomass production ranged from 2.73 to 6.53 $g \cdot L^{-1}$, with the highest value recorded for strain 6P. Protein content varied between 41.02% and 57.92% dry weight, with strains 2P and 4P showing the highest levels. The strongest total antioxidant activity was observed in strains 1P and 2P. Superoxide dismutase activity reached its maximum in strains 3P and 4P, while catalase activity was highest in strain 9P, isolated from commercial sourdough. Regarding technological performance, strains 9P and 10P exhibited superior dough leavening ability, characterized by rapid fermentation and efficient CO_2 retention within 90–120 minutes. Overall, artisanal sourdough isolates demonstrated better performance than commercial strains in most evaluated parameters, highlighting their potential for sustainable applications in baking and microbial biotechnology.

Keywords: *antioxidant activity, carbohydrates, catalase, dough leavening, proteins, superoxiddismutase, yeasts.*

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INTRODUCTION

Artisanal sourdough, obtained through the spontaneous fermentation of local flours, represents a rich source of complex microbial consortia in which yeasts of the *Saccharomyces* genus coexist with lactic acid bacteria, providing bakery products with distinctive aromatic profiles and enhanced technological properties [1, 2].

The use of sourdoughs in the bakery industry contributes to increased mineral bioavailability and to the generation of peptides with antioxidant activity and preservative properties, possessing a beneficial effect on the nutritional quality and shelf life of the products [3].

Artisanal sourdough is usually developed under semi-controlled conditions, through periodic refreshments with flour and water, a process that leads, over time, to the stabilization of microbial consortia well-adapted to the specific substrate and medium. These consortia influence the rheological properties of the dough and the nutritional value of bakery products, through the degradation of anti-nutrients and the synthesis of bioactive compounds [4].

Thus, *S. cerevisiae* strains isolated from artisanal sourdough may exhibit high biotechnological potential, sometimes exceeding the performance of commercial strains, especially under specific technological conditions or when artisanal products are desired.

In the context of the increasing demand for artisanal products and with geographical indication, the selection and characterization of *S. cerevisiae* strains from local sourdoughs becomes a priority for the development of starter cultures with high biotechnological potential. *S. cerevisiae* is one of the most studied and used yeast species in the food biotechnology field, being the main microorganism involved in alcoholic fermentation, baking and numerous traditional fermentation processes. The use of strains adapted to local raw material conditions and technology represents a current direction in food biotechnology field, with an impact on the sensorial, nutraceutical qualities and identity of products. Research by winemakers from the Republic of Moldova has demonstrated that local yeast strains can present superior enzymatic activity and fermentation capacity to commercial ones, which provides them a particular interest for various industrial applications [5].

Studies conducted in various regions have shown that local strains can exhibit distinct physiological and technological properties compared to commercial strains, including fermentative capacity adapted to local raw materials, specific tolerance to stress factors, and unique aromatic profiles. Microbial consortia in artisanal sourdough, mainly consisting of yeasts and lactic acid bacteria, are recognized as valuable sources of genetic and physiological diversity, essential for the selection of new cultures with distinctive properties [6, 7].

Artisanal sourdough is also of interest as a sustainable source for the isolation and selection of yeast strains with high potential for non-food microbial biotechnologies, oriented towards the synthesis of biologically active substances used in medicine, cosmetology, agriculture and animal husbandry. Microbiological biosynthesis underlies many biotechnological processes, with bacteria, microalgae and yeasts being widely used as producers of such compounds [8]. Yeasts are among the most efficient producers of biologically active substances due to their advantages over other microorganisms: they metabolize a wide range of carbon sources, exhibit high biomass productivity,

demonstrate the resistance to contamination, can be easily separated from the culture medium, and can be cultivated on relatively inexpensive nutrient media, thereby reducing production costs [9].
From this perspective, local strains of *Saccharomyces cerevisiae* isolated from artisanal sourdoughs constitute a valuable microbial source for biotechnology. The research focused on their isolation, evaluation of biochemical composition and characterization of technological performances allows the identification of strains with advantageous fermentative and metabolic properties, which can subsequently be integrated into food biotechnologies or into processes involving the directed biosynthesis of biologically active substances.
The present study aimed to evaluate the productive, biochemical, antioxidant and leavening potential of eleven strains of *S. cerevisiae* isolated from artisanal and commercial sourdoughs, following cultivation on standard YPD medium. The main objectives were to determine biomass production, the proteins and carbohydrate contents, evaluate the total antioxidant activity, the activity of CAT and SOD enzymes, as well as to examine the leavening capacity of dough in the model samples.

MATERIALS AND METHODS

Object of the study

The object of the study consisted of eleven yeast strains of *S. cerevisiae*. Nine strains were isolated from artisanal sourdoughs prepared using regional flours: *S. cerevisiae* 1P from wheat flour “Lanul de aur” (Ukraine), *S. cerevisiae* 2P from wheat for “Cozonac” (Romania), *S. cerevisiae* 3P from wheat flour type 000 “Szatmari” (Romania), *S. cerevisiae* 4P from wheat flour “Oleineac” (Republic of Moldova), *S. cerevisiae* 5P from wheat flour ”Măcinătorul” (Republic of Moldova), *S. cerevisiae* 6P from homemade wheat flour (Republic of Moldova), *S. cerevisiae* 7P from rye flour (Ukraine), *S. cerevisiae* 8P from wheat flour type 000 ”Băneasa” (Romania), and *S. cerevisiae* 10P from homemade rye flour (Ukraine). Two additional strains were isolated from commercial sourdoughs: *S. cerevisiae* 9P from commercial dried sourdogh “O-Tentic-Durum” (Romania) and *S. cerevisiae* 11P from commercial dried sourdogh “4199 Spring Pane” (MILBO S.P.A., Italy). All flours and commercial sourdoughs used in this study are freely available in retail networks in the Republic of Moldova.

Media and fermentation conditions

The yeast strains were preserved on wort agar medium (6 °Bx) and stored at + 4 °C. The inoculum was obtained by cultivating the yeast strains in liquid YPD nutrient medium (1 % yeast extract, 2 % glucose, 2 % peptone, 1 L distilled water, *p*H 5.5) [10] for 48 hours, under agitation (200 rpm), at +27 - 28 °C. The inoculum ($2x10^6$ cells·mL^{-1}) represented 5 % of the total volume of the nutrient medium.
In the experiments, YPD medium was used for submerged cultivation of the strains in Erlenmeyer flasks containing 0.2 L of medium. The cultures were incubated under agitation (200 rpm), at + 27 - 28 °C, for 120 hours.

Research methods

Biomass was determined gravimetrically after centrifugation at 4000 rpm for 15 minutes. Dry biomass was assessed by drying the known amount of yeast biomass at 105 °C to constant weight.
Protein content was determined spectrophotometrically according to the Lowry method, using bovine serum albumin as a standard [11].
Total carbohydrate content was determined spectrophotometrically using the anthrone reagent, with D-glucose as the standard. Absorbance was measured at 620 nm [12].
Total antioxidant activity (TAA) was determined spectrophotometrically using the $ABTS^+$ radical cation method [13].
Catalase activity (CAT) was determined spectrophotometrically using hydrogen peroxide as the substrate [14].
Superoxide dismutase (SOD) activity was determined using a method based on the inhibition of nitroblue tetrazolium reduction in the presence of TEMED and riboflavin [15]. Spectrophotometric assays were performed using Shimadzu UV-1280 UV-VIS spectrophotometer (Shimadzu Corporation, Japan).

Preparation of dough samples

Biomass from eleven yeast strains, cultivated in standard YPD medium, was used to prepare dough samples for monitoring fermentation dynamics. The recipe was adapted to the experimental conditions and local raw materials [16], and included 108.3 g of Bunetto wheat flour (Vitalcomus LTD, Republic of Moldova), 70 mL of OM potable water (Gura Căinarului LTD, Republic of Moldova), 3.1 mL of oil, 1.07 g of salt, 1.55 g of active dry yeasts, and 3.0 g of sugar. After kneading all ingredients for 15 minutes, the dough was placed in 1000 mL Brezelius beakers and incubated at + 25 °C for 120 minutes. In order to evaluate fermentation dynamics, dough height in the beakers was recorded every 20 minutes.

Statistical analysis

All experiments were performed in triplicate. Data were analyzed using Microsoft Excel and Statistics 9.0 software. Results are presented as mean ± standard deviation, and confidence intervals were calculated based on three replicates. For the determination of statistical significance was used t-test. Differences were considered statistically significant at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

The production of a substantial amount of biomass, along with a strong capacity for dough leavening, constitutes essential indicators of the applicability of yeast strains in baking and other areas of microbial biotechnology [17].
These indices vary depending on the strain taxonomic affiliation, the composition of the nutrient medium, cultivation conditions (temperature, *p*H, aeration) and the duration of cultivation. During the research, it was found that the biomass production of yeast

strains, cultivated on YPD nutrient medium, under identical conditions (*p*H 5.5, + 28 °C, 120 hours, 200 rpm), varied significantly, ranging from 2.73 ± 0.28 to 6.53 ± 0.32 g·L^{-1} (Figure 1). Strains *S. cerevisiae* 9P and 11P, isolated from commercial dry sourdoughs, accumulated 3.67 ± 0.19 and 4.28 ± 0.16 g·L^{-1} of dry biomass, respectively. The maximum biomass yield of 6.53 ± 0.32 g·L^{-1} was recorded for strain 6P, isolated from artisanal sourdough prepared from local homemade wheat flour. This value was significantly higher ($p \leq 0.05$) than those of strains isolated from commercial dry sourdoughs as well as the other studied strains. Strain 2P also accumulated a relatively high biomass of 5.03 ± 0.29 g·L^{-1}. Most strains (six out of nine) isolated from artisanal sourdoughs exhibited values among 3.72 ± 0.31 and 4.98 ± 0.13 g·L^{-1}, comparable or even higher than those of commercial strains 9P and 11P (3.67 ± 0.19 and 4.28 ± 0.16 g·L^{-1}), however, no statistically significant differences were observed (Figure 1).

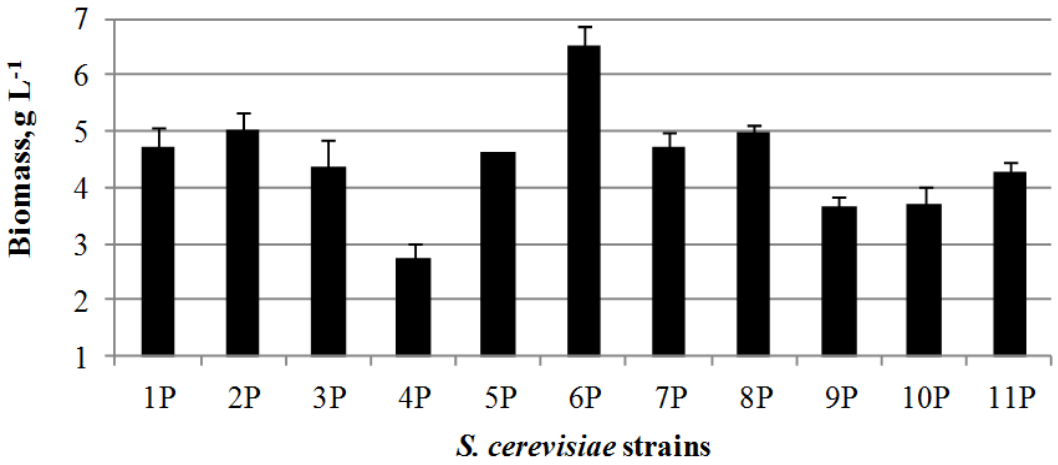

Figure 1. *Biomass production of S. cerevisiae strains cultivated in YPD medium*

The lowest biomass yield, of only 2.73 ± 0.28 g·L^{-1}, was recorded for the strain 4P, isolated from sourdough made with "Oleineac" wheat flour (Republic of Moldova). This low yield probable due to poor adaptation to the used medium and was significantly lower ($p \leq 0.05$) compared to the other strains evaluated in the study (Figure 1).

The obtained results indicate the more efficient adaptation of artisanal strains to YPD medium compared to strains isolated from commercial sourdoughs, highlighting the advantage of local isolates in biomass production. The obtained biomass values (2.73 - 6.53 g·L^{-1}) fall within the ranges reported for strains cultivated on molasses or similar substrates, where typical yields range from 0.33 g biomass per g substrate (equivalent to approximately 3 - 7 g·L^{-1} under batch conditions) to over 10 g·L^{-1} in industrially optimized fed-batch processes.

The strain 6P, with a biomass yield of 6.53 g·L^{-1}, exceeds the average yield of commercial strains (approximately 4 - 5 g·L^{-1}) in aerobic cultures cultivated on molasses [18], although it remains below the higher values (15 - 20 g·L^{-1}) reported for controlled bioreactor systems [19].

The *S. cerevisiae* 6P strain, isolated from sourdough prepared with homemade wheat flour, shows promising potential for sustainable artisanal applications and other areas of microbial biotechnology, as it produced biomass yield on standard YPD medium under laboratory conditions comparable to those obtained for commercial yeasts cultivated on

molasses. However, to achieve industrial-level efficiency, further research is required to optimize cultivation parameters, including temperature, *p*H, aeration, and process duration.

Yeast biomass is a rich source of proteins, which typically account for 40 – 60 % of the total cell dry weight [20]. Proteins derived from yeasts such as *S. cerevisiae* and *Pichia* spp. are generally recognized as safe for use in the food industry, as they are not associated with significant allergenicity or toxic effects in a food context [21]. *S. cerevisiae* proteins contain a wide range of essential and immunoactive amino acids, various enzymes and structural proteins [22]. The protein content of the yeast biomass analyzed in this study varied considerably, ranging from 41.02 ± 2.84 to 57.92 ± 2.13% d.w. These values are comparable to those reported in the scientific literature, where the protein content of *S. cerevisiae* biomass obtained on nutrient media based on fruit and vegetable wastes, molasses, or residues from the brewing industry ranged between 48 % and 54 % d.w. [20]. In our study the highest protein contents, 55.97 ± 4.89 and 57.92 ± 2.13 % d.w., were determined in strains 2P and 4P, both isolated from artisanal sourdoughs. The lowest values were recorded in strains 9P (from commercial sourdough) and 10P (from artisanal sourdough), with protein contents of 41.02 ± 2.84 and 41.53 ± 2.97 % d.w., respectively (Figure 2). Strain 11P (from commercial sourdough) exhibited a content of 52.02 ± 0.7 % d.w. proteins. Statistical analysis, performed both between individual strain values and the overall mean, as well as between the extreme values, confirmed the presence of significant differences ($p \leq 0.05$ and $p \leq 0.01$, respectively), suggesting variability in genetic potential and opportunities for strain selection. Strain 6P also showed a notable protein content of 45.18 ± 4.64 % d.w. (Figure 2).

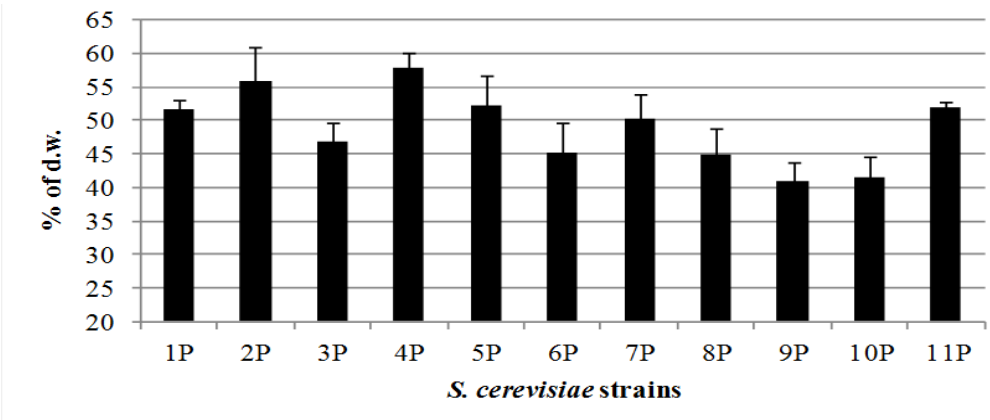

Figure 2. *Protein content of biomass from S. cerevisiae strains cultivated on YPD medium*

Strain 4P, although it accumulated the highest protein content, significantly higher ($p \leq 0.05$) than that of most strains, exhibited significantly lower biomass production ($p \leq 0.05$) compared to the reference strains. Thus, it can be mentioned that the strains isolated from artisanal sourdoughs showed a biomass production and a protein contents comparable to or even higher than those of strains isolated from commercial sourdoughs, in agreement with data reported in the scientific literature. Notably, strains 2P and 6P, after optimizing the cultivation parameters and conditions, demonstrated a promising potential as protein biomass producers.

The carbohydrate content of the biomass of the strains varied moderately, between 28.8 ± 4.62 and 35.58 ± 3.12 % d.w., without statistically significant differences, being highlighted between the strains ($p > 0.05$). The *S. cerevisiae* 7P strain, isolated from sourdough prepared from rye flour, accumulated the highest amount of carbohydrates (35.58 ± 3.12 % d.w.), but the differences from the other strains were not statistically significant (Figure 3). Among the strains with relatively high values of this parameter, strains 2P and 11P were notable, exhibiting carbohydrate contents of 33.92 ± 1.54 and 33.48 ± 2.73 % d.w., respectively (Figure 3).

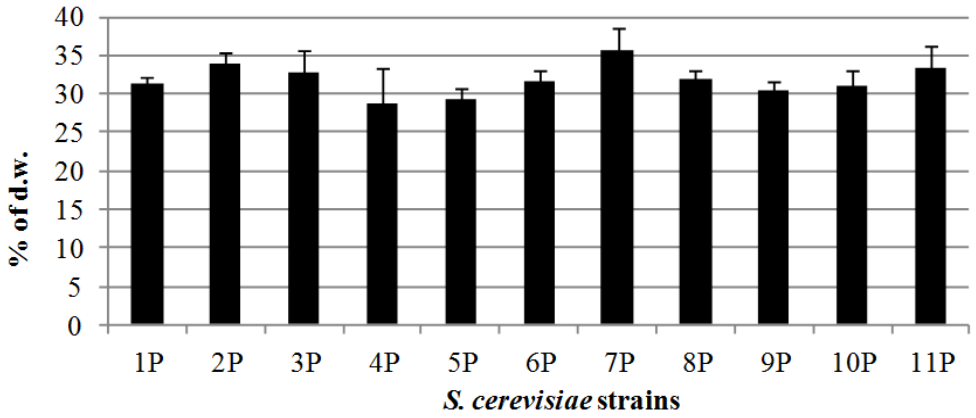

Figure 3. *Carbohydrate content of biomass from S. cerevisiae strains cultivated on YPD medium*

The obtained results allow us to conclude that most of the studied strains, isolated from both artisanal and commercial sourdoughs, accumulate carbohydrates in quantities comparable to those reported in other research [23, 24].

It should be noted that strains 2P (55.97 ± 4.89 % d.w. proteins, 33.92 ± 1.54 % d.w. carbohydrates) and 7P (50.16 ± 3.67 % d.w. proteins, 35.58 ± 3.12 % d.w. carbohydrates) show promising potential as producers of biologically active polymers, particularly β-glucans and mannans, which are structural components of the yeast cell wall with high functional value [25].

The analyzed *S. cerevisiae* strains exhibited moderate total antioxidant activity, with values ranging between 23.18 ± 2.16 and 36.48 ± 2.78 % inhibition (Figure 4). The biomass of strains 1P and 2P presented the highest values of total antioxidant activity, 36.48 ± 2.78 and 33.58 ± 2.79 % inhibition, respectively. Whereas strains 4P and 5P, recorded similar values of TAA of 29.94 ± 1.96 and 29.72 ± 2.54 % inhibition, respectively, however lower than those previously mentioned (Figure 4). The other strains showed a lower antioxidant activity, ranging between 23.18 ± 2.16 and 27.75 ± 2.58 % inhibition (Figure 4). The obtained values are comparable or slightly lower than those reported for pigmented yeasts of the genus *Rhodororula*, cultivated on YPD medium (21.46 ± 2.43 - 66.95 ± 3.72 % of inhibition) [26], and are significantly lower than those observed for *S. cerevisiae* extracts (2000 $\mu g \cdot mL^{-1}$), which showed inhibition of up to 92.2 % [27].

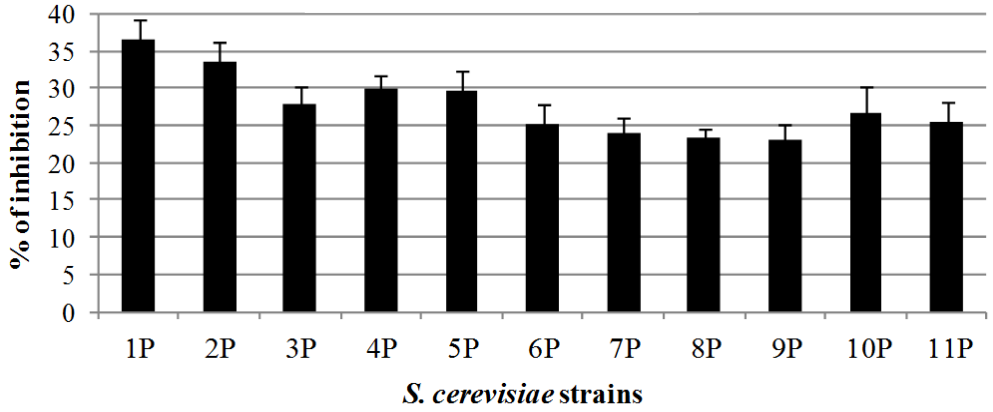

Figure 4. *Total antioxidant activity of biomass from S. cerevisiae strains cultivated on YPD medium*

The antioxidant activity of yeasts largely depends on the composition of the used nutrient medium. For example, the increase in antioxidant activity of *S. cerevisiae* products has been correlated with the high content of phenols in the nutrient media based on cornmeal [28].

S. cerevisiae yeasts are organisms known to possess highly important antioxidant enzyme systems, such as superoxide dismutase and catalase [29].

CAT activity in the strain biomasses varied significantly, ranging from 262.92 ± 47.22 to 448.21 ± 46.17 mmol·min^{-1}·mg^{-1} protein (Figure 5). The highest values were observed in two strains isolated from commercial sourdoughs (9P and 11P), with activities of 448.21 ± 46.17 and 374.33 ± 49.13 mmol·min^{-1}·mg^{-1} protein, respectively. High CAT activity was also recorded in two strains isolated from artisanal sourdoughs, *S. cerevisiae* 3P and 10P, with values of 391.63 ± 28.63 and 383.56 ± 43.14 mmol·min^{-1}·mg^{-1} protein, respectively. In the remaining seven strains isolated from artisanal sourdoughs, CAT activity was lower, strains 1P and 2P showing the lowest values of 288.91 ± 36.82 and 262.92 ± 47.22 mmol·min^{-1}·mg^{-1} protein, respectively (Figure 5).

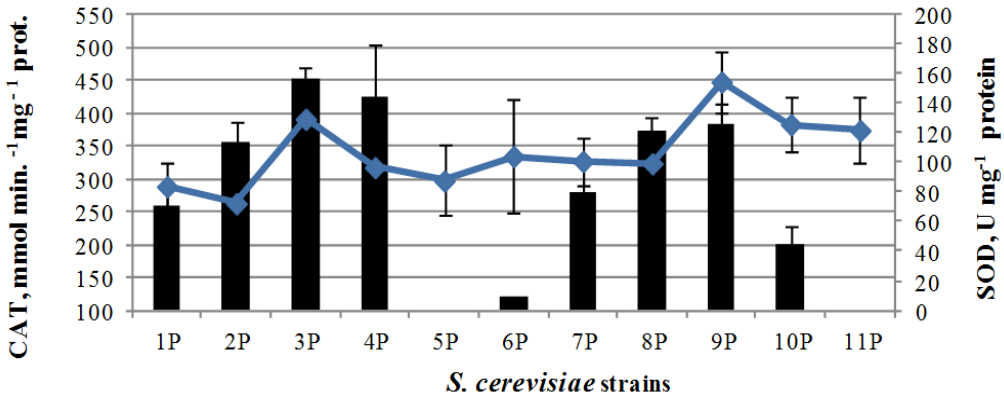

Figure 5. *CAT and SOD activities of biomass from S. cerevisiae strains cultivated on YPD medium*

These results are comparable to those reported for pigmented yeasts, in which CAT activity varies over a wider range, from 91.87 ± 8.18 to 738.00 ± 81.88 mmol·min^{-1}·mg^{-1} protein [26] and confirms that *Saccharomyces* yeasts are an excellent source of catalase [30].

The *S. cerevisiae* strains analyzed in this study showed significant variations in SOD activity, with values ranging from the lowest of 8.67 ± 0.16 U·mg^{-1} protein to the highest of 157.1 ± 6.71 U·mg^{-1} protein (Figure 5). The highest SOD activities were observed in the biomass of the strains 4P and 3P, with values of 145.01 ± 34.66 and 157.1 ± 6.71 U·mg^{-1} protein, respectively (Figure 5), comparable to the values reported for the *S. cerevisiae* TBRC657 strain cultivated on YPD medium (146.16 ± 10.87 and 208.18 ± 10.87 U·mL^{-1}), but lower than those obtained in strains, cultivated on molasses (329.91 ± 27.66 - 590.87 ± 39.36 U·mL^{-1}) [31].

Among the strains with lower SOD activity are strains 6P, 10P, 1P and 7P, with values of 8.67 ± 0.16, 45.05 ± 11.47, 71.34 ± 11.05 and 79.87 ± 20.99 U·mL^{-1} protein, respectively (Figure 5), with values similar those reported for other *Saccharomyces* strains [32, 33]. SOD activities were not detected in *S. cerevisiae* strains 5P and 11P (Figure 5). The scientific literature reports cases in which certain *S. cerevisiae* strains exhibit absent or undetectable SOD activity, supporting the observations obtained in our study [34 – 36].

The dough fermentation process is an essential stage in obtaining high-quality bread because yeast cells, by releasing CO_2, directly influence the dough rheology, volume, texture, and the taste of the finished product. The fermentation rate largely depends on the fermentative performance of the yeasts and the temperature conditions, which usually range between + 25 and 30 °C [16].

Dough volume is an important visual and quantitative indicator of yeast activity, providing a quantitative measure of yeast performance. It reflects both the capacity to produce CO_2 and the ability of the dough to retain gas bubbles, thus serving as a key parameter of fermentative activity [37].

The dynamics of dough fermentation with the strains investigated in this study revealed significant differences in dough expansion capacity. The *S. cerevisiae* 9P (commercial sourdough) and 10P (artisanal sourdough) strains stood out from the early stages of the process (20 minutes), exhibiting a rapid fermentation rate significantly higher ($p \leq 0.05$) than that of the other strains, which is advantageous for industrial processes with short maturation times. Dough expansion continued steadily at 40 and 60 minutes, reaching maximum values of 40 mm at 90 minutes (Figure 6).

The *S. cerevisiae* 7P (artisanal sourdough) and 11P (commercial sourdough) strains showed slower dynamics, with significantly lower dough heights compared to strains 9P and 10P at all time intervals, reaching 35 mm at 90 minutes (Figure 6). At 120 minutes, the height of the dough fermented by *S. cerevisiae* 9P decreased, reaching values comparable to those of dough fermented by strains 7P and 11P (Figure 6). In contrast, strain 10P maintained dough height at 120 minutes at the levels similar to the maximum recorded at 90 minutes, remaining higher than strains 7P, 9P, and 11P, although without statistically significant differences ($p > 0.05$) (Figure 6).

Thus, dough fermented with *S. cerevisiae* 10P exhibits superior CO_2 retention capacity compared to the strains 7P, 9P, and 11P. The other studied *S. cerevisiae* strains (1P-6P and 8P) showed reduced leavening potential and are of limited interest for industrial applications.

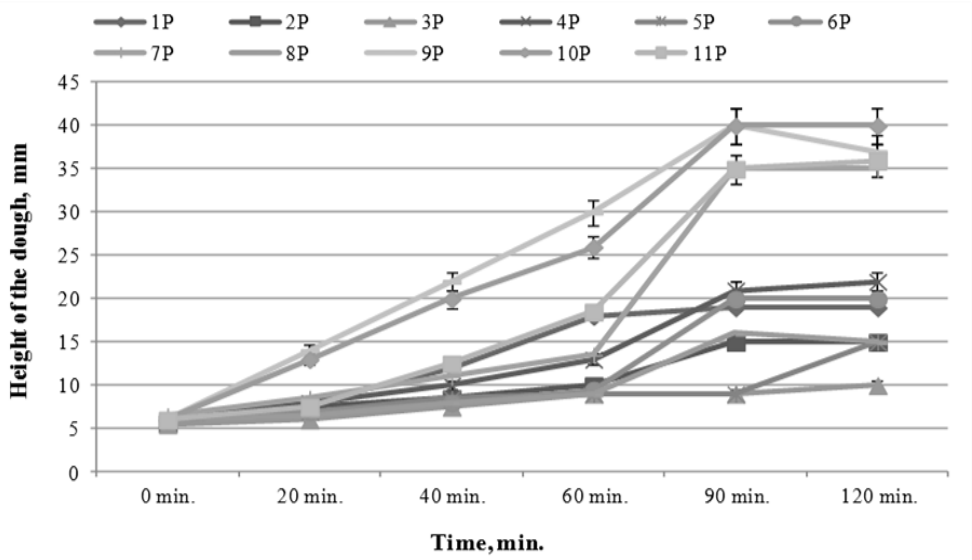

Figure 6. *Leavening potential at 25°C of S. cerevisiae strains cultivated on YPD medium*

S. cerevisiae strains 9P and 10P showed maximum efficiencies comparable to those of industrial baker's yeast strains, efficiently producing CO_2 at 25 °C [38] and reaching maximum fermentation volumes after approximately 90 minutes, consistent with results reported in other studies [39]. Comparative analysis of the eleven *S. cerevisiae* strains demonstrated significant differences in dough fermentation capacity, including variations in gas retention and rheological properties [40]. These results indicate that, while some commercial and artisanal strains (9P and 10P) exhibit similar performance in dough fermentation, most local strains show highly variable behavior, influencing both fermentation speed and the final volume and structure of the dough. Therefore, the selection of strains for industrial or laboratory applications must take these differences into account, and the potential of high-performance strains can be exploited to optimize bakery recipes and develop products with constant technological characteristics.

CONCLUSIONS

On balance, the results of the study show the variable potential of the *S. cerevisiae* strains isolated from artisanal and commercial sourdoughs for applications in bakery, protein biomass production and other microbial biotechnologies. The artisanal strain *S. cerevisiae* 6P accumulated the highest amount of biomass, indicating a high yield and potential for industrial scaling. The artisanal strains *S. cerevisiae* 2P and 4P, also artisanal, presented the highest protein content, surpassing the commercial strains, which recommends them as safe and efficient sources of food protein. The biomass of *S. cerevisiae* strains 1P and 2P exhibited moderate total antioxidant activity, supported by CAT and SOD enzyme activities, highlighting their additional functional value. The *S. cerevisiae* strains 9P (commercial) and 10P (artisanal) excelled in dough fermentation, with fast initial rise rates and superior CO_2 retention, offering technological advantages for industrial processes. Overall, these results emphasize the

diversity and differentiated performance of local strains and provide an essential basis for their optimization and sustainable application in various biotechnological fields.

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