biology and life sciences forum

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Proceeding Paper

Biomass Production and Use of *Saccharomyces cerevisiae* var. *boulardii* in a Beverage for Athletes †

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† Presented at the 4th International Electronic Conference on Foods, 15–30 October 2023; Available online: https://foods2023.sciforum.net/.

Abstract: *Saccharomyces cerevisiae* var. *boulardii* is the only yeast indicated as a probiotic. In recent decades, sports drinks have become very popular due to their peculiarities of providing health benefits. The main topic of this work was to study the effects of adding two commercial strains of *S. boulardii* to a beverage intended for athletes, focusing on their viability and consumer preference; as a preliminary step for biomass production, the effects of pH, glucose concentration and temperature were studied through Central Composite Design methodology. The results of this research suggest that the probiotic microorganism/food interaction needs to be carefully evaluated.

Keywords: *Saccharomyces boulardii*; desirability profiles; viability; sensory assessment

1. Introduction

The most used definition dates back to 2001 from an Expert Consultation of international scientists FAO/WHO defines the probiotics as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” [1,2], later revised and slightly modified [3]. Recent studies have shown that to exert positive physiological functions in the host, probiotics should be able to survive gastric transit and reach the small intestine in sufficient numbers to be effective, and they should maintain a minimum number cell level of 10^7 CFU/g of food at the time of consumption [4].

Although for a long time the concept of probiotic was applied only to bacteria because the human origin was a requisite essential for their selection and use, recent investigations have confirmed the probiotic potential of yeasts [5]. The list of potential probiotic yeasts includes various species, but currently the only yeast with a solid corpus of knowledge necessary for a regulatory framework is *Saccharomyces cerevisiae* var. *boulardii* [6]. *S. boulardii* resistance to bile acids of the gastrointestinal tract and positive modulation it exerts on gut microbiota are two main properties, which support its use as a biotherapeutic agent for humans [7].

For decades milk has been the main vehicle for probiotics but currently there is an increasing demand for non-dairy probiotic foods, especially non-fermented beverages [6]. The importance of functional beverages is due to their peculiarity of being very efficient carriers of nutrients and bioactive compounds. They also have an important commercial value, thanks to their easeness and practicality of use combined with the possibility of refrigeration, which makes them stable and consumable in the time [6,8]. Among functional beverages, sports drinks have become the most popular beverages. These are flavored drinks designed to prevent dehydration, provide carbohydrates, electrolytes (such as sodium, potassium, calcium, and magnesium) and sometimes vitamins or other nutrients, during exercise [8].

Citation: Accettulli, A.; Sinigaglia, M.; Racioppo, A. Biomass Production and Use of *Saccharomyces cerevisiae* var. *boulardii* in a Beverage for Athletes. *Biol. Life Sci. Forum* **2023**, *26*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: date

Therefore, the main topic of this research was to explore the possibility of adding two commercial strains of *S. boulardii*, with a known regulatory status, to a beverage intended for athletes, assessing both the viability of the probiotics and end-users' acceptability. In a preliminary stage, the effect of some intrinsic (pH and glucose concentration) and extrinsic (temperature) factors on *S. boulardii* biomass production was evaluated.

2. Materials and Methods

2.1. Yeasts

Two commercial strains of *S. cerevisiae* var. *boulardii* were used. The first strain was isolated from Enteroboulardi (SB1), a dietary supplement from Laboratori Legren srl (Bordighera, Italy). The second preparation is Codex 5 billion (SB2), a dietary supplement from Zambon Italia srl (Milan, Italy).

From the commercial preparations, *S. boulardii* strains were isolated using the following protocol: capsules and/or sachets containing the suspension powder were dissolved in 20 mL of distilled water. 100 μL were then taken and inoculated into tubes containing 5 mL of YPG broth. The tubes were then incubated at 25 °C for 48 h. Then from tubes, yeasts were plated and purified on YPG agar plates, incubated at 25 °C for 48 h; identification was confirmed through microscope examination and phenotypic tests.

2.2. Biomass Production

The effect of pH, glucose concentration, and temperature, on biomass production was studied through the methodology of Central Composite Design (CCD).

Yeasts were inoculated in YPG broth with variable concentrations of glucose or adjusted at different pHs at 5 log CFU/mL, and then incubated at 15, 20, 25, 30, and 35 °C. Glucose concentration, pH, and the incubation temperature were modulated through a five levels/three variable CCD.

Yeast growth was evaluated after 24 and 48 h through OD measurements at 600 nm. Data were processed with the DoE (Design of Experiments) option of the Statistica for Windows software (Statsoft, Tulsa, OK, USA).

The effect of each independent variable (pH, temperature, glucose) vs biomass (OD reading) was also evaluated through the desirability function.

2.3. Viability of Saccharomyces boulardii in Sport Beverages

The beverage was prepared from a commercial powder, containing proteins, derived from whey, vitamins, and amino acids. The preparation is recommended for athletes, as it contributes to the growth and maintenance of muscle mass.

25 g of freeze-dried powder were dissolved in 150 mL of water. Once the beverage was prepared, it was placed in 50 mL sterile single-use containers on which were marked the code SB1 or SB2. Yeasts were inoculated at 6 log CFU/mL; then, the samples were stored at a temperature of 4 °C, and analyzed after 2, 5, 7 and 9 days to assess viable count on YPG agar.

2.4. Sensory testing

Sport drinks were subjected to a sensory test with 56 untrained assessors. They were given three samples coded as A (control), B (sample inoculated with SB1) and C (sample inoculated with SB2) and were asked to answer a questionnaire to assess perceived differences between the samples.

3. Results

The first output of DoE is the table of standardized effects shown in Table 1, which indicates the individual, quadratic and interactive effects of pH, temperature, and glucose concentration on biomass production of the strains SB1 and SB2 after 24 and 48 h.

After 24 h for both SB1 and SB2 strains, temperature acted as a positive individual term, suggesting, therefore, that as temperature increased, biomass increased; the presence, however, of the negative quadratic term (of an opposite sign to the linear term) indicated that the correlation was not linear, but that there was a critical threshold beyond which a further increase in temperature resulted in a decrease in biomass.

A similar trend was found with glucose. Only for the strain SB1 there was also a positive interaction glucose x temperature, thus highlighting that biomass increased when both temperature and glucose increased.

After 48 h temperature acted only as a negative quadratic term, while for glucose both a positive linear term and a negative quadratic term were found; finally, both strains were influenced by the negative interaction glucose*pH.

Table 1. Table of standardized effects of the linear (L), quadratic (Q) and interactive terms of glucose, pH, and temperature on biomass of *S. boulardii* SB1 and SB2 after 24 and 48 h.

	SB1		**SB2**	
	24 h	**48 h**	**24 h**	**48 h**
T (L)	9.849		9.592	
T (Q)	−2.723	−4.071	−2.338	−3.836
pH (L)				
pH (Q)				
G (L)	6.298	17.369	6.089	14.229
G (Q)	−4.664	−5.179	−4.140	−4.748
T * pH				
T * G	2.282			
pH * G		−2.160		−2.303
R^2_{ad}	0.867	0.924	0.852	0.891

A table of standardized effects is a useful tool, clarifying the statistical weights of different variables; however, it does not show the individual effect of each factor; this output can be obtained through desirability profiles. The desirability is a dimensionless parameter, ranging from 0 to 1 and is the answer to question: how much is desired an output? If desirability is 0, it means that the outcome is not desired; conversely, a value of 1 indicates the best possible outcome.

Figures 1 and 2 show the desirability profiles related to temperature, pH, and glucose on the biomass production of strain SB1 after 24 h and 48 h. After 24 h, desirability increased for temperature values between 15 and 25 °C, while a further increase of temperature up to 30 and 35 °C did not affect biomass production; pH did not exert a significant effect, while desirability pointed out an optimal value of glucose at 10–15 g/L.

After 48 h, the optimal values of temperature were at 25–30 °C while at 35 °C a significant decrease of biomass was evidenced. On the other hand, the optimal values of glucose were 15–20 g/L, slightly higher than the threshold found after 24 h.

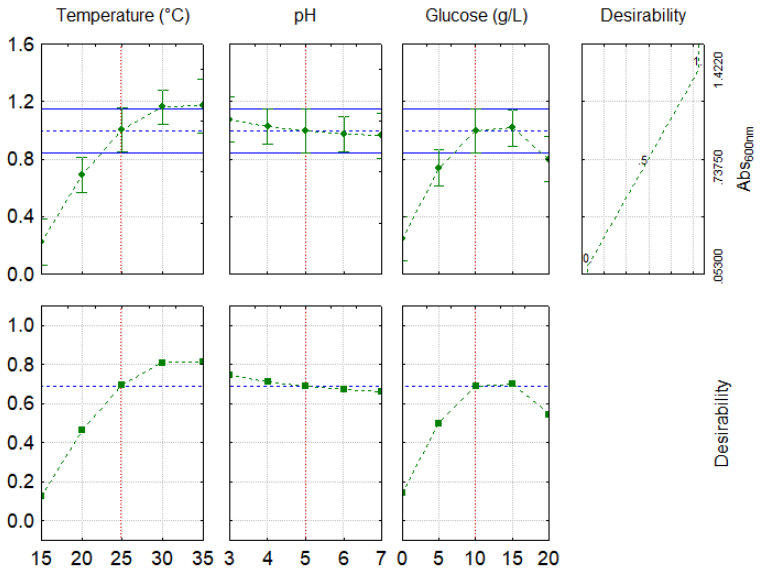

Figure 1. Desirability (lower part) and prediction profiles (upper part) for the individual effects of glucose, temperature, and pH on the biomass of the strain SB1 after 24 h.

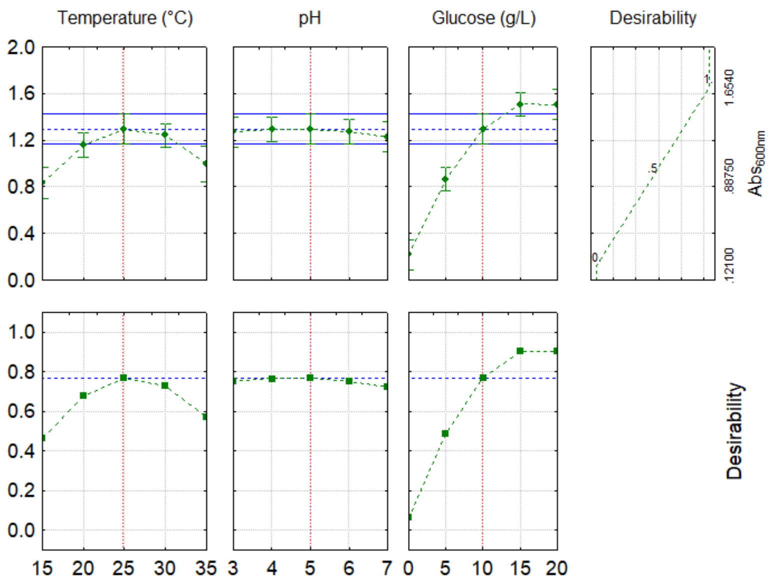

Figure 2. Desirability (lower part) and prediction profiles (upper part) for the individual effects of glucose, temperature, and pH on the biomass of the strain SB1 after 48 h.

Desirability profiles for the strains SB2 pointed out similar trends.

After assessing biomass production, the second step was on the viability of yeasts in sport drinks; once the beverages were prepared and inoculated with SB1 and SB2 strains, viable counts were assessed for 9 days under a refrigerated storage. Yeast viable count did not experience decreases or significant changes within time.

Beverages were also evaluated by 56 untrained assessors, that is athletes of a sport center. Assessors were asked to point out the control (uninoculated beverage) and then to express their preference for the two inoculated beverages.

As shown in Figure 3, 31 athletes were able to recognize, while 22 failed.

During the sensory test, a propensity toward the beverage containing strain SB2 was found. The beverage containing the strain SB1 was regarded as unpalatable, due to an attenuation of aromas, sour smell and a different color.

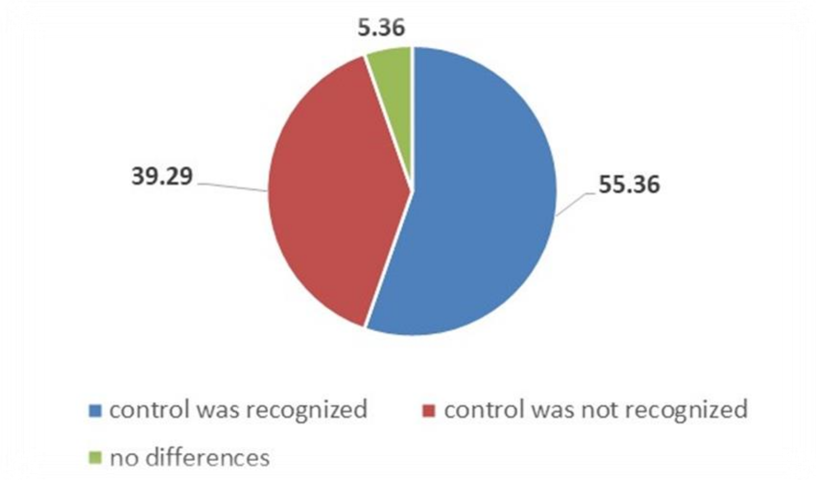

Figure 3. Do you recognize the control (uninoculated beverages)? Outputs of athletes (%).

4. Discussion

S. cerevisiae var. *boulardii* is a well-known probiotic yeast for its ability to maintain a balanced gut microbiota, as well as for its effect on the amelioration of lactose tolerance, stimulation of the immune system and prevention of the formation of carcinogenic substances in the intestine [9,10].

The supplementation of probiotics in foods is a challenge for several reasons, that is the production of biomass and the survival of the probiotic strains in the product, as well as the possible effects on the sensory scores. This paper addresses both these issues, focusing on commercial probiotic strains, elucidating the role of some factors on biomass production, and then proposing an exploratory approach for the use of *S. boulardii* in a beverage for athletes. Many variables could affect yeast growth under controlled conditions, resulting in enhanced or delayed kinetics. In this research, two intrinsic factors (pH, and glucose) and extrinsic variables (temperature) were assessed.

There are several approaches for the optimization of biomass production/growth kinetic, and the Central Composite Design is a suitable methodology. However, the optimization of the output through the desirability approach suggests that the individual effect of a variable could be modified through the interaction with other factors.

Generally, sugar concentrations up to 200 g/L were used in the past for the optimization of biomass production from *S. cerevisiae* [11,12], while lower concentrations were tested in this paper following preliminary experiments, which showed a delayed growth kinetic of the two strains of *S. boulardii* at high glucose concentrations.

Another step of this research was a focus on the possibility of using *S. cerevisiae* var *boulardii* strains in a beverage intended for athletes, as some data of the literature suggest the interest towards probiotic products from this niche of consumers [13].

This paper confirms the possibility of a supplementation of two commercial probiotic yeasts of *S. cerevisiae* var. *boulardii* in a beverage intended for athletes, as the strains survived in the product; however, the two strains had different effects on the sensory profile and the strain labelled as SB1 (from Enteroboulardi) was referred as not acceptable by some assessors, while the profile of the beverage with SB2 strain (Codex preparation) was judged as acceptable and pleasant.

This paper represents exploratory research on two probiotic *S. boulardii* strains, used as test or case-studies, and shows the importance of a multitarget approach (growth kinetic and biomass production, statistic treatment of the results, evaluation of effects on sensory scores) to implement and optimize new kinds of probiotic beverages.

Author Contributions: Conceptualization, A.R. and M.S.; methodology, A.A. and A.R.; formal analysis, A.A. and A.R.; investigation, A.A. and A.R.; resources, M.S.; data curation, A.A. and A.R.;

writing—original draft preparation, A.A.; writing—review and editing, A.R.; supervision, M.S.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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