Heliyon 9 (2023) e21190

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Physicochemical and microbiological parameters during the manufacturing of a beer-type fermented beverage using selenized *Saccharomyces boulardii*

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ARTICLE INFO

Keywords:
Selenium
Selenoamino acid
Beer
Saccharomyces boulardii

ABSTRACT

Selenium is an essential trace element in human health. However, it has been considered a widespread selenium deficiency worldwide, although the recommended daily intake is very low (55 μg per day). Strategies have been implemented to comply with the recommended doses, for example, through bioavailable selenium such as selenoamino acids. Thus, this research aimed to elaborate on a beer-type fermented beverage produced with previously selenized *Saccharomyces boulardii*. For this, the yeast was selenized by adding a minimum inhibitory concentration of Na_2SeO_3 (74 ppm) to YPD media. Subsequently, barley must fermentations were carried out for 120 h. Kinetic parameters of the fermentation and physicochemical parameters and selenium content of the beverage were measured. The yeast accumulated up to 25.12 mg/g of dry cell. Furthermore, selenization affected the fermentation rate, but the beverage's physicochemical parameters were not different from those of the control. Due to the final concentration of selenium in the beverage (0.378 mg/kg), it is considered a process that confers advantages for the safe intake of selenium with bioavailable potential. In conclusion, fermented beverages enriched with organic selenium could be produced through cell selenization to produce functional beverages and food.

1. Introduction

Selenium is a trace element essential for life. This trace element has been shown to have antioxidant effects, and its intake in adequate concentrations helps to prevent different diseases such as cancer and diabetes, as well as to improve thyroid function and male fertility [1]. The sources of selenium are diverse, such as celery, cucumber, broccoli, and some cereals, such as wheat and barley. Their selenium content will depend on the selenium in the soil where they grow [2]. Despite all sources of selenium, it is generally

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https://doi.org/10.1016/j.heliyon.2023.e21190
Received 13 May 2023; Received in revised form 13 September 2023; Accepted 18 October 2023
Available online 19 October 2023

found in its inorganic form as selenite or selenate, both of which are not fully bioavailable. For this reason, the necessary daily requirements for human beings are not fully satisfied (55 μg/day), especially in regions where this element is still scarce on Earth [3].

The beneficial effects of Se on human health are attributed to the presence of functional selenoproteins involved in physiological processes such as antioxidant defense (glutathione peroxidases), thyroid homeostasis (iodothyronine deiodinase), redox regulation of cellular processes (thioredoxin reductase), Se transport and delivery to peripheral tissues (selenoprotein P), protein folding, and endoplasmic stress (selenoprotein 15, selenoprotein M, selenoprotein N, and selenoprotein S). Thus, Se deficiencies have been associated with cardiovascular disease (Keshan disease), cancer (liver, colon, skin, and breast), infectious diseases, type 2 diabetes, thyroid disorders, and male infertility [4].

Due to the deficiencies of this element in the human body and its low absorption after ingestion, the selenization of microorganisms, such as yeasts, has been proposed, which are capable of biotransforming Se and expressing it organically in the form of proteins. These microorganisms later serve as food supplements or as ingredients in fermented foods. They introduce inorganic Se into the polypeptide chains of their ribosomes to subsequently express it as selenocysteine [5].

Yeast selenization benefits the conversion of inorganic selenium to selenoamino acids such as selenocysteine (SeC) and selenomethionine (SeM). The most studied yeast in selenization techniques is *Saccharomyces cerevisiae* [5–8]. However, new research supports using Saccharomyces boulardiii, the only yeast approved and used as a probiotic in humans. This yeast has been used to treat gastrointestinal diseases, such as acute diarrhea and traveler's diarrhea [9,10]. Additionally, it has been shown that *S. boulardii* is capable of metabolically producing selenoamino acids and selenium nanoparticles, enriching growth media with sodium selenite [1].

Currently, and intending to cover selenium deficiencies, research has focused on producing selenium-enriched foods and supplements. Companies such as Altech, Lallemand, Selko, Orffa, and Angel Co., offer selenium-enriched yeasts for use in livestock feed to obtain selenium-enriched products such as eggs, milk and meat [11–13]. Regarding beverages, such as beer, research has focused on enriching the soil to obtain barley and wheat with a high selenium content and use them in their production [14,15]. Another way to obtain selenium-enriched beer is to directly add sodium selenite to the barley wort before adding the yeast [16]. Since beer is one of the most consumed beverages in the world, after tea and water [17], selenium-enriched beer could be considered a vehicle to supplement selenium in the diet and cover its deficiencies. In this context, the objective of the present investigation is to take advantage of the prebiotic characteristics of *Saccharomyces boulardii* and use it in a selenized form to ferment barley must produce beer for its use as a vehicle in the intake of organic selenium.

2. Material and methods

2.1. Selenization of Saccharomyces boulardii

A study on the selenium accumulation capacity of *Saccharomyces boulardii* was carried out independently [1]. The working group has already reported the minimum inhibitory concentration, so the same protocol was followed. According to this study, it was determined that the minimum concentration of Na_2SeO_3 placed in the yeast growth medium was 74 ppm. Thus, selenized yeast was produced by enriching YPD broth with 74 ppm Na_2SeO_3, and 10^7 CFU/mL of *Saccharomyces boulardii* ATCC were inoculated. Then the inoculated medium was incubated at 37 °C. At the end of 24 h, it was centrifuged (7800×*g*) for 5 min (SORVALL-Fresh, Thermo Fisher Scientific, USA), and the biomass was separated from the supernatant.

2.2. Fermentation

2.2.1. Inoculum preparation and activation

After cell recovery, the supernatant was seeded onto YPD agar previously enriched with 74 ppm Na_2SeO_3, incubating under aerobic conditions at 37 °C for 48 h. Selenized *Saccharomyces boulardii* cells (reddish colonies) were scraped and transferred to YPD broth enriched with the same concentration of Na_2SeO_3, incubated at 37 °C for 24 h, and after fermentation, centrifuged (7800×*g*) for 30 min. The resulting biomass was washed with deionized water and finally lyophilized (Labconco) and saved for analysis and fermentation. Some lyophilized selenized *Saccharomyces boulardii* and *Saccharomyces boulardii* (control) were inoculated into YPD broth test tubes. They were incubated at 37 °C for 24 h. This broth was used for the fermentation of the barley must.

2.2.2. Wort preparation

For the preparation of wort, the recommendations of Castro et al. [18]. were followed, along with the experiences of the research group. A batch of beer wort was prepared on a laboratory scale in Erlenmeyer flasks of 1 L capacity. It was based on a style of Amber Ale beer. To prepare the wort, previously ground pale ale malt (Simpsons malt) was added to a mash vessel containing water heated to 65 °C. The malt/water ratio was 15 % weight/volume. The maceration was carried out for 60 min, maintaining the temperature at 65 °C. A filtration process removed the beer bagasse. The resulting wort was brought to a boil with stirring for 60 min to achieve a hot break. After 30 min of boiling, Yellow hops (Yakima Chief) were added in pellets with 9.8 % alpha-acids to achieve a total content of 20 IBUS. The added proportion was 0.08 % weight/volume. The wort was cooled to separate hop sediments and proteins at the end of the boiling and the incorporation of hops. The wort was separated into two flasks, and the lost volume of water was recovered. The original gravity (OG) was 1.050 for the control (*S. bulardii*) and 1.048 for the wort inoculated with selenized *S. boulardii*. The inoculation of the yeasts was carried out when the wort reached a temperature of 22 °C. Later, the inoculated wort was manually homogenized, and the fermentation process was carried out at 22 °C for 120 h. The viability and pH of both yeasts were monitored.

2.2.3. Kinetic parameters

The speed, the generation time, the growth kinetic constant, and the deceleration zone of the fermentation were calculated to establish the differences in the metabolism of each yeast. The specific growth rate constant (μ) was calculated according to Eq. 1. Equations 2 and 3 were used to determine the generation time (g) and the growth rate constant (K). The initial (N_0) and final (N_x) biomass concentrations correspond to the selected time interval within the logarithmic phase of growth, which were t_0 and t_x, respectively. The determination of the deceleration zone using the GeoGebra software (https://www.geogebra.org) using the Talmage and Fitch graphic method with modifications [19].

Ec 1. $\text{Ln}(N_x) - \text{Ln}(N_0) = \mu (t_x - t_0)$

Ec. 2. $g = 0.693 / \mu$

Ec. 3. $K = 1 / g$

2.3. Selenium content

Selenium content analysis was performed by ICP-OES according to the unmodified methodology of González-Salitre et al. [1]. The analysis was carried out on the biomass of selenized *S. boulardii*, on the fermented beverage after the separation of biomass resulting from fermentation and spent yeast.

2.4. Physicochemical parameters

The pH was measured in triplicate during the 120 h the fermentation lasted. The specific gravity was measured with the help of a QWORK hydrometer with a specific gravity scale of 0.99–1.16. For this, a sample of wort was taken in a 250 mL capacity test tube, the hydrometer was inserted, and the reading was taken. The procedure was carried out in triplicate. To determine the alcohol content, the Balling equation [20] was used by first multiplying the specific gravity by 1000:

$A_{v/v} = 0.125 * (OG - FG)$

2.5. Statistic analysis

Results were analyzed by one-way ANOVA ($p < 0.05$) and by Tukey's using the NCSS statistical software (NCSS 2007, v.0, Kaysville, UT, USA, 2007).

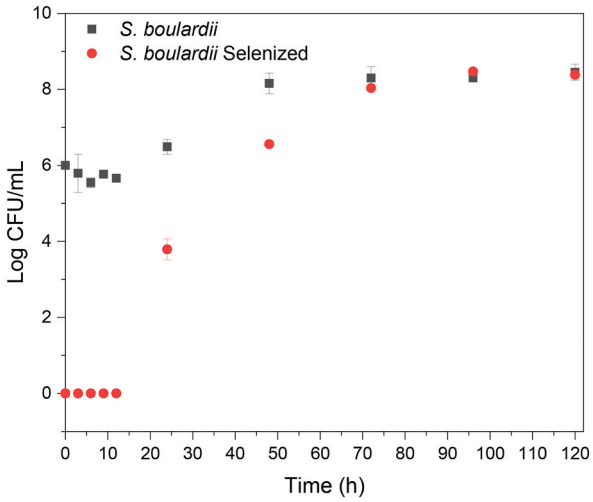

Fig. 1. Growth of *Saccharomyces boulardii* selenized (•) and Saccharomyces boulardii (■) in barley must. Results are expressed as the mean ± standard deviation (n = 3).

3. Results

3.1. Selenization of Saccharomyces boulardii and fermentation

The selenium-rich probiotic yeast produced a reddish yeast with a selenium accumulation of 25.12 mg/g of dried biomass. The technique used in this study resulted in adaptation and probably selenium dependence, which, when reseeded, was followed by selenium accumulation.

The fermentation was followed for 120 h, where viability (Fig. 1) and pH (Fig. 2) were monitored. The first figure shows an adaptation period of more than 12 h for the selenized yeast, reaching its stationary phase up to 72 h but reaching the same viability as the control yeast at 120 h. A consistent behavior concerning pH was observed, where pH decreased up to 24 h for the selenized yeast; both results indicate that the fermentation of selenized *Saccharomyces boulardii* in barley wort began up to 24 h later compared to control yeast. This more extended period of adaptation by the selenized yeast may be mainly because the selenized yeast is growing on a medium that does not contain selenium.

As shown in Table 1, there was a significant difference in all the kinetic parameters of both fermentations ($p < 0.05$). The selenized yeast has a lower speed (μ) concerning the control. This behavior coincided with the generations (g) per hour, which were lower than those observed in the control. Therefore, the constant (K) was lower in control than in the selenized yeast. Likewise, the deceleration phase (end of the logarithmic phase and beginning of the stationary) was reached faster in control yeast than in selenized yeast, and this is due to the correlation that exists with the growth rate and the acceleration of metabolism.

3.2. Physicochemical parameters of the fermented beverage

The physicochemical parameters between the fermented beverage using selenized yeast and the control fermentation varied remarkably (Table 2), and there was a significant difference in all the parameters analyzed ($p < 0.05$). One of the most critical parameters of a beer is the final gravity. Regarding the final gravity, a better attenuation was observed for the fermented with the control yeast. However, it is not ideal for a beer to obtain a better attenuation. The final gravity should be between 1.008 and 1.012, depending on the style of beer [21]. In addition, alcohol production was 1.25 % lower for the beer that used selenized yeast. In the case of pH, the decrease as a function of time was similar in both fermentations, reaching a value of 4.3.

3.3. Selenium content of the fermented beverage

Finally, this is the first report of brewing with a selenized probiotic yeast. A craft beer style was chosen since these beverages do not undergo filtration, clarification, or pasteurization, allowing yeast viability and a more significant amount of selenium in the beverage. The initial selenium content in selenized *S. boulardii* was 25.12 mg/g. For the starter culture, 230 ± 0.2 mg of yeast was weighed at the end of fermentation, and after the ICP-OES analysis, it was found that 0.378 mg/kg were present in the beer and 5.302 mg/kg in the spent yeast.

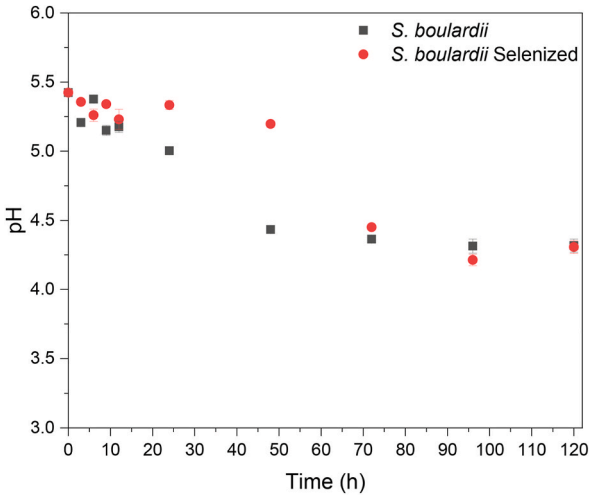

Fig. 2. pH of Saccharomyces boulardii selenized (●) and *Saccharomyces boulardii* (■) in barley must. Results are expressed as the mean ± standard deviation (n = 3).

Table 1
Kinetics parameters of *Saccharomyces boulardii* selenized and *Saccharomyces boulardii* (control).

	S. boulardii selenized	Control yeast
Velocity rate μ (h^{-1})	0.28 ± 0.03^a	0.14 ± 0.06^b
Generations (g)	2.39 ± 0.26^a	4.77 ± 0.78^b
Growth constant *k* (g/h)	0.41 ± 0.00^a	0.20 ± 0.01^b
Deceleration time (h)	113.84 ± 1.23^a	98.25 ± 0.98^b

Table 2
Physicochemical parameters, yeast viability and selenium content of beers produced by *S.boulardii* selenizase and *S. boulardii*.

Parameter	Saccharomyces boulardii selenized	Control yeast
OG	1.048 ± 0.00^a	1.050 ± 0.00^a
FG	1.029 ± 0.00^a	1.020 ± 0.00^a
%Alc. Vol.	2.5 ± 0.007^a	3.75 ± 0.006^b
pH	4.307 ± 0.047^a	4.317 ± 0.047^a
Log10 CFU/mL	8.385 ± 0.120^a	8.450 ± 0.212^a
Se (mg/kg)	0.378 mg/kg	–

Results are expressed as the mean ± standard deviation (n = 3).

4. Discussion

4.1. Selenization

Similar results to those obtained in this study have already been reported in a previous study by the working group during the selenization of *S. boullardii* [1]. The high accumulation of selenium has not been previously achieved; studies report an accumulation for *S. cerevisiae* of up to 1.19 mg/g of dried biomass when enriching the medium with 15 mg/L of selenite [8] and 3.4 mg/g for *S. boulardii* at 9 h of growth in a medium enriched with 74 ppm of selenite.

Different factors affect selenium uptake by yeast. Within these factors is the selenium concentration in the medium [22]. It is known that at concentrations close to 15 ppm, *S. cerevisiae* absorbs up to 20 % of the selenium in the medium. These data coincide with what Yoshinaga et al. [23] observed, who determined an absorption more significant than 10 % in *S cerevisiae* when used up to 60 ppm selenium in the medium.

4.2. Fermentation

Very few studies have realized the importance of fermentation kinetics in systems in which a selenized yeast is used or in the selenization of food products and beverages. However, it has been observed that in selenized microorganisms, there are differences in the kinetic parameters of each fermentation, identifying that both the generations and the duplication rate were lower in fermentations of selenized microorganisms than in those where the same microorganisms were used without selenized. Likewise, due to these decreases, the stationary phase was reached more slowly in fermentations with selenized microorganisms [24]. This same behavior has been observed by Ye et al. [25], determining that in a YPD medium prepared for selenized *S. cerevisiae*, a logarithmic phase was found up to 32 h, while for the control, it was reached at 21 h.

Selenium's presence in the medium directly impacts the yeast replication rate. A similar experiment determined that the presence of selenium does not allow reaching more than 3.3 log CFU/mL and that the deceleration phases of the growth curve are reached in longer times. This is coincident with a study in which it was determined that the presence of 50 ppm of selenium in the medium has an effect of decreasing growth rate close to 85 % and that it increases up to 93 % if the selenium concentration is doubled [7,26,27]. This effect has recently been explained by Kieliszek et al. [28], who stated that this behavior is the effect of a detoxification process initiated by excess selenium in the environment.

4.3. Physicochemical parameters of the fermented beverage

A study by de Paula et al. [29] mentions that fermenting wheat beer wort with *Saccharomyces cerevisiae* var. *boulardii* also did not match the performance of *Saccharomyces cerevisiae* since it did not reach the same final gravity values. This is due to the preference of *S. boulardii* for glucose over other sugars, such as maltose, which is only consumed when glucose is scarce [30]. Therefore, the fermentation must take a longer time.

Previous investigations on the production of beer with *S. boulardii* have reported that the alcohol production (3.83 (%v/v)) is like the control yeast using *S. cerevisiae* (3.75 (%v/v)) [31]. In the same way, the authors mention that the attenuation is low, for which they have opted for mixed fermentations with *S. cerevisiae* for better attenuation and alcohol production. Other studies using *S. boulardii* for elaborating wheat beer report an alcohol production of 4.01–4.40 (% v/v). Therefore, the production of alcohol depends on the

amount of glucose in the medium and the attenuation mentioned above. However, in this study, the alcohol production by selenized yeast was affected. Although Sánchez-Martínez et al. [16] mention that, during beer production in the presence of sodium selenite, alcohol production was not affected. More studies would be needed to elucidate why alcohol production is decreased when the wort is fermented with selenized yeast. Similar results were reported by Mulero-Cerezo et al. [32] for an India Pale Ale-style beer. After fermentation with *S. boulardii*, the pH reached a value of 4.7. On the other hand, de Paula et al. [29] report a pH of 4.17 and 4.52 for wheat beer, and they conclude that this effect is due to the production of organic acids by the probiotic yeast, which has been reported by Chan and Liu [33].

4.4. Selenium content of the fermented beverage

Similar studies carried out with *S. cerevisiae* report that when enriching the wort with increasing concentrations of Na_2SeO_3 (0.2–20 μg/mL), the selenium concentration in the beer also increased with minimum values of 0.086 μg/mL and maximums of 6.00 μg/mL [16]. Another study was based on enriching barley with Na_2SeO3 (10 and 20 g/ha), of which 20.6 and 17.9 μg/kg were present in the beer [15]. This demonstrates a new way to obtain selenium-enriched beer, which allows more excellent retention of selenium in the beverage. In addition to the fact that selenium from selenized *S. boulardii* is present as selenoamino acids and selenium nanoparticles, as reported by our research group previously [1].

Due to selenium deficiency in humans, this has become an endemic problem worldwide. Although there are supplements on the market with adequate selenium content to meet daily requirements, this selenium is generally of inorganic species in the form of selenite or selenate. That is why this study is essential, as it provides an alternative for the development of a selenized probiotic yeast through which more bioavailable selenium could be consumed or in the form of nanoparticles [1]. That is why this probiotic yeast used in the study (*S. boullardii*) is a suitable microorganism to be used to transport organic selenium species.

When this type of microorganism is included in processes to produce beer-type fermented barley beverages, selenium is directed primarily to meet the requirements of humans, and, on the other hand, processed food would be a promising element with nutraceutical and functional capacity. However, different challenges and opportunities are revealed after analyzing the results. Among the most significant opportunities is to carry out in vivo studies to determine the bioactive efficiency of the drink and determine if selenium can fulfill its function. In addition, determining selenium species in the beverage and the probable presence of nanoparticles is a challenge. With this, the study would be completely open to new explorations determining the importance of selenium in fermented beverages such as beer.

5. Conclusion

This study observed that selenized *S. boulardii* could ferment barley wort under the same fermentation conditions used for control yeast. Likewise, the physicochemical parameters of the resulting drink did not vary from each other. This is a technological advantage since selenized yeast could be used as an ingredient in the preparation of beverages that do not have this benefit, keeping all its characteristics. However, it is necessary to carry out some studies to obtain the best fermentation kinetic controls and physicochemical parameters for a beer-type beverage made with a selenized yeast comparable to a non-selenized yeast to give greater technological value. Likewise, using a selenized probiotic yeast to prepare a beer-type drink is a great advantage. It represents an opportunity within the challenges of manufacturing functional foods. This has to do with the accumulation of selenium, which is probably more bioaccessible and bioavailable. Due to global selenium deficiencies, the presence of organic selenium represents an important source of this element, which has been obtained in a biogenic way. That is why the results obtained in this research represent a potential alternative to developing fermented beverages with a high selenium content, which could positively impact human health.

Data availability statement

Data included in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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