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Spectroscopic Analysis of Selenium Nanoparticles Synthesized by *Saccharomyces boulardii* for the Production of Craft Beer

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Abstract: Selenium is an essential micronutrient which is found in many foods and beverages in low concentrations. Craft beer, one of the most widely consumed fermented beverages globally, presents a strategic opportunity for selenium intake through organic nanoparticles. This study aimed to confirm the presence of selenium nanoparticles in the fermentation process of an ale-style beer using *S. boulardii* yeast selenized with Na_2SeO_3 (74 ppm), through spectroscopic analysis and TEM. The yeast accumulated 5.92 mg/g of dry cell mass, and the beer contained 0.642 mg/g of selenium. UV-VIS detected nanoparticles with a peak at 300 nm and FT-IR at a wavelength of 1398.85 cm^{-1}. The particle size ranged between 74 to 175 nm, with a maximum ζ-potential of −4.2 mV, an electrophoretic mobility of −0.3492 μm × cm Vs^{-1}, and a conductivity of 2.656 mS cm^{-1}. TEM analysis revealed that the nanoparticles exhibited circular/ovoid shapes. The fermentation process, combined with the ingredients used to produce ale-type craft beer, proved to be a feasible method for the biosynthesis of selenium nanoparticles using *S. boulardii*, offering a reliable option for developing and innovating functional craft beers.

Keywords: *S. boulardii*; nanoparticles; selenium; craft beer; spectroscopy

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1. Introduction

Selenium (Se) is an essential, functional micronutrient that is crucial to human health. According to the National Institute of Health (NIH), the recommended daily intake of Se for adults is 55 μg/day [1]. S exists in organic forms, such as selenomethionine (SeMet), selenocysteine (SeCys), selenocysteine (SeCys2), and methylselenocysteine (MeSeCys), as well as inorganic forms like SeVI and SeIV. Both forms exert various bioactive effects, including antioxidant, anticancer, fertility, reproductive, metabolic, and immune system functions [2–6]. Insufficient Se intake has been linked to chronic degenerative diseases. Se is now recognized as an essential trace element for human health, and extreme deficiencies have been observed worldwide, affecting individuals of all ages. Therefore, it is vital to address Se deficiency by supplementing with selenium-enriched foods or yeast biomasses.

Saccharomyces boulardii (*S. boulardii*) is a probiotic yeast that is tolerant to acidic pH and offers numerous health benefits, particularly in the management of gastrointestinal disorders. The recommended average consumption of probiotics is 10^7 CFU/g (100 mg/day) to achieve beneficial effects on the intestinal mucosa, combat pathogenic intestinal microbiota, prevent traveler's diarrhea, reduce symptoms of inflammatory bowel disease, irritable bowel syndrome, dyslipidemia, colic inflammation, and manage oxidative stress, among others health conditions [7–10]. Yeasts, like *S. boulardii*, can transform and incorporate inorganic Se into their cellular structures, enhancing its bioavailability and minimizing its toxicity. Research on *S. boulardii* as a biogenic yeast has demonstrated its ability to synthesize inorganic Se (Na_2SeO_3) into seleno-amino acids and selenium nanoparticles (SeNPs), with SeNPs becoming an area of growing interest [11,12].

The trend toward innovation and development of new food products is increasingly focused on SeNPs as a promising vehicle for the intake of organic Se, thereby supporting consumer health. However, these SeNPs still require instrumental analyses to better understand their behavior in complex food matrices. Fermented beverages such as craft ale beer are recognized for their health benefits, which arise from their ingredients (water, malt, hops, and yeast), and are among the moderately consumed beverages worldwide [13].

Lager-type craft beer, which ferments at temperatures between 8 to 15 °C, and ale-type beer, which ferments at temperatures ranging from 16 to 25 °C, have beneficial health properties. Ale-type beers, in particular, offer these benefits due to a unique blend of base ingredients, such as malt and hops, compared to lager-type beers. Both types are consumed moderately on a global scale [14].

The fermentation process for both ale and lager follows the same principle, which involves the extraction and hydrolysis of carbohydrates from key malted grains, including barley and wheat. This process generates a concentrated wort of sugars, free of α-glucosides, including maltose (55%), maltotriose (15%), and dextrins (α-glucosides-polysaccharide), in addition to a mixture of simple sugars (glucose, fructose, and sucrose), which collectively comprise about 20% [15].

The fermentation process begins with the addition of yeast. Yeast from the Saccharomyces genus, such as *Saccharomyces cerevisiae*, *S. paradoxus*, *S. mikatae*, *S. jurei*, *S. kudriavzevii*, *S. arboricola*, *S. eubayanus*, or *S. uvarum* is commonly used. However, industrial fermentations often involve hybrid yeasts, such as *S. cerevisiae*/*S. eubayanus* (*S. pastorianus*), *S. eubayanus*/*S. uvarum*, and *S. cerevisiae*/*S. kudriavzevii*, as well as probiotic yeasts such as *Saccharomyces boulardii*, which provide additional health benefits. Other yeast alternatives, such as those from the *Brettanomyces* and *Lachancea genera*, are also being explored for their beneficial properties [15–18].

During fermentation, glucose molecules enter the yeast cells by diffusion through membrane proteins. Maltose and maltotriose are transported into the cell through active transport via proton symport. This process allows the yeast to regulate maltose and maltotriose consumption based on the temperature and fermentation time, as well as the characteristics of the wort. Inside the cytosol, α-glucosides are hydrolyzed into glucose molecules by the maltase enzyme, which breaks down α1–4 bonds. The fermentation process also produces various metabolites, including alcohols, esters, volatile phenols, and aldehydes, all of which contribute to the aroma and flavors of the beer [15,19].

The use of selenized *S. boulardii* to produce functional craft beer enriched with SeNPs has been successfully demonstrated, resulting in fermented beverages with organic Se through cellular selenization [16]. However, beer is a complex, two-phase, foam-type colloid system. This study focuses on the presence and behavior of SeNPs, particularly in terms of their suspension or precipitation within the colloidal matrix. We aimed to analyze the behavior and presence of SeNPs produced by the selenized *S. boulardii* yeast

on functional craft beer, employing spectroscopic analytical methods, dynamic light scattering (DLS), ζ-potential measurements, and inductively coupled plasma optical emission spectrometry (ICP-OES) to confirm the presence of SeNPs in the beverage.

2. Materials and Methods

2.1. Selenization of S. boulardii

A study investigating the Se accumulation capacity of *S. boulardii* was carried out independently following the methodology outlined by González-Salitre et al. [11]. In the present study, *S. bourlardii* was inoculated in YPD with a Na_2SeO_3 concentration of 74 ppm and 1×10^7 CFU/mL and incubation at 37 °C for 24 h. After incubation, the culture was centrifuged at 7800× *g* for 5 min using a SORVALL-Fresh centrifuge (Thermo Fisher Scientific, Waltham, MA, USA), and the biomass was separated from the supernatant.

2.2. Beer Production

2.2.1. Wort

The wort preparation followed the recommendations of Castro et al. [20]. The GYPU brewery and the research group suggested the production process for an ale-type beer. A batch of ale-type beer wort was prepared on a laboratory scale in 1 L Erlenmeyer flasks. It was based on an amber ale beer style. To prepare the wort, previously ground pale ale malt (Simpson malt) was added to a mash vessel containing water heated to 65 °C. The malt/water ratio was 15% *w*/*v*. Mashing was performed for 60 min, maintaining a temperature of 65 °C. After mashing, a filtration process removed the beer grits. The resulting wort was brought to a boil and stirred for 60 min. After 30 min of boiling, yellow hops (Yakima Chief®, Central, Washington, DC, USA) in pellet form, containing 9.8% alpha acids, were added to achieve a total bitterness of 20 IBU (International Bitterness Unit). The addition rate was 0.08% *w*/*v*. The wort was then cooled to separate the hop sediments and proteins. The wort was divided into two flasks and the lost water volume was replaced. The original gravity (OG) of the control (*S. boulardii* without selenization) was 1.050, and the wort inoculated with selenized *S. boulardii* was 1.048. Yeast inoculation occurred once the wort reached 22 °C. The inoculated wort was then manually homogenized, and the fermentation process took place at 22 °C for 120 h. Yeast viability and pH were monitored throughout the process.

2.2.2. Fermentation

The yeast recovered from *S. boulardii* was plated onto YPD agar enriched with 74 ppm of Na_2SeO_3 and incubated under aerobic conditions at 37 °C for 48 h. After incubation, selenized *S. boulardii* cells, which formed reddish colonies, were scraped off and transferred to YPD broth containing the same Na_2SeO_3 concentration. The culture was incubated at 37 °C for 24 h, and after fermentation, the culture was centrifuged at 7800× *g* for 30 min. The resulting biomass was washed with deionized water, then lyophilized (Labconco, Marshall, Scientific, Hampton, NH, USA) and stored for fermentation. The lyophilized *S. boulardii* was inoculated into YPD broth in test tubes and incubated at 37 °C for 24 h. This broth was then used for the fermentation of barley wort.

2.2.3. Physicochemical Parameters of Beer

The pH was determined using a pH meter from Mettler Toledo (Columbus, OH, USA). Initially, the samples were placed in a beaker and subjected to a digital ultrasonic bath (Scientific CS-UB 32, Victorville, CA, USA) for 30 min to remove carbon dioxide and ensure

accurate pH readings. The alcohol content was determined following the methodology outlined by Cutaia et al. [21], using the Balling equation (Equation (1).

$$\%\text{Alc. Vol.} = f \times (OG - PG) \quad (1)$$

where OG and PG are the original and present (apparent) gravities multiplied by 1000 and f is a "mandatory" factor that varies from 0.125 to 0.135 since OG–PG varies from 6.9 to 100.7.

2.3. UV-Vis Spectroscopy

The nanoparticles synthesized by *S. boulardii* were characterized using a DR6000™ Hach UV VIS Spectrophotometer (Loveland, CO, USA) with a wavelength range of 200–500 nm and a resolution of 5 nm. The maximum absorbance (λmax) was identified, confirming the production of selenium nanoparticles.

2.4. Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra of the extracts were recorded between 4000 and 500 cm^{-1} using an Agilent 4300 FTIR (Santa Clara, CA, USA), with 40 scans and a resolution of 4 cm^{-1}. The spectra were obtained using the ATR module in transmission mode, with 64 scans. The data were smoothed using the "auto-smoothing" feature of the software, applying the OriginLab algorithm v9.0, (30-point moving second degree polynomial). Then, baseline correction was performed using the "auto baseline correction" feature. All FTIR measurements were repeated three times for each sample.

2.5. Dynamic Light Scattering (DLS) and ζ-Potential

The particle size distribution of SeNPs was determined by laser diffraction with static light scattering (DLS). The electrostatic stability of the particle distribution was assessed using a Litesizer 500 laser diffractometer (Anton Paar®, Graz, Austria).

2.6. Plasma Optical Emission Spectrometry (ICP-OES)

The Se content was analyzed by ICP-OES according to the methodology described by González-Salitre et al. [16]. The analysis was conducted on the selenized *S. boulardii* biomass and the fermented beverage after separating the biomass resulting from the fermentation.

2.7. Transmission Electron Microscopy (TEM)

Selenium nanoparticles produced by selenized yeast were characterized using transmission electron microscopy (TEM). The fermented beverage was degassed through sonication (Scientific CS-UB 32, CA, USA) for 30 min to remove carbon dioxide. The sample was centrifuged at 8000 RPM for 15 min, and the supernatant was decanted. The sediment (yeast) was lyophilized and plated onto carbon-coated copper grids of 3.05 mm diameter. In brightfield TEM mode, TEM images were acquired using a JEOL transmission electron microscope (JEOL-6010LA, Peabody, MA, USA), operated at 200 kV acceleration voltage.

2.8. Statistical Analysis

The results were analyzed using a one-way analysis of variance (ANOVA) with Origin software (v9.0, Northampton, MA, USA) at a 95% confidence level. The Tukey test was applied to compare means, with statistical significance set at $p \leq 0.05$.

3. Results

Natural resources, such as plants, bacteria, fungi, and yeasts, can biologically synthesize SeNPs at the intracellular and extracellular levels. These organisms utilize biomolecules as reducing and stabilizing agents, facilitating SeNP production without relying on toxic or

harmful chemical reagents. In this study, *S. boulardii* synthesized an initial Se content of 28.48 mg/g under in vitro conditions using YPD broth. However, during fermentation with beer ingredients, the Se concentration decreased. At the end of fermentation, 250 ± 0.5 mg of the spent yeast was harvested for ICP-OES analysis, revealing 5.92 mg/g of Se in the yeast and 0.642 mg/g in the final beer. The resulting craft beer did not undergo any additives, pasteurization, filtration, or clarification process, allowing the yeast and beneficial components to remain in the final product.

The fermented beverage with selenized yeast had an alcohol content of 2.4% and a pH of 4.5, while the control yeast produced a beer with 3.5% alcohol and a pH of 4.8 (Table 1). According to Mexican regulations for alcoholic beverages with nutritional or health properties, the beer meets the required alcohol content (above 2%) and pH range (2.5 to 5), suggesting its potential as a probiotic beverage [22]. Previous studies on *S. boulardii* have demonstrated its adaptability to different wort compositions, such as growth temperature, nutritional properties of the wort, alcohol tolerance capacity, effect of α and β acids provided by hops, and its potential for SeNP biosynthesis [23–25]. This research highlights the emerging biotechnological potential of *S. boulardii*, offering a promising path for enhancing functional foods with essential minerals and health benefits.

Table 1. Physicochemical parameters of beers produced by selenized and non-selenized *S. boulardii*.

Parameter	*S. boulardii* (selenized)	*S. boulardii*
pH	4.5 ± 0.022 [a]	4.8 ± 0.031 [b]
%Alc. Vol.	2.40 ± 0.004 [a]	3.51 ± 0.009 [b]

Means with different letters between columns showed significant differences ($p \leq 0.05$) using the Tukey test.

UV-vis analysis of SeNPs (see Figure 1) indicated a broad emission peak (λmax) at 300 nm, confirming the presence of Se synthesized by *S. boulardii* yeast. SeNPs produced by yeasts typically exhibit a broad emission peak (λmax) within the 250 to 350 nm range, which can be detected using a spectrophotometer. This variation in wavelength is attributed to the concentration of Se salt used during synthesis [26]. While the UV-Vis method effectively indicates the presence of Se, the peak's exact wavelength may vary depending on the specific biosynthesis technique and the concentration of the SeNPs involved.

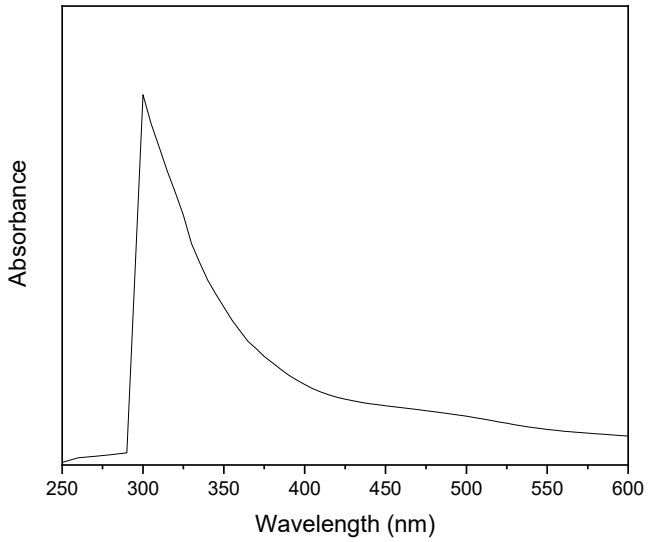

Figure 1. UV-Vis spectrum of SeNPs in beer.

FT-IR is a quantifiable surface chemical analysis technique that measures infrared light intensity as a function of wavenumber. It helps evaluate the interaction between

the synthesizing source components and SeNPs, providing insight into the functional groups responsible for Se reduction. The FT-IR spectra of the samples were compared with those of the corresponding biosynthesizing material. The results of these spectra are shown in Figure 2. The FT-IR spectrum of the sample without SeNPs (Figure 2a) displayed lower peaks than that of SeNPs (Figure 2b) synthesized by *S. boulardii* yeast. Both samples exhibited a peak at 3274.68 cm^{-1}, corresponding to O–H and N–H groups, which are associated with amide A in proteins. The peaks in the 3000 to 2800 cm^{-1} range in both samples could be attributed to C–H groups in CH_3, $>CH_2$, and $–CH_3$, indicating the presence of aliphatic chains from fatty acid residues [27]. A peak at 1634.25 cm^{-1} was observed in both samples, corresponding to strong scissor mode vibration bands of H–O–H within the amide I region of the proteins [28].

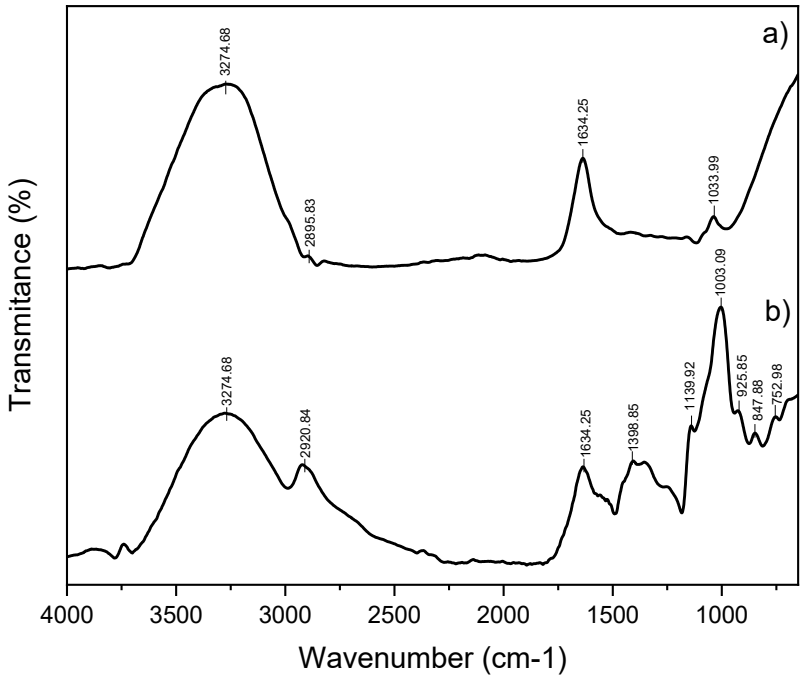

Figure 2. FT-IR spectrum of beer samples: (**a**) beer without SeNPs, (**b**) beer with SeNPs.

However, the sample containing SeNPs exhibited an additional peak at 1398.85 cm^{-1}, attributed to the COO– vibration, corresponding to side chains of amino acids and carboxylated polysaccharides. This peak, associated with the presence of SeNPs, was absent in the spectrum of the sample without SeNPs, suggesting that it may have masked the presence of both Se and the COO– υS group. The typical vibration region of polysaccharides (1200–1000 cm^{-1}) showed strong C–O and C–C stretching absorption and C–H–O/C–O–C bending absorption, indicating the presence of polysaccharides and related functional groups in proteins and polyesters. Additionally, the bands between 950 and 750 cm^{-1} displayed the fingerprint of the COO– δ group.

This study observed higher molecular component vibrations in the FT-IR spectra of the sample with SeNPs compared to the sample without, highlighting the production of other nutritional elements. These findings contribute to the growing body of research on *S. boulardii* and its potential to enhance the capabilities of probiotics, prebiotics, paraprobiotics or postbiotics.

Figure 3 shows the particle size distribution of SeNPs, which range from 74 to 175 nm, with an average particle size of 125 nm. The maximum ζ-potential was measured at −4.2 mV, with an electrophoretic mobility of −0.3492 μm × cm Vs^{-1} and a conductivity of 2.656 mS cm^{-1}. The SeNPs exhibited a spherical/ovoid shape, demonstrating varied sizes

with minimal surface energy and maximum stability. Previous studies on SeNP biosynthesis using *S. cerevisiae* revealed the presence of particle sizes ranging from 4 to 709 nm, with variations attributed to extrinsic and intrinsic factors, such as the concentrations and type of salts (e.g., SeO_2 and Na_2SeO_3) used in the biosynthesis process [26]. Additionally, the maximum ζ-potential of SeNPs synthesized by *S. cerevisiae* has been reported to range from −7.50 to −10.3 mV; these values were used to assess the surface charge and stability of the SeNPs [26].

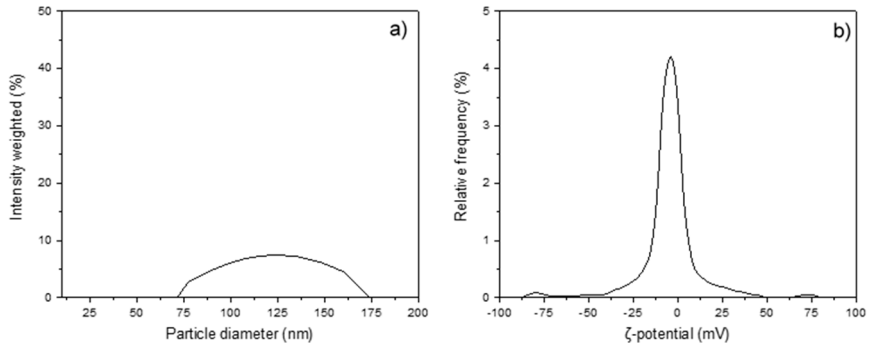

Figure 3. SeNPs synthesized by *S. boulardii*: (**a**) Particle size distribution and (**b**) ζ-potential.

TEM images of the SeNPs revealed the formation of partially spherical, ovoid-type particles during the biosynthesis process, with a size ranging from 75 to 175 nm (Figure 4). In Figure 4c, it can be observed that the smaller particles merged due to diffusibility, forming larger particles as a result of their high surface energy. This observation is consistent with the ζ-potential and DLS results from this research. TEM analysis confirmed the Se accumulation capacity of a probiotic yeast, with inorganic Se being bioconverted into SeNPs.

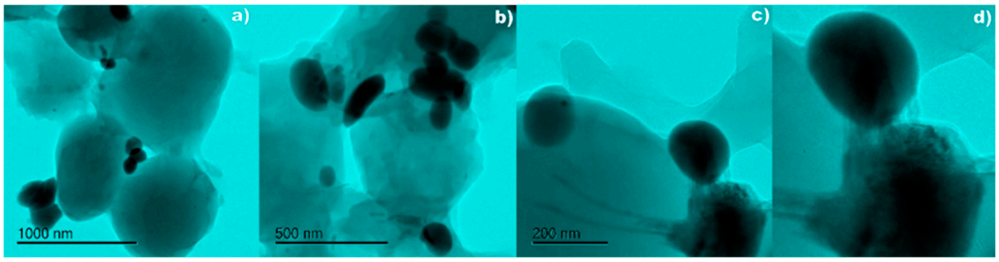

Figure 4. TEM micrographs of SeNps produced by *S. boulardii*: (**a**) 1000 nm, (**b**) 500 nm, (**c**) 200 nm, (**d**) 200 nm, amplitude of micrograph (**c**).

However, further research is needed to investigate the interactions between the external components of the yeast cell wall, such as mannans, β-1,3-glucan, β-1,6-glucan and chitin, in the formation of SeNPs. This could potentially enhance various biological activities, including antioxidant, antimicrobial, anticancer, immune regulation, and hyperlipidemia reduction, as well as compounds with potential value for multiple sclerosis treatment and improvement of intestinal health.

4. Discussion

Probiotic microorganisms have become a growing trend in food technology due to their beneficial effects on health and overall well-being, helping to prevent chronic degenerative diseases and intestinal tract disorders. While lactic acid bacteria are the most commonly used probiotics in dairy products, over 75% of the global population is lactose intolerant, limiting the use of these microorganisms. This has paved the way for developing non-dairy

probiotic alternatives, such as fermented beverages made from cereals [29,30]. In this context, the present research demonstrates the presence of SeNPs in a probiotic ale-style beer produced using *S. boulardii*, as identified through spectroscopic analysis.

Among functional beers, probiotic beer is a new development incorporating probiotic microorganisms such as *S. boulardii*. In the present investigation, similar results were observed to those reported by González-Salitre et al. [16], with initial Se values in yeast of 25.12 mg/g, as well as SeNP concentrations of 0.378 mg/g in the beer and 5302 mg/mg in the spent yeast. González-Salitre et al. [11] noted that the concentration of Na_2SeO_3 (ranging from 0 to 200 ppm) directly influences yeast metabolism, impacting the adaptation, development, and Se accumulation of *S. boulardii* during fermentation. They reported Se accumulation values ranging 0.509 ± 0.2 at 3 h to 3.402 ± 0.3 mg/g at 9 h of fermentation. These results were attributed to factors such as Se dependence, metabolic differences, Se concentration in the experimental medium, and nutritional components of the wort. However, there is still limited information on the biogenic activity of *S. boulardii* in the synthesis of SeNPs. A study conducted by Sánchez-Martínez et al. [31] investigated the biotransformation of Se at varying concentrations of Na_2SeO_3 (0, 0.2, 1, 2, 10, and 20 g Se mL^{-1}) in the production of ale and lager-type beers using *S. cerevisiae* and *Saccharomyces uvarum*, respectively. After twelve days of fermentation, they found values Se concentrations from 0.086 to 6.0 g Se mL^{-1}, with inhibition observed at levels exceeding 20 g Se mL^{-1}. Hyrslova et al. [12] demonstrated that an optimal concentration of 10 mg/L of SeNP could influence Se accumulation and metabolism under stress conditions (72 h at 25 °C). Another study by Martiniano et al. [32] used sugarcane bagasse and corn bran hydrolysates as substrates for SeNP production, with *S. cerevisiae* growing in the presence of 15 mg/L of Se, yielding 7.97 g/L of biomass and 1193 ppm of Se. Corn bran hydrolysate supported 4.25 g/L of biomass enriched with 167 ppm of Se. Capece et al. [33] examined mixed cultures of *S. boulardii* and *S. cerevisiae* during fermentation, finding that the probiotic yeast remained viable at the end of the fermentation process. In all mixed fermentations, *S. boulardii* predominated over *S. cerevisiae*, with the final beer concentration of *S. boulardii* cells ranging from 8×10^6 to 7×10^7 UFC/mL. The study also reported no significant effect on the aromatic profile of the beer, along with an increase in antioxidant capacity and total polyphenols, highlighting the potential to enhance the health benefits of probiotic beer.

Nanoparticles produced by yeast primarily accumulate inside the cells. Due to their surface plasmon resonance (SPR), they exhibit strong absorption in the ultraviolet and visible light spectrum, making them easily detectable [26]. In a study involving *Saccharomyces cerevisiae* yeast, the SeNPs that formed at varying Na_2SeO_3 concentrations (5, 10, 15, 20, and 25 µg) exhibited a maximum absorption peak (λmax) at 340 nm at 25 µg concentration, confirming the formation of SeNPs [26]. However, in extracts, bacteria, and fungi, the maximum absorbance peak due to SeNP typically ranges from 200 to 400 nm [34]. A FT-IR spectroscopy analysis of the SeNPs produced by *A. brasilense* showed varying intensities at different frequencies, indicative of proteins, polysaccharides, and lipids associated with the nanoparticles [35]. FT-IR studies have identified several functional groups on the yeast surface, with characteristic peaks at 3408.22 cm^{-1}, corresponding to the superposition of OH and NH bond stretching vibrations. Peaks at 2924.09 and 2854.65 cm^{-1} are associated with C–H stretching vibrations in lipids and proteins, while carbonyl group (C=O) stretching vibrations are observed at 1637.56 and 1716.65 cm^{-1}. The wavenumbers at 1240.23 and 1074.35 cm^{-1} correspond to the stretching vibration of the C–N and C–O bonds in yeast cell wall polysaccharides, respectively. A peak between 578.64 to 900 cm^{-1} indicates CH_3–Se and Se–O–C bonds of selenoproteins in the yeast structure [36,37]. SeNPs have also been reported in bacteria, such as *Lactiplantibacillus plantarum* CXG4 and *Bacillus* sp EKT1 using FTIR spectroscopy. These studies showed absorption peaks related to hydroxyl stretching

vibration (υ(O–H)) and the amide band (υ(NH)) at 3395 to 3380 cm^{-1}. Specific absorption peaks at 1543 cm^{-1} (amide II in protein), 1230 cm^{-1} (amide III in protein), 1077 cm^{-1} (pyranose), and the 850 cm^{-1} (cell wall polysaccharides) suggest an interaction among Se, proteins, and polysaccharides in the bacteria, contributing to their resistance to selenite and facilitating the formation of organic Se and SeNP [38,39]. The biomass obtained from *S. cerevisiae* yeast used for beer production after fermentation was initially discarded but is now valued for its beneficial properties, including as a source of β-glucans, proteins, and functional peptides, and its potential for reuse in fermentation with recognized wort, as well as for synthesizing organic minerals [40,41]. With biogenic yeasts, additional benefits can be derived, particularly in terms of mineral contributions, enabling the formation of complex NPs. The size of the SeNPs, as determined by DLS, was influenced by their metallic cores and the biomaterials deposited on their surfaces as stabilizers [42]. During NP formation, the electrostatic capacity of the components fluctuated, increasing or decreasing depending on their composition. Higher ζ-potential values, whether positive or negative, indicate better stability of the particles due to their electrostatic repulsive forces. ζ-potential values between −30 mV to +30 mV generally indicate good stability, while values closer to 0 can lead to instability and particle aggregation, as reported in [43–46]. Studies on bacteria have shown that low molecular weight molecules such as proteins and carbohydrates on biomacromolecules (SeNPs) can influence nanoparticle size regulation and facilitate their transfer outside the cell [47]. Variations in SeNP sizes during synthesis are attributed to aggregation mechanisms related to the concentration of Na_2SeO_3 used in the process [11]. The formation and sizes of the nanoparticles are influenced by factors such as the electron configuration of the trace element and the presence of nitrogenous or carbonated nutritional molecules. The initial formation of ovoid-shaped SeNPs occurs through aggregation by nucleation, where the coalescence process occurs [47,48]. There is evidence of SeNP formation using *S. boulardii* through TEM; a study by Tugarova et al. [49] characterized *Azospirillum thiophilum* BV-S biomass grown aerobically to synthesize SeNPs from selenite. TEM images revealed the presence of spherical NPs, predominantly extracellular, with a reddish color in the medium, indicating SeNP formation due to selenite reduction. In a study by Ge et al. [50], *Metasolibacillus* sp. and *Oceanobacillus* sp., both with high efficiency in selenite reduction, were compared for their ability to remove selenite and recover SeNP. TEM analysis showed SeNP formation in the cytoplasm, which was later released into the extracellular space through cell lysis, highlighting the efficient microbial reduction of selenite to SeNP as part of a detoxification process. Li et al. [38] demonstrated that *Lactiplantibacillus plantarum* CXG-4 efficiently transforms Se(IV) into SeNPs, with an enrichment rate and Se content of 64.11% and 1874 ± 14 μg/g, respectively. Transmission and scanning electron microscopy showed SeNPs with diameters between 35 to 240 nm, consistent with the results of this study. These studies illustrate that variability in SeNP sizes and shapes, influenced by factors such as concentration, physicochemical conditions, and the metabolism of each microorganism, affects the potential to transform Se(IV) to SeNPs, which is essential for successful nanoparticle production and development.

A new trend in functional beer is the emergence of probiotic beer, a novel product that synthesizes organic Se by incorporating probiotic microorganisms such as *S. boulardii*. The presence and quantification of Se in the biogenic yeast *S. boulardii* were determined using various instrumental analyses, confirming mineral's presence in the fermented beverage. This presents a significant challenge, as analytical techniques for such complex matrices are critical. Given the growing production of Se-enriched foods, it is essential to develop fast, efficient, cost-effective, and environmentally friendly methods to identify trace elements synthesized by microorganisms like *S. boulardii*. As Se is synthesized in complex products

like craft beer, it is crucial to present research utilizing spectroscopic analysis to detect this essential element accurately.

5. Conclusions

The biological production of SeNPs using *S. boulardii* is an important research area in nanobiotechnology, with significant potential for various sectors, including health, food, pharmaceutical, and agricultural. Instrumental analytical methods, particularly spectroscopic techniques, are pivotal for characterizing and verifying the presence of SeNPs synthesized by *S. boulardii* during the fermentation process, such as in producing craft ale beer. Spectroscopic analyses effectively confirmed NP formation and their chemical composition, both of which are crucial for advancing research on their biological activities and applications. Transmission electron microscopy (TEM) further revealed that SeNPs form through electrostatic attraction and diffusibility, leading to their aggregation into nanoparticles. This process demonstrates the ability of *S. boulardii* to bioaccumulate Se in the form of SeNPs. Incorporating selenized *S. boulardii* into ale-type craft beer presents an innovative approach to creating functional beverages. These beers serve as a reliable source of organic Se, a trace element with potential health benefits, offering a controlled means of Se intake within safe consumption limits. This research opens new avenues for developing functional fermented products with enhanced nutritional and therapeutic properties, contributing to the growing demand for health-promoting functional foods.

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