

Extraction and Characterization of β -glucans from Brewer Spent Yeast

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Abstract— Brewer's spent yeast (BSY), a byproduct of the beer fermentation process, has traditionally been utilized in animal feed but has gained increasing attention for its potential applications in food, pharmaceutical, and nutraceutical industries. Rich in proteins, vitamins, minerals, and bioactive compounds such as β -glucans, BSY offers a sustainable source of functional ingredients with antioxidant, immunomodulatory, and prebiotic properties. The extraction of β -glucan from spent yeast presents a promising avenue for value addition while addressing waste management challenges in the brewing industry. This review comprehensively explores the composition, extraction methods, and functional properties of β -glucan derived from BSY, with a focus on its applications in food science, healthcare, and microbiome modulation. Additionally, this paper highlights advancements in processing techniques aimed at improving the purity and bioavailability of β -glucans. The increasing interest in BSY-derived β -glucan as a natural, sustainable, and multifunctional ingredient underscores its potential to contribute to health and wellness while promoting circular economy principles in food production.

Index Terms— Brewer's Spent Yeast (BSY), β -Glucan Extraction, Antioxidant Activity, Brewery Waste Valorization, FTIR Spectroscopy

I. INTRODUCTION

The brewing industry generates substantial byproducts, with brewer's spent yeast (BSY) being one of the most abundant residues. Traditionally, BSY has been used as animal feed due to its high protein content (45-60%), but recent research has highlighted its potential as a valuable ingredient in food, pharmaceutical, and nutraceutical applications (Avramia & Amariei, 2021). As the food industry shifts towards sustainability and waste valorization, BSY is gaining recognition as a functional ingredient with significant health benefits. It contains bioactive compounds such as β -glucans, mannoproteins, and polyphenols, which contribute to its antioxidant, immunomodulatory, and prebiotic properties (Rakowska et al., n.d.). β -Glucan, in particular, has emerged as a key component due to its numerous biological activities, including cholesterol-lowering, immune-enhancing, and gut microbiota-modulating effects (Camilli et al., 2018).

β -Glucans are natural polysaccharides composed of glucose units linked through β -(1,3) and β -(1,6) glycosidic bonds, commonly found in fungi, yeast, and cereal grains such as oats and barley (Chan et al., 2009). The physicochemical properties of β -glucans, including solubility, molecular weight, and branching structure, influence their functional benefits, making them valuable ingredients in functional foods, pharmaceuticals, and dietary supplements (Davani-Davari et al., 2019). Studies have demonstrated that β -glucans derived from yeast, including BSY, possess superior immune-boosting properties compared to their cereal-derived counterparts due to their specific structural characteristics (Amer et al., 2021a). The potential applications of yeast β -glucan extend to cancer therapy,

cardiovascular health, metabolic regulation, and gastrointestinal health, making it an attractive candidate for incorporation into health-promoting food products.

The extraction and purification of β -glucan from BSY present both opportunities and challenges. While conventional methods such as hot water, alkali, enzymatic, and ultrasound-assisted extractions have been explored, optimizing these techniques to enhance yield, purity, and bioavailability remains a key research focus (Chioru & Chirsanova, 2023). The development of cost-effective and scalable extraction processes is critical to maximizing the commercial viability of BSY-derived β -glucan. Additionally, recent studies have explored innovative processing methods, such as modified acid-base extractions, to enhance the biological activity of β -glucans, further expanding their functional applications (Amer et al., 2021a).

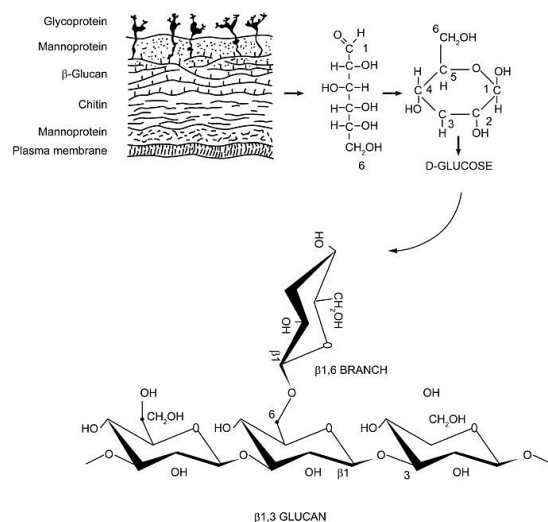


Fig 1: β -1,3 glucan (Chan et al., 2009)

Beyond human nutrition, β -glucans have been studied as potential antibiotic alternatives in animal feed, particularly in poultry production, where they have been shown to enhance immune response and reduce reliance on synthetic antibiotics (Schwartz & Vetvicka, 2021). Furthermore, their role as prebiotics has gained attention for their ability to selectively stimulate beneficial gut microbiota, such as *Bifidobacterium* and *Lactobacillus* species, leading to improved digestive health and overall well-being (Wang et al., 2004).

This review aims to provide a comprehensive overview of the properties, extraction methods, and functional applications of β -glucan derived from BSY. It will also highlight recent advancements in processing techniques, potential industrial applications, and the future outlook of this underutilized resource. By integrating BSY-derived β -glucan into the food and healthcare industries, there is an opportunity to reduce industrial waste while enhancing public health through sustainable and functional nutrition solutions.

Table1: Literature Review

Serial No.	Microorganism Species	Substrate Source	Carbon and Nitrogen Profile	Biomass Production	References
1	β -glucans are found in the cell walls of bacteria and fungi, including species from <i>Ganoderma lucidum</i> , <i>Lentinus edodes</i> , <i>Grifola frondosa</i> , and <i>Schizophyllum commune</i>	These β -glucans are primarily derived from medicinal mushrooms and bacterial cell walls	not mentioned	not mentioned	Chan et al. (2009)
2	<i>Saccharomyces cerevisiae</i> (HII31 strain showed the highest β -glucan content)	not mentioned	not mentioned	not mentioned	Pengkumsri et al., 2016
3	<i>Saccharomyces cerevisiae</i>	Spent brewer's yeast from beer production	High protein (45-55% dry weight) and nitrogenous compounds, 7% ash	Derived from spent yeast	Rakowska et al., 2017
4	<i>Saccharomyces cerevisiae</i>	Derived from brewing processes, yeast ferments sugars from grain (e.g., wort) into alcohol	Low carbon-to-nitrogen ratio, high protein content (45-60%), rich in essential amino acids and carbohydrates (e.g., B-glucans)	BSY is the biomass generated post-fermentation with significant nutritional potential	Jaeger et al., 2020
5	<i>Saccharomyces cerevisiae</i>	Glucose-based media	High glucose, minimal nitrogen input	~2.0 mg/5 mL dry weight	Utama et al., 2020
6	<i>Saccharomyces cerevisiae</i>	Spent brewer's yeast (by-product of beer production)	Yeast cell wall: 50-55% B-1,3-glucans, 10-15% B-1,6-glucans, and minor polysaccharides (mannoproteins, chitin). Nitrogen from proteins, reduced by alkaline treatments	Residual yeast biomass: 0.9 million tons/year in the EU; glucan content varies by strain and conditions	Avramia & Amariei, 2021

7	<i>Saccharomyces cerevisiae</i> (yeast)	Glucose-based liquid growth media containing K ₂ HPO ₄ , (NH ₄) ₂ SO ₄ , NaH ₂ PO ₄ , MgSO ₄ ·7H ₂ O, CaCl ₂ ·2H ₂ O, and yeast extract	Carbon from glucose (20 g/L); Nitrogen from (NH ₄) ₂ SO ₄ (3.35 g/L) and yeast extract (6 g/L).	Not explicitly detailed, but yeast cells were cultivated and harvested for extraction procedures.	Karslioglu et al., 2021.
8	<i>Saccharomyces cerevisiae</i>	baker's yeast	not mentioned	it is indirectly linked	Mahmoud Amer et al., 2021
9	<i>Saccharomyces cerevisiae</i>	Yeast, fungi, algae, oats, barley	not mentioned	not mentioned	Zhang et al., 2021
10	<i>Saccharomyces cerevisiae</i> (Brewer's Spent Yeast - BSY)	By-product of beer production	not specified	Approximately 1.5–3 kg of BSY per 100 L of beer	Gautério et al., 2022
11	<i>Saccharomyces cerevisiae</i>	Brewer's spent yeast (by-product of brewing industry)	not specified	not specified	Thomas et al., 2022
12	<i>Saccharomyces cerevisiae</i> is the primary microorganism for β-glucan extraction	Spent brewer's yeast, baker's yeast, and residual yeast from beer production are mentioned as common sources	Glucose: 20–50 g/L in submerged fermentation, Sucrose: 30–40 g/L, depending on the organism, Ammonium sulfate: 2–5 g/L	not mentioned	Chioru & Chirsanova, 2023
13	<i>Saccharomyces cerevisiae</i> , <i>Aspergillus</i> sp., <i>Xanthomonas campestris</i>	Complex substrates from food industry waste	Carbon-rich waste materials, nitrogen not explicitly detailed	~1.9 mg/5 mL dry weight	Khadam et al., 2023

The literature survey highlights various microorganisms and substrates used for β-glucan production, mainly focusing on *Saccharomyces cerevisiae* and other fungi. Most studies emphasize optimizing yield or enhancing functional properties. In contrast, this study adopts a more systematic extraction and purification approach using a feed-spent yeast system, aiming to maximize yield while preserving bioactive properties. By optimizing the removal of residual wort components, it provides a more efficient and scalable solution compared to previous methodologies.

II. MATERIALS & METHODOLOGY

This study aimed to extract and analyze β-glucan from spent yeast, optimizing its yield and assessing its antioxidant, antimicrobial, and prebiotic properties. The research was carried out in several sequential phases, ensuring methodical precision in each step.

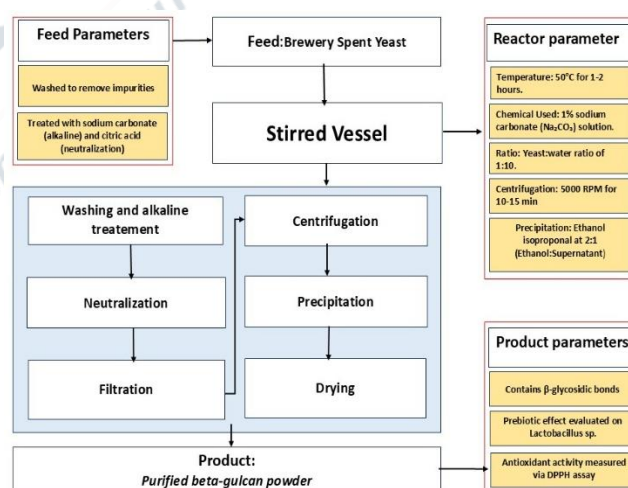


Fig2: Schematic representation of extracting and purifying beta-glucan from spent brewer's yeast

2.1 Collection and Pre-Treatment of Spent Yeast

Spent brewer's yeast was obtained from United Breweries Pvt. Ltd. and initially subjected to a rigorous washing process using distilled water. The purpose of this step was to remove residual wort components and impurities that could interfere with downstream extraction and characterization.

2.2 Extraction of β -Glucan

The extraction process involved alkaline treatment, where the washed yeast was suspended in a 1% sodium carbonate (Na_2CO_3) solution at a ratio of 1:10 (yeast to water). This mixture was maintained at 50°C for 1-2 hours to solubilize non- β -glucan components and weaken the yeast cell walls. The extracted solution was then neutralized using citric acid, carefully adjusting the pH to 7.0 to precipitate unwanted debris.

Following neutralization, the extract underwent filtration using cheesecloth or filter paper to remove large particles, after which the filtrate was centrifuged at 5000 rpm for 10–15 minutes. The resulting supernatant, containing β -glucan, was subjected to precipitation using ethanol or isopropanol in a 2:1 ratio. The mixture was left undisturbed for 2-3 hours (or overnight) to facilitate the precipitation process.

After precipitation, the β -glucan pellet was collected by centrifugation, washed with distilled water to remove residual impurities, and subsequently dried at 40°C in an oven or using a freeze-drying technique for optimal preservation. The final product was ground into a fine powder and stored for further analysis.

2.3 Characterization of Extracted β -Glucan Using FTIR Spectroscopy

To determine the chemical structure and composition of the extracted β -glucan, Fourier Transform Infrared (FTIR) spectroscopy was performed. FTIR analysis was conducted at the Department of Polymer Science and Technology at JSS-STU University. The β -glucan sample was analyzed for characteristic absorption peaks corresponding to functional groups such as hydroxyl ($-\text{OH}$), carbonyl ($\text{C}=\text{O}$), and glycosidic bonds that confirm the β -glucan structure.

2.4 Antioxidant Activity Assessment via DPPH Assay

The antioxidant potential of β -glucan was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, which measures the ability of β -glucan to neutralize free radicals.

A standard stock solution of ascorbic acid (10 mg/mL) was prepared as a positive control, while β -glucan solutions were formulated at varying concentrations (10–50 mg/mL).

Similarly, a DPPH solution (0.1 mM in methanol) was prepared. Equal volumes (4 mL) of DPPH were added to the test tubes containing ascorbic acid or β -glucan, incubated in darkness for 30 minutes, and absorbance was recorded at 517 nm using a UV-visible spectrophotometer. The radical scavenging ability was calculated using the standard formula:

$$\% \text{Inhibition} = \frac{(A - A_1)}{A} * 100$$

A = Control absorbance
 A_1 = Sample absorbance

→ Equation (1)

2.5 Antimicrobial Activity Evaluation

The antimicrobial efficacy of β -glucan was assessed using an agar well diffusion assay against various bacterial strains isolated from soil. Nutrient agar plates were prepared, and microbial isolates were sub-cultured before being evenly spread across the agar surface. Wells (3 mm and 6 mm in diameter) were punched into the agar, and each was filled with β -glucan extract at varying concentrations (10–100 mg/mL).

To compare the antimicrobial properties, control wells containing antibiotics such as methicillin, clindamycin, ampicillin, erythromycin, and ciprofloxacin were included. The plates were incubated at 35°C for 18-24 hours, after which inhibition zones were observed and recorded. The results were analyzed to determine the extent of β -glucan's antimicrobial action.

2.6 Prebiotic Activity and Lactobacillus Growth Evaluation

To assess the prebiotic potential of the extracted β -glucan, the study investigated its ability to support the growth of *Lactobacillus* sp., a beneficial gut bacterium. Pure *Lactobacillus* cultures were isolated from a curd sample, serially diluted in a sterile saline solution, and plated on MRS agar to obtain distinct colonies.

For the prebiotic evaluation, MRS broth was prepared, and five experimental groups were set up: a control group (0 mg/mL β -glucan) and four test groups supplemented with different β -glucan concentrations (10, 20, 30, and 40 mg/mL). Each culture flask was inoculated with a standard *Lactobacillus* suspension and incubated anaerobically at 37°C for 24 hours.

Bacterial growth was monitored at 600 nm using a spectrophotometer, with optical density (OD) values recorded every 2 hours over 48 hours. Growth curves were generated, and specific growth rates (μ) were calculated using the equation:

$$\mu = \frac{\ln(X) - \ln(X_0)}{\Delta t} \longrightarrow \text{Equation (2)}$$

Where X represents OD at the end of the exponential phase, X_0 is OD at the beginning, and Δt is the time interval. This analysis helped determine the extent to which β -glucan enhanced *Lactobacillus* growth, reinforcing its potential as a prebiotic.

III. RESULTS & DISCUSSION

3.1 Antioxidant Assay of β -Glucan and Ascorbic Acid.



Fig 3: The resulting β -glucan emerged as a finely ground, brown powder with water solubility. It was collected by extraction process mentioned in the methodology section

This study investigates the extraction, characterization, and bioactivity assessment of β -glucan, a polysaccharide derived from brewery spent yeast. The research aims to optimize the extraction process and evaluate the antioxidant and prebiotic potential of the extracted β -glucan. Various concentrations of β -glucan were tested for their ability to scavenge free radicals (DPPH assay) and enhance the growth of *Lactobacillus* bacteria, demonstrating its potential as an antioxidant and prebiotic compound.

The study focuses on β -glucan extraction from brewery spent yeast and its functional applications. It explores different extraction methods and their efficiency in obtaining β -glucan. The extracted compound is then subjected to Fourier Transform Infrared (FTIR) spectroscopy to confirm its chemical composition. Furthermore, the study assesses the antioxidant properties of β -glucan using the DPPH radical scavenging assay, comparing its activity with ascorbic acid. Additionally, it examines its prebiotic potential by evaluating its effect on *Lactobacillus* growth, an essential aspect of gut microbiota health.

Absorbance of test solutions at 517 nm (UV-Visible spectrophotometer) was used to calculate percentage inhibition via a standard formula,

Tables 1 and 2 provide numerical data on DPPH radical scavenging activity for all tested concentrations (10-50 mg/mL) of ascorbic acid and β -glucan, complementing the graphical data.

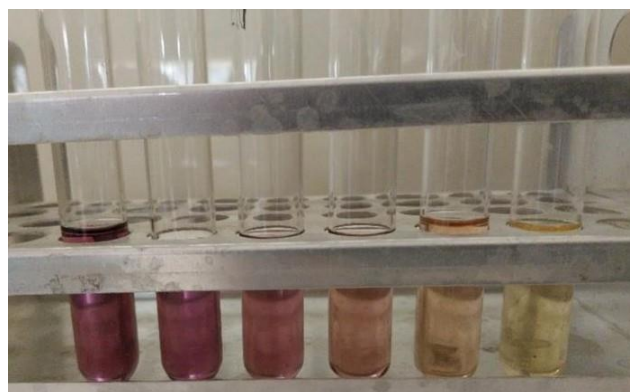


Fig 4: Ascorbic acid solutions (10-50 me /ml) in test tubes after 31 min dark incubation for DPPH assay

DPPH Assay for Antioxidant Activity of Ascorbic Acid

The antioxidant potential of ascorbic acid (vitamin C) was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely used and reliable method for evaluating free radical inhibition capacity. The assay involved testing various concentrations of ascorbic acid solutions, specifically ranging from 10 mg/ml to 50 mg/ml, to observe their efficacy in neutralizing DPPH radicals.

In **Figure 4**, a qualitative observation of the antioxidant activity is depicted through the visual appearance of test tubes containing different concentrations of ascorbic acid after a **31-minute incubation in the dark**. The characteristic deep purple color of the DPPH solution arises from the presence of unreacted DPPH free radicals. As antioxidants donate hydrogen atoms or electrons to the DPPH radicals, the solution's purple color fades, turning pale yellow or colorless depending on the extent of reduction. Thus, the observed **color fading** is a direct visual indicator of antioxidant activity. The degree of decolorization increases with higher ascorbic acid concentrations, confirming its **dose-dependent radical scavenging behavior**. At lower concentrations (e.g., 10 mg/ml), the purple color remains relatively intact, while at higher concentrations (e.g., 50 mg/ml), the purple hue significantly diminishes, suggesting that more radicals were neutralized.

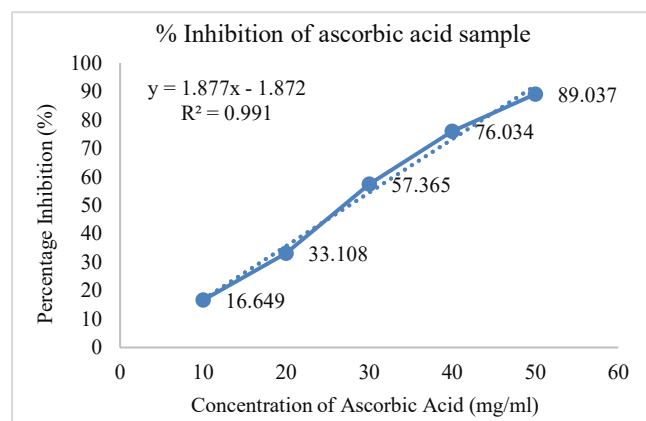


Fig 5: Graphical representation of percentage inhibition of ascorbic acid

To quantitatively assess this scavenging effect, **Figure 5** presents a graphical plot of **percentage inhibition of DPPH radicals** versus **ascorbic acid concentration (mg/ml)**. The data clearly demonstrate a **strong linear relationship**, where inhibition increases proportionally with concentration. This trend is mathematically described by the regression equation: $y = 1.877x - 1.872$, where y is the percentage inhibition and x is the concentration in mg/ml. The **correlation coefficient** ($R^2 = 0.991$) indicates a nearly perfect linear fit, reinforcing the reliability of the observed trend.

The percentage inhibition values ranged from **16.649% at 10 mg/ml** to **89.037% at 50 mg/ml**, showcasing a significant enhancement in antioxidant performance with increasing concentration. These results highlight that **ascorbic acid is a potent antioxidant**, capable of effectively scavenging free radicals in a concentration-dependent manner. The high level of inhibition at elevated concentrations confirms its potential for therapeutic and nutritional applications where oxidative stress reduction is desired.

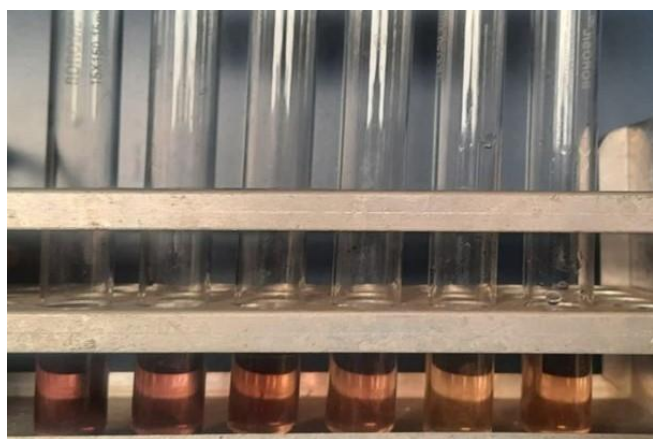


Fig 6: B-glucan solutions (10-50 mg/mL) in test tubes after 30 min dark incubation for DPPH assay

DPPH Assay for Evaluating the Antioxidant Activity of β-Glucan

The antioxidant potential of **β-glucan**, a biologically active polysaccharide known for its health-promoting properties, was investigated using the **DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay**. This widely accepted method assesses the capacity of a compound to donate electrons or hydrogen atoms to neutralize free radicals, thereby reducing oxidative stress. In this study, β-glucan solutions were prepared at concentrations ranging from **10 to 50 mg/mL**, and their antioxidant activity was evaluated both visually and quantitatively.

In **Figure 6**, qualitative results are demonstrated through a series of test tubes containing β-glucan solutions that were incubated in the dark for **30 minutes**. This incubation period allowed sufficient time for the interaction between β-glucan and the DPPH radicals. The characteristic deep purple color of the DPPH solution gradually faded in the presence of increasing β-glucan concentrations, indicating radical scavenging activity. A **lighter color signifies a higher**

degree of DPPH radical reduction, as the unpaired electrons in DPPH are neutralized by the antioxidant molecules present in β-glucan. At lower concentrations (10 mg/mL), the solution retains much of its purple hue, whereas higher concentrations (50 mg/mL) result in noticeably lighter shades, visually confirming β-glucan's dose-dependent antioxidant action.

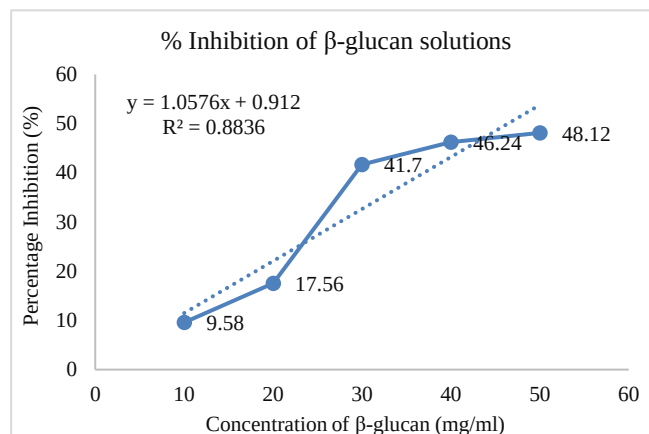


Fig 7: Graphical representation of percentage inhibition of β-glucan solution

To quantitatively assess this antioxidant potential, **Figure 7** presents the **percentage inhibition of DPPH radicals** plotted against **β-glucan concentration (mg/mL)**. The graph demonstrates a **moderate positive linear trend**, described by the regression equation: $y = 1.0576x + 0.912$, with a correlation coefficient ($R^2 = 0.8836$). This R^2 value reflects a reasonably strong linear relationship between concentration and DPPH inhibition, though slightly less consistent than that observed with ascorbic acid (which showed $R^2 = 0.991$). The data reveal a progressive increase in antioxidant activity, with inhibition rising from **5.58% at 10 mg/mL** to **48.12% at 50 mg/mL**.

These findings confirm that **β-glucan exhibits antioxidant activity** by scavenging free radicals, although its efficacy is **significantly lower than that of ascorbic acid** at equivalent concentrations. This could be attributed to the structural nature of β-glucan, a complex polysaccharide, which may not donate electrons as readily as the smaller, highly reactive ascorbic acid molecule. Nonetheless, the moderate scavenging activity observed, especially at higher concentrations, supports β-glucan's potential role as a **functional bioactive compound** in nutraceuticals and therapeutic applications targeting oxidative stress.

The study reveals that β-glucan exhibits antioxidant activity, though lower than that of ascorbic acid. As shown in Table 1, the percentage inhibition of ascorbic acid increases significantly with concentration, reaching a peak of 89.037% at 50 mg/ml. In contrast, Table 2 demonstrates that β-glucan shows moderate but stable antioxidant properties, with percentage inhibition increasing to 48.12% at 50 mg/ml. This indicates that while β-glucan possesses antioxidant activity, its efficacy is lower than that of ascorbic acid.

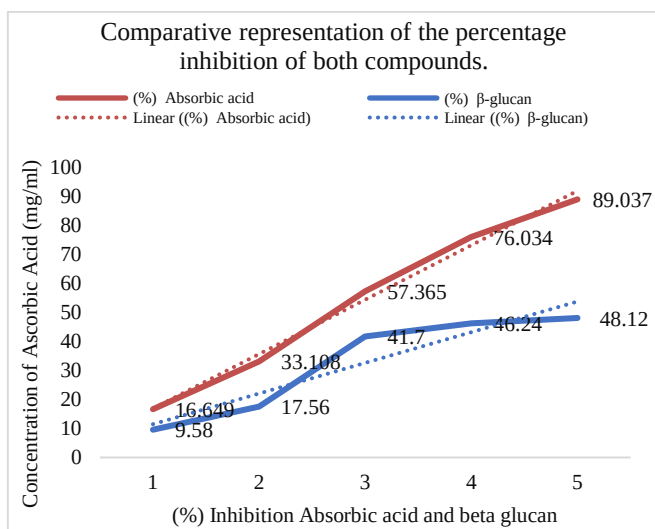


Fig 8: Comparative representation of the percentage inhibition of both compounds.

While ascorbic acid follows a steep upward trajectory, β -glucan demonstrates a relatively moderate increase, suggesting a lower efficacy in neutralizing free radicals. Figures 5 and 7 separately illustrate the antioxidant activity of each compound, confirming the dose-dependent behavior of DPPH inhibition.

3.2 Evaluation of prebiotic activity of extracted β -glucan

The prebiotic potential of β -glucan was assessed by cultivating *Lactobacillus* sp. isolated from curd in MRS media supplemented with varying β -glucan concentrations (0, 10, 20, 30, and 40 mg/mL). After 24 hours of incubation, bacterial growth was evaluated by measuring optical density (OD) at 600 nm every 2 hours for 48 hours. Growth curves generated for each concentration displayed a typical microbial growth pattern, consisting of a lag phase (2–8 hours), exponential phase (8–24 hours), and stationary phase (>24 hours). A closer analysis of the first 12 hours highlighted variations in the lag phase across treatments.

Specific growth rates for all β -glucan concentrations showed minimal variation (0.197–0.202), yet all cultures exhibited positive growth, with a slight enhancement observed at 40 mg/mL β -glucan. This suggests that β -glucan supports *Lactobacillus* growth, reinforcing its potential as a prebiotic. Further studies with broader concentration ranges and longer incubation times could help clarify a possible dose-dependent effect.

Regarding its prebiotic potential, results indicate that *Lactobacillus* growth is enhanced in the presence of β -glucan, suggesting its capability to act as a prebiotic. The growth rates calculated for different β -glucan concentrations suggest a positive correlation, reinforcing its potential to support beneficial gut bacteria. Specific growth rates were calculated for each bacterial culture using the formula, $\mu = [\ln(X) - \ln(X_0)] / \Delta t$

where X is the optical density (OD) at the end of the exponential phase (24 hours), X_0 is the OD at the beginning

of the exponential phase (8 hours), and Δt is the time interval (16 hours) between measurements

3.3 Antimicrobial activity of β -glucan

The antimicrobial activity of extracted yeast β -glucan was tested using agar plates with control antibiotic discs (methicillin, clindamycin, ampicillin, erythromycin, and ciprofloxacin). Wells containing β -glucan (10–100 mg) were compared to control wells. After 24 hours, inhibition zones formed around antibiotic discs, but none appeared around β -glucan wells, indicating minimal direct antimicrobial activity. This suggests that yeast β -glucan influences immune cells rather than directly targeting microbes as shown in Fig - 9

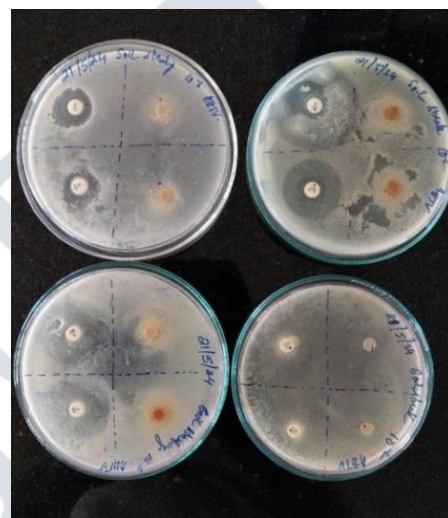


Fig 9: Antimicrobial activity of β -glucan

The study also contributes significantly to functional food development by highlighting the dual benefits of β -glucan—its antioxidant and prebiotic properties. The findings suggest that β -glucan could be incorporated into nutraceuticals or functional foods to support gut health and reduce oxidative stress. Furthermore, its extraction from brewery-spent yeast promotes sustainable waste utilization, making the process economically and environmentally beneficial.

IV. CONCLUSION

The extraction and utilization of β -glucan from brewer's spent yeast (BSY) present a sustainable approach to valorizing this industrial by-product. Our study optimized the extraction process by modifying solvent use and alkaline treatment ratios, leading to a notable increase in yield. The extracted β -glucan exhibited promising antioxidant activity, as demonstrated by DPPH radical scavenging assays, though its direct antimicrobial activity was minimal. This suggests that its biological function may be more aligned with immune modulation rather than direct pathogen inhibition. Moreover, our preliminary findings indicate that β -glucan has potential prebiotic properties, supporting the growth of *Lactobacillus* species in vitro. These results align with existing literature highlighting β -glucan as a dietary fiber with functional benefits, including gut microbiota modulation, immune

support, and metabolic regulation. However, further research is warranted to elucidate its precise mechanisms of action, optimize its incorporation into functional food formulations, and explore its synergistic effects with probiotics. Future studies should focus on long-term in vivo trials, bioavailability assessments, and the impact of β -glucan-enriched diets on human health. Expanding research in these areas will contribute to the development of nutritionally enhanced food products and therapeutic applications of β -glucan in gut health management and immune function..

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