

VALORIZATION OF BREWER'S SPENT YEAST FOR THE EXTRACTION OF β -GLUCAN

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ABSTRACT

Brewer spent yeast (BSY) is an underutilized byproduct of brewery industry that can be used as a renewable source for β -glucan extraction, contributing to a circular economy. The present study was aimed to extract β -glucan from indigenous and commercial brewing yeasts using two different extraction methods. Pilsner beer was brewed using *Saccharomyces cerevisiae* (MK680910) for the collection of indigenous BSY and commercial BSY was collected from Underdoggs Brewery and Kitchen, Ludhiana (India). The BSY derivatives, obtained from cell lysis and extraction, were assessed for their proximate analysis. β -glucan content in indigenous BSY was found to be 16.6 mg g⁻¹ by acid-base extraction and 15.1 mg g⁻¹ by water extraction methods, while commercial BSY yielded 19.1 and 16.5 mg g⁻¹ by the respective methods. β -glucan was characterized using FTIR, with evidence provided by the bands recorded around 1034-1150 and 889 cm⁻¹, which corresponded to C-O-C stretching in polysaccharides. The acid-base extraction method was more efficient than water extraction, producing higher yields with fewer impurities. The study paves the way for cost-effective, scalable extraction process that can benefit the brewing industry and other sectors relying on β -glucan.

Keywords: Acid-base extraction, β -glucan, brewer's spent yeast, FTIR, water extraction

INTRODUCTION

Brewing sector produces a considerable amount of by-products, with spent yeast as the most prominent one. The brewing industry is a key economic sector in many countries, and the 5th most consumed beverage globally, after tea, carbonated drinks, milk and coffee (Fillaudeau *et al.*, 2007). Increasing population, booming economy, rapid urbanization and rise in living standards have accelerated waste generation in developing countries. Brewers use many ways to reduce semi-solid waste produced during beer brewing process, and but there is urgent need to develop safe cost-effective methods to treat brewer's spent yeast for reuse (Simate *et al.*, 2011). This biomass is mainly composed of typically discarded *Saccharomyces cerevisiae*, resulting in environmental contamination and financial losses (Jaeger *et al.*, 2020). However, spent yeast is a valuable source of essential compounds, like β -glucans, mannoproteins and vitamins, which have enticed significant interest because of its various health advantages and functional qualities (Suwannarong *et al.*, 2020).

The type of yeast used in beer production plays a crucial role in imparting its quality, aroma and flavour, which affects the organoleptic profile of beer. However, the type of yeast strains used in beer production significantly affects the process itself including fermentation time and ultimately the chemical composition of produced beer (Svoboda *et al.*, 2022). Therefore, selection of appropriate brewing yeast is a crucial in beer-making process. At the end of fermentation, yeast is separated from the residual contents by flocculation process. During this process, yeast cells form clumps which

either float atop or settle at the bottom of container. It is critical for yeast to have excellent flocculation properties so that it can be reused in future fermentation. At industrial scale, this method ensures some economical saving. Re-fermentation is the process of initiating new fermentation with a tiny amount of yeast from prior batch. Brewer's yeast is frequently reused for fermentation cycles, however, the number of times it is reused is limited so as to maintain beer quality. Brewer's spent yeast (BSY), discarded as waste, is no longer used for beer production. The amount of waste generated depends on the brewery's practices, especially the number of times the yeast is reused (Ionut and Sonia, 2021).

Globally, 1.86 billion hectoliter beer was produced in 2021, and as per conservative estimates 0.00015-0.0003 kg BSY is produced per hectoliter beer with total BSY production of 279-558 tonnes in 2021 (Statista, 2023). The BSY market was valued at US \$ 1289.3 million in 2021, and with average compound annual growth rate of 6.12% it is predicted to reach US \$ 1841.92 million by 2027. BSY is separated by sedimentation before the beer reaches full maturity during fermentation which can be used for β -glucan extraction (Avramia and Amariei 2021). β -glucan is a primary non-starch polysaccharide consisting of a linear chain of β -D-glucose units linked by β -(1-3), (1-4) and (1-6) D-glycosidic linkages consisting of over 25000 D-glucose units either in branched or unbranched form (Barsanti *et al.*, 2011). β -glucan is the most prevalent polysaccharide in yeast cell wall, comprising 65-90% of total polysaccharides (Teparić *et al.*, 2020). Within the cell wall, 1,6- β -glucans and 1,3- β -glucans account for 12 and 55% of polysaccharide content, respectively, reflecting their major structural role (Thomas *et al.*, 2022). In addition to β -glucan, the yeast cell wall is made up of 50-60% β -glucans, 35-40% mannoproteins, 1-3% chitin and 1-23% glycogen (Nakashima *et al.*, 2018). The chemicals used in commercial production of β -glucan from cereal crops like wheat, rye and oats pose a threat to both the environment and food consumption. Therefore, microbial production of β -glucan is a viable alternative due to its sustainability, efficiency and seasonality. The biological properties of β -glucan vary depending on its source (fungi, bacteria, algae and cereals), structure, solubility, conformation formation in solution, molecular weight and polymer charge. Yeast β -glucans can be categorized into three distinct groups based on their solubility in aqueous solutions, namely water-soluble β -(1-6) glucans, alkali-soluble β -(1-3) glucans, and alkali-insoluble β -(1-6) glucans (Bastos *et al.*, 2022).

The European Food Safety Authority thoroughly evaluated yeast β -glucans and recommended their use as food components (EFSA, 2011), also the US Food and Drug Administration (FDA, 2008) classified them as generally recognized safe. In addition, β -glucans have been linked to several health benefits, including anti-inflammatory, anti-diabetic, anti-cholesterol, anti-aging, blood glucose regulation and anti-tumour properties. These beneficial properties make valuable in food industry, where they are used as stabilizers, thickeners, oil binding agents and emulsifiers in the production of soups, sauces, beverages and other food products (Rakowska *et al.*, 2017).

BSY has received little attention as a profitable commodity and its disposal is an environmental problem. As a result, researchers have focused on extraction techniques to isolate β -glucans. To analyse the structure of resulting yeast β -glucan, various methods are employed which include assessing the total sugar & protein contents and FTIR (Fourier-transform infrared spectroscopy) analysis, etc. (Bikmurzin *et al.*, 2022). These techniques provided valuable insights into the composition and structural characteristics of yeast β -glucan, shedding light on its potential applications and purification functionality (Zheng *et al.*, 2019). This study was aimed to explore the use of BSY for the extraction of β -glucan using acid-base and water extraction methods and characterize the extracted β -glucan.

MATERIALS AND METHODS

Fermentation was carried out by using *Saccharomyces cerevisiae* [NCBI GenBank Accession No. MK680910]. The yeast culture was procured from the Fermentation Laboratory, Department of Microbiology, PAU, Ludhiana, Punjab (India). The commercial brewer's spent yeast, Pilsner malt of '2RS' variety and hops were procured from Underdogs Brewery and Kitchen, Gurdev Nagar,

Ludhiana, Punjab, India. Media components for microbial growth were purchased from HiMedia Laboratories, India. Glass beads of 425–600 μm (30–40 U.S. sieve), Congo red and other reagents were purchased from Sigma Aldrich Chemicals Pvt. Ltd (St Louis, MO, USA).

Brewing process

The beer was brewed using pre-optimized parameters (Anju *et al.*, 2024). During mashing process, starch was gelatinized by adding water to the malt in 3:1 ratio and cooking it on a hot plate. Different mash-in-temperature stands were held at 60–65°C for 40 min for β -amylase activation and with final mash-off temperature at 75–78°C for 20 min. β -amylase was rendered inactive at 72°C, but α -amylase continues to hydrolyse starch despite its activity, it significantly decreased at 78°C. Initial wort was 22°Bx, which was adjusted to 11°Bx. Wort was boiled at 100°C for 1 h with hops. Bitter hops (Willamette 0.8 g) were added at the start of boiling and aroma hops (Perle 0.8 g) were added 10 min before the end of boiling. The wort was cooled to 20°C with the help of a plate heat exchanger. Next, 6.5% of *S. cerevisiae* yeast was pitched into the wort and fermented at 25°C for 4 days in glass conical fermenter. Heavy debris and yeast cells settled at the bottom of fermentation flask. The yeast separation process lasted for 2 days, followed by a 5-day maturation at 4°C. Various physicochemical parameters of beer were analysed and residual yeast cells in Pilsner beer were removed to produce a clarified beer that was subsequently bottled. The beer was pasteurized at 63°C for 30 min, followed by storage at 4°C. Finally, beer was assessed based on its sensory profile (Amerine *et al.*, 1965). The beer production process has been outlined in Fig. 1.

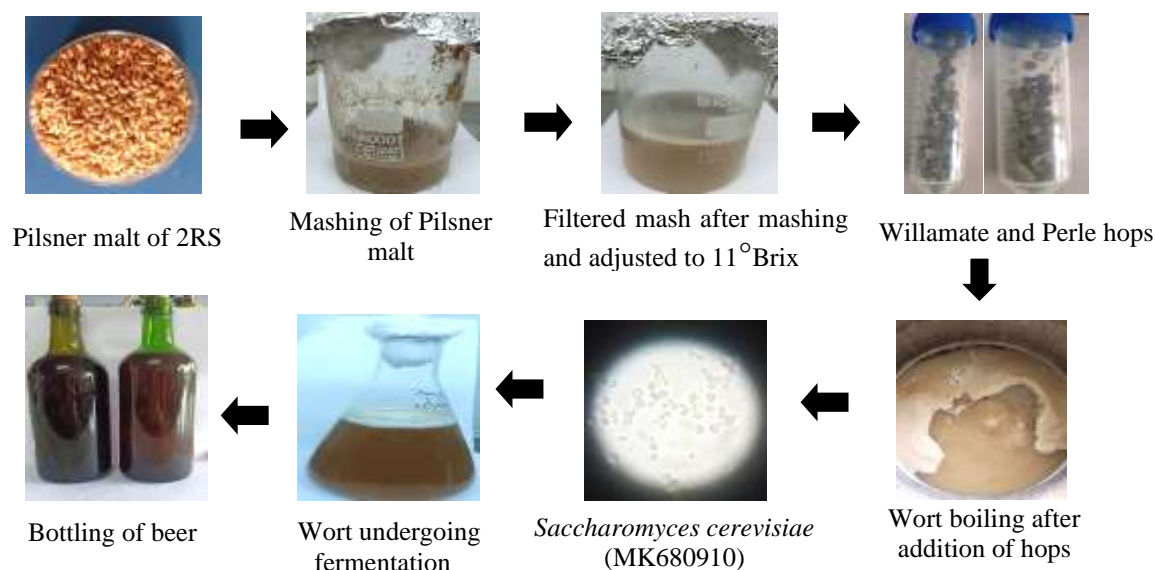


Fig. 1: Flow sheet depicting the process of Pilsner beer production

Analytical methods

The physicochemical analysis of beers was carried out with respect to alcohol, pH (pH meter, Hanna HI96107), titratable acidity (AOAC, 1999), total soluble sugars (Dubois *et al.*, 1956), total reducing sugars (Miller, 1959), ethanol estimation (Caputi *et al.*, 1968).

Extraction of β -glucan from brewer spent yeast

Preliminary filtration: After beer production, indigenous BSY was recovered by centrifugation of sediment prior to full maturation at the final stage of fermentation. The spent yeast slurry was also collected from the brewery and stored in sterilized jars. The slurry was refrigerated at 4°C overnight to allow the beer liquor to separate. The liquid on top was discarded, leaving the slurry at bottom. To ensure the microbiological purity of yeast waste, BSY slurry was examined under a binocular microscope (Labomed Opticx) and cultured on nutrient agar, MRS agar and GYE agar media before further processing. Prior to processing, the yeast cells were washed with distilled water, followed by

washing with 0.5% NaCl and 0.1% tween 80 at 1:3 (w/w) ratio of yeast to solvent. Prior to processing, the cream yeast was rinsed with solvents. The cream yeast was gently stirred with a glass rod and centrifuged at 3500 rpm for 15 min at 4°C (Xuan *et al.*, 2023). Washing of cells were examined under a compound microscope at 40x magnification.

Autolysis of yeast cells: After removing impurities, yeast cell autolysis was performed as per Pengkumsri *et al.* (2016). The yeast cell suspension was mixed with distilled water at 15:100 (w/v) ratio, adjusted to pH 5.0 using 1.0 M HCl, and incubated at 50°C for 48 h with shaking at 120 rpm. The autolysis process was stopped by heating the mixture to 80°C for 15 min in a water bath. After incubation, yeast cells were collected by centrifugation at 4°C for 10 min at 5000 rpm. The resulting pellets were dried at 60°C and stored at 4°C until they were ready for further extraction.

Acid-base extraction: A combination of acid and base was employed for acid-base extraction of β -glucan (Amer *et al.*, 2021). The autolyzed yeast cells were mixed with 1 M NaOH and incubated at 80°C with stirring for 2 h. After centrifugation, the cell pellets were dissolved in 1 M acetic acid and incubated with stirring at 80°C for another 2 h. The autolyzed cell pellets were collected by centrifugation at 6700 rpm for 25 min at 4°C. The resulting pellets were rinsed 3 times with distilled water.

Water extraction: Yeast cells (100 g) were mixed with 50 mL of 10 mM phosphate buffer (pH 8.0) and then sonicated for 5 min, followed by continuous shaking with glass beads for 4 h. The centrifugation was done at 6100 rpm for 15 min, followed by 2 washes with phosphate buffer. To separate protein substances, 1% sodium dodecyl sulphate was added to the remaining mixture and shaken for 3 h at 30°C. The resulting preparation was dried using absolute ethanol (Amer *et al.*, 2021).

Proximate analysis of pellet

Before proximate analysis, lyophilization of sample was done at -40°C. The moisture content was determined as per AOAC (2005). Ash content and wet weight content were determined using standard methods (AOAC, 1999). The physiochemical analysis of pellet was performed for total soluble sugars (Dubois *et al.*, 1956), total reducing sugars (Miller, 1959), total soluble protein (Lowry *et al.*, 1951) and total lipids (as per sulpho-phospho-vanillin assay) [Mishra *et al.*, 2014].

Estimation of β -glucan

Congo red {1 mL = 52.2 ppm} and 5 mL (500 μ L) of 50 mM sodium acetate buffer (pH 5.0) were added to 1 mL sample. The intensity of orange colour was measured at 545 nm relative to a reagent blank (Ishikawa *et al.*, 1998). The β -glucan content was determined using following formula:

$$\frac{60 \mu\text{g}}{0.164} \times \frac{\text{OD of sample}}{\text{volume of sample taken}} \times \frac{\text{Volume after centrifuge}}{\text{fw}}$$

Where, 60 μ g = concentration of standard; 0.164 = OD of standard; fw = fresh weight of sample

Based on the most effective extraction technique resulting in the highest yield of β -glucan, acid-base extraction technique was identified as the most suitable for upscaling of β -glucan extraction.

Characterization of β -glucan

FTIR spectra of lyophilized BSY samples were recorded using Agilent Cary 630 FTIR spectrometer. The instrument employs a high-throughput Michelson interferometer with a KBr/ZnSe beam splitter, a solid-state laser, a wire-wound infrared source and a thermoelectrically cooled deuterated triglycine sulphate detector. Spectra were collected over the range of 4000-250 cm^{-1} at a resolution of 1 cm^{-1} under ambient conditions. Measurements were performed using a diamond attenuated total reflectance accessory, which is well suited for powdered biological samples and enables effective detection of polysaccharide functional groups.

Statistical analysis

The data generated were summarized as means and standard deviations. The experimental design was a completely randomized design with all measurements taken in triplicate. The experimental data were statistically analysed using STATISTIX 10.0 software with one-way analysis of variance.

RESULTS AND DISCUSSION

Brewing beer and its physicochemical analysis

Pilsner malt of 2RS variety was selected for mashing due to its extensive processing, as it contains four amylolytic enzymes (α -amylase, β -amylase, α -glucosidase and limit dextrinase) that effectively breakdown starch into fermentable sugars. After mashing, the wort was boiled with hops to impart beer with its characteristic bitterness, aroma and antimicrobial properties, which not only helps inhibit the growth of certain bacteria and wild yeast but also contributes to the stability and shelf life of the beer. During boiling, the alpha acids in beer undergo isomerization to become iso-alpha acids, which are bittering compounds (Prado *et al.*, 2023). Boiling wort removes off flavors like dimethyl sulphide and sanitizes it while causing denaturation and aggregation of proteins (Thesseling *et al.*, 2019). The dissolved solids (sugar concentration) were adjusted from 22°Bx to 11°Bx to ensure optimal yeast performance and to achieve the desired alcohol content. The yeast converts two-thirds of sugars into alcohol, with the remaining one-third is oxidized to produce carbon dioxide. This process makes a final product containing both ethanol and carbon dioxide (Alba-Lois and Segal-Kischinevzky, 2010).

After 4 days of fermentation, an alcohol content of 6.3% was observed in the beer (Table 1). The pH of wort decreased sharply during the initial stages of fermentation, while the decline in later stages became more gradual. Li and Liu (2015) reported that beers with a low pH become more 'sharp' and exhibit an elevated sour flavour. The fermentation byproducts of yeast, hops and malt include organic and carbonic acids, which play a role in determining the beer's acidity (Buiatti, 2009). The rise in acidity is attributed to the production of organic acids, such as lactic acid and acetic acid, by yeast throughout fermentation. The sugar content was higher during the initial period of fermentation and as the yeast consumed sugars, the sugar content gradually decreased. By the final

Table 1: Periodic physicochemical analysis of Pilsner beer

Fermentation time (days)	Brix (°Bx)	Total soluble sugars (g 100 mL ⁻¹)	Total reducing sugars (g 100 mL ⁻¹)	pH	Titrateable acidity (%)	Alcohol content (% v/v)
0	11.0 ± 0 ^a	1.17 ± 0.01 ^a	0.66 ± 0.02 ^a	5.40 ± 0.10 ^a	0.60 ± 0.43 ^a	0.00 ± 0.1 ^c
1	8.0 ± 1.0 ^b	1.03 ± 0.01 ^b	0.58 ± 0.09 ^b	4.60 ± 0.01 ^b	0.18 ± 0.01 ^a	2.10 ± 0.12 ^d
2	4.0 ± 1.0 ^c	0.51 ± 0.02 ^c	0.40 ± 0.01 ^c	4.44 ± 0.07 ^{b-c}	0.24 ± 0.01 ^a	3.10 ± 0.1 ^c
3	3.5 ± 1.2 ^c	0.48 ± 0.5 ^c	0.20 ± 0.03 ^d	4.17 ± 0.07 ^{c-d}	0.29 ± 0.01 ^a	4.40 ± 0.1 ^b
4	1.0 ± 0.0 ^d	0.13 ± 0.05 ^d	0.02 ± 0.01 ^e	3.90 ± 0.05 ^d	0.31 ± 0.01 ^a	6.30 ± 0.2 ^a

Values are mean of 3 replicates ± standard deviation (SD) and were analysed using one-way ANOVA followed by Tukey's test. Values with different superscripts in the same column differ significantly at $P_{0.05}$.

day, most sugars were converted to alcohol and CO₂, leading to a significant reduction in sugar concentration in beer. The level of efficiency during fermentation is entirely dependent on the capacity of yeast strains to adjust to different stress factors, including high ethanol levels and nutrient availability (Sharma and Sharma, 2017). Additional colour development takes place during mashing, wort boiling and through oxidation reactions during wort production and bottling. The color decreased during fermentation due to a drop in pH and further reductions appeared to occur during filtration. After complete fermentation, the colour of beer was pale yellow to dark brown.

Extraction of β -glucan from brewer spent yeast

After beer production, 37 g indigenous BSY *S. cerevisiae* (MK680910) was obtained from 1 L beer by centrifuging the sediment before beer maturation. Further, 37 g commercial BSY *S. cerevisiae* (BF16) was procured from Underdoggs Brewery and Kitchen, Ludhiana and stored at 4°C. BSY had a brownish, viscous, gel-like texture, high moisture content, slightly sour and yeasty odour. Washing of BSY was done to remove the contaminants (Fig. 2). BSY is often underutilized due to impurities like bitterness from hop resins. The debittering process through washing reduces bitterness, making BSY suitable for animal and human consumption. Higher temperatures enhance debittering and

tween 80 is four times more effective than water and 0.5% NaCl solution in removing tannins (Jacob *et al.*, 2019). However, excessive chemical use can alter BSY's nutritional composition and cause environmental issues. Tween 80 (0.1%) is ideal for safely removing bitterness without compromising BSY's quality (Xuan *et al.*, 2023). Prior to washing, the yeast cells appeared aggregated and clumped (Fig. 2A), likely due to the presence of residual fermentation byproducts and cellular debris. After washing, the yeast cells were widely dispersed and exhibited a greater differentiation, with each cell displaying distinct individual characteristics (Fig. 2B). Furthermore, the colour of the supernatant changed from pale yellow to white following centrifugation of BSY.

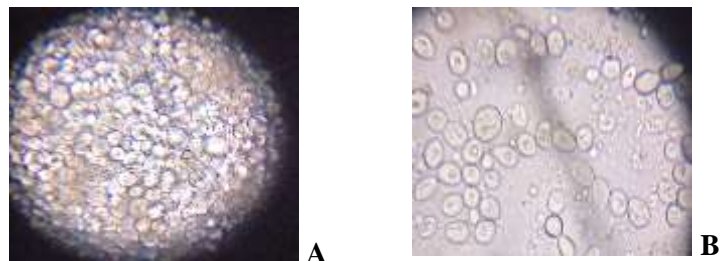


Fig. 2: BSY cells before washing (A) aggregated and clumped; and after washing (B) clear and dispersed as observed under 40x magnification



Fig. 3: Autolysis of spent yeast cells as observed under 40x magnification

The autolysis of cells was done to break the rigidity of yeast cell wall. The autolyzed cells appeared ruptured, with irregular shapes and damaged cell walls (Fig. 3), revealing a significant breakdown of cellular structures. Further, the cytoplasmic content appeared as diffused material in the surrounding medium. Cell debris and small vesicles were observed with the cells lacking defined boundaries and exhibiting roughness and holes, indicating the process of autolysis. Autolysis occurs when yeast cells die at temperatures $>45^{\circ}\text{C}$, during which autolytic enzymes breakdown the cell wall and release yeast extract (Avramia and Amariei, 2021; Xuan *et al.*, 2023). In present study, induced autolysis occurred at 50°C for 48 h, which is in line with Pengkumsri *et al.* (2016). The thick cell wall of *S. cerevisiae* resists lytic activity; therefore, combined treatments like heat and ultrasound are used to enhance autolysis and reduce cellular stiffness (Magnani *et al.*, 2009)

Extraction of β -glucan

Mannoproteins, found in yeast cell wall, protect yeast cells due to their gel-like properties. Two extraction methods namely water extraction and acid–base extraction, were evaluated for high-purity β -glucans. Water extraction causes swelling of yeast cell wall, thereby weakening its structure and making the cells more susceptible to disruption (Liu *et al.*, 2008). This method is eco-friendly and cost-effective and removes mannoproteins, proteins and polysaccharides for β -glucan production. Autolyzed cells were heated at 80°C for 15 min to deactivate enzymes (Varelas *et al.*, 2016).

In acid–base extraction, acetic acid is used due to its mild acidity which targets β -1, 6-linked glucans without significantly hydrolysing β -1, 3 linkages (Zohri *et al.*, 2019). After extraction, the insoluble β -glucan was separated from the supernatant and precipitated using ethanol (Pengkumsri *et al.*, 2016). Proximate analysis for total ash, wet weight and total moisture showed significant differences in the wet weight of samples obtained by water extraction and acid-base extraction methods (Table 2). Lyophilization of samples was carried out and the freeze-dried samples depicted irregular

Table 2: Proximate analysis of BSY pellet after acid-base and water extraction

Techniques used	Samples	Total ash (%)	Wet weight (g)	Total moisture (%)
Acid-base extraction	Indigenous pellet	0.73 ± 0.01^a	10.00 ± 0.49^c	11.4 ± 0.28^a
	Commercial pellet	0.80 ± 0.02^a	12.50 ± 0.16^a	13.1 ± 0.33^a
Water extraction	Indigenous pellet	0.66 ± 0.02^a	$11.09 \pm 0.46^{b-c}$	10.2 ± 0.28^a
	Commercial pellet	0.79 ± 0.02^a	$12.20 \pm 0.49^{a-b}$	9.9 ± 0.35^a

Values are the mean of 3 replicates \pm SD. The data was analysed by using one-way analysis of variance followed by Tukey's test. The values with different superscripts in the same column differ significantly at $P_{0.05}$.

Table 3: Physiochemical analysis of lyophilized pellets using two extraction methods

Techniques used	Samples	Total sugars (mg g ⁻¹)	Total reducing sugars (mg g ⁻¹)	Total soluble protein (mg g ⁻¹)	Total lipid (%)	β -glucan (mg g ⁻¹)
Acid-base extraction	Indigenous pellet	2.79 ± 0.01 ^a	1.25 ± 0.07 ^b	2.94 ± 0.34 ^a	1.61 ± 0.1 ^b	16.6 ± 0.03 ^b
	Commercial pellet	2.65 ± 0.01 ^{a-b}	1.6 ± 0.05 ^{a-b}	2.84 ± 0.26 ^{a-b}	2.17 ± 0.8 ^a	19.1 ± 0.05 ^a
Water extraction	Indigenous pellet	2.46 ± 0.03 ^b	1.23 ± 0.08 ^b	2.34 ± 0.16 ^c	1.50 ± 0.02 ^b	15.1 ± 0.02 ^b
	Commercial pellet	2.24 ± 0.03 ^c	1.70 ± 0.03 ^a	2.67 ± 0.15 ^b	1.05 ± 0.05 ^c	16.5 ± 0.03 ^b

Values are the mean of 3 replicates ± SD; The data was analysed by using one-way of variance followed by Tukey's test. Values with different superscripts in the same column differ significantly from each other at P_{0.05}.

structure, being compressed into sheet-like layers with a porous surface. A light brown colour and powder-like form was observed after lyophilization of pellets. Freeze-drying helps to preserve the characteristics of β -glucan by maintaining its chemical structure, physical properties and biological activity, ensuring long-term storage and stability without altering sensitive compounds. Physiochemical analysis of both samples for sugar, reducing sugars, protein, lipids and β -glucan is given in Table 3.

Autolysis of yeast cells when combined with mechanical methods such as sonication results in a significant release of proteins (Bzducha-Wrobel *et al.*, 2014). Marinescu and Stoicescu (2009) found lower levels of β -glucan (29.5-38.1%) extracted from spent brewer's yeast cells using bead milling. This could be due to differences in bead diameter, material (stainless steel) and intermittent mixing process. Depending on the yeast source, the amount of β -glucan in yeast cell wall varies significantly making up 30-60% dry weight (Gautério *et al.*, 2022). Wang *et al.* (2016) recommended processing severely repitched yeast quickly for β -glucan extraction, as delaying this can lead to autolysis, damaging polymer chains in yeast cell wall. Yeast can increase glucan storage by 49% during beer fermentation in response to stress, with β -glucan levels rising during maturation, especially under cold fermentation. Freimund *et al.* (2003) reported that 26% lipids get depleted due to the presence of water soluble components in lipid fraction like phospholipid after extractions.

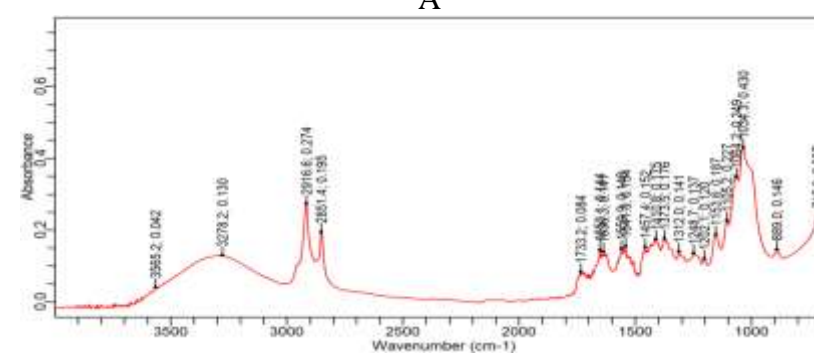
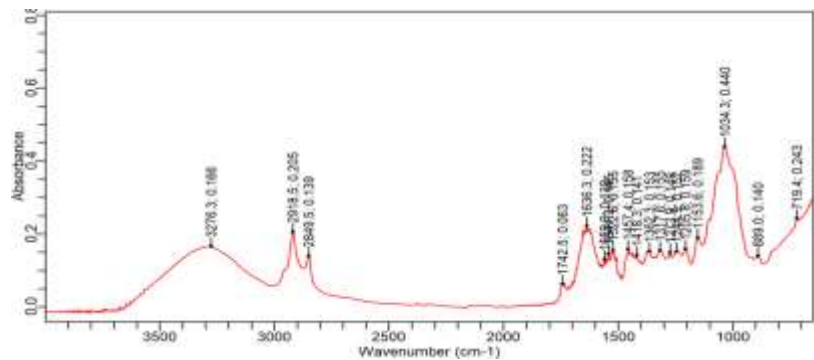


Fig. 4: FTIR analysis of extracted β -glucan from indigenous BSY by A) Acid-base extraction and B) Water extraction

Characterization of β -glucan

The characterization of β -glucan by FTIR analysis showed that the samples displayed distinctive bands, suggesting the presence of glycosidic bonds (Fig. 4; Table 4). The β -1,3 and β -1,4 linkages were validated by the absorption bands observed between 1064-1153 cm⁻¹, which are related to C-O-C stretching in polysaccharide. These results confirm the presence of β -glucan in BSY. Acid-base extraction method yielded β -glucan with higher purity as compared to water extraction, with fewer impurities and

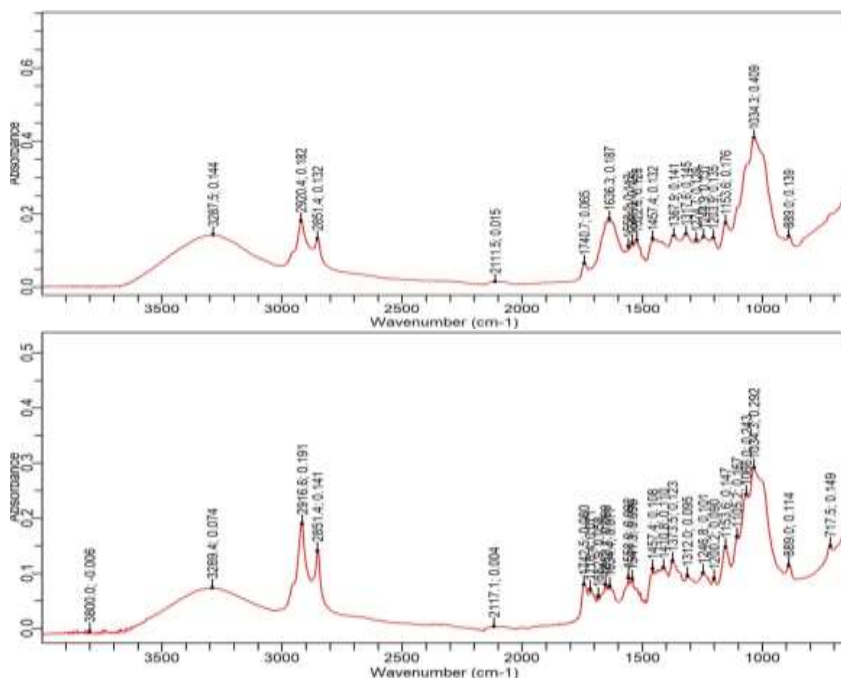


Fig. 5: FTIR analysis of extracted β -glucan from commercial BSY by A) Acid-base extraction and B) Water extraction

unidentified peaks (Fig. 5). This difference could be due to human error during cell wall preparation or washing (Bikmurzin *et al.*, 2022). Acid-base extraction separates lipids, proteins and starch, while water extraction may co-extract proteins and polysaccharides. FTIR analysis of water extraction is challenging due to overlapping absorption bands (Plata *et al.*, 2013). β -glucan peaks from commercial *S. cerevisiae* (lager beer BF16) showed slight differences as compared to indigenous *S. cerevisiae* (MK680910)

Table 4: Band distribution of extracted β -glucan from BSY after FTIR analysis

Functional groups	Spectra range indigenous pellet (cm ⁻¹)		Spectra range of commercial pellet (cm ⁻¹)	
	Acid-base extraction	Water extraction	Acid-base extraction	Water extraction
O-H stretching	3267	3278	3287	3289
C-H stretching	2850-2920	2850-2920	2850-2920	2850-2920
C-C & C-O stretching	889-1153	889-1153	880-1200	1034-1200
C=O stretching	1742	1733	1636-1740	1653
C-O-C stretching	889	889	1034-1078	1034-1150
C-H bending	1457	1457	1257	1410

due to strain-related structural differences. Acid-base extraction offers cleaner, more specific β -glucan extraction, making it preferred for industrial applications.

Conclusion: The results emphasize the potential of brewer's spent yeast (BSY) as a sustainable source for β -glucan extraction, supporting the circular economy by minimizing waste and encouraging the reuse of industrial byproducts. On the basis of highest yield of β -glucan, the upscale experiment for β -glucan extraction from 250 g BSY was carried out to check the potential of process for the benefit of brewing industry and other sectors that utilize this valuable biopolymer. However, more research is needed to develop new green methods for β -glucan extraction which may contribute to harness its properties and applications in eco-friendly manner.

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