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#### SPECIALTY SECTION

This article was submitted to Food Characterization, a section of the journal Frontiers in Food Science and Technology

RECEIVED 19 October 2022 ACCEPTED 22 November 2022 PUBLISHED 01 December 2022

### CITATION

Gautério GV, Silvério SIDC, Egea MB and Lemes AC (2022),  $\beta$ -glucan from brewer's spent yeast as a technofunctional food ingredient. *Front. Food. Sci. Technol.* 2:1074505. doi: 10.3389/frfst.2022.1074505

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# β-glucan from brewer's spent yeast as a techno-functional food ingredient

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Brewer's spent yeast (BSY) is a by-product generated during beer production. After heat inactivation, large quantities of BSY are discarded or sold as a low-cost animal feed supplement. Fortunately, BSY can be a good source of valuable compounds such as  $\beta$ -glucan, which has several biological and techno-functional properties for application as a food ingredient. Practical application of  $\beta$ -glucan from BSY requires disruption cell wall and purification steps that significantly influences the yield, cost, biological, physic-chemical, and technological characteristics of this compound. This mini-review presents the use of BSY as a source of  $\beta$ -glucan, the available methods to extract it, and its biological and techno-functional properties.

### KEYWORDS

polysaccharide, yeast cell wall, disruption methods, biological properties, technological properties

# Introduction

Beer is a beverage obtained by the alcoholic fermentation of brewer's wort from barley malt and water, with the addition of hops (Farber and Barth, 2019). In the brewing process, yeasts utilize fermentable sugars to produce ethanol and carbon dioxide (Mohammadi et al., 2011). Some by-products are generated during beer production, which includes malt bagasse, brewer's spent yeast (BSY), and trub (particles denser than the wort that settle at the bottom, e.g., coagulated proteins, hop residue, tannins, among others) (Onofre et al., 2017; Liu et al., 2021).

Yeasts are reused in new fermentations within a limit that considers inoculum contamination and cell vitality/viability. After reaching the maximum potential for use in fermentation, the BSY must be processed due to its complex composition and high biochemical oxygen demand. BSY is often destined for animal feed, but in other cases, it is still improperly discarded (Jaeger et al., 2020; Marson et al., 2020).

Fortunately, BSY presents a rich composition in terms of proteins (about 40–60%) and carbohydrates (about 29–54%) (Onofre et al., 2017; Liu et al., 2021), which encourages its reuse as a potential source of macro compounds for food application

(Bacha et al., 2017). Approximately 1.5 kg–3 kg of BSY is produced for every 100 L of beer, reaching an annual production of up to 400 million kilograms of biomass available to obtain valuable compounds (Marson et al., 2020; Olajire, 2020). Among the macro components in yeasts are  $\beta$ -glucan, a polysaccharide formed by branched glucose molecules interconnected by glycosidic bonds and with several biological and technological activities (Nakashima et al., 2018; Bastos et al., 2022).

Although yeast contains an appreciable amount of  $\beta$ -glucan (30–60% of the dry weight) (Yang and Huang, 2021), the recovery and isolation of this compound could be difficult due to the compact and rigid cell wall (Bacha et al., 2017). Several disruption cell wall methods have been developed to extract  $\beta$ -glucan (Tian et al., 2019; Zheng et al., 2019; Takalloo et al., 2020), considering its yield, purity, and biological and technological properties (Figure 1). In this mini-review, the potential of BSY  $\beta$ -glucan as a food ingredient will be highlighted by discussing the

different extraction methods to obtain it and presenting its techno-functional and biological properties.

### Yeast β-glucan

Yeast  $\beta$ -glucan is a polysaccharide formed by branched glucose molecules interconnected by other glucose chains, especially in the  $\beta$ -(1,3) and  $\beta$ -(1,6) bonds. About 50–55% of  $\beta$ -(1,3) bonds are found in the  $\beta$ -glucan structure, which are responsible for cell elasticity, and 10–15% of  $\beta$ -(1,6), which serves as an anchor for cell structure and integrity (Teparić et al., 2020).

The  $\beta$ -glucan is abundant in the yeast cell wall (i.e., accounting for 30–60% of the dry weight) (Yang and Huang, 2021) and associated with other compounds as chitin and mannoproteins. The amount of  $\beta$ -glucan and the type of binding can be highly variable according to yeast type, usage



cycle, cell vitality and viability, and exposure to cellular depletion factors, which include as follows: temperature shock, osmotic and oxidative stress, oxygen availability, hydrostatic pressure, ethanol concentration, internal acidification, and nutrient limitation (Bastos et al., 2022).

The biological and technological activities of  $\beta$ -glucan vary in relation to the molecular weight, types of bonds, degree of branching, and structural arrangement, which can impact  $\beta$ -glucan solubility (Du et al., 2019; Bastos et al., 2022). Generally,  $\beta$ -glucan is classified with respect to its solubility in aqueous solution, including water-soluble, alkali-soluble, and alkali-insoluble  $\beta$ -glucan (Bastos et al., 2022). In addition, its properties can be influenced by the disruption/extraction methods applied.

# Cell disruption methods for $\beta$ -glucan extraction

Cell rupture methods for  $\beta$ -glucan include mechanical and non-mechanical strategies usually applied as sole (Takalloo et al., 2020), combined (Tian et al., 2019), or in sequence (Zheng et al., 2019). The choice of the rupture method depends partly on the disruption effectiveness and efficiency (Jacob et al., 2019), and it considerably affects the yield, purity, physicochemical, and functional properties of the  $\beta$ -glucan obtained (Fu et al., 2022).

Generally, the methods used to  $\beta$ -glucan extraction from BSY are the same as those applied to disrupt and lysis yeast's cells. Mechanical methods favor scalability and present low operational cost but are not selective and can result in fragments of  $\beta$ -glucan with low purity. Some examples include agitation with glass beads (also known as milling) (Avramia and Amariei, 2022), homogenization at high pressure (Tian et al., 2019; Dimopoulos et al., 2020), and ultrasound (Bzducha-Wróbel et al., 2014; Zheng et al., 2019; Dimopoulos et al., 2020). Non-mechanical methods, in turn, are mostly selective but limited regarding the potential for scale-up (Liu et al., 2016). These include pulsed electric field (Ganeva et al., 2020), alkaline/ acid extraction (Bacha et al., 2017), hot water extraction (Bzducha-Wróbel et al., 2020), autolysis (Vieira et al., 2017), and hydrolysis with commercial enzymes (Marson et al., 2019). Emerging technologies involving ionic liquids are also reported to release  $\beta$ -glucan (Khanh et al., 2020).

Alkaline and alkaline-acid extractions are extensively applied to recover  $\beta$ -glucan—the first consists of using an alkaline solution combined with heating and time. For example, a typical alkaline extraction utilizes sodium hydroxide as a solvent at 90°C for 2 h (Bacha et al., 2017) or potassium hydroxide with different concentrations at room temperature for 2 h (Pinto et al., 2015). Parameters such as alkali concentration, extraction time, and temperature can be evaluated during  $\beta$ -glucan extractions (Varelas et al., 2016;

Vaithanomsat et al., 2022). The extraction results in the precipitate (insoluble  $\beta$ -glucan) and supernatant fractions after a centrifugation step, where the last one is commonly mixed with ethanol to obtain soluble  $\beta$ -glucan. Sometimes the alkali method is followed by acid extraction (Pengkumsri et al., 2016; Dimopoulos et al., 2020; Mahmoud Amer et al., 2021), where the insoluble fraction is mixed with hydrochloric acid, acetic acid, and phosphoric acid (Krpan et al., 2010; Bzducha-Wróbel et al., 2020; Mahmoud Amer et al., 2021), among others.

Hot water extraction is another common method for  $\beta$ glucan extraction that utilizes pressurized steam to release protein and some carbohydrates to the soluble phase while maintaining  $\beta$ -glucan in the solid phase. Some reports mention that hot water extraction occurs with a biomass suspension in water/buffer in a steam autoclave at 121°C and 1.1 atm for 1–5 h (Borchani et al., 2014; Bzducha-Wróbel et al., 2020). At the end of the process, phases are separated, and the sediment containing insoluble  $\beta$ -glucan is recovered; the liquid phase one can be added with ethanol to precipitate soluble  $\beta$ -glucan.

Among enzymatic approaches for cell wall disintegration, autolysis is an extensively applied treatment that utilizes endogenous enzymes in the yeast biomass and usually occurs between 50 and 60°C for 24-48 h (Pengkumsri et al., 2016; Bertolo et al., 2019; Jacob et al., 2019; Dimopoulos et al., 2020; Takalloo et al., 2020). It can be performed by adding some lytic promoters, such as inorganic salts (e.g., sodium chloride) (Bertolo et al., 2019; Jacob et al., 2019) and organic solvents (e.g., ethanol) (Takalloo et al., 2020), and optimized regarding operational parameters (e.g., temperature and autolysis time) (Vieira et al., 2017). Enzymatic hydrolysis, in turn, applies endo or exoproteases (Alcalase®, Flavourzyme<sup>®</sup>, Protamex<sup>®</sup>, Savinase<sup>®</sup>, Neutrase<sup>®</sup>) (Borchani et al., 2014; Lee et al., 2015; Marson et al., 2019; Vaithanomsat et al., 2022) to hydrolyze β-glucan-associated proteins, resulting in both β-glucan fraction and a proteinrich hydrolysate. Sometimes carbohydrases are applied to hydrolyze polysaccharides in the yeast cell wall, which could result in soluble  $\beta\mbox{-glucan}$  fragments (Zheng et al., 2019). In both cases, parameters, including enzyme amount, temperature, pH, time, and yeast biomass concentration, can be evaluated during  $\beta$ -glucan extraction (Marson et al., 2019; Zheng et al., 2019). Enzymatic procedures are completed by heating (above 80°C) the final medium to inactivate enzymes (Bertolo et al., 2019; Vaithanomsat et al., 2022), followed by a solid-liquid separation step (e.g., centrifugation).

Steps such as purification, chemical modification (for insoluble  $\beta$ -glucan), and polishing commonly occur after extraction (Pinto et al., 2015). The characterization of  $\beta$ -glucan in terms of structure and biological and technological properties is also recommended (Pinto et al.,

2015; Bacha et al., 2017; Mahmoud Amer et al., 2021) to better knowledge for future applications.

# Technological properties and applications of BSY $\beta$ -glucan as an ingredient

The food industry is continuously looking for novel healthy ingredients to enhance the products' nutritional and functional value and reduce production costs. Yeast  $\beta$ -glucans are already considered safe food ingredients by the European Food Safety Authority (EFSA. Scientific, 2011) and received the generally recognized as safe (GRAS) status from the United States Food and Drug Administration (FDA Services DoHH, 2008). In fact, several food products have been proposed with the addition of yeast  $\beta$ -glucans (Krpan et al., 2009) and this carbohydrate is commercially available for food supplementation (e.g., Wellmune<sup>®</sup>, Goldcell<sup>®</sup> or Yestimun<sup>®</sup>). In particular, the  $\beta$ -glucans extracted from the BSY have been used in feed applications as alternative diets or supplements for shrimps (Suphantharika et al., 2003) or pigs (Bo et al., 2020).

 $\beta$ -glucan can be used as an ingredient and potentiator for other ingredients promoting the characteristics of foods and beverages.  $\beta$ -glucan can also act as a substitute for components, e.g., fat, with an effect on the nutritional, techno-functional, and sensory properties; and provide thickening and assist in the emulsification, stabilization, and gelation of different foods, which include bakery, meat, and dairy products (Mykhalevych et al., 2022; Sengul and Ufuk, 2022). Additionally,  $\beta$ -glucans act as a water retainer and are a suitable fat replacer, having a good mouthfeel similar to fat (Thammakiti et al., 2004) and enabling the reduction or exclusion of fat levels in foods. This compound can change the sensory properties, viscosity, and rheology of added products, even in small concentrations (Sengul and Ufuk, 2022).

The technological potential of  $\beta$ -glucan in dairy products includes the increasing of overrun ice cream, binding of free moisture, milk fat mimetic, structure formation, and increasing the yield of cheeses, even if low concentrations of  $\beta$ -glucan in the formulations (in the range of 0.5–3.0%) (Mykhalevych et al., 2022). Furthermore, BSY  $\beta$ -glucan as a fat replacer in skim milk yogurt has already resulted in better rheological properties and physical stability (Mejri et al., 2014).

In bakery products, the inclusion of  $\beta$ -glucan can modify the expansion rate, reduce the volume, increase the firmness of the bread, promote an adverse effect on the gluten matrix, increase the water consumption of the dough due to the increase in the fibrous fraction, and increase the number of pores in bread, among other effects. Also, the incorporation of BSY  $\beta$ -glucan in bread preparations improved the nutritional/health-promoting properties of the product (Martins et al., 2018) and enhanced the quality and shelf life during the chilled storage (Suwannarong

et al., 2020). Thus, it is essential to establish the consequences of adding  $\beta$ -glucan and evaluate its suitable concentration that results in less impact on the product and, whenever possible, to correlate with its biological properties (Andrzej et al., 2019).

In meat products,  $\beta$ -glucan can reduce fat content, acting as a substitute ingredient. The  $\beta$ -glucan also acts as an agent to minimize cooking losses of emulsions and increase viscosity and retention of moisture and fat due to its ability to create a three-dimensional network. Additionally, a reduction in textural parameters is verified, indicating the use of  $\beta$ -glucan in meat products with a softer texture, for example (Álvarez and Barbut, 2013).

BSY  $\beta$ -glucans can be incorporated in mayonnaises as a fat replacer, contributing to higher storage stability and a lower caloric value (Worrasinchai et al., 2006; Marinescu et al., 2011). It also exhibit a protective effect of lactobacilli during freeze-drying, refrigerated storage and exposure to simulated gastrointestinal conditions, which make them interesting additives for functional foods containing probiotics (Guedes et al., 2019).

Due to its characteristics, yeast  $\beta$ -glucan can be used as a substitute for traditional ingredients applied at an industrial scale, such as alginates, gum arabic, pectin, and carboxymethylcellulose, with similar or improved properties (Sengul and Ufuk, 2022). However, as yeast  $\beta$ -glucan is a new ingredient, recently applied at industrial scale in food products, it is essential to evaluate the impact of its incorporation in food matrices. The same applies to BSY, which industrial use is still limited but has shown a technological potential to be inserted into different food categories with distinct purposes, as discussed above.

# Biological properties of BSY β-glucan

Several studies have reported the multi-bioactivity of yeast  $\beta$ -glucans, considering them as interesting compounds for food, pharmaceutical and biomedical applications (Geller et al., 2019; Caruso et al., 2022). Nevertheless, only a few numbers of works described the biological effect of  $\beta$ -glucans extracted from BSY as by-product of the brewing industry (Table 1).

BSY  $\beta$ -glucans positively affected the immunogenic activity in different animals. This bioactivity was demonstrated both *in vitro* and *in vivo* by increasing of some important immune index indicators, such as the phenoloxidase activity in black tiger shrimps (*Penaeus monodon*) fed with BSY  $\beta$ -glucan (Thanardkit et al., 2002; Suphantharika et al., 2003).

Furthermore, an increase in the number of haemocytes and in the antibacterial activity against the pathogen *Vibrio harveyi* was also achieved for the shrimps (Thanardkit et al., 2002). The immunogenic activity was also demonstrated in murine peritoneal macrophages after a carboxymethylation reaction to obtain soluble BSY  $\beta$ -glucan (Liepins et al., 2015). The carboxymethylated  $\beta$ -glucan was able to mediate the induction of

TABLE 1	Bioactivity	of	β-glucan	extracted	from	brewer	Ś	spent yeast.	
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Type of glucan	Bioactivity	Specific effect	References
Human tested			
Carboxymethyl-glucan	Antioxidant activity	$\downarrow$ malondial dehyde levels in healthy men	Araújo et al. (2015)
Animal tested			
β-glucan	Immunogenic and antibacterial activity	↑ phenoloxidase activity, the number of haemocytes and the antibacterial activity against <i>Vibrio harveyi</i> in black tiger shrimp	Thanardkit et al. (2002)
β-glucan	Immunogenic activity	$\uparrow$ phenoloxidase activity in haemocytes of black tiger shrimp	Suphantharika et al. (2003)
Carboxymethyl-glucan and β-glucan	Hypocholesterolemic activity	$\ensuremath{\downarrow}\xspace$ cholesterol concentration in blood and reduce the lipid concentration in liver of mice	Waszkiewicz-Robak and Bartnikowska, (2009)
	Immunogenic activity	↑ induction of tumor necrosis factor alfa in mice	Liepins et al. (2015)

tumor necrosis factor-alpha (TNF- $\alpha$ ). The hypocholesterolemic activity of dried BSY, as well as the soluble and insoluble  $\beta$ glucans isolated from it, was reported by Waszkiewicz-Robak and Bartnikowska (Waszkiewicz-Robak and Bartnikowska, 2009). In this study, 6 weeks of a supplemented diet containing dried BSY or BSY  $\beta$ -glucans positively affected the lipid metabolism in blood and liver of mice by reducing the cholesterol and triacylglycerols. The bioactivity of BSY  $\beta$ -glucan, namely the antioxidant potential, was also demonstrated in humans (Araújo et al., 2015). The oral administration of carboxymethylated BSY  $\beta$ -glucan promoted a significant reduction of malondialdehyde levels in healthy men, thus suggesting the positive action of this carbohydrate in preventing oxidative damage.

Overall, it is expected that the promising bioactivity results already obtained for BSY  $\beta$ -glucan could lead to additional studies where the specific structure of the extracted carbohydrates would be directly associated with their biological effect. These studies would greatly contribute to defining and establishing suitable methodologies for  $\beta$ -glucan extraction from BSY.

### **Conclusion and perspectives**

BSY is an important source of  $\beta$ -glucan with already demonstrated biological and technological properties. For application in foods and beverages,  $\beta$ -glucan needs to be extracted, purified, and fully characterized to a better knowledge of its potential applications. The available scientific information is not enough to establish an exact interconnection between the extraction strategy, molecular structure, biological and techno-functional properties. Therefore, additional studies

are needed to define suitable strategies for BSY valorization and  $\beta$ -glucan application.

Governmental disposal policies are needed to implement reuse practices in the beer industry, promoting the efficient conversion of by-products into value-added compounds (circular economy).

### Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

# Acknowledgments

The authors acknowledge the CNPq, CAPES, FAPEG, IF Goiano and UFRJ by support. SS acknowledges the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS—Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/ 0029/2020.

### Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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