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Preparation of Yeast Lysate from Brewer's Spent Yeast

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Abstract

Brewer's spent yeast (BSY) is considered a valuable by-product obtained from the fermentation process. This study aimed to investigate the extraction methods for preparing yeast lysate extract from BSY using conventional shaking, hot steam, and enzyme-assisted extractions. In addition, the effect of sample per ethanol ratio on harvesting the yeast lysate was also determined. Protein and total carbohydrate content was evaluated by using Bradford and phenol-sulfuric assay. The results showed that hot steam-based extraction showed a superior yield of 21.27±0.26 and 21.61±1.13 %w/w when using 1:1 v/v and 1:5 v/v ethanol ratios, respectively. Moreover, hot steam also gave β-glucan at 17.35 ± 0.81% w/w, which was higher than enzyme-assisted extraction (15.4 ± 1.17% w/w). The highest total carbohydrate content (TCC) was obtained at 757.53 ± 0.1 mg/g by conventional extraction methods with sample per ethanol ratio of 1:1 v/v. Fourier-transform infrared spectroscopy (FTIR) confirmed the compositional uniformity of all yeast lysate extracted by the different methods. Scanning electron microscope (SEM) study revealed smoother surfaces of yeast lysate extracted by hot steam and enzyme-assisted methods than the one from conventional extraction. However, other parameters are necessary to study other parameters for optimizing the extraction of yeast lysate from BSY that could have potential for commercial applications.

Keywords: By-product, Enzyme Assisted-Extraction, Carbohydrate, Protein, β-glucan

Introduction

The global rise in beer production has significantly increased the demand for yeast fermentation. This process utilizes strains such as like *Saccharomyces cerevisiae* (ale yeast) and *Saccharomyces pastorianus* (lager yeast) to convert sugars in wort into ethanol, carbon dioxide, and other key compounds that contribute to the sistinct characteristics of beer (Bamforth, 2017). This process generates large amounts of brewer's spent yeast (BSY) as a byproduct, with approximately 10-20 kg of BSY produced per 1,000 L of beer, resulting in millions of tons globally each year (Ferreira et al., 2010). The disposal of BSY poses environmental challenges and costs due to the volume of waste generated (Ferreira et al., 2010). However, BSY is rich in proteins (45-60%), carbohydrates (35-45%), lipids, vitamins, and minerals, making it a potentially valuable resource for upcycling, especially in the animal feed, food, and cosmetic industries (Ivanova, 2020; Ferreira et al., 2010; Jaeger et al., 2020).

Brewer’s spent yeast (BSY) contains valuable biomolecules such as β-glucan and mannoproteins, which have functional properties beneficial for food and cosmetics. β-glucan, a polysaccharide making up 50-60% of the yeast cell wall, has thickening and gelling properties that are useful in food products and can enhance texture and stability in cosmetic formulations as a water-binding agent (Ferreira et al., 2010; Thammakiti et al., 2004). Mannoproteins also offer potential as non-synthetic food emulsifiers and stabilizers, which could translate to similar benefits in cosmetics (Marson, 2020). While methods such as enzymatic, alkaline, and acidic extractions exist to isolate these components, most research has focused on β-glucan due to its commercial value (Mussatto & Mancilha, 2007; Jaeger et al., 2020). This study aims to address this gap by exploring methods for the simultaneous extraction of polysaccharides and proteins from BSY and assessing their potential application in cosmetic formulations based on their functional properties.

Research Objectives

1. To investigate and optimize the preparation methods for extracting of polysaccharides and proteins from Brewer's Spent Yeast (BSY).

2. To characterize the composition of polysaccharides and proteins isolated from BSY using various extraction methods.

Scope of The Study

1. Extracting polysaccharides and proteins from brewer's spent yeast using three different methods: conventional shaking method, hot steam extraction, and the enzyme-assisted extraction method.

2. Evaluating the contents of total polysaccharides, β-glucan, protein, and peptide.

3. Characterizing physicochemical properties, and structural properties of the extracts by FTIR and SEM.

Literature Review

Yeast, particularly *Saccharomyces cerevisiae,* is a single-celled fungus with extensive applications in fermentation and biotechnology due to its metabolic versatility and well-characterized genetic makeup (Goffeau et al., 1996; Boulton & Quain, 2001). This yeast species is not only pivotal in producing fermented beverages but also serves as a model organism for studying biological processes, such as cell cycle regulation and stress response (Botstein et al., 1997). Yeast's structural integrity is maintained by a cell wall composed of polysaccharides like β-glucans, mannans, and chitin, along with proteins and lipids, which have diverse functional roles (Klis et al., 2002; Lenardon et al., 2010). Beyond its role in fermentation, yeast biomass, including brewer’s spent yeast (BSY), is an abundant by-product rich in proteins, lipids, vitamins, and bioactive compounds such as β-glucans and mannoproteins, which are valuable for applications in food, pharmaceuticals, and cosmetics (Ivanova, 2020; Jaeger et al., 2020).

The BSY represents a sustainable by-product of beer production, comprising essential proteins, amino acids, lipids, and bioactive polysaccharides, making it a promising resource for animal feed, functional foods, and cosmetic formulations

(Ferreira et al., 2010; Gabriela, 2020). Various extraction methods, including conventional shaking, hot steam, and enzyme-assisted approaches, have been developed to recover valuable compounds like β-glucans, mannoproteins, and proteins from BSY. These methods differ in their effectiveness based on factors such as temperature, enzyme type, and pressure, thus allowing tailored applications across multiple industries, from animal nutrition to pharmaceuticals (Thammakiti et al., 2004; Chen et al., 2021). Continued research into optimizing these extraction methods is crucial for fully leveraging BSY's potential to promote sustainability and innovation in various sectors.

Research Methodology

1. Preparation of yeast lysate from BSY by conventional shaking extraction

Conventional shaking extraction that was used in this study is modified from Pengkumsri et al. (2016). Fifteen g of powdered brewer's spent BSY were mixed with 150 ml of deionized (DI) water in a 1:10 (w/v) solid-to-liquid ratio. The mixture was shaken at 150 rpm, 50°C for 3 hours, then filtered and centrifuged at 6,000 rpm for 30 minutes (Pengkumsri et al., 2016). The supernatant was concentrated and split into two flasks, where cooled ethanol was added at 1:1 and 1:5 (v/v) ratios. After overnight refrigeration, the precipitates were dried at 50°C in a hot air oven.

2. Preparation of yeast lysate from BSY by hot steam extraction

Hot steam extraction that was used in this study is following Jaeger et al. (2020) with modifications, 15 g of BSY was mixed with 150 ml DI water and autoclaved at 121°C for 15 minutes. The mixture was filtered, and the filtrate was centrifuged at 6,000 rpm for 30 minutes. Ethanol was added at 1:1 and 1:5 (v/v) ratios to the supernatant, and after overnight refrigeration, the precipitates were dried at 50°C.

3. Preparation of yeast lysate from BSY by enzyme-assisted extraction

Enzyme-assisted extraction that was used in this study is based on Podpora et al. (2016), 15 g of BSY was mixed with 0.1 M phosphate buffer (pH 6) in a 1:10 (w/v) ratio, flash was sealed, and treated with papain enzyme (150, 350, or 450 units) at 40°C for 1 hour. The reaction was terminated at 90°C for 15 minutes, and the

mixture was filtered and centrifuged at 6,000 rpm. Ethanol precipitation (1:1 and 1:5 v/v ratios) was followed by overnight refrigeration and drying at 50°C.

4. Determination of total protein

Bradford reagent was used for protein quantification (Bradford, 1970). BSA standards were prepared, and yeast lysate extracts were mixed with Bradford reagent and incubated for 15 minutes. Absorbance was measured at 595 nm, and protein content was calculated using the BSA standard curve.

5. Determination of total carbohydrate content (TCC)

The phenol-sulfuric acid method (Dubois et al., 1956; Nielsen, 2017) was used. Yeast lysate samples (1 mg/ml) were mixed with 5% phenol and sulfuric acid, incubated at 50°C for 10 minutes, and cooled. Absorbance at 490 nm was measured to determine carbohydrate content using a glucose standard curve.

6. Determination of β-Glucan

For the analysis of total glucan content, 90 mg of yeast lysate was treated with sulfuric acid, boiled, adjusted with buffer, treated with enzymes, and analyzed at 510 nm. For α-glucan, a similar procedure was followed with sodium hydroxide and acetate buffer. β-glucan was calculated as the difference between total glucan and α-glucan.

7. Functional group characterization by FTIR analysis

FTIR-ATR was used to analyze the functional groups in yeast lysate, following methods by Smith (2018) and Jones et al. (2019). Spectra were compared with reference data to identify groups like hydroxyl, carbonyl, amine, and alkyl (Brown & White, 2020).

8. Scanning electron microscope (SEM) study

Samples were coated with conductive material to enhance conductivity (Joy & Joy, 1996). In a vacuum chamber, the samples were bombarded with electrons, generating signals like secondary electrons, backscattered electrons, and characteristic X-rays (Reimer & Kohl, 2008).

Results and Discussion

1. Extraction of yeast lysate

Three methods—conventional shaking, hot steam, and enzyme-assisted extraction—were compared for extracting yeast lysate from brewer's spent yeast (BSY). The hot steam method yielded the highest lysate content, with 21.27±0.26% (1:1 ethanol ratio) and 21.61±1.13% (1:5 ethanol ratio) due to effective cell wall disruption by heat (Jaeger et al., 2020). The conventional shaking method yielded the lowest lysate (8.83±0.58% at 1:1 ratio), likely because it relies only on physical agitation, which is less effective in breaking cell walls (Pengkumsri et al., 2016). Enzyme-assisted extraction with papain (150, 350, and 450 units) provided intermediate yields, as the enzyme helped decompose mannoproteins in the cell wall. However, extending the enzyme incubation time could further improve the extraction yield (Podpora et al., 2016).

Table 1 Percentage of yeast lysate extracts from three different methods

Extraction method	**Yield of yeast lysate (%w/w)**	
	1:1 (v/v)	**1:5 (v/v)**
Conventional-Shaking	8.83±0.58^{dB}	10.57±1.12^{dA}
Hot Steam	21.27±0.26^{aA}	21.61±1.13^{aA}
Enzyme-Assisted (150 Units)	15.92±3.77^{cB}	18.49±2.03^{cA}
Enzyme-Assisted (350 Units)	17.26±3.32^{cB}	19.41±1.88^{bA}
Enzyme-Assisted (450 Units)	19.56±1.48^{bB}	20.79±0.07^{bA}

Note Value is expressed as means ± SD (n = 3). Different small letters compared in the same column indicate significant differences ($p<0.05$) and different capital letters compared in the same row indicate significant different ($p<0.05$).

2. Protein content

Protein content, measured by the Bradford assay (Bradford, 1976), was highest in lysates from the hot steam method, yielding 33.62±0.04 mg/g (1:1 ethanol ratio) and 39.01±0.04 mg/g (1:5 ethanol ratio), consistent with studies showing steam-assisted methods effectively release intracellular components (Lee et al., 2016). In contrast, enzyme-assisted extraction resulted in lower protein contents due to proteolytic activity from papain breaking down proteins (Park et al., 2017). The

conventional method yielded moderate protein levels, suggesting that it better preserves proteins compared to enzyme-assisted methods.

Table 2 Protein content in yeast lysate extracts from three different methods

Extraction Method	Protein content (mg/g extract)	
	1:1 (v/v)	**1:5 (v/v)**
Conventional-Shaking	21.51 ± 0.03^{bA}	21.37 ± 0.05^{bB}
Hot Steam	33.62 ± 0.04^{aB}	39.01 ± 0.04^{aA}
Enzyme-Assisted (150 Units)	7.24 ± 0.01^{eB}	12.67 ± 0.02^{cA}
Enzyme-Assisted (350 Units)	9.39 ± 0.04^{cB}	9.73 ± 0.03^{eA}
Enzyme-Assisted (450 Units)	9.02 ± 0.03^{dB}	11.74 ± 0.04^{dA}

Note Value is expressed as means ± SD (n = 3). Different small letters compared in the same column indicate significant differences ($p<0.05$) and different capital letters compared in the same row indicate significant different ($p<0.05$).

3. Total carbohydrate content (TCC)

Total carbohydrate content, determined using the phenol-sulfuric acid assay (Dubois et al., 1956; Nielsen, 2017), was highest in lysates obtained via conventional shaking (757.53±0.15 mg/g extract at 1:1 ethanol ratio), likely due to mechanical forces causing cell wall disruption and polysaccharide release (Smith J., 2020). Lower carbohydrate yields in hot steam and enzyme-assisted methods, especially at higher enzyme concentrations, suggest potential degradation or incomplete release of polysaccharides.

Table 3 Total carbohydrate content in yeast lysate extracts by three different methods

Extraction method	Total carbohydrate content (mg/g extract)	
	1:1 (v/v)	**1:5 (v/v)**
Conventional-shaking	757.53 ± 0.15^{aA}	575.14 ± 0.15^{aB}
Hot Steam	273.41 ± 1.54^{cB}	285.88 ± 0.27^{bA}
Enzyme-Assisted (150 Units)	346.44 ± 0.16^{bA}	250.17 ± 0.08^{cB}
Enzyme-Assisted (350 Units)	187.56 ± 0.27^{eB}	211.38 ± 0.08^{eA}

Table 3 (continued)

Extraction method	Total carbohydrate content (mg/g extract)	
	1:1 (v/v)	**1:5 (v/v)**
Enzyme-Assisted (450 Units)	213.92 ± 0.08^{dB}	215.88 ± 0.14^{dA}

Note Value is expressed as means ± SD (n = 3). Different small letters compared in the same column indicate significant differences ($p<0.05$) and different capital letters compared in the same row indicate significant different ($p<0.05$).

4. β-Glucan Content

The highest β-glucan content was observed in lysates from hot steam extraction, at 18.81±1.86% (1:1 ratio), which aligns with effective polysaccharide extraction through heat treatment (Jaeger et al., 2020). The enzyme-assisted method, particularly at higher enzyme units (450 units), resulted in the lowest β-glucan content (6.94±0.90%), potentially due to papain's activity degrading glucans (Park et al., 2017).

Table 4 β-Glucan in yeast lysate extracted by three different methods.

Extraction method	β-Glucan (%w/w)	
	1:1 (v/v)	**1:5 (v/v)**
Conventional-shaking	17.35 ± 0.81^{aA}	15.4 ± 1.17^{bB}
Hot Steam	18.81 ± 1.86^{aB}	18.99 ± 0.74^{aA}
Enzyme-Assisted (150 Units)	10.22 ± 1.16^{bA}	8.24 ± 0.94^{cB}
Enzyme-Assisted (350 Units)	7.67 ± 1.20^{bcA}	6.14 ± 0.12^{cB}
Enzyme-Assisted (450 Units)	6.94 ± 0.90^{cB}	8.32 ± 2.56^{cA}

Note Value is expressed as means ± SD (n = 3). Different small letters compared in the same column indicate significant differences ($p<0.05$) and different capital letters compared in the same row indicate significant different ($p<0.05$).

5. Functional Group Characterization by FTIR

FTIR-ATR analysis identified the consistent functional groups across all samples, including hydroxyl, carbonyl, and amine groups, confirming the presence of proteins and polysaccharides (Hromádkováin, 2003; Amer, 2021). The similarity in

spectra among different extraction methods indicates effective precipitation of these components with both ethanol ratios.

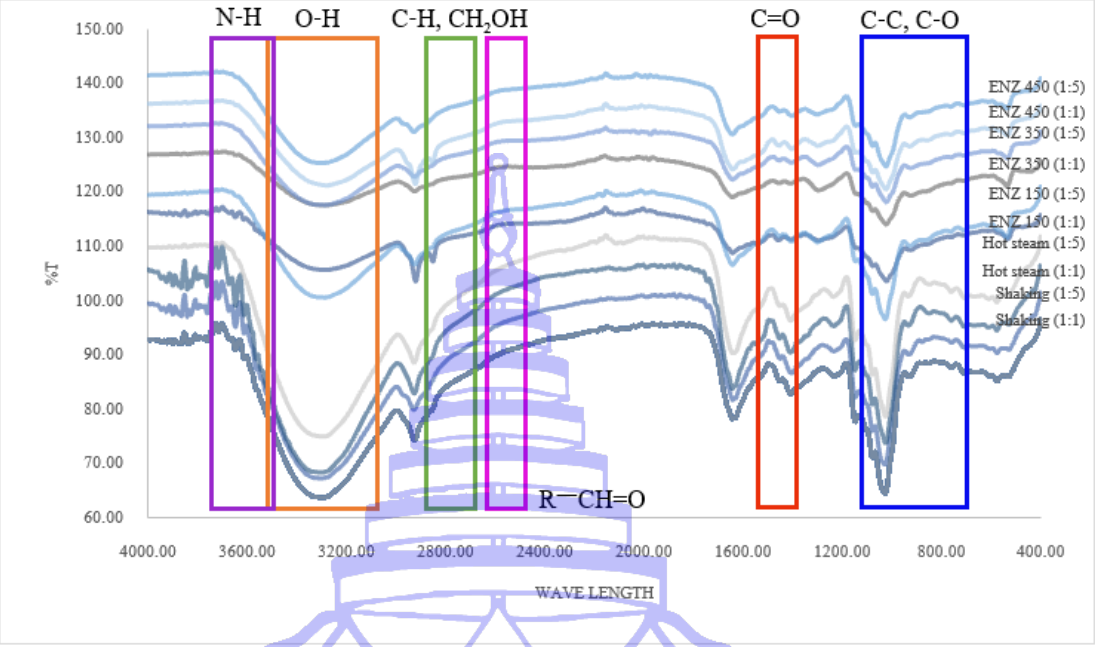

Figure 1 FTIR spectra of yeast lysate's functional groups

6. Morphological Study

Microscopy revealed that yeast cell size decreased, and cell walls became less distinct across extraction methods. SEM images showed rough and porous surfaces in lysates from conventional extraction, while those from hot steam and enzyme-assisted methods were smoother, affecting potential applications where texture and surface morphology are critical (Kaur & Dhillon, 2014).

Conclusion and Suggestion

This study demonstrated that the hot steam method was the most effective in terms of yield and composition of yeast lysate. The hot steam extraction produced the highest yield of yeast lysate, protein, and β-glucan. However, the enzyme-assisted extraction method, yielded lower amounts of protein, glucose, and β-glucan. Specifically, the enzyme-assisted method at 150, 350, and 450 units showed lower protein yields compared to the conventional and hot steam methods. The compositional analysis using FTIR confirmed the presence of polysaccharide and protein. Despite this, the enzyme-assisted and hot steam method produced lysates with smoother and less porous surfaces, as evidenced by SEM analysis. The ratios of ethanol that used for precipitation, which were 1:1 and 1:5 v/v, could precipitate both of protein and polysaccharide.

Further research on yeast lysate extraction methods has been highlighted by this study. Surface differences from various extraction methods should be clarified by studying brewer's spent yeast using SEM. Exploring different temperatures for hot steam extraction could be explored to optimize costs. Additionally, enzyme concentrations and conditions should be adjusted to refine enzyme-assisted extraction, thereby increasing yield without compromising quality. The use of different enzymes or solvents might be used to enhance efficiency. While ethanol precipitation has been the main focus, varying ethanol ratios and alternative solvents should be examined in future research to better isolate valuable components. Furthermore, long-term stability and optimal storage conditions for yeast lysates should be investigated to ensure their quality and efficacy in practical applications.

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