A Simple and Efficient Mechanical Cell Disruption Method Using Glass Beads to Extract β-Glucans from Spent Brewer’s Yeast

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Abstract: β-glucan extraction from spent brewer’s yeast is a long process that starts with the lysis of yeast cells, this step lasting up to 36 h and can be disadvantageous when working on a small scale. In this study, a rapid cell rupture method was selected for the lysis of spent brewer’s yeast to obtain β-glucans. Optimal parameters were determined for the lysis of a cellular suspension of spent brewer’s yeast by vortexing with glass beads. Thus, parameters such as the number of 10 min vortex cycles from 1 to 3, the concentration of cell suspension (5, 10, and 15%), and the ratio of yeast/glass beads (1:1, 1:2, and 1:3) were varied in a Box-Behnken design. A cell lysis mechanism using glass beads allows the cell to rupture and permits the removal of intracellular content. An increase in yeast suspension concentration decreased the disruption efficiency, while a proportional increase was observed with the yeast/glass beads ratio and the increasing number of vortexing cycles. The optimal parameters for cell lysis were found to be a cell suspension concentration of 5%, a ratio of yeast/glass beads of 1:2, and a vortexing cycle of 3, with a disruption efficiency of 99.8%. The β-glucan fraction extracted from the optimal sample showed characteristic absorption bands at 1370.77 and 1153.92 cm⁻¹, the content of β-glucan being 78.53%.

Keywords: glass beads; β-glucan; mechanical disruption; spent brewer’s yeast; Saccharomyces cerevisiae

1. Introduction

The beer production industry generates large amounts of valuable by-products with many biological and functional values. Spent brewer’s yeast is such by-product that could be used as raw material for β-glucan extraction since the most important polysaccharides found in cell walls are glucan and mannan with linear bonds at β-(1-3)/(1-6), the most frequent being the β-(1-3) [1].

In the literature, there are various methods of cell lysis that can be implemented for yeast extraction, depending on the cellular components that should be isolated. Thus, for total protein extraction, improved methods, such as the one described by Zhang et al. (2011) using lithium acetate (LiAc) in combination with NaOH, which enhances the permeability of yeast cell walls, releasing proteins from cells, could be used [2]. In contrast, for lipid extraction, the homogenization of a cell suspension in the presence of organic solvents and glass beads with a diameter of 0.25–0.30 mm can be effective, according to the data presented by Schneiter and Daum (2006) [3]. The authors showed that the method allows cell disruption over four cycles of 30 s at 1700 rpm with a pause of 30 s between cycles.

Regarding the extraction of polysaccharides that are mainly found in cell walls, first, it should be considered whether whole particles or fragments of β-glucans should be obtained. One of the methods to extract β-glucans involves cell fragmentation that occurs by using glass beads or ultrasound. Some more complex methods include the use of ultrasound, alkaline substances, and enzymes in combination for the extraction of β-glucans [4].

The extraction from spent brewer’s yeast is slightly different compared to extraction from native yeast. The environment in which the yeast lives and the growing conditions have special implications in the resistance and polysaccharide content of the cell wall.
Thus, a deficiency of nutrients in the environment can lead to an increase in β-glucans in the yeast cell wall [5]. In general, each one of 13–25 serial repitchings of yeast cells involves 2–3 rounds of cell division [6]. Considering the number of successive replications, as well as the composition of the environment in which cell division takes place, yeast will develop a resistance to the environment that is expressed by an increase in thickness and a deformation caused by budding scars on the cell surface. Such changes in the thickness of the cell wall of spent brewer’s yeast are observed by using transmission electron microscopy (TEM) [7]. One of the most efficient and reliable methods of breaking the cell wall is agitation with glass beads, which achieves breakage in a relatively short time, and the continuous kinetic energy generates sufficient mechanical energy to disrupt yeast cell walls [8,9]. This complex process allows a rapid release of cytoplasmic content in the reaction phase and fragments of cell walls can be easily separated by centrifugation [10].

The critical parameters, such as mechanical agitation speed, glass bead loading, suspension concentration, and glass bead size, are factors that influence the rate of cell rupture with direct effect on the concentration in the final β-glucan content. Kakko, Ivanona, and Rantasalo (2016) stated in a brief presentation of cell rupture methods that the optimal size of glass beads for yeast cell lysis would be a large diameter of 0.5 mm because beads with small diameters are used especially for bacteria [11]. Beads smaller than 0.25 mm in diameter are not suitable for yeast, and a particular diameter between 0.1–0.15 mm is used for microbial cells [12]. In a more elaborate study investigating the kinetic effect of cell disruption, which admits as variables the operating time (0–3 min), bead loading (0.3–0.85 volume ratio), bead diameter (0.375–0.875 mm) and agitation speed (2000–6000 rpm), stated that an increase in bead loading up to 0.85 and a bead diameter of 0.375 mm will speed up the rate of cell rupture [13]. Also, by using the non-linear least squares method, the authors found that the effective disruption volume increased with the increase in bead diameter (the proportionality constant β increased from $3.45 \times 10^{-8}$ mm$^3$ at a bead diameter of 0.375 mm to $1.61 \times 10^{-7}$ mm$^3$ at a 0.625 mm diameter) [13].

In another paper, Bzducha-Wróbel et al. (2014), used glass beads with diameters between 0.5–1 mm, in addition to other methods of cell rupture. The study showed that the solubilized material content after cell rupture did not vary significantly, but the content in β-glucans clearly increased (14.10%) by using glass beads with a diameter of 0.5 mm, compared to an increase of 12.4% using beads 1 mm in diameter [14].

Considering these critical factors presented above, the aim of this study was to optimize the lysis of spent brewer’s yeast, *S. cerevisiae* cells, by using as variables three different yeast suspension concentrations, yeast/glass bead ratios, and vortexing times. Furthermore, we analyzed the molecular characteristics and the content of insoluble β-glucan extracted by the conventional alkaline acid method from the optimal sample.

2. Materials and Methods

2.1. Materials

To conduct this study, we used a fresh suspension of spent brewer’s yeast (a strain of *S. cerevisiae*) provided by a local brewery (S.C. Bermas S.A., Suceava, Romania). The yeast suspension required a preliminary purification treatment, described below in Section 2.2.1, to remove residues and beer liqueur from the fermentation process. The glass beads purchased from Sigma-Aldrich, St. Louis, MA, USA (Sigma G-9268) with diameters of 425–600 µm were chosen for an efficient breakage of the yeast cells.

Cell rupture was performed using a vortex stirrer (Vortexer, HSA 72432) operated at a stirring speed of 3000 rpm. The device was loaded symmetrically with two tubes in a volume of 50 mL securely fixed with elastic. The loading parameters of the symmetrical tubes, such as weight, solid/liquid ratio, and samples volume, were the same to prevent any mass imbalance that might affect the cell rupture.
2.2. Methods

2.2.1. Yeast Pretreatment and Preparation of Cell Suspension

Before cell lysis, a pretreatment of spent brewer’s yeast is necessary to produce food-grade yeast with no residues or alcohol. Thus, to remove the wort components from the beer, the yeast suspension was centrifuged at 3000 rpm for 10 min. The obtained sediment containing yeast and small impurities from the fermentation process (wheat bran, malt, and cereal fractions) was washed with distilled water and then passed through a nylon mesh filter with a pore size of 80 μm (180 mesh) and centrifuged at 2000 rpm for 5 min for separation. Subsequently, about 10 g of yeast was collected to determine the dry weight and the remaining sediment was placed in a refrigerator. Knowing the amount of dry substances composing the purified yeast (22.7%), for the preparation of the solutions of 5%, 10%, and 15%, respectively, the cell suspension was adjusted with distilled water at desired volumes. The debittering of the yeast suspension in this process was excluded to avoid the loss of cell viability [15].

2.2.2. Cell Lysis Procedure

The lysis of yeast cells was performed in conical centrifuge tubes with volumes of 50 mL (95 × 45 mm) and inner diameters of 27 mm. Centrifuge tubes were filled with glass beads up to a volume of 85% of the total substances to avoid possible overheating [16]. Cell rupture was achieved between 1 and 3 cycles of 10 min each with 15-min breaks in the refrigerator between cycles. The concentration of the cell suspension was between 5 and 15% and the ratio of yeast to glass beads was 1:1, 1:2, or 1:3, according to the experimental design presented in Section 2.4.

2.2.3. Determination of Cell Rupture

While the protein dilution procedure failed to provide satisfactory results [17] and microscopic observations are not a common criterion for counting disrupted cells [18], cell disruption was determined by the difference between the 5 mL suspension weight after drying, before and after cell lysis (Equation (1)):

\[
\text{Disruption efficiency} = \frac{m_0 - m_1}{m_0} \times 100
\]

where \(m_0\) is the initial dried biomass before lysis and \(m_1\) is the residual dried biomass after lysis.

The amount of solids released during lysis into the yeast suspension is defined as the autolysis rate [19,20]. Thus, beside the indirect quantification method for cell disruption evaluated by the loss of dried biomass, microscopic examinations) assess the degree of cell breakage at different yeast suspension concentrations and vortexing cycles. After lysis, samples were diluted at an equivalent of 1% cell slurry according to Wenger et al. (2008), stained with methylene blue, and examined at 400 × magnification [21].

2.3. Extraction and Analysis of β-Glucan from Optimal Sample

After optimizing the cell lysis parameters, the optimal sample was subjected to β-glucan extraction using an alkaline-acid extraction (Figure 1).

Thus, after cell breakage the cell suspension was separated from the glass beads by centrifugation under the nylon mesh filter (80 μm). The autolysate was mixed with five volumes of 1 N NaOH in a water bath at 90 ± 5 °C under continuous stirring [22]. After cooling, cell walls were separated through centrifugation and washed three times with distilled water, then mixed with 0.5 N acetic acid in a 1:5 ratio at 75 ± 5 °C for 1 h. The pellet was washed twice with distilled water, centrifuged at 3500 rpm for 10 min, and dried in a heating oven at 50 ± 5 °C [23].
2.3.1. β-Glucan Determination

The β-glucan content was evaluated by means of a K-EBHLG 02/17 enzymatic kit (Megazyme). The β-glucan fraction (about 20 mg) was mixed with 2 M KOH in an ice bath, then the solution obtained was mixed with a Glucazyme™ enzyme mix and kept for 16 h at 40 °C. After dilution and centrifugation, an aliquot was used for glucose determination by using a GOPOD reagent on a UV–VIS–NIR Shimadzu 3600 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 510 nm against a reagent blank. β-glucan content was determined in duplicate by using the following equation described by [14]:

$$\beta\text{-glucan} = \Delta E \times F \times \frac{10.836}{W};$$

where $\Delta E$ is the absorbance against the blank, $F$ is the conversion from absorbance to the µg (150 µg of D-glucose) standard divided by the GOPOD absorbance of this 150 µg, and $W$ is the weight of the sample analyzed in mg, 10.836-constant.

2.3.2. β-Glucan FT-IR Spectra

The FT-IR spectra of the fine-ground β-glucan from the optimal sample was achieved at a spectral resolution of 4 cm$^{-1}$ by 32 scans recorded between 400 cm$^{-1}$ and 4000 cm$^{-1}$, using a Thermo Scientific Nicolet iS20 (Waltham, MA, USA) spectrometer equipped with an ATR module. A background spectrum was collected prior to analysis with the ZnSe ATR module. An ATR correction was applied to compensate for the fractional penetration depth of the wavelength between the contact with β-glucan and the ZnSe crystal.

2.4. Statistics, Experimental Design, and Optimization of Factors

The differences among the samples were checked with the Tukey test at a significance level of 95% by using XLSTAT 2021 version software (Addinsoft, New York, NY, USA).

The effects of yeast cell suspension concentration, the amount of glass beads, and the number of vortexing cycles of 10 min factors varied at three levels on the cell disruption efficiency response were investigated by means of a Response Surface Methodology with a Box-Behnken design, by using Design Expert (trial version) software (Stat-Ease, Inc., Minneapolis, MN, USA). Five center points and one block were selected, resulting in 17 runs. The actual and coded values of the factors are presented in Table 1. The data were fitted to the quadratic model, adequacy of which was evaluated through Analysis of Variance (ANOVA) by considering the Fisher coefficient (F), the coefficient of determination...
\( R^2 \), the adjusted coefficient of determination \( (adj.-R^2) \), and the lack of fit values. The significance level considered was \( p < 0.05 \).

Table 1. Actual vs. coded values of the factors.

| Run | Yeast Suspension Concentration (%) | Yeast/Glass Bead Ratio \((v/w)\) | Vortexing Cycles | A | B | C |
|-----|-----------------------------------|-------------------------------|-----------------|---|---|---|
| 1   | 5                                 | 1:1                           | 2               | −1.00 | −1.00 | 0.00 |
| 2   | 5                                 | 1:2                           | 1               | −1.00 | 0.00  | −1.00 |
| 3   | 5                                 | 1:2                           | 3               | −1.00 | 0.00  | 1.00  |
| 4   | 5                                 | 1:3                           | 2               | −1.00 | 1.00  | 0.00  |
| 5   | 10                                | 1:1                           | 1               | 0.00  | −1.00 | −1.00 |
| 6   | 10                                | 1:1                           | 3               | 0.00  | −1.00 | 1.00  |
| 7   | 10                                | 1:2                           | 2               | 0.00  | 0.00  | 0.00  |
| 8   | 10                                | 1:2                           | 2               | 0.00  | 0.00  | 0.00  |
| 9   | 10                                | 1:2                           | 2               | 0.00  | 0.00  | 0.00  |
| 10  | 10                                | 1:2                           | 2               | 0.00  | 0.00  | 0.00  |
| 11  | 10                                | 1:2                           | 2               | 0.00  | 0.00  | 0.00  |
| 12  | 10                                | 1:3                           | 1               | 0.00  | 1.00  | −1.00 |
| 13  | 10                                | 1:3                           | 3               | 0.00  | 1.00  | 1.00  |
| 14  | 15                                | 1:1                           | 2               | 1.00  | −1.00 | 0.00  |
| 15  | 15                                | 1:2                           | 1               | 1.00  | 0.00  | −1.00 |
| 16  | 15                                | 1:2                           | 3               | 1.00  | 0.00  | 1.00  |
| 17  | 15                                | 1:3                           | 2               | 1.00  | 1.00  | 0.00  |

For factor optimization, the desirability function was used and the yeast suspension concentrations, the yeast/glass bead ratios, and the vortexing cycle factors were kept in range, while the cell disruption efficiency was set at maximum.

3. Results and Discussion

3.1. Effect of Parameters and Optimization of Cell Lysis

The cell lysis parameters are key factors that influence the breakage of the cell wall. The effects of yeast suspension concentrations, yeast/glass bead ratios, and the number of vortexing cycles on spent brewer’s yeast cell disruption are presented in Table 2. The disruption efficiency varied from 14.5 to 99.8%, depending on the conditions applied, with significant differences \( (p < 0.05) \) among samples. Liu et al. (2008) reported an autolysis ratio of 5 to 49% when spent brewer’s yeast was lysed in a 3% sodium chloride solution at 55 \( ^\circ \)C and a pH of 5.5. The efficiency of cell lysis depends on the method selected, a fact also supported by the findings of Bzducha-Wróbel et al. (2014), who stated that the highest efficiency was observed after cell disruption through homogenization in a bead mill and combined autolysis with bead milling.

Table 2. Effects of method parameters on spent brewer’s yeast cell disruption efficiency.

| Yeast Suspension Concentration (%) | Yeast/Glass Bead Ratio \((v/w)\) | Vortexing Cycles | Disruption Efficiency (%) |
|-----------------------------------|-------------------------------|-----------------|---------------------------|
| 5                                 | 1:1                           | 2               | 34.8 \( ^b \)           |
| 5                                 | 1:2                           | 1               | 45.6 \( ^c \)           |
| 5                                 | 1:2                           | 3               | 99.8 \( ^a \)           |
| 5                                 | 1:3                           | 2               | 78.9 \( ^c \)           |
| 10                                | 1:1                           | 1               | 21.5 \( ^l \)           |
| 10                                | 1:1                           | 3               | 44.0 \( ^f \)           |
| 10                                | 1:2                           | 2               | 41.6 \( ^s \)           |
| 10                                | 1:3                           | 1               | 33.4 \( ^l \)           |
| 10                                | 1:3                           | 3               | 93.9 \( ^b \)           |
| 15                                | 1:1                           | 2               | 14.5 \( ^l \)           |
| 15                                | 1:2                           | 1               | 15.1 \( ^k \)           |
Table 2. Cont.

| Yeast Suspension Concentration (%) | Yeast/Glass Bead Ratio (v/w) | Vortexing Cycles | Disruption Efficiency (%) |
|------------------------------------|-------------------------------|------------------|---------------------------|
| 15                                 | 1:2                           | 3                | 49.0 d                    |
| 15                                 | 1:3                           | 2                | 21.5 j                    |

a–l—different letters in the same column show significant differences among samples ($p < 0.05$).

The data obtained were fitted to the quadratic model (Equation (3)), which showed significance at the $p < 0.05$ level ($F$-value = 393.43) and described 99.80% of data variation (Table 3).

Disruption efficiency (%)  

$$ \text{Disruption efficiency} = 41.66 - 19.87A + 14.12B + 9.26AB - 5.07AC + 9.50BC - 0.01A^2 - 4.18B^2 + 10.77C^2 $$  \hspace{1cm} (3)

Table 3. ANOVA results for the effects of factors on cell disruption efficiency.

| Model                                | Sum of Squares | Mean Square | $F$-Value | $p$-Value |
|--------------------------------------|----------------|-------------|-----------|-----------|
| Quadratic                            | 9768.51        | 1085.39     | 393.43    | <0.0001   |
| A-Yeast suspension concentration      | 3157.34        | 3157.34     | 1144.46   | <0.0001   |
| B-Yeast/glass bead ratio              | 1594.71        | 1594.71     | 578.05    | <0.0001   |
| C-Number of cycles                   | 3665.39        | 3665.39     | 1328.62   | <0.0001   |
| AB                                   | 342.81         | 342.81      | 124.26    | <0.0001   |
| AC                                   | 102.82         | 102.82      | 37.27     | 0.0005    |
| BC                                   | 361.38         | 361.38      | 130.99    | <0.0001   |
| $A^2$                                 | 0.0002         | 0.0002      | 0.0001    | ns        |
| $B^2$                                 | 73.44          | 73.44       | 26.62     | 0.0013    |
| $C^2$                                 | 488.50         | 488.50      | 177.07    | <0.0001   |
| Residual                              | 19.31          | 2.76        |           |           |
| Lack of fit                          | 19.31          | 6.44        |           |           |

Fit statistics

| Mean                     | 44.76          |
| Standard deviation       | 1.66           |
| C.V. (%)                 | 3.71           |
| $R^2$                    | 0.9980         |
| $Adj.-R^2$               | 0.9955         |
| Predicted $R^2$          | 0.9684         |
| Adeq. Precision          | 67.5219        |

ns—not significant.

The predicted $R^2$ of 0.9684 is in reasonable agreement with the $adj.-R^2$ of 0.9955, the difference being less than 0.2, which supports model adequacy. Furthermore, the lack of fit value was not significant ($p > 0.05$) and the Adeq. precision was $>4$ for the considered response, indicating an adequate signal-to-noise ratio.

According to the data presented in Table 3, the yeast suspension concentration (A), yeast/glass bead ratio (B), and number of vortexing cycles (C) factors and their interactions influenced disruption efficiency response in a significant way ($p < 0.05$), except the quadratic term of yeast suspension concentration ($A^2$). The highest negative influence was observed for the linear term of the yeast suspension concentration factor, while the vortexing cycles factor showed the greatest positive influence on the response.

The response surface plots of the combined effects of the factors on spent brewer’s yeast cell disruption efficiency are presented in Figure 2. Yeast cell suspension concentration determined the decrease of disruption efficiency. On the other hand, the increase of the yeast/glass bead ratio and the number of vortexing cycles led to an increase in cell wall disruption efficiency. These observations were supported by previous results obtained by Mao and Moo-Young (1990), who reported that an increase in cell concentration decreases the efficiency of cell rupture [24]. This could have been caused by an insufficient collision between the glass beads and the yeast cells in the effective disruption region as a result of the
yeast concentration and cell wall fragments in suspension. Furthermore, Heim et al. (2007) investigated whether a decrease in cell wall disruption rate is caused by a lack of action of the breaking surface formed by the glass beads and high yeast concentration [10]. Vieira et al. (2017) underlined the significant effects ($p < 0.05$) of spent brewer's yeast cell lysis temperature and time on the amount of bioactive compounds released [25]. Another study conducted by Ardiyanti and Guntoro (2018) revealed that the highest disruption efficiency (99.5%) was obtained after freezing-defreezing, combined with glass-bead treatment of a 15% spent brewer's yeast suspension, the results being in line with our data [26].

![3-D plots of combined effects of factors](image)

**Figure 2.** 3-D plots of combined effects of factors: (a) yeast suspension concentration (A) and yeast/glass beads ratio (B), (b) yeast/glass bead ratio (B) and number of cycles (C), (c) yeast suspension concentration (A) and number of cycles (C) on cell disruption efficiency.
The optimization of spent brewer’s yeast cell lysis is essential and advantageous for subsequent β-glucan extraction. Thus, after investigating the effects of the factors on cell disruption efficiency, an optimization process was performed by using a desirability function that implies the transformation of the predicted response occurs in a dimensionless partial desirability function (d_i), which comprises the researcher’s particular requirements. The partial desirability functions obtained are put together into an overall desirability function, D, which is the geometric mean of the d_i values [27]. The combination that showed the highest D value was considered the optimal one (Figure 3).

The results obtained showed that a yeast suspension concentration of 5%, a yeast/glass bead ratio of 1:2, and three vortexing cycles of 10 min each would be the optimal parameters for spent brewer’s yeast cell lysis that produce a disruption efficiency of 99.8% (Figure 3).

Different results in the rate of cell lysis were found both for spent brewer’s yeast and fresh yeast in the β-glucan isolation protocol. Thus, Liu et al. (2008), after autolysis of a 15% (w/w) spent brewer’s yeast followed by an autoclavation of a 10% (w/w) cell suspension with glass beads in a ratio of 5:1 yeast/glass beads (bead diameters 0.3–0.4 cm), managed to release about 48% (w/w) cellular substances [19]. Magnani et al. (2009), under the same conditions but this time using a 30% (w/w) cell suspension, reported an autolysis rate of 53%, which is also lower than our results, probably because of the different processing conditions (glass bead diameter of 0.4 mm, temperature, slurry concentration, etc.). The experiment used fresh yeast as raw material and in the context of cell lysis demonstrated that a concentrated mixture of 30% fresh yeast requires special cell lysis conditions (autolysis followed by heating with glass beads in an autoclave at 121 °C) [28].

Ardiyanti and Guntoro (2018) managed to disrupt 79.7% of total cells in similar conditions of cell lysis by using a 15% spent brewer’s yeast suspension, with a 1:1 yeast/glass bead ratio and five vortexing cycles of 30 min each [26]. Their findings suggest that an efficient cell rupture under a 1:1 yeast/glass bead ratio requires a higher number of vortexing cycles and implicitly a much longer time (about 150 min) at highly concentrated yeast cell suspensions.

3.2. Degree of Cell Rupture

Figure 4a–c show the degree of cell rupture of samples with three different yeast suspension concentrations at each agitation cycle. We observed a high number of broken cells with released intracellular content, such as cytoplasm and organelles, as described by D. Liu et al. (2016) in a previous study [29]. Melendres et al. (1991) introduced for the first time the concept of effective disruption volume. This concept implies that disruption occurs only in a specific region where beads collide [13]. Therefore, the black arrows indicate the broken cells with irregular cell walls caused by the collision of yeast in the effective disruption region between glass beads, while the red arrows show dead cells stained inside with methylene blue. These stained cells still hold their intracellular content because they are undamaged by glass beads and cannot be dissolved in the continuous phase [30].
A number of small fragments of the broken cell wall and cell debris can be observed behind the whole yeast cells. Similar results were presented by Bzducha-Wróbel et al. (2014) for *S. cerevisiae*, disrupted cells with no intracellular components being observed [14]. The efficiency of the bead mill lysis method is indicated by the degree of cell fragmentation that contributes to the leaching of cytosol components. Another cell disruption technique using a horizontal bead mill highlighted that a 5% (w/v) yeast suspension under a rotational speed of 2500 rpm resulted in asymmetrical deformation of the spherical cell shape [30]. After methylene blue staining, microscopic analysis at 0, 1.5, and 4.5 min revealed that a high number of cell walls and cells with intracellular content released were present in the suspension with the longest duration of milling. This is in agreement with the results obtained and suggests that an increased duration of vortexing cycles has a major influence on cell rupture and implicitly on the elimination of intracellular content.

### 3.3. β-Glucan Content

The β-glucan was extracted from the optimal sample with the highest degree of cell rupture and showed a value of 78.53% (Figure 5a), which was higher than the results obtained by Bzducha-Wróbel et al. (2014) for β-glucan extracted from *S. cerevisiae* baker yeast in similar conditions. The β-glucan extraction yield is directly influenced by yeast conditioning and lysis method. Suphantharika et al. (2003) reported β-glucan contents between 50.5 and 71.2%, depending on the yeast strain, the highest value being that of spent brewer’s yeast [22]. The data presented by Marinescu and Stoicescu (2009) showed lower values of β-glucan (29.5–38.1%) extracted from spent brewer’s yeast cells lysed by the bead milling method compared to our study, most likely because of bead diameter (12 mm), the different material (stainless steel), and an intermittent mixing process [31]. To observe if there were any significant differences from the optimal sample, further investigations were conducted on the yeast samples at the lowest concentration of 5% with different degrees of cell rupture at three different vortexing cycles and yeast/glass bead ratios. Thus, by using the alkaline-acid method described in Section 2.3 on samples with disruption efficiencies between 34.8 and 99.8%, the β-glucan content was determined. The results of the effect of cell breakage efficiency on β-glucan content (Figure 5a) indicates that incomplete cell rupture leads to a decrease in β-glucan after the alkaline-acid extraction (67.38% β-glucan at a disruption efficiency of 34.8% compared to 78.53% β-glucan at 99.8% cell breakage). This is mainly due to an incomplete extraction achieved by cells that still
have intracellular content after the lysis procedure (these cells are indicated by red arrows in Figure 4). Bacon et al. (1969) noted that the extraction process is made more difficult when using the whole cells in alkali-extractions because of remaining residues from the cell contents [32]. Additionally, Figure 5b shows significant correlation ($R^2 = 0.93$) between the disruption efficiency and β-glucan content after the alkaline-acid extraction protocol and supports the assumption that a high degree of cell breakage will provide a large amount of β-glucans. The molecular characteristics of the β-glucan extracted from spent brewer’s yeast after optimization of cell lysis is shown in Figure 6. The polysaccharide absorption band can be observed at 3330.31 cm$^{-1}$, while the peak at 2918.23 cm$^{-1}$ could be given by the C-H stretching vibrations of the CH$_2$ and CH$_3$ groups [33]. These data are in agreement with those reported by Magnani et al. (2009). The particular bands are attributed to the axial deformation of OH, being also characteristic for β-D-glucans in addition to the vibrations of the CH bonds, the peak at 2918.23 indicating the frequencies of the functional groups of these polysaccharides.

![Figure 5](image1.png)

**Figure 5.** (a) Disruption efficiency and β-glucan content of samples at 5% concentration and various yeast/glass bead ratios and vortexing cycles; (b) correlation between different degrees of disruption efficiency of samples at 5% concentration and β-glucan content after the alkaline-acid extraction.

![Figure 6](image2.png)

**Figure 6.** ATR–FTIR spectra of spent brewer’s yeast β-glucan.

The absorption band found at 1635.26 cm$^{-1}$ can be caused by the stretching vibrations of C=O groups. The absorption bands characteristic to β-glucan fractions were observed at 1370.77, 1153.92, 1076.26, and 1042.85 cm$^{-1}$, respectively, the first one being attributed
to β-(1,3) linkage [33,34]. The band observed at 1076.26 cm$^{-1}$ could be attributed to the characteristic C-O-C linkage to glycosidic bonds with cyclic structures, demonstrating that the β-glucan is not broken [35].

4. Conclusions

The yeast cell lysis protocol is an important step for further β-glucan extraction that depends on the origins of the raw material, one of the most convenient being the use of glass beads. The cell wall disruption method applied to spent brewer’s yeast using glass beads should be adapted and optimized before further processing of this valuable by-product. The results obtained in this study underline the significant effects of yeast cell suspension concentration, yeast/glass bead ratio, and the number of vortexing cycles of 10 min each on cell disruption efficiency. Microscopic examination validates the yeast lysis method and evaluates the degree of cell disruption. The optimal conditions found were a cell suspension concentration of 5%, a ratio of yeast/glass bead loading of 1:2, and a vortexing cycle of 3. The β-glucan content extracted from the optimal sample amounted to 78.53% and showed characteristic FT-IR absorption bands at 1370.77 and 1153.92 cm$^{-1}$. We also observed that β-glucan content after extraction is directly correlated with the degree of cell rupture. These results show the adequacy of the glass bead cell lysis method for the extraction of β-glucan from spent brewer’s yeast, underlying the possibility to valorize such valuable by-products generated by the beer production industry.

Compared to previous studies, we also observed that optimized parameters such as a low yeast concentration, increased vortexing cycles, and a yeast to glass bead ratio provide a suitable degree of cell breakage.

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