# OPTIMIZATION OF BETA-GLUCAN EXTRACTION FROM WASTE BREWER'S YEAST *SACCHAROMYCES CEREVISIAE* USING AUTOLYSIS, ENZYME, ULTRASONIC AND COMBINED ENZYME – ULTRASONIC TREATMENT

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## ABSTRACT

Beta-glucans are complex, high molecular (100 – 200 kDa) polysaccharides, found in the cell wall of many yeasts and cereals. Yeast beta-glucans differ from their cereal counterparts in that they comprise a mixture of beta-1,3- and 1,6-glucans, compared to the cereal derivatives which are a mixture of beta-1,3- and 1,4-glucans. In this study, beta-glucan was prepared from waste beer yeast by enzymatic, ultrasonic and combined method. In the ultrasound-assisted extraction, estimated optimum conditions were as follows: treatment time of 11.91 minutes. Cell disruption yield of beta-glucans has inferior recovery enzyme method. Maximum cell disruption yield by enzyme-ultrasonic is 72.06% at power 28,9w/g.

**Keywords:** β-glucan, Saccharomyces cerevisiae, enzymatic hydrolysis, sonication

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## 1. INTRODUCTION

The natural sources of β-glucans are bacteria, yeast, algae, mushrooms, barley as well as oat. Th e native chemical structure of β-glucans depends on the source they are isolated from. Each type of β-glucan, generally derived from different sources, has an unique structure in which glucose units are linked together in different ways (Stone and Clarke, 1992; Stone, 2009). β-Glucans from different sources have different chemical structures [10].

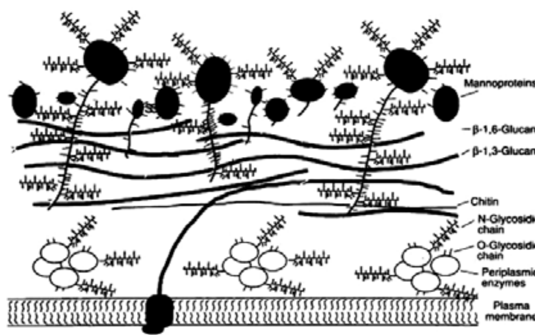


**Figure 1. Structure of the cell wall of yeast Saccharomyces cerevisiae (Osumi, 1998).**

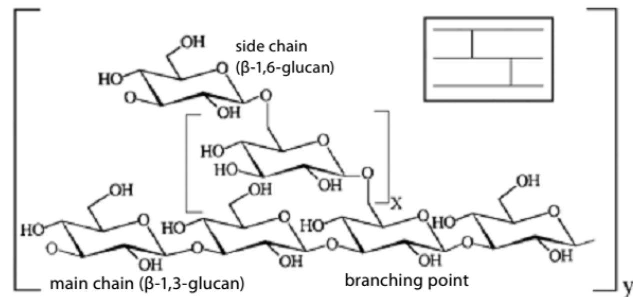


**Figure 2. Chemical structure of β-glucan in yeast *Saccharomyces cerevisiae* (Kath, 1999).**

Yeast is a well known microorganism that is used in biotechnology since ancient times [2]. Therefore it is a good source of β-glucan. β-Glucans in yeast cell walls are branch-onbranch molecules containing linear (1,3)-β-glucosyl chains that are joined through (1,6)-linkages (Osumi, 1998; Kath and Kulicke, 1999; Clavaud et al., 2009) (Figure 1). These molecules occur as complexes with other polysaccharides and proteins (Osumi, 1998) [5].

β-Glucans, polymers of glucose linked by β-glycosidic bonds, are widely distributed in the cell walls of microorganisms, mushrooms and plants. β-Glucans from various sources has a wide range of potential applications in food production such as thickening agent, fat substitute, and emulsifier [4]. Particularly, β-glucan draws attention as an immunostimulant for human use [1, 3, 9]. Yeast cell wall is one of the most common sources for β-glucan production. Some studies showed that β-glucan from brewer's yeast exhibits good ability to improve immune system [11]. β-Glucans isolated from baker's or brewer's yeast can be used in the production of salad toppings (dressings), frozen deserts, sauces, yogurts and other milk products, softdoughs and paning doughs, conditories and mixture for cake filling (Seeley, 1977; Read and Nagodawithana, 1991). The ability of β-glucan to retain water can be also used in the production of sausages and other meat products (Th ammakiti et al., 2004). Its gelling, water-holding and oil-binding characteristics make it suitable for many food products (Reed and Nagodawithana, 1991; Lazzari, 2000; Wylie-Rosett, 2002; Th ammakiti et al., 2004), such as the production of mayonnaise and sausages [13]. The possible use of yeast β-glucans in the different food products is illustrated in table 1.

**Table 1. The application of β-glucan in food products [13]**

| Food products | References |
|---|---|
| gelling thickeners for functional food products | Shukla and Halpern, 2005a; Laroche and Michaud, 2007 |
| biscuits and cookies | Seeley,1977; Shukla and Halpern, 2005b |
| meat products | Thammakiti et al., 2004; Shukla and Halpern, 2005c |
| soft cheese | Shukla and Halpern, 2005d |
| bread, bread mixture, pancakes, toast, dough | Shukla and Halpern, 2005e |
| nibbling food (salty and sweety) | Shukla and Halpern, 2005f |
| ice creams, yogurts, milk drinks | Shukla and Halpern, 2005g,h; Tudorica et al., 2004 |
| salad dressings (creamy, vinegar, mayonnaise) and their ready mixture for use | Shukla and Halpern, 2005i,j Worrashinchai et al., 2006 |
| sauces and mixture for their preparation | Shukla and Halpern, 2005k,l |
| soups and mixture for soups, concentrates for soups | Shukla and Halpern, 2005m,n,o,p |
| beverages, including juices and dairy drinks | Neumann et. al., 2006 |

Spent brewer's yeast is produced in huge amounts as a secondary product in breweries all around the world. Most of it is usually sold after heat inactivation as a cheap feed supplement. The rest of it ends in waste water disposal and pollutes the natural water sources with organic material (Thammakiti et al., 2004; Seeley, 1977; Suphantharika et al., 2003; Thammakiti et al., 2004; Liu et al., 2008) [15].

β-Glucan preparations extracted from spent brewer's yeast is the way to obtain high valuable product from a cheap raw-material (Worrasinchai et al., 2005), that showed high apparent viscosity, water holding, oil binding, and emulsion stabilizing capacities (Thammakiti et al., 2004) and could be used in food products as a thickener and fat replacer. Few authors such as Worrasinchai et al. (2006), Santipanichwong and Suphantharika (2007) and Satrapai and Suphantharika (2007) performed their research using β-glucan isolated by Thammakiti et al. (2004) and applied it later in different food systems (for example mayonnaises with reduced fat amounts) [12].

There are some researches mentioned to extraction of beta-Glucan from Brewer's Yeast [14]. Vesna Zechner-Krpan et al. (2009) conducted two different procedures to isolate water-insolubleb-glucans from brewer's yeast: alkaline-acidic isolation (AA) and alkaline-acidic isolation with mannoprotein removal (AAM). The obtained beta-glucans were then dried by air-drying, lyophilization and combination of sonication and spray-drying. b-Glucan preparations obtained by AA and AAM isolations had similar values of dry mass, total polysaccharides, proteins and organic elemental microanalysis. The mass fractions ofb-glucan in total polysaccharides were significantly affected by different isolation procedures. Fourier transform infrared (FTIR) spectra of all preparations had the appearance typical for (1→3)-β-D-glucan. Lyophilization and especially air-drying caused a higher degree of agglomeration and changes in β-glucan microstructure. Sonication followed by spray-drying resulted in minimal structural changes and negligible formation of agglomerates [13]. Vesna Zechner-

Krpan et al. (2010) investigated three different drying methods were used: air-drying, lyophilization and spray-drying. Air-drying and lyophilization caused β-glucan particles agglomeration and changes of their microstructure. Sonication combined with spray-drying resulted in minimal β-glucan structural changes and negligible formation of agglomerates. Reaggregation of spray-dried β-glucan particles was minimal even after resuspending in water [15].

The purpose of the present study was to find out the optimum conditions for β-glucan extraction from *S. cerevisiae* cell wall by autolysis, enzyme, ultrasound and combined enzyme-ultrasound methods.

## 2. EXPERIMENTAL

### 2.1. Materials

Spent brewer's yeast slurry (a strain of *S. cerevisiae*), a by-product from brewery, was provided by Saigon Beer Alcohol Beverage Corporation (SABECO), Ho Chi Minh City, Vietnam. Alcalase® 2.4 LFG was obtained from Novozymes Co. All other chemicals were of analytical grade.

### 2.2. Procedures for preparing β-glucan from spent yeast

Spent brewer's yeast was washed in distilled water using a 1:3 of the yeast slurry: water (g/g) ratio. Natural sedimentation was performed for 1 hour and followed by decantation step. The sediment was centrifuged at 3000 rpm for 15 minutes for wet yeast biomass recovery. Wet biomass was kept in cool condition (4$^{o}$C) for 2-3 days. Biomass was adjusted to 15% (w/w) suspension in phosphate-citrate buffer (pH 7.0) and subjected to the cell wall disruption step assisted by enzyme and ultrasound. The acquired mixtures was heated to 121$^{o}$C by autoclave for 4 hours and centrifuged at 3000 rpm for 15 minutes to separate the liquid phase. The insoluble residues were washed twice with distilled water. The obtained cell walls were suspended in organic solvent with solvent: solid ratio of 1:4 (w/w). The wet β-glucan residues were centrifuged and dried at an air temperature of 40$^{o}$C for 1 hour.

### 2.3. Optimization of cell wall disruption

**Table 2. Core value and disruption steep in autolysis cell wall disruption**

| Coefficents | Time of autolysis $X_1$ (h) | pH $X_2$ |
|---|---|---|
| Core value | 30 | 5 |
| Disruption step | 5 | 0.5 |

To investigate the optimum conditions of β-glucan extraction assisted by enzyme and ultrasound, the rotatable central composite design with a quadratic model was selected (Table ). The model is expressed as Eq. (1):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_{12} + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

where $Y$ = response, $X_1$ and $X_2$ = coded variables, $b$ = estimated coefficients in the response surface model.

**Table 3. Independent variables and their coded and actual values used in response surface design of enzyme and ultrasound-assisted condition**

| Independent variable | Symbol | Coded levels | | |
|---|---|---|---|---|
| | | –1 | 0 | +1 |
| **Enzyme concentration (% w/w)** | $X_1$ | 0.5 | 0.75 | 1 |
| **Treatment time (hour)** | $X_2$ | 2 | 4 | 6 |
| **Sonication power (W/g)** | $X_3$ | 6.5 | 7.5 | 8.5 |
| **Treatment time (minute)** | $X_4$ | 8 | 10 | 12 |

## 2.4. Analytical methods

The β-glucan content in the preparations was determined enzymatically by the commercial assay ‘‘YEAST BETA GLUCAN ASSAY KIT’’ (Megazyme Int., Bray, Ireland). Crude protein content of the cell wall fractions was determined by a Kjeldahl method. Soluble protein content was determined according to the procedure described by Lowry (1951) [6].

## 2.5. Calculation of cell wall disruption yield

The cell disruption yield was defined as the ratio of soluble protein content in the extract to total protein content in the yeast slurry.

β-glucan = Total glucan − α-glucan − oligomer[7]

Cell wall discruption yield:

$$H = \frac{P_D - P_B}{P_T - P_B} \times 100 \quad [8]$$

Where:

$P_D$: soluble protein after cell wall disruption.

$P_B$: soluble protein before cell wall disruption.

$P_T$: total protein.

## 3. RESULTS AND DISCUSSION

### 3.1 Autolysis cell wall extraction

**Table 4. The regression equation of autolysis cell wall extraction**

| | Test run no | $X_0$ | $X_1$ | $X_2$ | $X_1X_2$ | Y |
|---|---|---|---|---|---|---|
| **Test run at core: $2^k$** | 1 | +1 | -1 | -1 | +1 | 43.97 |
| | 2 | +1 | +1 | -1 | -1 | 45.20 |
| | 3 | +1 | -1 | +1 | -1 | 42.74 |
| | 4 | +1 | +1 | +1 | +1 | 38.88 |
| **Test run at cross points with two axis: 2k** | 5 | +1 | $-\sqrt{2}$ | 0 | 0 | 45.33 |
| | 6 | +1 | $+\sqrt{2}$ | 0 | 0 | 41.82 |
| | 7 | +1 | 0 | $-\sqrt{2}$ | 0 | 44.33 |
| | 8 | +1 | 0 | $+\sqrt{2}$ | 0 | 40.66 |
| **Test run at core** | 9 | +1 | 0 | 0 | 0 | 49.13 |
| | 10 | +1 | 0 | 0 | 0 | 52.51 |
| | 11 | +1 | 0 | 0 | 0 | 51.79 |

**Table 5. the regression coefficients of autolysis cell wall extraction**

| Coefficient | Value | Standard deviation | P | P < 0.05 |
|---|---|---|---|---|
| $b_0$ | 51.1434 | 0.727583 | 1.10364E-008 | Accept |
| $b_1$ | -0.949285 | 0.445586 | 0.086358 | Not accept |
| $b_2$ | -1.59266 | 0.445586 | 0.0159705 | Accept |
| $b_{11}$ | -3.86913 | 0.530421 | 0.000757944 | Accept |
| $b_{22}$ | -4.4093 | 0.530421 | 0.000411602 | Accept |
| $b_{12}$ | -1.2725 | 0.630105 | 0.0994332 | Not accept |

$R^2 = 0.959$, $Q^2 = 0.867$. The regression equation: $Y= 51.14-1.59X_2-3.87X_1^2-4.41X_2^2$

On 3-dimension scale, $Y = 51.32\%$, $X_1 = 29.53$ (h) and $X_2 = 4.92$.

### 3.2 Optimization of enzyme and ultrasound-assisted extraction

Table 6 shows the matrix of central composite design ($2^2$) with the level of variation and the dependent variable $Y$ (experimental response) expressed as % cell wall disruption for enzyme and ultrasound-assisted extraction.

**Table 6. Experimental design matrix used in response surface design of enzyme and ultrasound-assisted extraction**

| Test run [a] no. | Coded level of variable | | Response 1[b] | Response 2[c] |
|---|---|---|---|---|
| | $X_1$ | $X_2$ | | |
| **1** | –1 | –1 | 25.37 | 23.81 |
| **2** | +1 | –1 | 36.75 | 29.06 |
| **3** | –1 | +1 | 43.73 | 32.04 |
| **4** | +1 | +1 | 47.77 | 41.58 |
| **5** | –1.14 | 0 | 38.25 | 29.93 |
| **6** | +1.14 | 0 | 42.63 | 40.42 |
| **7** | 0 | –1.14 | 20.23 | 28.33 |
| **8** | 0 | +1.14 | 45.57 | 39.85 |
| **9** | 0 | 0 | 45.06 | 39.52 |
| **10** | 0 | 0 | 45.20 | 39.11 |
| **11** | 0 | 0 | 43.36 | 39.71 |

[a] **Test runs were performed in random order.**
[b] **Response 1 was expressed as disruption yield obtained in enzyme-assisted extraction.**
[c] **Response 2 was expressed as disruption yield obtained in ultrasound-assisted extraction.**

The results of multiple regression analysis of two independent variables on response along with the results of analysis of variance (ANOVA) were summarized in Table . The regression analysis showed a significant probability of P-value ($p< 0.05$) in estimating cell wall disruption yield, which means that the two independent variables had significant effects on response.

**Table 7. Statistical significance obtained for the regression coefficients of the enzyme and ultrasound-assisted extraction**

| Coefficient | Regression coefficient | P value |
|---|---|---|
| $b_0$ | 44.54 (39.45) | <0.01[a] (<0.01[a]) |
| $b_1$ | 2.70 (3.71) | 0.02 (<0.01[a]) |
| $b_2$ | 8.15 (4.63) | <0.01[a] (<0.01[a]) |
| $b_{11}$ | –1,62 (–2.89) | 0.14 (0.02) |
| $b_{22}$ | –5.39 (–3.43) | <0.01[a] (0.01) |
| $b_{12}$ | –1.84 (1.07) | 0.15 (0.34) |
| **Regression** | | |
| **R square** | 0.970 (0.947) | |
| **Q square** | 0.797 (0.628) | |

[a] **Significant at 1% level (p< 0.01).**
**Value in parentheses is of the ultrasound-assisted extraction.**

The correlation coefficient, $R^2$, which is a measure of how well the model can be made to fit the raw data, was more than 0.90, indicating an adequate model fit.

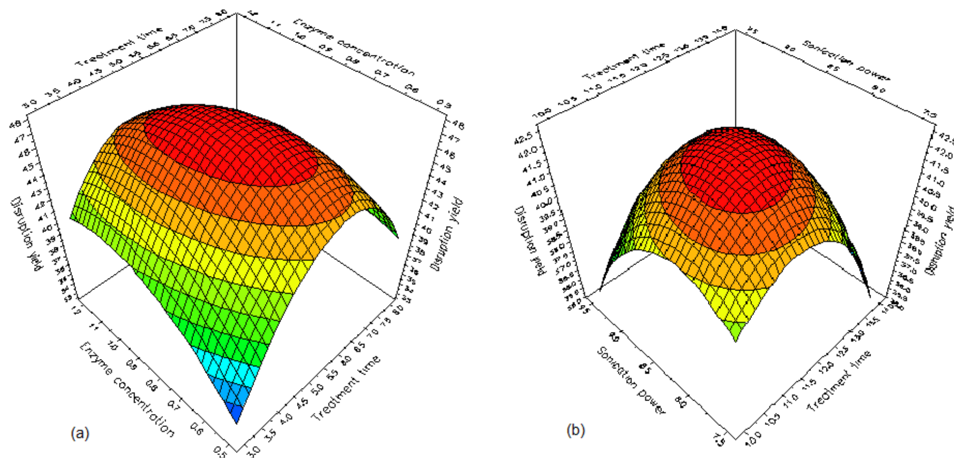


**Figure 3. Response surface for the effects of (a) enzyme and (b) ultrasound-assisted extraction conditions on the cell wall disruption yield.**

The model for enzyme-assisted extraction could be expressed as:

$$Y = 44.54 + 2.70X_1 + 8.15X_2 - 5.39X_2^2 \quad (2)$$

The set of optimum conditions was obtained by the use of RSM optimization and was as follows: enzyme concentration of 0.86% (w/w) and treatment time of 5.34 hours. Under the conditions, the predicted and practical response value were 47.92% and 46.79%, respectively.

The model for ultrasound-assisted extraction could be expressed as:

$$Y = 39.45 + 3.70X_1 + 4.63X_2 - 2.89X_1^2 - 3.43X_2^2 \quad (3)$$

The optimum conditions were as follows: ultrasound power of 8.29 W/g and extraction time of 11.60 minutes. The predicted cell wall disruption yield under the optimum condition was 42.76%. The experimental value of 42.55%, obtained from practical experiments, demonstrated the validation of the RSM model, indicating that the model was adequate for the extraction process.

### 3.3 The combined method for cell wall disruption

To improve the yeast disruption, we performed tests using combination of enzyme and ultrasound treatment. The cell wall disruption yield had maximum value (58.56%) in case of enzyme hydrolysis followed by sonication. When this step was carried out in the reverse order, the lower value was observed (51.95%). 8 summarized the composition of β-glucan preparations in various methods.

**Table 8. Composition of the β-glucan preparation in various methods**

| Sample | Water (%) | Protein (%) | Lipid (%) | Ash (%) | β-glucan (%) |
|---|---|---|---|---|---|
| **Control** | $78.23^{ab}\pm1.52$ | $52.72^{a}\pm2.08$ | $3.53^{d}\pm0.37$ | $6.50^{d}\pm0.65$ | $26.92^{a}\pm1.96$ |
| **Ultrasound** | $79.64^{bc}\pm0.61$ | $26.53^{b}\pm0.92$ | $2.50^{c}\pm0.28$ | $5.22^{b}\pm0.62$ | $56.50^{b}\pm1.28$ |
| **Enzyme** | $77.38^{a}\pm1.16$ | $23.33^{c}\pm1.18$ | $1.33^{a}\pm0.13$ | $3.62^{a}\pm0.21$ | $60.32^{c}\pm1.01$ |
| **Ultrasound-enzyme** | $80.63^{c}\pm0.71$ | $20.20^{d}\pm1.12$ | $1.25^{a}\pm0.09$ | $3.43^{a}\pm0.18$ | $65.43^{d}\pm1.93$ |
| **Enzyme-ultrasound** | $79.48^{bc}\pm1.20$ | $15.38^{e}\pm1.34$ | $1.10^{a}\pm0.07$ | $3.48^{a}\pm0.31$ | $72.06^{e}\pm1.23$ |

**Components based on dry basis.**

**Values which have the same superscript symbol in the same column have no significant difference ($p= 0.05$).**

Due to yeast cell wall's rigidity, ultrasonic treatment is not capable of breaking down it [7]. However, after hydrolyzed by enzyme, yeast cell became easy to be disrupted. Therefore, the highest yield in samples subjected to enzymatic hydrolysis followed by sonication can be inferred from that. Prior enzymatic hydrolysis decomposing mannoprotein component and reducing the regidity of cell wall could support ultrasound in further disruption. The higher extraction yield, the higher β-glucan and lower other components concentration.

## 4. CONCLUSION

A considerable history of safe use of beta-glucans also exists through the consumption of yeast, cereals and mushrooms among other foods, as well as food supplements. As a result of extensive research, one could expect increasing application of β-glucan in food production in the near future. β-Glucan obtained from spent brewer's yeast possesses properties that are beneficial for food production. The use of spent brewer's yeast for isolation of β-glucan intended for food industry would represent a payable technological and economical choice for breweries. With the aim of preserving the β-glucan structure and bioactivity, enzyme and ultrasound-assisted extraction are among the potential methods. Moreover, the cell wall disruption yield can be improved in case of combination of these methods in appropriate order. The yeast suspensions which are subjected to enzyme treatment followed by ultrasonic shows good result with cell wall disruption yield of 58.56% and β-glucan content of 72.06% on the dry basis.

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