Biotechnological β-glucan Production from Returned Baker´s Yeast and Yeast Remaining after Ethanol Fermentation

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Abstract

The aim of this work was performed to develop a milder and cost effective extraction procedure for Beta-glucan (BG) from two types of *S. cerevisiae*, the returned Baker's yeast "RBY" and yeast remaining after ethanol fermentation "EFY" using different chemical extraction steps. The current results revealed that the carbohydrate percent in yeast biomass was considerably increased by the different extraction steps (from 37.64 to 92.41% and from 41.37 to 93.46% in case of RBY and EFY, respectively). On the other hand, the percent of protein in yeast biomass was decreased by the different extraction steps (from 41.91 to 1.28% and from 36.32 to 1.15% in the two cases, respectively). The extracted dry biomass in the two cases were analysed by Fourier Transform Infrared (FTIR) spectra. This method of β-glucan extraction steps has been shown to produce great yields of β-glucan with maintaining their purity and native structure.

Keywords: Saccharomyces cerevisiae, Baker´s yeast, Ethanol fermentation yeast, β-glucan, production.

Introduction

The yeast cell wall accounts for up to 30% of cell dry mass and its difference in thickness is about 70-100 nm depending on growth conditions (growth medium, temperature, osmotic pressure, toxic metabolites and the time of harvesting) and genetic background **(1).** In another study, ***Klis et al.*****(2)** recorded that the cell wall of yeast remaining after ethanol fermentation as thick as 200 nm. The yeast

cell wall consists of 30-60% polysaccharides (β-glucan and mannan oligosaccharides), 15-30% proteins, 5-20% lipids and a small amount of chitin **(3)**. Most of the proteins linked to the mannan oligosaccharides and is referred to as the mannoprotein complex **(4,5).**

The yeast cell wall, especially of baker's and brewer's yeast (*Saccharomyces cerevisiae*), is an important source of β-D-glucans. Yeast β-D-glucan polysaccharide is a D-glucose biopolymer and it constitutes a major structural element of yeast cell wall. In yeast cell wall, two different types of β-D-glucans are found: β-1,3-D-glucan, a main component (85%) representing more than 50-55% of cell wall and β-1,6-D-glucan amounts (15%) **(6-8).**

Beta-glucan (BG) is a structural cell wall component of filamentous fungi and yeast. *Saccharomyces cerevisiae* is Generally Recognized as Safe (GRAS) by the **US Food and Drug Administration (9)**. BG isolated from ***Saccharomyces cerevisiae*** have several beneficial properties and have a wide variety of uses in human and veterinary medicine, immunopotentiation, pharmaceutical, cosmetic and chemical industries as well as food and feed production **(10,11).**

Yeast β-glucans have been proven as beneficial for many human and animal diseases and disorders**(8).** β-1,3-D-glucans and 1,6-D-glucans are called biological response modifiers (BRMs) due to their ability to enhance and stimulate the human immune system **(12).** β-Glucans obtained from yeasts cell wall can also be used in food industry as dietary fibers **(13)**, fat replacers and emulsifiers due to their ability for holding water, oil and fat **(14).** Their incorporation in food helps in lowering blood sugar. Yeast β-glucan extract is considered as safe for oral applications and recognized as GRAS **(15)**

The purpose of this study was performed to develop a milder and cost effective extraction procedure for BG from *S. cerevisiae* **(**the returned Baker's yeast (RBY) and yeast remaining after ethanol fermentation (EFY) produced by Chemical and Distillery Factories, respectively, at Egyptian Sugar and Integrated Industrial Company, El-Hawamdia, Giza, Egypt using different chemical extraction steps. Furthermore, the production of β-glucans, as added value product

from the two types of yeasts as a part of an integrated, green and environmentally oriented industry was also assessed.

Materials and Methods

Yeasts

Industrial *Saccharomyces cerevisiae* of returned Baker's yeast (RBY) and yeast remaining after ethanol fermentation (EFY) produced by Chemical and Distillery Factories, respectively, at Egyptian Sugar and Integrated Industrial Company, El-Hawamdia, Giza, Egypt were collected and used for extraction of β-glucans from their cells wall using different chemical extraction steps.

Yeast pre-treatment

Yeasts were pre-treated as the method described by ***Simard and Bouksaim*** **(16).** A total of 100 g of each of RBY and EFY debittering using alkaline wash (NaOH, pH=10, at 50°C for 30 min) was followed by centrifugation (4000*g* for 20 min) and washing three times in distilled water.

Autolysis

Autolysis procedures were achieved according to the method described by ***Liu et al.*****(17)**. as following: The pre-treated yeast cells slurry were adjusted to 15% (w/v) solids content at pH 5.0 and then added 3% sodium chloride as the autolysis promoter and incubated at 55°C for 24 h with agitation at 120 rpm. The autolysate was then heated at 80°C for 15 min, cooled to room temperature and centrifuged at 4000*g* for 10 min to separate the residual autolyzed cells from yeast extractions. The autolyzed yeast cells were stored at 4°C for the next step.

Alkaline treatment

The autolyzed yeast cells were mixed with five folds of 1.0 M NaOH and incubated at 90°C in stirrer for 2 h. Then, the cell pellet was collected by centrifugation at 4000*g* for 25 min. and suspended in 3 folds of distilled water. After thorough mixing, cells were centrifuged at 4000*g* for 25 min. The pellets were stored at 4°C for next step. This method previously recorded by ***Penjkumsri, et al.*** **(18)**.

Acid treatment

The cell pellets were dissolved in 5 fold of 1.0 M acetic acid and incubated at 80°C in stirrer for 2 h. Then the pellets were collected by centrifugation at 4000*g* for 25 min. The obtained pellets were washed 3 times with water. The pellets were stored at 4°C for next step **(18) .**

Organic extractions

The obtained pellets subjected to six organic extractions as method of ***Jamas, et al.*(19)**. The organic extraction steps including n-hexane, petroleum ether, isopropyl alcohol, n-butyl alcohol, ethanol, methanol, or acetone**.** The first four extractions carried out in isopropanol. The solids collected by centrifugation and then subjected to two acetone extractions. The pellets were stored at 4°C for next step.

Drying

The resulting wet solids were dried in a vacuum oven at 65°C for 48 h to yield a white powder of β-glucan with small amount of impurities.

Chemical analysis

Total fats, ash content, moisture content and dry mass were determined using AOAC Official Methods **(20)** . Total carbohydrate was measured by the phenol–sulphuric acid method according to method described by ***Dubois, et al.*(21)**. Total protein was measured by Biuret method[22].

Analysis of ß-glucans

Glucan extracted from the two strains of *Saccharomyces cerevisiae* (RBY and EFY) were analyzed by Infrared spectra recorded on 8001-PC FTIR Shimadzu Spectrophotometer using KBr pellets at Micro Analytical Center, Cairo University. An auto of scanning speed (2 mm/sec) with a spectral range from 450 to 4,000 cm^{-1} at 4 cm^{-1} resolution.

Statistical analysis

The obtained data were expressed as mean standard deviation. Statistical evaluations were performed by ***t*-test (23)** . *P* value< 0.05 was considered as statistically non-significant.

Results and Discussion

Pre-treatment

The effect of pre-treatment step on protein and carbohydrates content of RBY and EFY was recorded in Table (1) and illustrated in Figure (1). The results revealed that protein percent was slightly decreased while carbohydrate percent was increased in the biomass of both RBY and EFY. Yeasts especially brewer's yeast contains hops constituents such as humulone and lupulone (hops acids) would be mainly responsible for bitterness, alkaline washing (pH=10, at 50°C for 30 min) was used for the removal of these constituents without any deleterious effects in its nutritive value **(16).**

Autolysis

Autolysis has been defined as the hydrolysis of intracellular biopolymers under the effect of hydrolytic enzymes such as proteinases, ribonucleases and glucanases **(24-26)** . During autolysis the cell endo-structures degrade, releasing vascular proteases in the cytoplasm, enzymes hydrolyse the intracellular polymer components, the hydrolysis products accumulating in the space restricted by the cell wall and finally the pores in the cell wall **(27).**

As shown in Table (1) and Figure (1) compared hydrolytic products are released when their molecular masses are low enough to cross with fresh yeast biomass, about 70 and 69% of proteins had been removed by induced autolysis, whereas the content of total carbohydrates was increased by about 60 and 51% in RBY and EFY, respectively. The temperature, pH, cells concentration and autolysis promoter should have profound effects on cell autolysis. ***Liu et al.*(17).** found that the best autolysis conditions were adjusted yeast cells slurry to 15% (w/v) solids content and pH 5.0 in presence of 3% sodium chloride as autolysis promoter, and then incubated at 55°C for 24 h with agitation at 120 rpm. So, the content of protein in supernatant continued to decrease and total carbohydrates content in the residue cell wall continued to increase **(17)** .

Alkaline treatment

The data in Table (1) and Figure (1) illustrate the effect of alkaline treatment step on protein and carbohydrate contents of yeast

cell wall. The results revealed that there was an increase in the carbohydrate percent and decrease in the proteins percent after the alkaline treatment of RBY and EFY autolysis products. ***Liu et al.*(17).** reported that the yeast cell wall from autolysis consisted of about 60-62% polysaccharides and 11-13% proteins (most of the proteins were covalently linked to the mannans formed mannoproteins). In order to get rid of the mannoproteins and obtain high purity β-D-glucans, a suitable concentration of aqueous alkaline solution such as sodium or potassium hydroxide must be applied. ***Penjkumsri et al.*(18).** found that the best alkaline treatment condition was five-fold of 1.0 M NaOH and incubated at 90°C in stirrer for 2h.

Treatment with alkali leads to hydrolyse and solubilise the intracellular mannoproteins, nucleic acids, mannans and polar lipids into supernatant fractions. Also, chitin de-acetylated into chitosan in the cell wall with alkaline treatment. Alkali-hydroxide insoluble whole glucan particles having primarily β-l, 6 and β-l, 3-linkages also formed in presence of alkali medium **(19).**

Acid treatment

In general, alkaline-insoluble β-glucan contains predominantly β-1, 3-linkages with a small amount of β-1, 6-glucan. The whole glucan particles can be treated with an acid to decrease the amount of β-1, 6-linkages and thus change the hydrodynamic properties of β-glucans **(28)**. The results in Table (1) and Figure (1) showed increase in the carbohydrate percent after acid treatment to 91.31% and 91.93% in case of RBY and EFY, respectively. In the same time, proteins percent were decreased after acid treatment by 45.8% in case of RBY and 46.2% in case of EFY from the previous step.

In acid treatment, strong acids such as HCl or H_2SO_4 can be used but acetic acid is preferred, due to its mild acidity, easy in handling, low toxicity, low cost and availability. Generally these acids should be mild enough to limit hydrolysis of the β-l, 3-linkages, and substantially only affect the β-1, 6-linked glucans, where the β-1, 6-glucan molecules interconnect the largest class of covalently linked cell wall proteins **(29**, **31).**

The acid treatment is preferably carried out at a temperature from 55 to 85°C, acid concentration from 0.1 to 0.5 mol, concentration of β-D-glucan from 0.1 to 10.0 g/l. This treatment may remove from about 3 to 20% by weight of acid soluble material based on total weight of the whole glucan particles before treatment **(19)** .

Organic solvent treatment

Concerning the protein percent after the consequent treatment of BY and EFY with organic solvents including n-hexane, petroleum ether, isopropyl alcohol, n-butyl alcohol, ethanol, methanol, and acetone, the data in Table (1) and Figure (1) showed that a decrease in protein percent and consequently increase in the total carbohydrates content were detected.

After acid treatment, obtained yeast cell walls still had some lipids mainly nonpolar, the lipids were located in the cell wall and linked to mannoproteins *via* phosphate that prevented the removal of some mannoproteins. In order to remove most mannoproteins to obtain high purity β-D-glucans, the lipids should be removed before drying to eliminate nonpolar lipid and hydrophobic proteins **(19, 32).** The results recorded in table (2) showed that the organic solvent treatment removed most of lipids.

Drying

The β-D-glucan particles can be further processed and/or further purified; the BG can be dried to a fine powder by drying in an oven at 65°C±5°C, to remove any traces or organic-soluble materials, additional proteins or other impurities which may be present **(29)**. The composition of final β-glucan extracted from RBY and EFY showed in the Table (2) and illustrated in Figure (2)**.**

Composition analysis

The composition analysis, protein, carbohydrates, fat and ash of BG extracted from RBY or EFY, that presented in Table (2) and Figure (2) showed that there was non-significant difference between BG extracted from RBY or EFY.

The BG that extracted from RBY and EFY by this method was similar in the protein, carbohydrates, fat, ash contents and yield. Also BG extracted from RBY or EFY showed the same IR spectra and

have the same impurities as α-glucan and chitin according to peaks intensity as in Figure (3 a, b).

Although RBY is often used as a raw material for BG extraction, this study shown that EFY can also be successfully used as a source of β-glucan. This means that cell wall of yeast *Saccharomyces cerevisiae* is nearly similar of both RBY and EFY as a sources of β-glucan. Nearly similar conclusion was recorded by ***Zechner-Krpan et al.*****(33)** , they found that the cell wall of yeast *Saccharomyces cerevisiae* is one of the most common sources of β-glucan.

Analysis of ß-glucans

The IR spectra of extracted β-glucan from BY and EFY were achieved and shown in Figure (3a&b). The strong broad IR band at 3430 cm^{-1} indicates OH stretching vibration of hydroxyls and water. IR band at 2922 cm^{-1} have contribution of CH_2 stretching vibrations of CH_2OH groups in sugars, IR bands at 1641 cm^{-1} (amide I and in-plane bending of water) and near 1560 cm^{-1} (the amide II), these peaks indicate the presence of chitin, as a minor component and probably some products of protein degradation. The IR features in the region of 1200–1440 cm^{-1} were assigned mainly to in-plane ring deformation including CH and OH bending modes. Intense highly IR bands in the region of 990–1200 cm^{-1} (COC and CC stretching vibrations) are characteristic for polysaccharides and can be used for their identification **(34, 35).** IR bands at near 1160, 1080, 1040, 994, 960 and 893 cm^{-1} are characteristic for β-glucan. The vibration band near 890 cm^{-1} are sensitive to anomeric structure around glycosidic bonds and confirmed β-configuration of the polysaccharides **(36 ' 37).** IR bands at 1022, 933, 853, 760, 708, 577 and 532 cm^{-1} are characteristic for α-glucan **(38).** From the measured spectra that were estimated can observe that, the β-glucan extracted from BY and EFY contains β-glucan as the main component, while chitin, α-glucan and proteins are also present but in smaller amounts.

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Table 1. Effect of different β-glucan extraction steps on protein and carbohydrate contents of RBY and EFY.

Steps	Proteins%		Carbohydrates%	
	RBY	EFY	RBY	EFY
Yeast cell	42.91	36.32	37.64	41.37
Pre-treatment	41.70	35.94	38.61	44.62
Autolysis	12.92	11.18	60.11	62.70
Alkaline treatment	5.72	5.33	87.31	88.47
Acid treatment	3.10	2.87	91.31	91.93
Organic solvent treatment	1.36	1.28	92.17	92.80
Drying	1.28	1.15	92.41	93.46

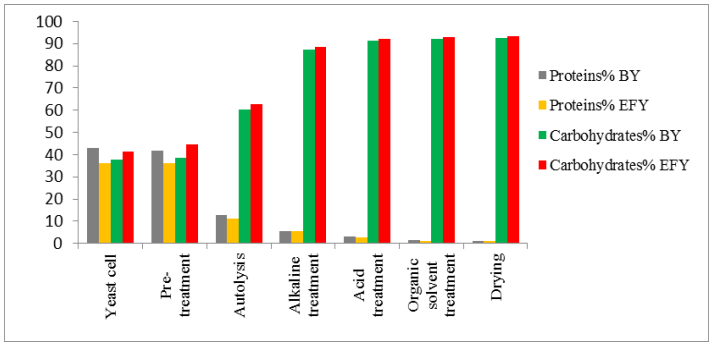
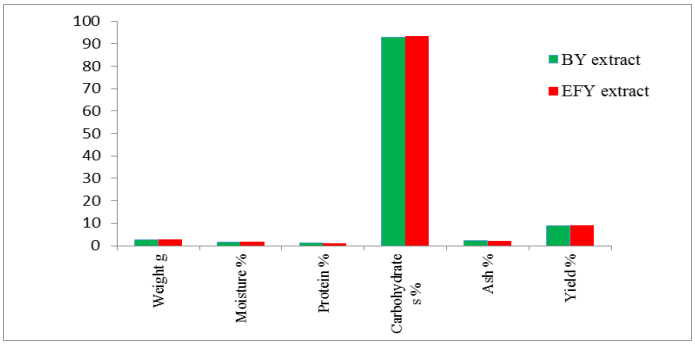


Figure (1): Effect of different β-glucan extraction steps on protein and carbohydrate content of RBY and EFY.

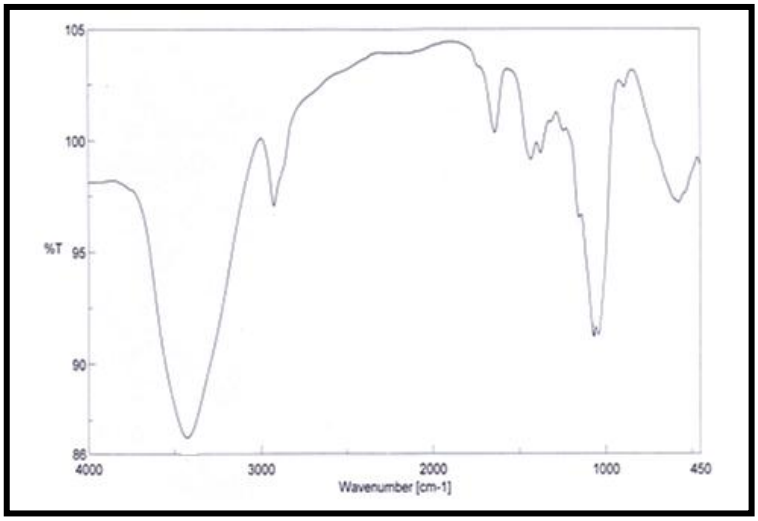
Table 2. Composition of final BG extract of RBY and EFY

Sample	RBY extract	EFY extract
Weight g	2.48	2.71 N.S
Moisture %	1.68	1.63 N.S
Protein %	1.28	1.15 N.S
Carbohydrates %	92.80	93.46 N.S
Fat %	traces	traces
Ash %	2.16	2.19 N.S
Yield %	8.75	9.16 N.S
*Significant at $P < 0.05$; N.S means non-significant.		



Figure(2): Composition of final BG extracted from RBY and EFY

(a)



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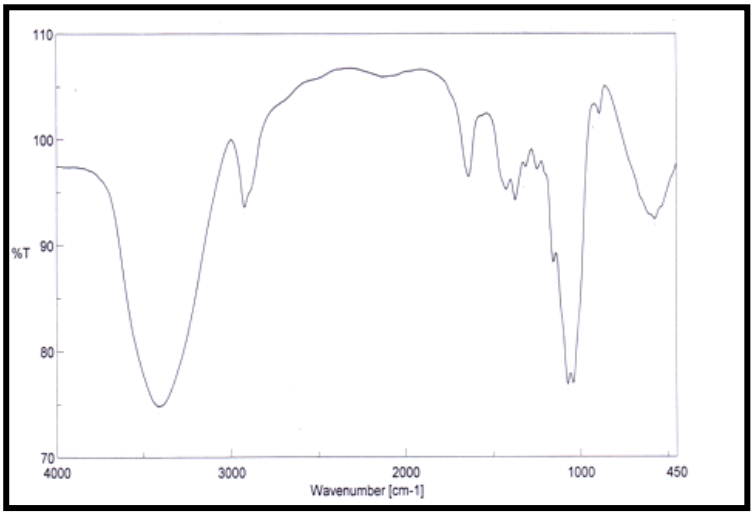

Figure (3): IR spectra of final extracted β-glucan: (a) RBY and (b) EFY.

الملخص العربي

إنتاج البيتا- جلوكان بالتقنية الحيوي من خميرة الخباز المرتجعة والخميرة المتبقية بعد تخمير الإيثانول

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الهدف من هذ ه الهراسة هوالبحث عن طريقة استخلاص لمركب البيتا- جلوكان من الخميرة تكون بسيطة و أكثر فاعلية واقل تكلفة. تم استخلاص البيتا- جلوكان في هذه الداسة من خميرة الخباز المرتجعة "RBY" والخميرة المتبقية بعد تقطير الإيثانول "EFY" باستخدام طرق وخطوات استخلاص كيميائية مختلفة. و لقد أظهرت النتائج زيادة نسبة المواد الكربوهيدراتية في الكتلة الحيوية المستخلصة من الخمائر زيادة كبيرة مع تدرج خطوات الاستخلاص من 37.64 إلى 2.41٪ فى حالة RBY ومن 1.37 إلى 93.46٪ في حالة EFY. هذا وقد انخفضت نسبة البروتين في الكتلة الحيوية المستخلصة من الخمائر قيد الدراسة مع تدرج خطوات الاستخلاص من 41.91 إلى 1.28٪ ومن 36.32 إلى 1.15٪ في من RBY و EFY بواسطة أطياف الأشعة تحت الحمراء(FTIR) وثبت أن كمية المواد نوعين الخمائر المستخدمة على التوالي. وتم في الدراسة تحليل الكتلة الحيوية الجافة المستخرجة من الخمائر في كلا الكربوهيدراتية المستخلصة بال طرق المستخدمة في هذه الدراسة معظمها بيتا- جلوكان بنسبة كبيرة مع الحفاظ على نقاء البيتا-جلوكان وبنيته الأصلية.