Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 34, Issue 2, 2023 DOI: http://doi.org/10.23851/mjs.v34i2.1298

Research Article **Open Access**

Determination the Optimum Conditions for β-glucan Production Extracted from *Saccharomyces cerevisiae*

Alyaa Abdulhussein Khadam[1*], Jehan Abdul Sattar Salman[1], Mokhtar Hijri[2]

[1]Department of Biology, College of Science, Mustansiriyah University, 10052 Baghdad, IRAQ.
[2]Department of Physics, College of Sciences, Imam Mohammad Ibn Saud Islamic University (IMISU), Riyadh 11623, SAUDI ARABIA.

[*]Correspondent contact: alyaaabdulhussein.ph.d.mic.2020@uomustansiriyah.edu.iq

ArticleInfo

Received
31/01/2023

Accepted
05/04/2023

Published
30/06/2023

ABSTRACT

B-glucan is a natural polysaccharide composed of a group of glucose monomers linked by beta glycoside bonds that yeasts can be synthesized. The effect of different temperatures, inoculum size, glucose concentration, yeast extract concentration, pH, and culture media on the β-glucan production from *saccharomyces cerevisiae* was studied. In addition, the dry weight of biomass containing β-glucan was measured. The results showed that the optimum conditions for β-glucan production were when the cells were grown at 30 ºc for 48 hours and pH = 7 with 10 % inoculum size, 2% glucose, and 0.1% yeast extract. Furthermore, the best culture medium for β-glucan production was yeast extract-glucose medium with a dry weight of glucan 2.5mg/5ml. In addition, this study aimed to determine the best method for β-glucan extraction with acceptable purity and concentration. B-glucan was extracted using two methods autolysis and alkali-acids extraction. The dried and weighed crude β-glucan was 6 gm/l, and 8 gm/l for autolysis of yeast cells, and alkali-acids extraction, respectively. The analysis of glucan by FT-IR and HPLC showed that the method of extraction by autolysis of yeast cells was successful to obtain β-glucan without the presence of the α-glucan with a concentration of β-glucan 10.8mg/gm, while in the extraction by alkali-acid were obtained mixtures of α-glucan and β-glucan with the concentration of β-glucan 7.4 mg/gm. The current study revealed that the optimum conditions of β-glucan production and the best culture medium for β-glucan production was yeast extract-glucose medium and the best method for β-glucan extraction was autolysis extraction.

KEYWORDS β-glucan, *saccharomyces cerevisiae*, *HPLC*, FTIR, extraction methods.

الخلاصة

بيتا كلوكان عبارة عن عديد السكاريد الطبيعي يتكون من مجموعة من الجلوكوز المرتبطة بروابط بيتا كليكوزيد. والظروف المثلى لإنتاجه من خميرة الخبز عند النمو بدرجة حرارة ٣٠م ومدة حضن ٤٨ ساعة عند الرقم الهيدروجيني ٧ وبنسبة لقاح ٤٪، وتركيز كلوكوز ٢٪، مستخلص خميرة أقل من ١٪ وأفضل وسط لإنتاج بيتا كلوكان هو وسط مستخلص الخميرة-جلوكوز وبلغ وزن الكلوكان الجاف في هذه الظروف المثلى ٢,٥ مجم / ٥ مل. كما هدفت هذه الدراسة إلى تحديد أفضل طريقة لاستخراج بيتا جلوكان حيث تم استخلاص بيتا كلوكان باستخدام طريقتين للتحلل الذاتي واستخلاص القاعدة والحامض، وكان وزن بيتا كلوكان الخام المجفف ٦ جم / لتر، ٨ جم / لتر بطريقة التحلل الذاتي لخلايا الخميرة، واستخلاص القاعدة والحامض على التوالي. تم توصيفه باستعمال جهاز التحليل الطيفي بالاشعة تحت الحمراء FTIRو HPLC وبلغت كمية الكلوكان ١٠,٨, ٧,٤ ملغم/غم بطريقة الاستخلاص للتحلل الذاتي واستخلاص القاعدة والحامض على التوالي. واظهرت النتائج بان الكلوكان المستخلص بطريقة للتحلل الذاتي يتكون من β- glucanفقط بينما الاستخلاص القاعدة والحامض يتكون من β- glucan و α- glucan.

INTRODUCTION

Yeast glucans are polysaccharides that constitute structurally different D-glucose polymers and they are divided into α-D-glucans, β-D-glucans, and α, β-D-glucans based on the glucose anomeric structure [1]. β-D-glucan biopolymer is located in the cell wall of various organisms like yeast, molds, bacteria, algae, and plants. The cell wall of *Saccharomyces cerevisiae* (*S. cerevisiae*) is composed of β-glucan about 55-65%, (1→3)-β-D-glucan backbone which gives the cell wall strength and links to the chitin, mannoprotein, and (1→6)-β-D-glucan side chains [2]. Yeast β-glucan

has been shown beneficial for both human and animal health systems as it serves as an immunostimulatory [3]. The β-glucan production by yeast is influenced by many growths and environmental factors such as pH, temperature, and glucose concentration [4]. β-glucan is biopolymer a composed of monosugar units, and it is associated with their secondary metabolism. The biosynthesis and structure of β-glucan can be influenced by many factors, which include the composition of the culture medium and fermentation conditions such as temperature, pH, and oxygen concentration [5]. Byrtusová [6] tested to optimize the culture conditions for β-glucan production such as temperature to nitrogen, osmotic stress, and carbon sources. The addition of numerous types and concentration of carbon sources significantly can be influencing the increase in yeast number, fungi number, β-glucan content, and pH [7]. The two main processes in the extraction of β-glucan from *S. cerevisiae* are yeast cell lysis (the separation of the cell wall from the cytoplasm) and β-glucan extraction from the insoluble cell wall [8]. Several studies discussed the enzymatic (lytic enzymes) [9], physical (sonication, high pressure) [10] and chemical (NaOH, HCl, acetic acid, citric acid) lysis of yeast cells [11]. The current study aimed to detect the optimal conditions for production β-glucan from *S. cerevisiae* and extraction of β-glucan by two extraction methods, and comparison between these methods.

MATERIALS AND METHODS

Saccharomyces cerevisiae isolates collection

Nine commercial Baker's yeast were collected from the commercial market, the yeasts were activated by dissolving (0.1) g of yeast in (10) ml sterilized distilled water for (30) min at 30°C, streaked on the Sabouraud dextrose agar, and incubated for (24-48) h at 37°C. Then, they were identified through cultural, microscopical, biochemical tests, and VITEK 2 system [12].

Screening of β-glucan production from *S. cerevisiae* by using aniline blue assay

A- Using agar medium

The β-glucan screening medium yeast extract-glucose medium was prepared according to [13] as the following (0.5 g\L yeast extract, 15 g\L of glucose, 0.12 g\L $MgSO_4$, 3.18 g\L KH_2PO_4, 5.2 g\L of K_2HPO_4, 0.54 g\L NH_4CL, 20 g\L agar agar) and aniline blue dye (0.005%) was added after cooling the media to 50°C). the medium was inoculated with 24h old culture of *S. cerevisiae* isolates and incubated at 30°C for 1-6 day. The appearance of blue colonies of the isolates was recorded as β-glucan producer.

B- Using broth medium

This process was carried out by the procedure described by [14], yeast extract glucose broth was prepared as mentioned above [13] and was inoculated with *S. cerevisiae* suspension containing (3×10^7) CFU /ml (absorbance at a wavelength of 600nm about 1) with inoculum size 10 % and incubated at 30 °C for 48 h with shaking at 200 rpm. After incubation, yeast cells were collected by centrifugation at 4 °C for 10 min at 75000 rpm. Then, 2ml of PBS and 2ml of aniline blue solution (0.02%) were added to precipitate the cells, transferred to a water bath at 50°C for 30 min to from β-glucan aniline complex and the tubes were left at room temperature for 24h to decolorize the unbound aniline blue dye. The O.D was measured at 510 nm using spectrophotometer, then cell pellet was collected by centrifugation at 4,000 × g for 20 min at 4 °C, and washed with distilled water twice. The colored blue pellets were dried at 40 °C and the dry weight of cell biomass containing β-glucan was recorded.

Determination of optimum conditions for β-glucan production

Effect of temperature

This process was carried out in accordance with the procedure described above using broth medium at various temperatures (25, 30 and 35) °C for 24 h. The O.D and the dry weight of biomass contained β-glucan were measured for each temperature.

Effect of the incubation time

This process was done with the procedure described above using broth medium with various incubation time (24, 48 ,72 and 96) h, at the best temperature. O.D and the dry weight of biomass contained β-glucan were measured for each incubation time.

Effect of inoculum size

The effect of inoculum size on the β-glucan production was examined by inoculating (5, 10, 15 and 20) % of inoculum (3×10^7) CFU / ml. The culture was incubated at the best incubation

time and best temperature. The O.D and the dry weight of biomass containing β-glucan were measured for each inoculum size with procedure as mentioned above.

Effect of glucose concentration

The effect of glucose concentration on β-glucan production was studied. The culture medium was adjusted at different glucose concentrations (1, 1.5, and 2) %. The cultivation media were incubated at the best temperature, incubation time, and inoculum size. The O.D and the dry weight of biomass contained β-glucan were measured for each concentration of glucose.

Effect of yeast extract concentration

The effect of yeast extract concentration on β-glucan production was studied, the culture medium was adjusted at different yeast extract concentrations, (0.02, 0.05, and 0.1) %. Incubation of the cultivation media at the best conditions with same procedure describe above. The O.D and dry weight of cell biomass contained β-glucan were estimated for each concentration of yeast extract.

Effect of pH

The influence of pH medium on β-glucan production was studied as following: the culture medium was adjusted at different pH included (5, 7 and 9). Incubation was performed on the cultivation medium at the best conditions mentioned above, the O.D and dry weight of cell biomass contained β-glucan were estimated for each pH value with the same procedure described above.

Effect of culture media

Date extract medium was prepared according to [15], 100g of Al-khastawy date (without pits) was added to 500ml distilled water and boiled with constant stirring for 10min, after that it was cooled and stored at 4°C for overnight. Then, it was mixed again and filtered through many layers of cheese cloth and Whatman No.1 filter paper. The filtrate was centrifuged twice at 3000 rpm and the pH adjusted to 7 then sterilized by autoclave. The isolate of *S. cerevisiae* was inoculating in date extract medium. This medium was used to examine the effect of culture medium on β-glucan production by measured O.D and the dry weight of cell biomass containing β-glucan.

β-glucan Extraction from *S. cerevisiae*

1- Alkali-acid method

β-glucan was extracted from the cell walls of *S. cerevisiae* according to [16]. 150 ml of NaOH (1.0 M) was added to 4g of dry yeast, heated at 100 °C for 15 min, mixed by a magnetic stirrer and left for 1 hour to cool. Then, it was centrifuged at 5000 rpm for 5 min, and 50 ml of distilled water was added to precipitate and centrifugated at the same speed and time. Washing process was carried out with distilled water and the pH of precipitate was adjusted to pH 7 using 1.0 M of HCl or 1.0 M of NaOH. Next step, 125 ml of H_3PO_4 (1.0 M) was added to the precipitate and heated at a temperature of 100 °C for 15 min and mixed by a magnetic stirrer. The samples were centrifuged at a speed of 5000 rpm for 5 minutes, and washed the precipitate with distilled water. The precipitate was treated with 50 ml of ethyl alcohol, heated for 15 min at 80 °C and centrifuged at 7000 rpm for 5 min. The precipitate was then submerged in 50 ml of distilled water. Centrifugation was done again and the precipitate was taken, washed well with distilled water, and 50 ml of distilled water was added. The samples were heated for 15 min, and left to cool. Centrifugation was carried out at the same speed and time, the precipitate was kept, and distilled water was added until the pH reaches 9.6-7. The centrifugation process was done again at the same speed and time, and the formed precipitate was collected and dried at 60°C.

2-Autolysis of yeast cell

The suspension of *S. cerevisiae* was prepared at the optimum condition detected above and incubated at 30°C for 48 h with shaking at 200 rpm. After incubation, yeast cells were collected by centrifugation at 4 °C for 10 min at 75000 rpm. The suspension of 15%w/v of yeast cells in distilled water with pH 5.0 was incubated at 50 °C for 48 h with shaking at 120 rpm. The autolysis reaction was terminated by incubating at 80 °C for 15 min in a water bath. Next step, yeast cells were collected by centrifugation at 4 °C for 10 min at 5,000 rpm. The autolyzed yeast cells were mixed with 5-fold of 1.0 M NaOH and incubated at 80 °C with stirrer for 2 h. Then, the cell pellet was collected by centrifugation at 6,000 rpm for 25 min at 4 °C and suspended in 3-fold of distilled water. After thorough mixing, cells were centrifuged at 6,000 rpm for 25 min at 4 °C. Then,

the cell pellet was dissolved in 5-fold 1.0 CH_3COOH and incubated at 80 °C with a stirrer for 2 h. The final step, the pellet was collected by centrifugation at 6,000 rpm for 25 min at 4 °C. The obtained pellet was washed with water 3 times and dried in hot air oven at 60 °C [17].

Analysis of glucan by Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed in the Chemistry department/ College of Science/ Mustansiriyah University. The instrument operated within the range of wave number (400-4000) cm-1 measuring the amount of IR radiation reflected or transmitted by sampling. The result obtained in the graphic chart, whereas the X-axis represents the variety of wave numbers, while the Y-axis represents the percentage of transmittance.

Analysis of β-glucan by High-Performance Liquid Chromatography (HPLC)

Auto sampler high performance liquid chromatography system (SYKAMN – Germany) supplied with C18- NH analytical column (250 mm x 4.6mm, 5µn) was used for analysis. Mobile phase composition was D.W: MeOH (98: 2). The flow rate was 0.7 mL/min.; the injected volume was 100 µL with Detector RI (reflective index detector). The obtained sample was then dissolved with D.W: MeOH (98: 2) for HPLC analysis supplied with C18- NH analytical column (250 mm x 4.6mm, 5µn) was used for analysis [18].

RESULTS AND DISCUSSION

Isolation and Identification of *Saccharomyces cerevisiae*

Nine isolates of dry baker's yeast belonged to *S. cerevisiae* were obtained, activated, and re-identified. The result showed that all isolates were able to grow on Sabarued dextrose agar and appeared as white, cream-colored, or tinged with brownish pigments. Microscopically examination showed that the *S. cerevisiae* which were isolated from suspected colonies, stained with lactophenol cotton blue dye, and observed under a microscope. In addition, *S. cerevisiae* was subjected to VITEK 2 YST ID card which confirmed the identification [19].

Screening of *S. cerevisiae* for β-glucan production using aniline blue assay

A-Using agar medium

The aniline blue dye was used to detect the β-glucan production from the *S. cerevisiae* isolates. All isolates of *S. cerevisiae* produced blue-colored colonies after 3 day of incubation (Figure 1). Abd El Ghany *et al.* showed that the use of Aniline blue to detect 1,3-glucans, formation of the blue color indicates the presence of β-glucan [13]. Aniline blue was used for the detection of β-glucan because aniline blue interacts with β-glucan, the visual observation of colony color used to screen β-glucan production in different environments [20].

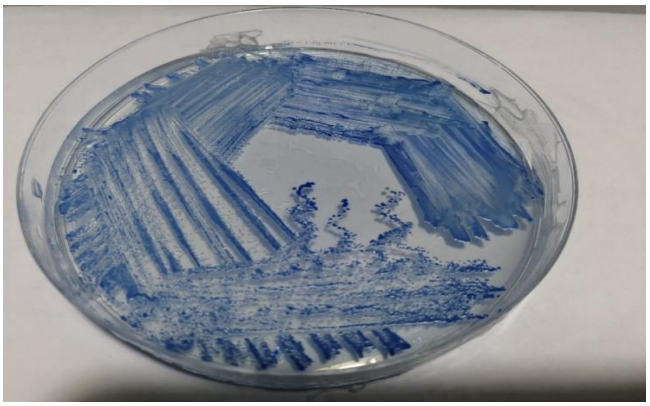


Figure 1. *S. cerevisiae* colonies on yeast extract glucose medium with aniline blue dye.

B-Using broth medium

Yeast isolates were screened according to O.D at 510 nm and the amount of dry weight of the cells contained β-glucan was recorded. The results in Table 1 showed that the isolate (*S. cerevisiae* Sc9) gave the highest amount of biomass of the cells at 1.9 mg/5ml. Furthermore, it is noted from the results that there is a correlation between the dry weight of the biomass of the cells and the O.D measured by spectrophotometer. Utama *et al*. examined total population of yeast cells by optical density [4]. The differences biomass of the cells may be due to the differences in the physiological activity of each isolate of *S. cerevisiae*, and different metabolic activities, the activity of its enzymes, and its ability to consume nutrients in its surroundings, which have a direct impact on the reproduction process and the synthesis of cellular structures [20] used aniline blue for detection of β-glucan.

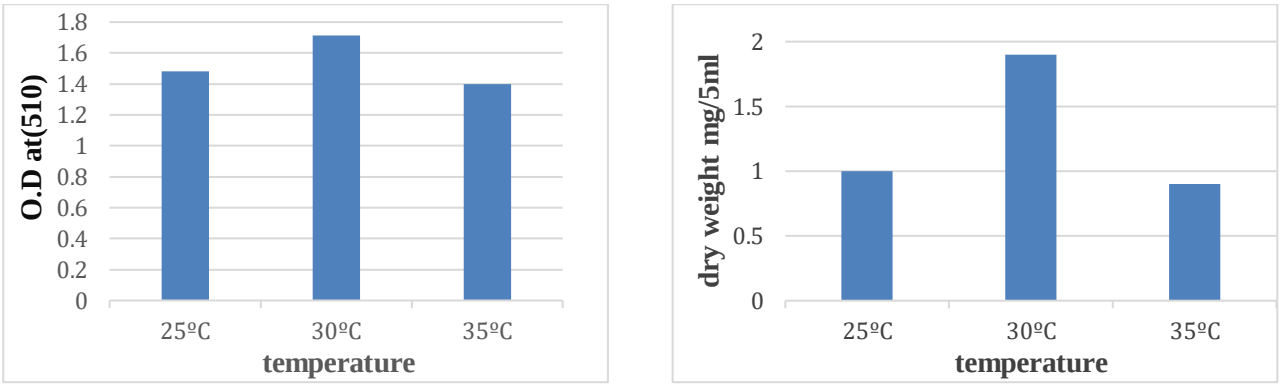
Table 1. Dry weight of cell biomass and O.D. obtained from *S. cerevisiae.*

Yeast isolate	O. D. (510 nm)	Dry weight mg/5ml
S. cerevisiae (SC1)	1.524	1.2
S. cerevisiae (SC 2)	1.533	1.6
S. cerevisiae (SC 3)	1.436	1.0
S. cerevisiae (SC 4)	1.599	1.7
S. cerevisiae (SC 5)	1.469	1.1
S. cerevisiae (SC 6)	1.596	1.7
S. cerevisiae (SC 7)	1.636	1.8
S. cerevisiae (SC 8)	1.684	1.8
S. cerevisiae (SC 9)	1.712	1.9

Determination the Optimum Conditions of β-glucan Production from *S. cerevisiae*

Effect of Temperature

The isolate *S. cerevisiae Sc9* was cultured at three temperatures (25,30 and 35) ºC, incubation at 30ºC gave dry weight of glucan as 1.9 mg/5ml, while at 25 ºC, and 35ºC as 1 mg/5ml, and 0.8 mg/5ml of dry weight, respectively. This study showed that there is a correlation between dry weight and optical density (Figures 2a and 2b). These results were similar to the results of other studies in which the highest cell biomass was obtained at 30°C [23].



(a) (b)

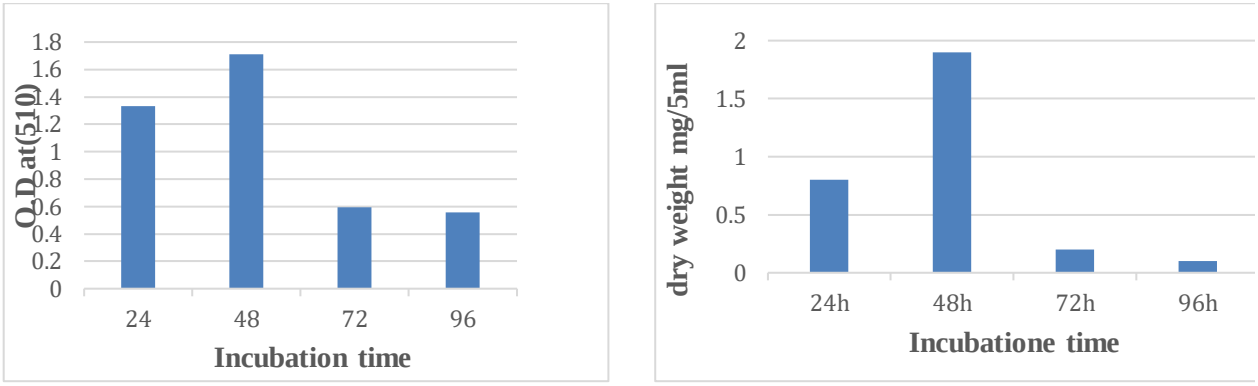
Figure 2. Effect of temperature on β glucan production of *Saccharomyces. cerevisiae* (a) Optical Density (O.D.) (b) dry weight.

Effect of incubation time

The β-glucan content of *S. cerevisiae* is affected by incubation time at different periods (24, 48 ,72, and 96) h. The results indicated that the high dry weight of 1.9 mg/5ml was obtained at 48 h, while 0.8 mg/5ml at 24 h, that decrease was observed to 0.2 mg/5ml, 0.1 mg/5ml, at 72 h, 96 h, respectively (Figures 3a and 3b). The dry weight of cell biomass decreased when the incubation period was increased to 72 and 96h, which may indicate that the cells are entering the stationary phase, and the reason for this may be due to the depletion of nutrients in the culture medium and the increase in the concentration of metabolites resulting from the cellular activity of cells, which have a negative effect on cell growth. Moreover, the pH value of the medium changes during incubation time that it prevents cell proliferation or create substances may be toxic to the cells themselves [4]. Byrtusová mentioned that when yeast is grown for 48h the percentage of glucan and the biomass of the cells increases [6].

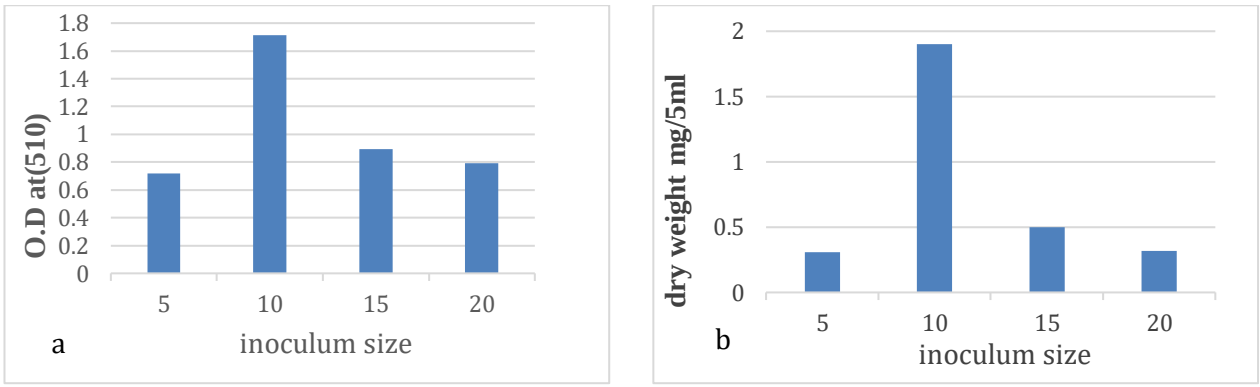
Effect of inoculum size

The β-glucan content of *S. cerevisiae* is influenced by the size of the inoculum, the highest dry weight was obtained 1.9 mg/5ml at 10% inoculum sizes while deacreased at 5 ,15,20 % inoculum size with dry weight (0.3,0.5,0.3mg/5ml) respectively (Figures 4a and 4b). Wróbela and Janowicz mentioned that the use 10% of inoculum size have a positive effect on the β-glucan yield [26].



(a) (b)

Figure 3. Effect of incubation time on β-glucan from *S. cerevisiae.* (a) Optical Density (O.D.) (b) Dry weight.

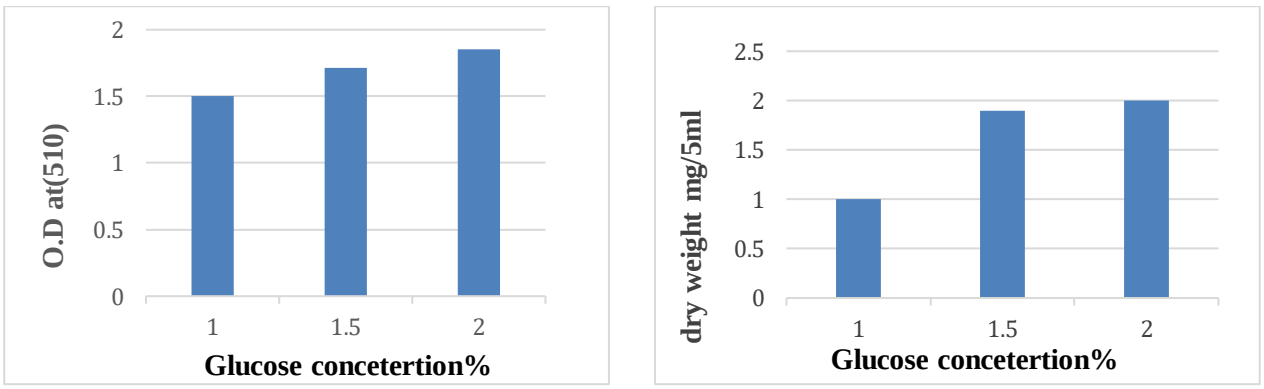


(a) (b)

Figure 4. Effect of inoculum size on β-glucan. (a) Optical Density (O.D.) (b) Dry weight.

Effect of glucose concentration

As shown in (Figures 5a and 5b), the highest dry weight was 2 mg/5ml at a concentration 2% of glucose, while 1.9 mg/5ml was recorded at 1.5 % and the lowest dry weight recorded as 1 mg/5ml at 1 % glucose. *S. cerevisiae* can consume glucose better than other types of sugars such as (fructose, maltose, galactose, and sucrose). Glucose is the optimal carbon source and glucose has a hormonal-like effect on yeast cells by activating cell growth and stimulating cell growth [16]. Shukla and Goyal [27] observed the effects of glucose as cosubstrate on glucansucrase and glucan production. According to Study by [28] exhibited when media were supplied with glucose as sources of carbon, most *S. cerevisiae* strains could grow. It was declared that medium containing glucose at specific concentration led to increasing in β-glucan production [6].

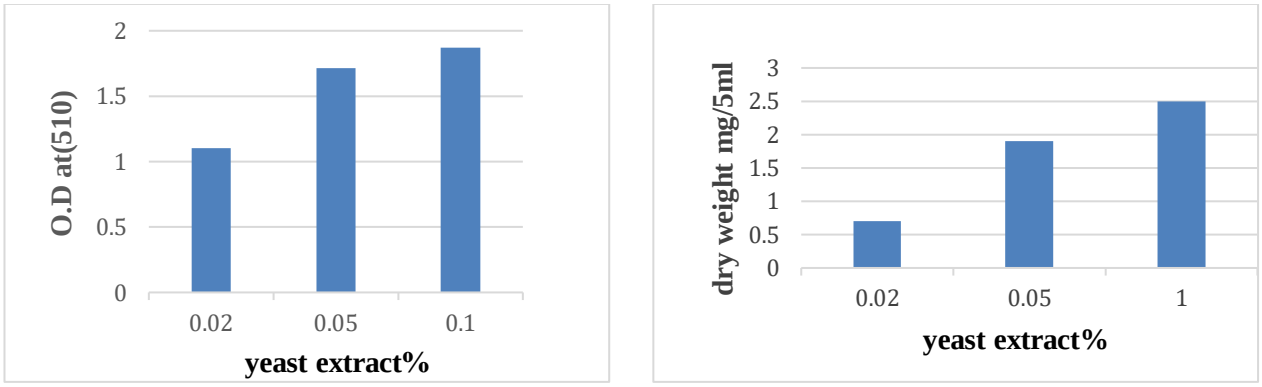


(a) (b)

Figure 5. Effect of glucose concentration on β-glucan production from *S. cerevisiae*. (a) Optical Density (O.D.) (b) Dry weight.

Effect of yeast extract concentration

The results of yeast extract concentration effect on β-glucan production were shown in **(**Figures 6a and 6b). The highest dry weight was obtained by using 0.1% of yeast extract concentration, which amounted to 2.5mg/5ml of the dry weight of the cells. Shukla & Goyal, was observed the effects of yeast extract, on glucan production [23]. Byrtusová, showed that medium containing yeast extract concentration at 0.5 g/L led to increasing in β-glucan production from *S. cerevisiae* [6].

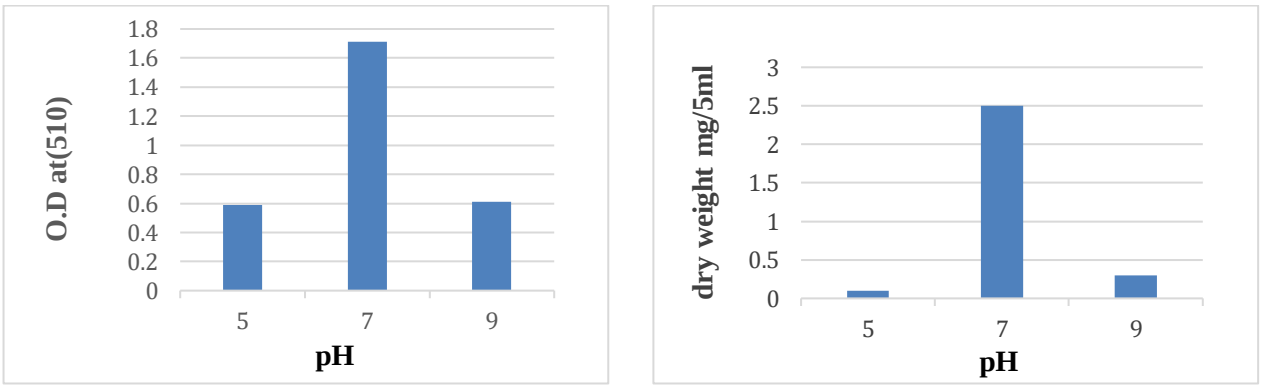


(a) (b)

Figure 6. Effect of yeast extract concentration on β-glucan from *S. cerevisiae.* (a) Optical Density (O.D.) (b) Dry weight.

Effect of pH

The results of the pH effect test were shown in (Figures 7a and 7b). It was showed that the highest dry weight was obtained at pH=7, which was 2.5mg/5ml of the dry weight of the cells, while 0.3 mg/5ml was recorded at pH= 9 and the lowest dry weight recorded 0.1 mg/5ml at pH= 5. Similar to study Byrtusová mentioned that the used pH 7 and recorded the glucan and the biomass of the cells increased [6].



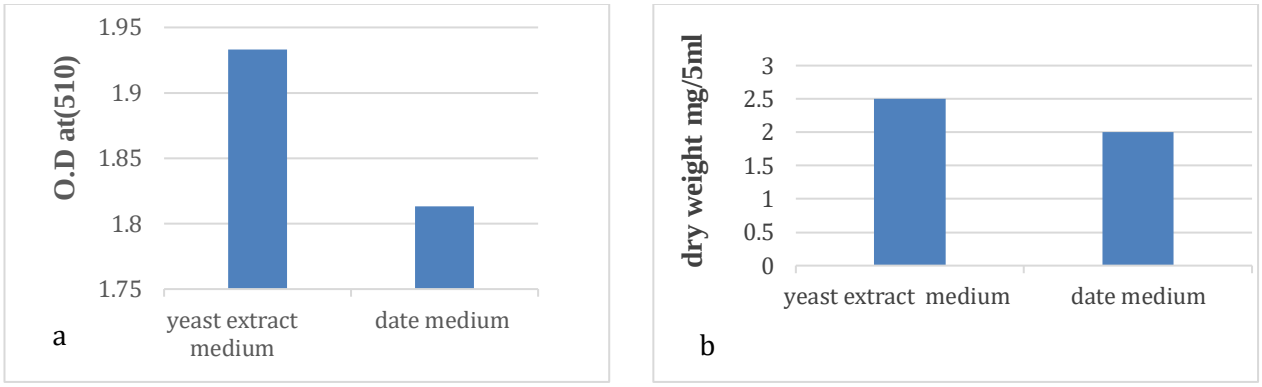
(a) (b)

Figure 7. Effect of pH on β-glucan from *S. cerevisiae.* (a) Optical Density (O.D.) (b) Dry weight.

Effect of the culture medium

The isolate of *S. cerevisiae* sc_9 was grown on two culture media: yeast extract –glucose and date extract medium, which can be used in a wide range and achieve economic returns. The results in (Figures 8a and 8b) showed that the growth of yeast-on-yeast extract-glucose medium gave the highest dry weight of the cells contained β-glucan, which was 2.5mg/5ml of the dry weight, while the growth in date extract medium gave 2mg/ml of the dry weight of the cells. The reason for this may be due to the nutrients in the yeast extract – glucose medium which suitable for yeast growth and the increase in its glucan content, and indicted this medium increase on the β-glucan yield from *S. cerevisiae* [21]. Dates contain moderate amounts of vitamin A, small amounts of vitamin B1, B2, and C, and a large amount of Nicotinic acid, in addition to containing appropriate amounts of calcium, copper, magnesium, sulfur, phosphorous, iron, and potassium. These salts and minerals are necessary for the growth of yeast

[24]. There are many studies that confirmed the suitability of date extract medium for *S. cerevisiae*. Chotigavin *et al* mentioned that the use of molasses led to increase the β-glucan yield [25].



(a) (b)

Figure 8. Effect of culture media on β-glucan from *S. cerevisiae.* (a) Optical Density (O.D.) (b) Dry weight.

Extraction of β-glucan from *S. cerevisiae*

In this study, two methods were used to extract glucan from yeast. Autolysis method was the best one to obtaining high concentration of glucan extracts compared to the Alkali-acid extraction method. Crude β-glucan was dried and weighed as (6 gm/L, and 8gm / L) for autolysis of yeast cells and Alkali-acids extraction respectively. In addition to the progress, the method of extraction by autolysis is time-consuming, taking several days and consuming the materials used for extraction, and the amount of yield was less but purer. However, the method of extraction with Alkali-acids was the least consuming of time and materials, but it gave less purity, and the amount of yield was more.

Analysis of glucan by (FTIR)

FTIR spectra analysis was used to detect functional groups of glucan extraction by autolysis of yeast cell and alkali-acid methods. The band in the region of 3391.21 - 3398.92 cm^{-1} for glucan extraction by autolysis of yeast cell and alkali-acid respectively was due to V(OH) stretching vibration of the polysaccharide, while the band in the region of 2921.63cm^{-1} and 2921.63$cm^{-1}$for glucan extraction by autolysis of yeast cell and Alkali-acid respectively, was due to C-H stretching vibration. The band region found in 1041.37 cm^{-1} and 1024.98 cm^{-1} for glucan extraction by autolysis of yeast cell and alkali-acid was assigned to vibration of C-O-C (glycosidic bond), whereas the region in 889.987 cm^{-1} and 1041.37 cm^{-1} for glucan extracted by autolysis of yeast cell was assigned to around the β-(1-6) glycosidic bond, β-(1-3) glycosidic bond for β-glucan responsible to the great chain flexibility in glucan. while in glucan extracted by alkali-acid, the region in 887.095 cm^{-1} was due to the great chain flexibility in glucan around the β-(1-6) glycosidic bond, 844.669 cm^{-1} and 1024.98 cm^{-1} was due to α-(1-3) glycosidic bond. The presence of the peak at region 844.669 cm^{-1}indicated the presence of small amount of mannan, the band region in1642.09cm^{-1} and 1641.13 cm^{-1} for glucan extracted by autolysis of yeast cell and alkali-acid, respectively was due to amide group (protein) (Figures 9a and 9b). FTIR spectra analysis showed that glucan extracted by alkali-acid had α-glucan, β-glucan and small amount mannan, while that extracted by autolysis of yeast cells showed presence only β-glucan. Broad region between 3000–2800 cm^{-1} can be assigned to CH bond and N-H bond at 3500–3100 cm^{-1} and O-H bond at 3600–2600 cm^{-1}, Characteristic amide regions were found at 1570–1470 cm^{-1} (amide I),1720–1620 cm^{-1} (amide II), and 1350–1250 cm^{-1} (amide III), at 1200–900 cm^{-1} region can be represented Sugar compounds. FTIR spectra analysis of glucan from *S. cerevisiae* which contain a functional group include a ROR group (ether)– a –CCC- group and OH group [27]. There are many studies that are compatible with result both spectra of autolysis of yeast cells and alkali-acid extracted β-glucans have characteristic β-glucan bands at 1065, 1038 and 890 cm^{-1} [28]. Galichet *et al* found that the appearance of β-glucan at the region 890 cm^{-1} and α-glucan at the region 841 cm^{-1} [29] while, the band of β-glucan observed at 894 cm^{-1} [21]

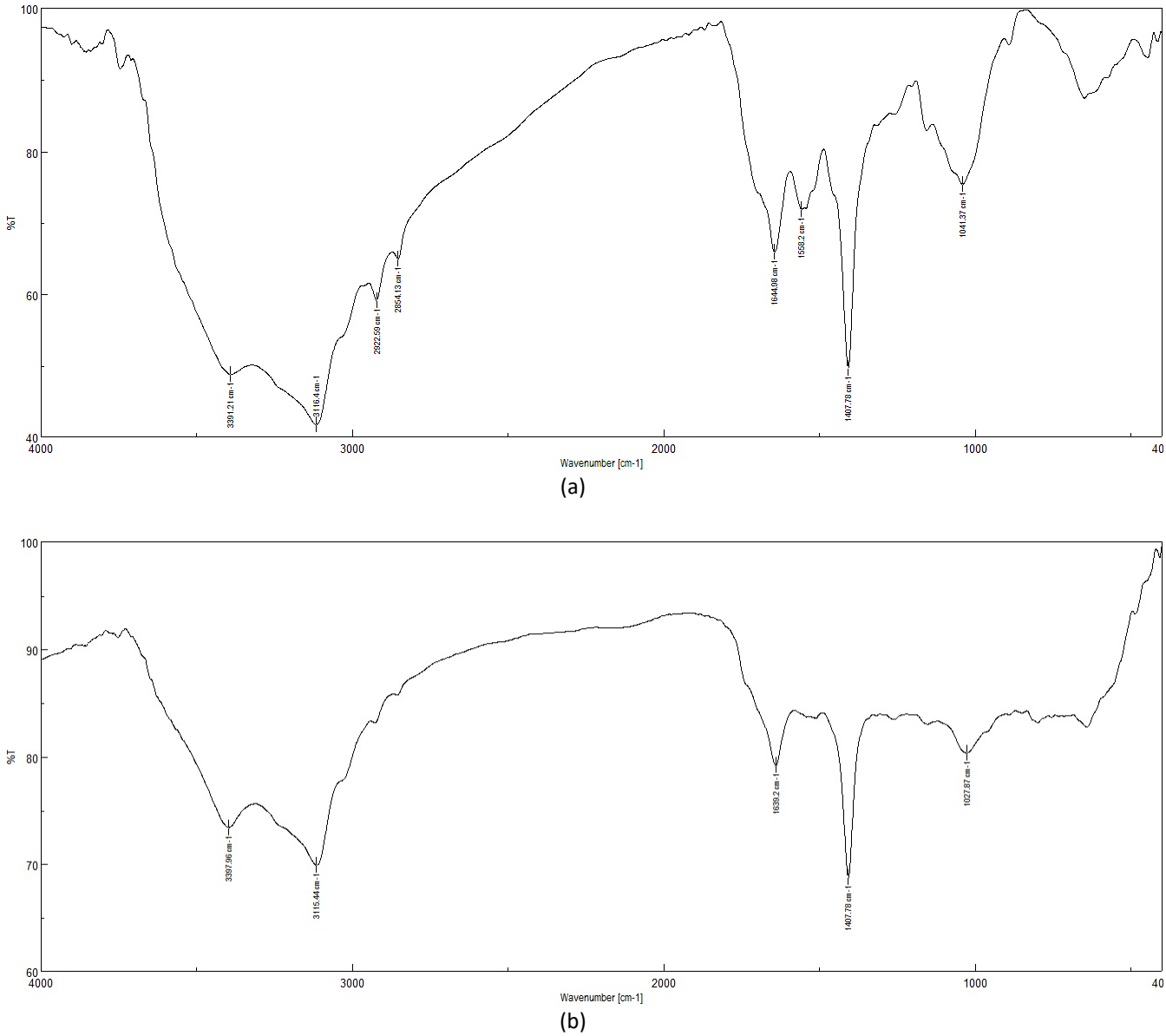


Figure 9. Fourier Transform Infrared Spectroscopy (FTIR) of glucan from *S. cerevisiae.*
(a) glucan extracted by autolysis of yeast cell. (b) glucan extracted by Alkali-acid.

Analysis of glucan by HPLC

The HPLC technique was used to determine the concentration of glucan present in the samples of the extract by using the two extraction methods and this was confirmed by injection of glucan standard. The major peak of glucan appeared after 30 min, and the result showed that the highest concentration of glucan 10.8mg/gm obtained by autolysis method, while for Alkali-acid method the glucan concentration was 7.4 mg/gm (Figure.10a and Figure 10b). Characterization of crude β-glucan derived from *S. cerevisiae* isolates by FTIR showed that glucan extracted by Alkali-acid had α- glucan, β-glucan and small amount mannan, while extraction by autolysis of yeast cells showed presence of only β-glucan 1.5. Glucan can be produced from baker's yeast with varying degrees of purity with great variation in its physical, chemical properties, and biological activity depending on the extraction method used [30]. On alkali-acid extraction, the alkali works to dissolve or remove most of the cellular proteins, nucleic acids, glycogen, mannan and polar lipids as well as the separate of glucan from chitin to chitosan and reduction of nonpolar lipids and hydrophobic proteins [31]. The reason for obtaining high-purity extracts by autolysis may be

due to the isolation of the cell wall and its use as a starting material in the extraction process, which avoids the presence of many cellular contents such as proteins, carbohydrates, and fats. However, the extraction by Alkali-acid, which uses the whole cells, you mean the attached cellular contents can be obtained with the extract. This study agrees with [21] that the autolysis method was good in obtaining high purity glucan extracts compared with other extraction methods. Pengkumsri *et al* [17] indicated that when a strong base (NaOH) and a weak acid (CH3CooH) were used for extraction, it was shown to recover large levels of polysaccharide and glucan, and that yeast autolysis led to the release of 49% of the contents of the cells after 48 h. In addition, in similar study by [32], β-glucan isolation was performed in a two-step process, yeast autolysis and alkali extraction with Nao autolysis at 55ºC/pH 5.0/24 h with use of acetic acid. Krisdaphong *et al.* [33] explained that yeast cell wall was broken down by the autolysis process (Step 1) to separate the inner protein liquid from the insoluble cell wall through 48 h at pH= 5 and 50°C, with the separation producing the insoluble sediment cell wall 34.9% (Step 2) process involving the NaOH extract breaking layers of protein.

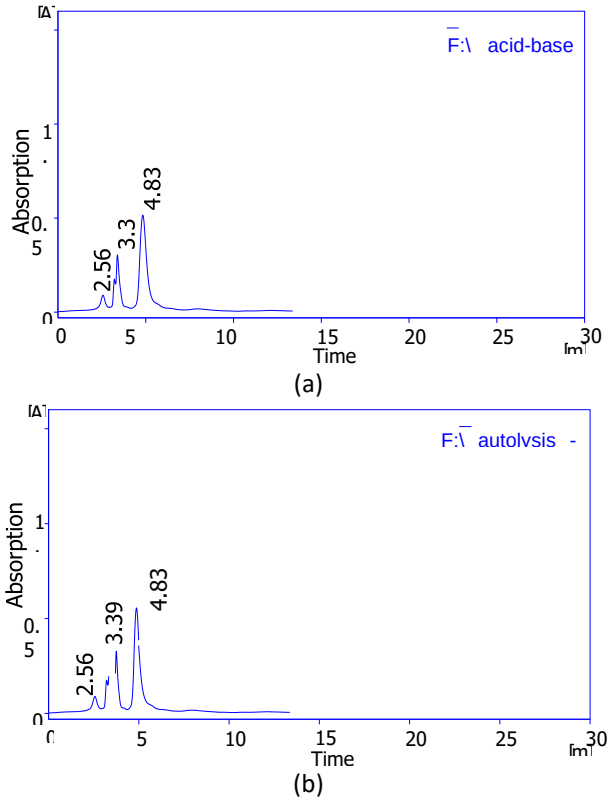


Figure 10. HPLC of glucan from *S. cerevisiae.* (a) glucan extracted by Alkali-acid. (b) glucan extracted by autolysis of yeast cell.

CONCLUSIONS

Based on the study, it was concluded that the optimum conditions of β-glucan production were 30 ºC for 48 h at pH 7 with 10 % inoculum size, 2% glucose, 0.1% yeast extract and the best culture medium for β-glucan production was yeast extract -glucose medium. The best method for β-glucan extraction was autolysis extraction method.

ACKNOWLEDGMENT

The authors would like to thank Mustansiriyah University (https://uomustansiriyah.edu.iq/), Baghdad, Iraq for its support to complete this work.

Disclosure and Conflict of Interest: The authors declare that they have no conflicts of interest.

REFERENCES

[1] A. Synytsya and M. Novák, "Structural diversity of fungal glucans," Carbohydr. Polym., vol. 92, no. 1, pp. 792-809, 2013. https://doi.org/10.1016/j.carbpol.2012.09.077

[2] B. Han, K. Baruah, E. Cox, D. Vanrompay, and P. Bossier, "Structure-functional activity relationship of β-glucans from the perspective of immunomodulation: a mini-review," Front. Immunol., vol. 11, p. 658, 2020. https://doi.org/10.3389/fimmu.2020.00658

[3] E. J. Murphy, E. Rezoagli, I. Major, N. J. Rowan, and J. G. Laffey, "β-glucan metabolic and immunomodulatory properties and potential for clinical application," J. Fungi, vol. 6, no. 4, p. 356, 2020. https://doi.org/10.3390/jof6040356

[4] G. L. Utama, C. Dio, J. Sulistiyo, F. Y. Chye, E. Lembong, Y. Cahyana, & S. Singh, "Evaluating comparative β-glucan production aptitude of Saccharomyces cerevisiae, Aspergillus oryzae, Xanthomonas campestris, and Bacillus natto," Saudi J. Biol. Sci., vol. 28, no. 12, pp. 6765-6773, 2021. https://doi.org/10.1016/j.sjbs.2021.07.051

[5] I. Gientka, S. Błażejak, L. Stasiak-Różańska, and A. Chlebowska-Śmigiel, "Exopolysaccharides from yeast: insight into optimal conditions for biosynthesis, chemical composition and functional properties? review," Acta Sci. Pol. Technol. Aliment., vol. 14, no. 4, pp. 283-292, 2015. https://doi.org/10.17306/J.AFS.2015.4.29

[6] D. Byrtusová, "Production of Beta-Glucans and Other Polysaccharides by yeast and microalgae". PhD thesis. Brno University of Technology.2020.

[7] S. Rizal, M. Murhadi, M. E. Kustyawati, and U. Hasanudin, "Growth optimization of Saccharomyces cerevisiae and Rhizopus oligosporus during fermentation to produce tempeh with high?-glucan content," Biodiversitas J. Biol. Divers., vol. 21, no. 6,

2020.DOI:10.13057/biodiv/d210639 https://doi.org/10.13057/biodiv/d210639

[8] S. C. Jaehrig S. Rohn, L. W. Kroh, F. X. Wildenauer, F. Lisdat, L. G. Fleischer, & T. Kurz, "Antioxidative activity of (1→3), (1→6)-β-d-glucan from Saccharomyces cerevisiae grown on different media," LWT-Food Sci. Technol., vol. 41, no. 5, pp. 868-877, 2008. https://doi.org/10.1016/j.lwt.2007.06.004

[9] A. C. Pelizon, R. Kaneno, A. Soares, D. A. Meira, and A. Sartori, "Immunomodulatory activities associated with β-glucan derived from Saccharomyces cerevisiae," Physiol. Res., pp. 557-564, 2005. https://doi.org/10.33549/physiolres.930686

[10] M. D. Wenger, P. DePhillips, and D. G. Bracewell, "A microscale yeast cell disruption technique for integrated process development strategies," Biotechnol. Prog., vol. 24, no. 3, pp. 606-614, 2008. https://doi.org/10.1021/bp070359s

[11] Y.-H. Kim, S.W. Kang, J.H Lee, H.L. Chang, C.W. Yun, H.D. Paik, C.W. Kang, and S.W. Kim. "High cell density fermentation of Saccharomyces cerevisiae JUL3 in fed-batch culture for the production of β-Glucan," J. Ind. Eng. Chem., vol. 13, no. 1, pp. 153-158, 2007. DOI: 10.1016/j.enzmictec.2005.12.020

[12] E. H. Abdulhameed . "Inhibition of Some Diarrheal Causative Bacteria by purified Dextran from Baker's yeast Saccharomyces cerevisiae" Ms.c. the faculty of Science - Mustansiriyah University .2020

[13] K. Abd El Ghany, R. A. Hamouda, H. A. Mahrous, S. M. Sharaf, and H. A. Hamza, "Molecular characterization using gtf gene detection and medium optimization of β-d-glucan production from Pediococcus parvulus F1030 isolated from local Egyptian boza," J. Pharm. Res. Int, pp. 1-8, 2017. https://doi.org/10.9734/JPRI/2017/39079

[14] N. A. Mohd Jamil, N. Rahmad, N. Mohd Nor Rashid, M. H. Y. Mohd Yusoff, N. S. Shaharuddin, and N. Mohd Saleh, "LCMS-QTOF Determination of Lentinan-like β-D-glucan content isolated by hot water and alkaline solution from tiger's milk mushroom, termite mushroom, and selected local market mushrooms," J. Mycol., vol. 2013, 2013. https://doi.org/10.1155/2013/718963

[15] Z.M. N.Al-Zabaedy,. Extraction and purification of inhibitory substance from yeast Saccharomyces Cerevisiae against some parts of bacteria, yeast. PhD, Faculty of Science - Mustansiriya University. (2001).

[16] S. Asare, Optimized acid/base extraction and structural characterization of β-glucan from S. cerevisiae. Ms.c. Thesis the faculty of the Dep. Chemi. East Tennessee State University75 p. 2015.

[17] P. Ferrer, "Revisiting the Cellulosimicrobium cellulans yeast-lytic β-1, 3-glucanases toolbox: A review," Microb. Cell Fact., vol. 5, no. 1, pp. 1-8, 2006. https://doi.org/10.1186/1475-2859-5-10

[18] A. M. Pérez-Vendrell, J. Guasch, M. Francesch, J. L. Molina-Cano, and J. Brufau, "Determination of β-(1-3), (1-4)-D-glucans in barley by reversed-phase high-performance liquid chromatography," J. Chromatogr. A, vol. 718, no. 2, pp. 291-297, 1995. https://doi.org/10.1016/0021-9673(95)00694-X

[19] S. S. Rahman, "Isolation and characterization of Saccharomyces cerevisiae for the production of ethanol from organic sources." Doctoral dissertation, BRAC University, 2013.

[20] I. Nakanishi, K. Kimura, T. Suzuki, M. Ishikawa, I. Banno, T. Sakane, & T. Harada, "Demonstration of curdlan-type polysaccharide and some other β-1, 3-glucan in microorganisms with aniline blue," J. Gen. Appl. Microbiol., vol. 22, no. 1, pp. 1-11, 1976. https://doi.org/10.2323/jgam.22.1

[21] A.A.W. A. AlRabie, Production and extraction of glucans from Saccharomyces cerevisiae and study of its effect on stimulating macrophages ex vivo. Ms.c. the faculty of Science - Mustansiriya University. (2009).

[22] A. Bzducha-Wróbel, S. Błażejak, M. Kieliszek, K. Pobiega, K. Falana, and M. Janowicz, "Modification of the cell wall structure of Saccharomyces cerevisiae strains during cultivation on waste potato juice water and glycerol towards biosynthesis of functional polysaccharides," J. Biotechnol., vol. 281, pp. 1-10, 2018. https://doi.org/10.1016/j.jbiotec.2018.06.305

[23] S. Shukla and A. Goyal, "Optimization of fermentation medium for enhanced glucansucrase and glucan production from Weissella confusa," Brazilian Arch. Biol. Technol., vol. 54, pp. 1117-1124, 2011. https://doi.org/10.1590/S1516-89132011000600006

[24] M. Kara Ali et al., "Optimization of baker's yeast production on date extract using Response Surface Methodology (RSM)," Foods, vol. 6, no. 8, p. 64, 2017. https://doi.org/10.3390/foods6080064

[25] N. Chotigavin, W. Sriphochanart, S. Yaiyen, and S. Kudan, "Increasing the Production of β-Glucan from Saccharomyces carlsbergensis RU01 by Using Tannic Acid," Appl. Biochem. Biotechnol., vol. 193, pp. 2591-2601, 2021. https://doi.org/10.1007/s12010-021-03553-5

[26] J. Liu, X. Zhang, J. Zhang, M. Yan, D.Li, , S. Zhou, , and Y. Liu, "Research on extraction, structure characterization and immunostimulatory activity of cell wall polysaccharides from Sparassis latifolia," Polymers (Basel)., vol. 14, no. 3, p. 549, 2022. https://doi.org/10.3390/polym14030549

[27] D. L. Indriati Ramadhani, Y. Yuliani, and M. Amir, "Extraction, characterization, and biological toxicity of β-glucans from Saccharomyces cerevisiae isolated from ragi," J. Microb. Syst. Biotechnol., vol. 2, no. 2, pp. 35-43, 2020. https://doi.org/10.37604/jmsb.v2i2.62

[28] A. Maneikis and R. Bikmurzin, "FT-IR Method Limitations for β -Glucan Analysis ¯," 2022. https://doi.org/10.3390/molecules27144616

[29] J. A. Boutros, A. S. Magee, and D. Cox, "Comparison of structural differences between yeast β-glucan sourced from different strains of Saccharomyces cerevisiae and processed using proprietary manufacturing processes," Food Chem., vol. 367, p. 130708, 2022. https://doi.org/10.1016/j.foodchem.2021.130708

[30] P. Courie, S. Patelakis, and A. Miles, "A Method of Producing an Economical and Ecologically Sound Natural Immunobiotic Extract for Use as a Health Management Instrument and a Replacement for Growth Promotion Antibiotics in Livestock and Companion Animals." Google Patents, Jan. 27, 2005.

[31] S. Jamas, D. D. Easson, and G. Ostroff, "Underivatized, aqueous soluble beta (1-3) glucan, composition and method of making same." Google Patents, Jun. 17, 2004.

[32] V. Varelas, E. Sotiropoulou, M. Liouni, and E. T. Nerantzis, "Production Of B-Glucan from Winery Yeast Waste Biomass". Int. J. Food. Sci. Tech. 39, 21-29,2015 https://doi:10.4172/2155-9600.1000133.

[33] I. Avramia and S. Amariei, "Spent Brewer's yeast as a source of insoluble β-glucans," Int. J. Mol. Sci., vol. 22, no. 2, p. 825, 2021. https://doi.org/10.3390/ijms22020825

How to Cite

A. A. . Khadam, J. A. S. . Salman, and M. . Hijri, "Determination the Optimum Conditions for β-glucan Production Extracted from Saccharomyces cerevisiae", *Al-Mustansiriyah Journal of Science*, vol. 34, no. 2, pp. 32–43, Jun. 2023.