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Investigation of extraction method effect on yeast beta glucan production

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Abstract: Nowadays, due to the stressful, tiring and busy lives of humans, the immune system becomes weak and can get sick easily. Therefore, scientists have been doing researches about new, natural and healthy products that can strengthen their immune systems and can provide to adapt life standards. One of these products is the beta glucan. It is a polysaccharide molecule that consists of D-glucose monomers bonding with beta glycosidic bonds. Beta (β) glucans have being produced from different sources (microorganisms, cereals and mushrooms) so they have different branched structures such as (1→3) (1→6), (1→3) (1→4), (1→3) (1→2). Thus, different branched beta glucans show different physicochemical properties and biological activities that designate their usage purposes. Especially yeast beta glucan has lots of biological activities. On the other hand, it is fact that the extraction method affects the molecular weight, yield, purity and other properties of beta glucans. The main purpose of this study is to compare the performances of ultrasonically assisted alkali-acidic and autolysis extraction methods to produce a high yield of beta glucan. Also, it was aimed to determine the effect of extraction method on the molecular weight of yeast beta glucan. As a result of this work, it was found that the yeast beta glucan yield for ultrasonic supported autolysis extraction (12 %) is higher than that of the ultrasonic supported alkali-acidic extraction (8 %). On the other hand, having the lower molecular weight of yeast beta glucan (87 kDa) was produced by using an ultrasonic supported autolysis extraction method.

Keywords: Beta glucan, *Saccharomyces cerevisiae*, yeast, alkali-acidic extraction, autolysis, molecular weight

1. Introduction

Humans have struggled with a lot of illnesses from past to present. Today world is fighting the Covid-19 pandemic. There is no doubt that the immune systems of humans should be strengthened for preventing and overcoming illnesses. Therefore, different natural and synthetic substances have been used to stimulate the immune system, modulating humoral and cellular immunity due to their beneficial effect in fighting infections (bacterial, viral, fungal and parasitic) (Mantovani 2008). Beta glucan is one of them (Murphy et al. 2020). It is a polysaccharide molecule which consist of D-glucose monomers bonding with beta glycosidic bonds. Different sources (microorganisms, cereals and mushrooms) consist of different branching structures and quantities of beta glucans. They have main three branching structures as (1→3) (1→6)-β-Glucan, (1→3) (1→4)-β-Glucan and (1→3) (1→2)-β-Glucan. Microorganisms and mushrooms contain the branching structures of (1→3) (1→6) and (1→3) (1→2) whereas cereals, grasses, and mushrooms involve (1→3) (1→4) branching structure (Stone 2009; Synytsya and Novak 2014). Beta glucan is generally found in cell walls of sources. 2 – 4.83 % total dry mass of cereals, 11.26 – 14.57 % total dry mass of mushrooms and 8 – 16 % total dry mass of baker yeast consist of beta glucan (Klis et al. 2002; Maheshwari et al. 2017; Özcan and Ertan 2018; Sapirstein 2016). In comparison to other sources, *S.cerevisiae* yeast has the highest beta glucan content and it shows most of the biological activities of beta glucans. It is important to emphasize that properties of beta glucan changes from source to source due to differing branching structure of beta glucan. For this reason, beta glucan is used in different areas such as food, medicine, cosmetics. Moreover, usage areas of beta glucan have been increasing day by day thanks to new researches.

It is a well-known fact that the properties and yield of beta glucan produced by using different extraction methods from sources are affected by both source and extraction methods. Hence, extraction method should be optimized in order to obtain the beta glucan with requested properties such as yield, purity, molecular weight, antioxidant and antitumoral activity, swelling, fat binding, emulsion and foam capacity,

viscosity, etc. (Adachi et al. 2013; Du et al. 2014; Karimi et al. 2019; Khan et al. 2017; Volpato et al. 2018; Yılmaz 2010; Zhu et al. 2016). Khan et al. (2016) reported that decreased molecular weight by using irradiation resulted in an increase in the antibacterial activity against both gram-positive and gram-negative bacteria. This is because of the low molecular weight yeast β-d-glucan enters into the cell, disturbing the metabolism of microbes which in turn cause the lysis of the microbial cell (Khan et al. 2016; Kim et al. 2013; Kofuji et al. 2011; Valasquez-Junior et al. 2014). Alzorqi et al. (2014), it is aimed to compare the different extraction methods (Soxhlet, hot water and ultrasonic assested extraction) used to obtain beta glucan from *G.lucidum* mushroom. Beta glucan obtained from *G.lucidum* mushroom by ultrasonic assisted extraction method was found higher molecular weight, antioxidant activity, and appropriate branching degree compared to beta glucans obtained by the other two methods. Karimi et al. (2019), the aim of this study was to examine the effects of different enzymatic extractions used in the production of beta glucan from barley on the physicochemical properties of beta glucan and concluded that using several different enzymes increases the purity of beta glucan and decreases the molecular weight since they also cause degradation of beta glucan.

The main purpose of this study is to compare the performances of ultrasound assisted alkali-acidic and autolysis extraction methods to produce high yield of beta glucan and determine the effect of extraction method on the molecular weight of it.

2. Materials and Method

2.1. Chemicals and yeast culture strains

NaOH, acetic acid, absolute ethanol, sodium phosphate dibasic, sodium phosphate monobasic and all media components were purchased from Sigma-Aldrich, Merck and Oxoid. A pure strain of *Saccharomyces cerevisiae* NRRL Y-567 yeast was provided Northern Regional Research Laboratory, Peoria USA.

2.2. Yeast cultivation

Saccharomyces cerevisiae NRRL Y-567 yeast was incubated in the liquid growth media consisting of 20 g/L glucose, 3 g/L K_2HPO_4, 3.35 g/L $(NH_4)_2SO_4$, 3.76 g/L NaH_2PO_4, 0.52 g/L $MgSO_4.7H_2O$, 0.017 g/L $CaCl_2.2H_2O$ and 6 g/L yeast extract (Sabuncu, 2016). Yeast was inoculated from agar to 60 mL growth medium and incubated at 32°C, 150 rpm for 12 hours (Incubator / Shel Lab S16/SI6R). After the growth of yeast cells they were separated from the liquid medium by centrifugation at 3000 rpm for 15 min (Rotofix 2800) Then they were inoculated to the fresh liquid medium of 600 mL in the laminar flow cabinet (NuAire, Biological Safety Cabinet Class II) and incubated at 32°C, 150 rpm for 8 hours by using an orbital shaker. The growth medium was divided in to 50 mL falcon tubes and yeast cells were collected by using centrifugation at 5865 rpm for 15 min (HERMLE Z 326 K). Wet yeast cells were portioned as four equal weights to perform extraction procedures.

2.3. Ultrasound assisted alkali-acidic extraction (UA-AA)

Yeast cells into two falcon tubes were extracted by using an ultrasound assisted alkali-acidic extraction method. They were treated with 2 M NaOH solution (0.008 v/w) at 90°C for 1 hour in a heated ultrasonic water bath (40 kHz, 6 L, AZİM) applied ultrasonic waves at 40 kHz for 15 min at the beginning. Suspension was centrifuged at 5865 rpm, 4°C for 15 min. The precipitate was washed with pure water and centrifuged at 5865 rpm, 4°C for 5 min. After removing the supernatant 3 % acetic acid solution (0.008 v/w) was added and waited at 85°C for 1 hour in a water bath. Suspension was centrifuged at 5865 rpm, 4°C for 15 min. again. The precipitate was washed with pure water and centrifuged at 5865 rpm, 4°C for 5 min. The supernatant was removed and the precipitate was washed with absolute ethanol (0.008 v/w). The last centrifugation was conducted at 5865 rpm, 4°C for 15 min and the precipitate was dried in the oven (THERMOMAC FDO 30) until the samples reach constant weight. All these experimental procedures were realized as three replicated.

2.4. Ultrasound assisted autolysis extraction (UA-A)

Sodium phosphate buffer solution (pH 7.3, 30 % w/v) was added into the two falcon tubes contains the yeast cells. Suspensions were autolyzed at 50°C, 200 rpm for 24 hours in the incubator. After autolysis, ultrasonic wave was applied at 40 kHz for 15 min to the suspensions by using an ultrasonic bath. Then they were centrifuged at 5865 rpm, 4°C for 15 min. Precipitates were washed with pure water and were centrifuged at 5865 rpm, 4°C for 5 min. By the addition of 3 % acetic acid solution (0.008 v/w) they waited at 85°C for 1 hour in water bath. Suspensions were centrifuged at 5865 rpm, 4°C for 15 min. Precipitates were washed with pure water and centrifuged at 5865 rpm, 4°C for 5 min. Precipitates were washed with absolute ethanol (0.008 v/w). Suspensions were centrifuged at 5865 rpm, 4°C for 15 min. Precipitates were dried in the oven till they reach constant weight. Experiments were performed three replicated.

2.5. Determination of molecular weight

Beta glucans produced by two extraction methods were dissolved using tetrahydrofuran (THF, Riedel-de Haën, > 99 puriss, stabilized) in certain solid/solvent ratio (5 %) to obtain diluted polymer solutions in room temperature (0.05, 0.04, 0.03 and 0.02 mg/mL). Viscosity average molecular weights of the samples were determined by using Ubbelohde viscometer and Mark-Houwing equation (Equation 1). K and α constants of (1→3) (1→6)-β-Glucan is not present in the literature. So, K and α constants of cellulose nitrate were used in this study because the molecular structure of cellulose nitrate is similar to the (1→3) (1→6)-β-Glucan. K and α values for cellulose nitrate are $25x10^{-2}$ mL/g and 1.00, respectively. Firstly, the relative viscosities of beta glucan-THF solutions were measured by Ubbelohde viscometer in room temperature. These values were used to calculate the relative (ηr), specific (ηsp) and reduced viscosities (ηsp/C) of the solutions. In order to obtain the intrinsic viscosity (η), reduced viscosities were plotted versus the polymer solution concentration. Finally,

the viscosity average molecular weight of beta glucan samples was evaluated by using the Equation 1 is known as the Mark-Houwing equation.

$$[\eta]=K\cdot M_v^{\alpha} \quad (1)$$

2.6. Fourier Transform Infrared Spectroscopy (FTIR)

Pellets prepared with beta glucan sample and potassium bromide (KBr) by weigt 1:140 were analysed by Shimadzu FTIR-8400 S at room temperature between 4000-400 cm^{-1} wave number region. Beta glucan from Sigma was used as the reference sample (Glucan from baker's yeast (*S.cerevisiae*) CAS Number: 9012-72-0)

3. Results

3.1. Comparison of beta glucan yields produced from different extraction methods

Results were obtained from three replicated experiments realized for both extraction methods. As seen in Table 1, beta glucan yields obtained with UA-AA are lower than those obtained with the UA-A method. On the other hand, beta glucan yields of UA-AA-1 and UA-A-1 are higher than the others because yeast cells were harvested at the end of the 12 h of growth time however, yeast cell growth was retained for 8 h for the other experiments (UA-AA-2, 3 and UA-A-2,3).

3.2. The effect of extraction method on the molecular weight of beta glucan

Relative, specific and intrinsic viscosities obtained for different concentrations of beta glucan-THF solutions which are used for the calculation of viscosity average molecular weight were given in Table 2. Beta glucan-THF solutions were prepared with produced by both UA-AA and UA-A extraction methods. According to Table 2, as expected, viscosities decreases as the concentrations are lowered.

Table 1 Beta glucan yields obtained by two extraction methods

Extraction method	Beta glucan yield (g dry beta glucan/g dry yeast)
UA-AA-1	0.1500
UA-AA-2	0.0610
UA-AA-3	0.0486
UA-A-1	0.1708
UA-A-2	0.0774
UA-A-3	0.1278

UA-AA: Ultrasound assisted alkali-acidic extraction

UA-A: Ultrasound assisted autolysis extraction

Table 2 Relative, specific and intrinsic viscosities for different concentrations of beta glucan-THF solutions

Extraction method	Concentration (mg/mL)	Relative viscosity	Specific viscosity	Intrinsic viscosity
UA-AA-1	0.05	1.0476	0.0476	0.952
	0.04	1.0350	0.0350	0.876
	0.03	1.0201	0.0201	0.671
	0.02	1.0073	0.0073	0.364
UA-AA-2	0.05	1.0460	0.0460	0.920
	0.04	1.0345	0.0345	0.863
	0.03	1.0195	0.0195	0.650
	0.02	1.0071	0.0071	0.353
UA-AA-3	0.05	1.0472	0.0472	0.944
	0.04	1.0353	0.0353	0.883
	0.03	1.0202	0.0202	0.673
	0.02	1.0072	0.0072	0.360
UA-A-1	0.05	1.0511	0.0511	1.022
	0.04	1.0317	0.0317	0.793
	0.03	1.0207	0.0207	0.692
	0.02	1.0078	0.0078	0.388
UA-A-2	0.05	1.0516	0.0516	1.032
	0.04	1.0320	0.0320	0.800
	0.03	1.0212	0.0212	0.706
	0.02	1.0077	0.0077	0.387
UA-A-3	0.05	1.0504	0.0504	1.008
	0.04	1.0336	0.0336	0.840
	0.03	1.0228	0.0228	0.760
	0.02	1.0079	0.0079	0.395

Viscosity average molecular weights of the produced beta glucans by UA-AA and UA-A methods were given in Figure 1. It is seen from the Figure 1 that molecular weights of beta glucans produced by using UA-AA methods were higher than that molecular weights of beta glucans produced by using UA-A methods. By using UA-AA method viscosity average molecular weight was obtain about 110 kDa whereas was obtained 87 kDa by UA-A.

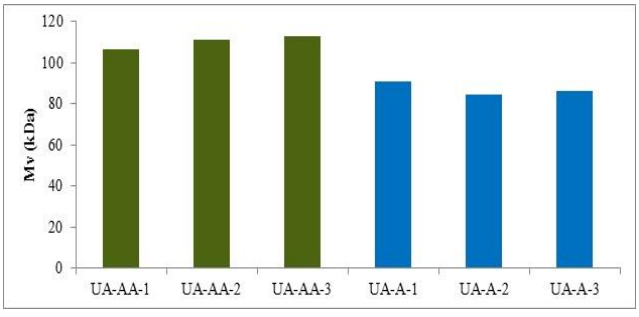

Figure 1 Molecular weights (M_v) of beta glucans produced by using UA-AA and UA-A methods

3.3. FTIR results

Figure 2 shows the FTIR spectra for the beta glucans produced by two different extraction methods. This analysis is also important in terms of produced beta glucan characterization because the characteristic peaks in this spectra determine the identity of the sample. Beta glucan from Sigma (Glucan from baker's yeast (*S.cerevisiae*) cas Number: 9012-72-0) was used as the reference sample. Peaks on 1030-1250 cm^{-1} wave number show the beta glycosidic bond for both produced beta glucans by different extraction methods and the reference sample.

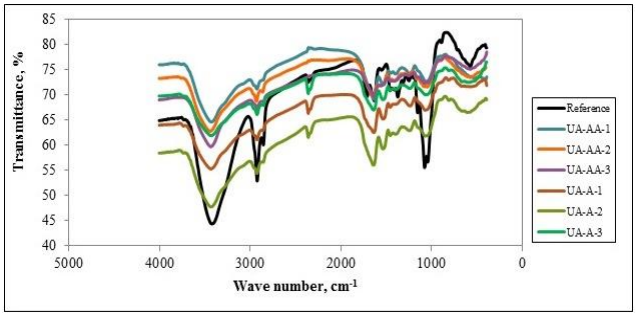

Figure 2 FTIR spectras of beta glucans

4. Discussion

S.cerevisiae yeast was used as a source in this study due to having highest beta glucan content and extensive biological activities of beta glucans. Freimund et al. (2003) used beta glucans produced from baker yeast using hot water and enzymatic extraction methods and beta glucan yield was found as 25-26%. Liu et al. (2008), produced the beta glucans from spent baker yeast using autolysis, hot water extraction, homogenization and enzymatic extraction methods and beta glucan yield was observed as 11%. Khan et al. (2016) by using alkaline-acidic extraction method yeast beta glucan yield was noticed 38.5%. Tang et al. (2017) beta glucan extracted yield as 19% from baker yeast using alkaline extraction method. Beta glucan is 8-16 % of dry cell wall of baker yeast. Normally, beta glucan yield obtained in the previous studies should be between 8 and 16%. Presented in the literature these extreme values of yield can not be comparable due to the different definition of it. Even beta glucan yield was not defined in most of the articles.

Effects of extraction methods on beta glucan production and properties were investigated in this study. The results show that higher yield of beta glucan was obtained by using UA-A method than UA-AA. A reason for the results are possible that UA-AA contains two-step cell wall disruption whereas UA-A has only one. Thus, because the beta glucan is a cell wall component of the yeast cell, a higher extraction yield was achieved by the extraction method containing the more efficient cell wall disruption. Otherwise, it was showed that the prolonged yeast growth can promote the beta glucan yield using different prolonged yeast growth time, but it should be confirmed by more of a replicated experimentation. Viscosity average molecular weights of beta glucans were determined to research effect of extraction methods on molecular weight of them. Lack of Mark-Houwing equation's coefficients for beta-glucan was led to an assumption of usage of cellulose nitrate coefficients in the Mark-Houwing equation due to the structural similarities of beta-glucan and cellulose nitrate to determine the viscosity average molecular weight of beta-glucan. Molecular weights of beta glucans produced by using autolysis extraction were found lower than the molecular weights of beta glucans produced by using alkali-acidic extraction since beta glucanase enzyme in yeast cell acts in autolysis extraction break up beta glucan molecule and cause of decreasing molecular weight of it. It is an obvious fact that extraction method is an important and critical parameter should be considered in beta glucan production and properties in light of these results.

5. Conclusion

Nowadays, beta glucan has been an important product according to its antitumoral, antimicrobial and antioxidant properties. Knowledge and usage areas about beta glucans increased by means of multidisciplinary researches (Murphy et al. 2020). The results show that extraction method affects both yield and molecular weight of beta glucan. Thus, the extraction method should be selected according to the requested interval value of molecular weight and providing high beta glucan yield. If lower molecular weight values besides high yields are required, the ultrasound assisted autolysis extraction method should be preferred. It can be concluded that beta glucan having the characteristic bonds is produced by both extraction methods.

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Authors' contributions:

SE planned and designed the experiments; FK carried out the experiments, SE and BA analyzed the data, SE and FK wrote the manuscript, SE and ZYH reviewed and editted the written manuscript.

Conflict of interest disclosure:

There is no conflict of interest.

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