

Application of Different Drying Methods on β -Glucan Isolated from Spent Brewer's Yeast Using Alkaline Procedure

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Summary

Water-insoluble (particulate) β -glucan was isolated from the cell walls of spent brewer's yeast using a single-step alkaline treatment. To stabilize β -glucan water suspensions, sonication was successfully applied. 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.

Key words

β -glucan, drying, sonication, spent brewer's yeast

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Introduction

Spent brewer's yeast is a by-product of beer production. Usually it is inactivated by heat and sold as animal feed supplement (Wheatcroft et al., 2002; Cook et al., 2003) but the rest is considered as industrial waste. It can be also used as active dry biomass, biologically active preparation containing dried living cells or as a raw-material for yeast extract and nucleotide production (Reed and Nagodawithana, 1991). Unfortunately, most of liquid brewer's waste containing yeast, ending in waste water disposal, leads to the pollution of natural water sources with organic material (Thammakiti et al., 2004). Therefore, spent brewer's yeast, as a good source of β -glucan and other byproducts, became an important raw material for the production of valuable substances (Supphantharika et al., 2003; Thammakiti et al., 2004; Liu et al., 2008). In that case, breweries could earn an extra income from extracting β -glucan as a high-value product that can improve human and animal health and immune system.

Yeast β -glucans are localized in the innermost layer of the cell wall. They are supporting the cell rigidity and maintain its shape. The cell wall consists of β -glucans, mannans, proteins, lipids and chitin (Klis 1994; Lipke and Ovalle 1998; Osumi 1998).

In this study, water-insoluble (particulate) β -D-glucan was isolated from spent brewer's yeast using a simple method. The purpose was to investigate the influence of ultrasonic treatment on the agglomeration and structural properties of isolated glucan suspensions. The further purpose was to investigate the influence of different drying methods on dimensions and microstructure of dried β -glucan particles.

Materials and methods

Isolation of yeast glucan. The starting material was spent brewer's yeast *Saccharomyces cerevisiae*, from Zagreb Brewery (Zagreb, Croatia). Briefly, after pretreatment debittered yeast was autolyzed and the obtained yeast cell walls served as a raw material for isolation.

During pretreatment, spent brewer's yeast was suspended in water and sieved through analytical sieve (125 μ m), followed by vacuum filtration. Yeast was then debittered by addition of sodium hydroxide (pH 10 / 50 °C / 10 min), followed by centrifugation (6000 rpm / 10 min) and washing three times with distilled water (Simard and Bouksaim 1998).

Debittered yeast was autolyzed (50 °C / 36 h) and yeast extract was removed by centrifugation (6000 rpm / 4 °C / 10 min) to remove yeast extract. The remaining yeast cell walls were washed three times with distilled water and then subjected to alkaline treatment (1M NaOH / 90 °C / 2 h) (Supphantharika et al., 2003). After centrifugation (6000 rpm / 4 °C / 10 min), supernatant was discarded and β -glucan sediment was washed three times with distilled water and then subjected to drying. Yield of the applied alkaline isolation procedure, calculated as a percent from dry weight of wet β -glucan and dry weight of spent brewer's yeast, was 13.64 % (Petravić-Tominac et al., 2009). Before drying, wet β -glucan was divided into three equal portions. The first portion was subjected to air drying, the second was lyophilized and the rest of the harvested wet material was used for sedimentation test, sonication and spray-drying. Preparations obtained by all

three drying methods were collected in bottles, weighted and stored in desiccator for further analyses.

Air-drying. Approximately one third of the isolated wet β -glucan was washed with ethanol. Ratio between wet weight of β -glucan and ethanol volume was 1:4 (w/v), i.e. 4 mL of ethanol was added to 1 g of wet β -glucan. After vacuum-filtration using Büchner funnel and filter paper (21/N, 80 g/m², Munktell & Filtrak GmbH, Germany), β -glucan was placed on the Petri dish and simply air-dried at room temperature.

Lyophilization. The second third of the harvested wet β -glucan was dispersed in several Petri dishes to form a layer of about a half a centimeter, frozen and then lyophilized (-50 °C / vacuum / 24 h) using Christ Gefriertrocknungsanlagen Freeze Dryer ALPHA 1-4 with controller LDC-1, (Martin Christ Gefriertrocknungsanlagen GmbH, Germany).

Sedimentation of β -glucan preparations. Wet β -glucan sediment, containing 7.33 % of dry weight, was suspended in water to dry matter concentration of 1.5 % (w/v), as described by Hunter et al. (2002). The resulting suspension was homogenized by vigorous stirring for one minute and divided into six 50-ml Falcon conical tubes. The tubes were vortexed for 20 seconds and allowed to settle for 0, 2, 5, 10, 30 and 60 minutes. Photographs of sedimentation procedure were taken by digital camera.

Sonication of β -glucan preparations. β -Glucan suspension, containing 1.5 % (w/v) of wet β -glucan, was prepared as described in previous section and homogenized by vigorous stirring for one minute. Sonication was performed as described by Hunter et al. (2002). The glass containing 250 mL of suspension was immersed in ice and water mixture and sonicated via 6-mm probe utilizing Bandelin Sonoplus HD 2070 with probe UV 2200 (Bandelin Electronic, Germany) for 15 min (48-s sonic cycles with a 12-s pause between cycles), using an ultrasonic output frequency of 12 kHz/s at 192 Watts (Hunter et al., 2002). After vigorous stirring for one minute, the sonicated suspension was equally distributed into six 50-ml Falcon conical tubes. Each tube was vortexed for 20 seconds and sedimentation test of the treated suspension was performed in 0, 2, 5, 10, 30 and 60 minutes intervals, as described in previous section. Sedimentation properties of sonicated and non-sonicated suspensions were compared.

Spray-drying. Sonicated β -glucan material was spray-dried using Büchi 190 Mini Spray Dryer (BÜCHI Labortechnik AG, Flawil, Switzerland) with an inlet air temperature of 146 °C and an outlet air temperature of 82 °C. The sparging velocity was 0.25 L/h, and the flow of compressed air was 700 L/h.

Analyses. To determine microscopic morphology of β -glucan particles, preparations were suspended in distilled water, observed under bright-field illumination and the photographs were taken by digital camera. Size distribution of dried particles was measured, based on laser beams diffraction principle, using laser particle sizer Analysette 22, Nano Tec, Fritsch (Germany) for air-dried samples during preliminary experiments and Malvern Mastersizer 2000 with HydroUnit S (Malvern Instruments Ltd., Worcestershire, UK) for lyophilized and spray-dried samples in our later experiments. Dry weight was determined by drying to constant weight (Mousdale, 1997). Polysaccharides in wet β -glucan sediment were hydrolyzed using sulfuric acid (Dallies et al., 1998) and trifluoroacetic acid (TFA) (Freimund et al.,

2005). Dry β -glucan samples were hydrolyzed only according to Freimund et al. (2005). After acidic hydrolysis, anthrone method (Mousdale, 1997) was used to analyze total carbohydrates in the hydrolyzates. Proteins were determined by Kjeldahl method (Mousdale, 1997). Organic elemental microanalysis (carbon, hydrogen and nitrogen content) of lyophilized β -glucan sample was performed using Perkin Elmer 2400 Series II CHNS Analyser. All analyses were done in triplicate.

Results and discussion

The most of the published procedures for isolation of particulate β -glucan from yeast *Saccharomyces cerevisiae* involved hot alkaline or acidic treatment or the combination of both (Williams et al., 1991; Müller et al., 1997; Chorvatovičová et al., 1999; Šandula et al., 1999; Freimund et al., 2003; Hromádková et al., 2003; Thammakiti et al., 2004; Machová et al., 2005; Zechner-Krpan et al., 2010). Organic solvent treatment is sometimes used for residual lipid removal (Donzis 1993; Šandula et al., 1995; Müller et al., 1997; Hunter et al., 2002; Jordan et al., 2002). Most of the mentioned procedures are multi-step processes, because both alkaline and acidic treatments are often performed several times. In this work, optimized parameters of single-step alkaline isolation procedure (Suphantharika et al., 2003) were applied and combined with three different drying methods.

Sedimentation properties and morphology of β -glucan

The appearance of wet β -glucan was examined by light microscopy (Fig. 1). Although the morphology of single glucan particles resembled yeast cell wall morphology, they showed significant agglomeration and formed big clumps.

The macrophage phagocytosis is more enhanced by micro-particulate β -glucan than by its aggregated form (Hunter et al., 2002). Biological activity of β -glucan can be improved by reducing the size of its particles. Agglomerates can be disrupted mechanically (Donzis 1995; Donzis 1998; Hromádková et al., 2003) or by ultrasound (Hunter et al., 2002). In our work, sonication treatment was applied to eliminate β -glucan agglomeration and to stabilize its water suspension. Lower sedimentation rate of sonicated glucan particles was proved according to Hunter et al. (2002). Before ultrasonic treatment, β -glucan suspension showed clearly visible settling immediately after homogenization, while sedimentation was almost fully completed after 5 - 10 minutes (Fig. 2a). Sonicated suspensions of wet β -glucan were stabilized, showing no settling tendency even after 60 minutes (Fig. 2b). Such sonicated solid particles remained dispersed in water and because of that can have beneficial characteristics for potential application in the pharmaceutical preparations.

The choice of drying method is very important for particle dimensions, their structure and biological activity (Hromádková et al., 2003). Spray-drying keeps biological activity of β -glucan to a greater extent and has advantage in comparison with air-drying and lyophilization. Air-drying of sonicated β -glucan from baker's yeast leads to reaggregation. But, sonication applied prior to spray-drying enables production of fine powder with few small aggregates (Hunter et al., 2002). In our work, similar sonication procedure was combined with spray drying, resulting in a fine non-aggregated powder. Due to very fast water



Figure 1. Light microscopy of wet β -glucan material obtained after alkaline isolation (magnification 400 $\times$, bar = 1 μ m)

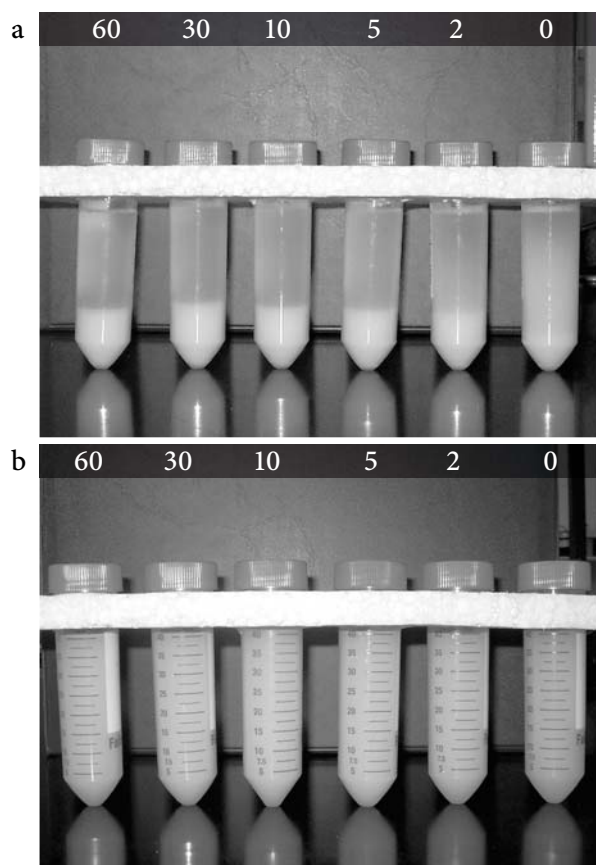


Figure 2. (a and b) Sedimentation of alkaline isolated β -glucan, measured at different time periods (0; 2; 5; 10; 30 and 60 min): a) before sonication; b) after sonication

evaporation, the original microstructure of β -glucan particles was preserved and they kept the oval to elliptical shape of the yeast cells (Fig. 3a). There was no agglomeration tendency even when such obtained, spray-dried particulate material was re-

Table 1. Chemical composition of alkaline isolated β -glucan, dried by three different methods

Samples	% Dry weight (g dry weight/100 g of sample)	% Total carbohydrates ^a (g carbohydrates/100 g dry weight)	% Proteins ^d (g proteins /100 g dry weight)	Elemental analysis ^f (%)		
				C	H	N
Wet β -glucan	7.33 $\pm$ 0.479	90.54 $\pm$ 4.590 ^b 93.73 $\pm$ 2.195 ^c	n.d. ^e		n.d. ^e	
Air-dried β -glucan	92.00 $\pm$ 0.209	91.73 $\pm$ 3.352 ^c	5.17 $\pm$ 0.023		n.d. ^e	
Lyophilized β -glucan	96.75 $\pm$ 0.364	91.12 $\pm$ 4.186 ^c	5.13 $\pm$ 0.029	44.33	7.08	0.87
Spray-dried β -glucan	98.24 $\pm$ 0.356	91.43 $\pm$ 3.949 ^c	3.16 $\pm$ 0.042		n.d. ^e	

^a expressed as glucose equivalent and analyzed using anthrone reagent (Mousdale 1997); ^b hydrolyses performed using sulphuric acid according to Dallies et al. (1998);

^c hydrolyses performed using trifluoroacetic acid (TFA) according to Freimund et al. (2005); ^d analyzed according to Kjeldahl method (Mousdale 1997);

^e not determined; ^f organic elemental analysis was performed using Perkin Elmer 2400 Series II CHNS Analyser.

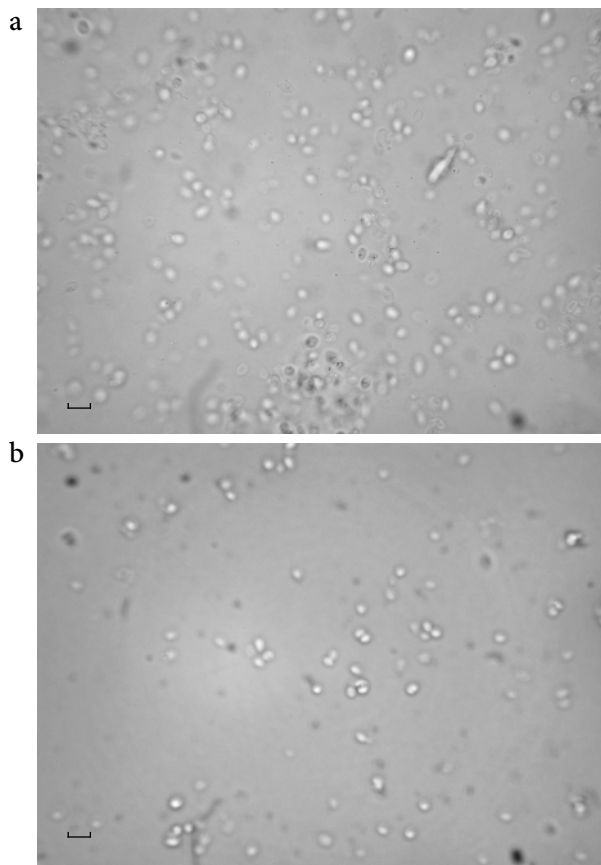


Figure 3. (a and b) Alkaline isolated β -glucan: (a) after ultrasonic treatment; (b) after ultrasonic treatment, spray-drying and resuspending in distilled water (magnification 400 $\times$, bar = 1 μ m)

suspended in water (Fig. 3b), which is in agreement with results published by Hunter et al. (2002).

Morphology of spray-dried and lyophilized β -glucan obtained in this work is in agreement with the morphology of preparations obtained by different isolation method from baker's yeast (Hromádková et al., 2003). During lyophilization process, water was frozen and removed as solid by sublimation and the particles were distorted and compressed into sheet-like layers with porous surface. The air-drying resulted in larger, granular β -glucan particles.

Table 2. Particle size distribution^a of β -glucan obtained after air-drying and mechanical grounding

Portion of particles [%]	Size interval of β -glucan particles [μ m]
5	4.744
10	10.202
20	22.729
30	26.892
40	30.320
45	31.946
50	33.572
60	37.073
70	40.688
80	45.568
90	52.625

^a measured by laser particle sizer Analysette 22, Nano Tec, Fritsch, Germany

Chemical composition of alkaline isolated β -glucan

Basic chemical composition of the obtained β -glucan preparations is shown in Table 1. Total carbohydrates, expressed as glucose equivalents, were higher than 90 %. Two methods of hydrolysis (Dallies et al., 1998; Freimund et al., 2005), previously optimized in analysis of yeast cell wall polysaccharides, were applied for analyses of wet β -glucan sediment. The obtained results corresponded well for both methods. For dry samples, hydrolysis using trifluoroacetic acid was chosen due to its simplicity and shortness (Freimund et al., 2005). The amounts of total carbohydrates in wet β -glucan preparations were in agreement with dried samples and with literature data as well (Supphantharika et al., 2003; Thammakiti et al., 2004; Liu et al., 2008).

Although in this study no differential characterization between β -1,3- and β -1,6-glucan linkages was done, it is already known that alkaline insoluble β -glucan contains predominantly 1,3- β -linkages with small amount of β -1,6-glucan (Manners et al., 1973).

Results of elemental analysis were similar to those published for β -glucan preparations isolated from baker's yeast (Sauter et al., 2004) and the present nitrogen amount corresponded well with protein content.

Particle dimensions in dried β -glucan preparations

After grounding, 90 % of air-dried particles belong to the class which size was smaller or equal to 52.625 μ m (Table 2). The particles having 1-2 μ m in diameter are optimally phagocytized by macrophages (Hunter et al., 2002).

Table 3. Dimension of β -glucan particles^a isolated by alkaline method and dried by lyophilization and spray-drying

Samples	d (0.1) ^b [μ m]	d (0.5) ^c [μ m]	d (0.9) ^d [μ m]	D [4,3] ^e [μ m]
Alkaline isolation+ lyophilization	2.663	4.26	6.729	4.516
Alkaline isolation+ sonication + spray-drying	4.49	9.609	19.253	10.927

^a measured by Malvern Mastersizer 2000 with HydroUnit S (Malvern Instruments Ltd., Worcestershire, UK); ^b d(0.1) – 10 % of the present particles has dimensions up to the stated value [μ m]; ^c d(0.5) – 50 % of the present particles has dimensions up to the stated value [μ m]; ^d d(0.9) – 90 % of the present particles has dimensions up to the stated value [μ m]; ^e D [4, 3] - diameter of the sphere whose volume is equal to an average particle volume [μ m]

Average dimensions of native brewer's yeast cells before β -glucan alkaline isolation, measured by light microscopy using measuring ocular and object micrometer, were 6.52 x 8.8 μ m (data not shown). Despite the presence of clumps formed during lyophilization, ultrasound treatment inside the measuring instrument disrupted their fragile aggregated structure before particle size measurement. Due to deformations and compression of its original structure (Hromádková et al., 2003), dimensions of single lyophilized particles were a bit diminished in comparison with dimensions of the yeast cell wall. It was shown that 50 % of lyophilized particles had dimensions up to 4.26 μ m and 90 % of them belong to class which dimensions were up to 6.729 μ m (Table 3). Sonication treatment combined with spray-drying resulted in preparations containing 50 % of particles up to 9.609 μ m (Table 3). Native shape of spray dried particles was conserved and they were about the same size as native yeast cells. Though, 90 % of spray-dried particles had dimensions up to 19.253 μ m, suggesting some extent of agglomeration.

The intention was to put our preparations in relation with other similar products, to foresee their possible biological activity and the potential applications in other fields of industry. Therefore the measured particle dimensions were compared with literature data.

Size of particles in our spray-dried samples was similar to those of spray-dried samples obtained from baker's yeast using other isolation methods (Hromádková et al., 2003; Sauter et al., 2004). It was also smaller than in some commercial β -glucan preparations isolated from baker's yeast and intended for immunostimulation.

β -Glucan isolated from brewer's yeast by Thammakiti et al. (2004) contained several times larger particles, whose dimensions were 32.89 and 36.77 μ m. By comparing particles size, we can suppose that our spray-dried preparation should probably have more favorable dimensions for immunostimulation (Hunter et al., 2002), but this should be additionally confirmed in the future experiments.

Good performance in food systems was confirmed for β -glucan particles having more than 30 μ m in diameter (Worrasinchai et al., 2006; Santipanichwong and Suphantharika, 2007), originating from brewer's yeast. Properties of our β -glucan preparations important for their application in food production will be investigated in our further studies.

Conclusions

A single-step alkaline treatment of spent brewer's yeast is a suitable procedure for effective isolation of water-insoluble, particulate β -glucan. Suspended β -glucan particles showed

high tendency of agglomeration. Sonication was successfully used to avoid agglomeration and to improve the stability of the β -glucan suspension. Air-drying and lyophilization provoked significant microstructure changes and agglomeration. When combination of sonication and spray-drying was applied, the original β -glucan microstructure retained and such obtained dried particles had smaller dimensions than other known glucans isolated from brewer's yeast. Reaggregation of spray-dried β -glucan particles was minimal even after resuspending in water. Additional characterization of the dried β -glucan preparations should be done to put these results into practice in medicine and in different fields of industry.

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