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ISSN 1330-9862
(FTB-2373)

original scientific paper

Characterization of β-Glucans Isolated from Brewer's Yeast and Dried by Different Methods

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Received: November 10, 2009
Accepted: January 27, 2010

Summary

Two different procedures have been used for isolation of water-insoluble β-glucans from brewer's yeast: alkaline-acidic isolation (AA) and alkaline-acidic isolation with mannoprotein removal (AAM). The obtained β-glucans were then dried by air-drying, lyophilization and combination of sonication and spray-drying. β-Glucan preparations obtained by AA and AAM isolations had similar values of dry mass, total polysaccharides, proteins and organic elemental microanalysis. The mass fractions of β-glucan in total polysaccharides were significantly affected by different isolation procedures. Fourier transform infrared (FTIR) spectra of all preparations had the appearance typical for (1→3)-β-D-glucan. Lyophilization and especially air-drying caused a higher degree of agglomeration and changes in β-glucan microstructure. Sonication followed by spray-drying resulted in minimal structural changes and negligible formation of agglomerates.

Key words: β-glucan, β-glucan isolation, brewer's yeast, *Saccharomyces cerevisiae*

Introduction

β-D-Glucans are found in bacteria, algae, yeasts, mushrooms, moulds and higher plants, and consequently, their structure depends on the source they were isolated from (*1–3*). Each of them possesses many interesting properties and can improve human and animal health and immune system (*1,2,4–11*). (1→3)-β-D-Glucans are classified as biological response modifiers (BRMs) (*4,12*). There are different opinions on which molecular mass, shape, structure, and source of β-glucans provide the greatest therapeutic benefit (*12,13*). According to the literature, the most biologically active form of β-glucans are those that contain 1,6-connected side-chains branching off from the longer β-1,3-glucan backbone and are referred to as β-1,3/1,6-glucan (*14*). Both soluble and insoluble β-glucans can find their beneficial applications. Their applications in pharmaceutical and chemical industries (*15*), and also in cosmetics (*16,17*) are well described.

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β-Glucans from different sources have potential application in food production as food thickeners or fat replacers, dietary fibres, viscosity imparting agents, emulsifiers, and films (*15,18–38*).

Cell wall of yeast *Saccharomyces cerevisiae* is one of the most common sources of β-1,3/1,6-glucan. Although baker's yeast is more often mentioned as a raw material for its isolation (*4,13,39–45*), it has been shown that brewer's yeast can also be successfully used as a source of β-glucan. According to the published literature, β-glucans isolated from brewer's yeast are mainly intended for food production (*10,23,46–48*), but there is also possibility of their use in immunostimulation (*9*).

Spent brewer's yeast is a by-product of beer production that could be used in different ways (*8,18,49,50*), but it is still not adequately exploited. At the same time, most of brewery liquid waste ends in wastewater disposal, leading to the pollution of natural water sources with organic material. There are a variety of components that can achieve a greater commercial value than the brewer's yeast itself and maximize the total profitability of the process (*20*). One of such compounds is yeast cell wall polysaccharide β-D-glucan.

In this paper, two isolation procedures are applied to isolate β-glucan from spent brewer's yeast: alkaline-acidic (AA) and alkaline-acidic isolation with mannoprotein removal (AAM). The preparations were dried using three different methods and characterized for composition and structure.

Materials and Methods

Isolation procedures

Spent brewer's yeast (6th generation), obtained from Zagrebačka pivovara (Zagreb, Croatia), was used as a raw material for isolation of water-insoluble β-glucan. Two different isolation procedures were used: alkaline-acidic isolation (AA) and alkaline-acidic isolation with mannoprotein removal (AAM). Wet sediments isolated by both procedures were subjected to drying. A short scheme of the performed procedures is shown in Fig. 1.

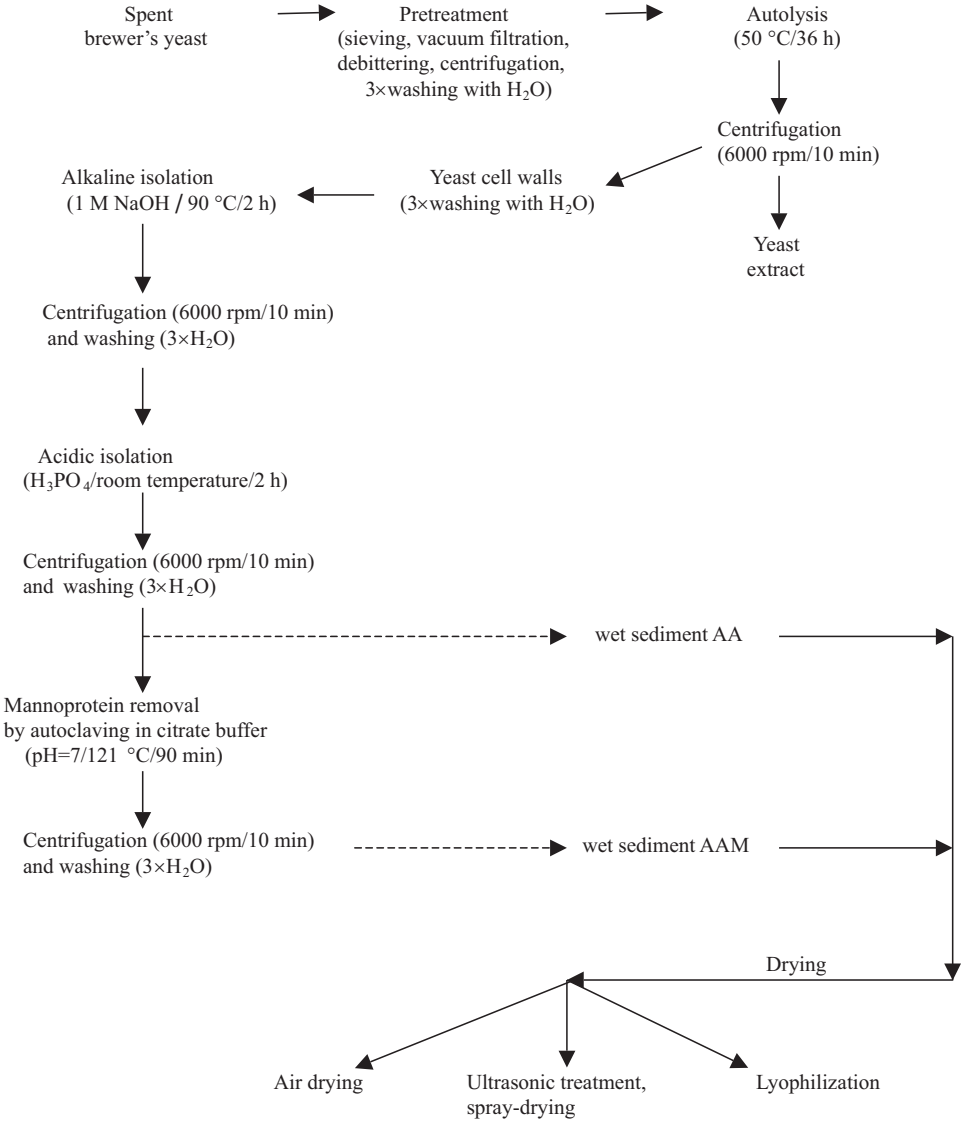

Fig. 1. Scheme of β-glucan isolation procedures: alkaline-acidic (AA) and alkaline-acidic with mannoprotein removal (AAM)

Yeast pretreatment and autolysis

Pretreatment began by sieving yeasts through analytical sieve (125 μm) and vacuum filtration. Yeast debittering using alkaline wash (NaOH, pH=10, at 50 °C for 10 min) (*51*) was followed by centrifugation (6000 rpm for 10 min) and washing three times in distilled water.

After the pretreatment, yeast autolysis (50 °C for 36 h) was performed (*20*). Yeast extract was separated by centrifugation (6000 rpm at 4 °C for 10 min) and the remaining cell walls were washed three times in distilled water and used for isolation of water-insoluble β-glucan.

Alkaline-acidic procedure (AA)

After alkaline treatment (1 M NaOH at 90 °C for 2 h) and centrifugation (6000 rpm at 4 °C for 10 min), the supernatant was discarded. Sediment was washed three times in distilled water, centrifuged (6000 rpm at 4 °C for 10 min) and subjected to acidic treatment (4 % phosphoric acid at room temperature for 2 h). The obtained material (AA sediment) was washed three times in distilled water and centrifuged (6000 rpm at 4 °C for 10 min).

Alkaline-acidic procedure with mannoprotein removal (AAM)

The sediment that remained after the described alkaline-acidic treatment was additionally treated to remove the residual mannoproteins according to Ballou *et al.* (*52*), by autoclaving in citric buffer (pH=7, 121 °C for 90 min). After cooling at room temperature, the sediment was separated by centrifugation and washed three times in distilled water.

Drying procedures

Upon isolation, wet β-glucans isolated by AA and AAM procedures were divided into three equal shares and used for three different drying procedures: air-drying (AD), lyophilization (L) and combination of ultrasonic treatment with spray-drying (SD). Dried β-glucans were collected into bottles, weighed and stored in a desiccator for further analysis.

Air-drying

Upon isolation, approximately one third of wet AA and AAM sediments were first washed with absolute ethanol. After vacuum-filtration performed using Büchner funnel, the sediments were put in a Petri dish and simply air dried at room temperature.

Lyophilization

One third of the wet AA and AAM sediments were dispersed in Petri dishes, frozen and then lyophilized (–50 °C, vacuum for 24 h) using Christ Gefriertrocknungsanlagen freeze dryer Alpha 1–4 with controller LDC-1 (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

Sonication and spray-drying

The rest of the isolated wet β-glucan sediments were suspended in water to dry matter concentration of 1.5 % (by mass per volume). Sedimentation properties of the suspensions were tested according to Hunter *et al.* (*43*). Before sonication, tubes containing homogenized β-glucan water suspensions were immersed in an ice and water mixture. Ultrasonic treatment was performed for 15 min (48-second sonic cycles with a 12-second pause between cycles) with 6-mm probe utilizing Bandelin Sonoplus HD 2070 with probe UV 2200 (Bandelin Electronic, Berlin, Germany), using an ultrasonic output frequency of 12 kHz/s at 192 W.

After ultrasound treatment, water suspensions of β-glucan were spray-dried using Büchi 190 Mini Spray Dryer (Büchi Labortechnik AG, Flawil, Schwitzerland) 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.

Sedimentation test

The sedimentation test was performed using wet β-glucan preparations before and after sonication. Suspensions containing 1.5 % (by mass per volume) of wet β-glucan sediment were vortexed for 10 s and then allowed to settle in 50-mL test tubes for 0, 2, 5, 10, 30 and 60 min (*43*). Sedimentation properties of suspensions containing sonicated and non-sonicated β-glucans were compared.

Analysis

Preparation A, obtained previously (*53*) by alkaline isolation, was used for comparison of chemical composition, particle dimensions and structural properties with β-glucan preparations obtained by AA and AAM isolation procedures. The mass fraction of β-glucan in preparation A, as well as the elemental composition and Fourier transform infrared (FTIR) spectra were additionally determined in this paper. The chemical composition, elemental analysis and infrared spectra of the isolated preparations were typical for β-glucans. Samples of analyzed β-glucans are shown in Table 1.

Table 1. Samples of β-glucans isolated, dried and analyzed in this work

Isolation procedure	Drying method	Sample designation
A (alkaline isolation)*	spray-drying	A-SD
	lyophilization	A-L
	air-drying	A-AD
AA (alkaline-acidic isolation)	spray-drying	AA-SD
	lyophilization	AA-L
	air-drying	AA-AD
AAM (alkaline-acidic isolation with mannoprotein removal)	spray-drying	AAM-SD
	lyophilization	AAM-L
	air-drying	AAM-AD

*samples isolated in our previous experiments (*53*) and used for comparison

Scanning electron microscope Philips XL30 ESEM Tungsten, Philips, Eindhoven, the Netherlands (Edax detector, type PV 9760/68 ME, resolution 134.30 eV; BSE detector, Philips PW 6848/00) and software EDAX Gene-

sis v. 5.21 were used for characterization of air-dried, lyophilized and spray-dried specimens. Photographs were taken at an accelerating voltage of 25.0 kV under recording time of 5 seconds. Diameter of observed area was 10 mm.

Size distribution of dried particles was measured based on the laser beam diffraction principle, using Malvern Mastersizer 2000 with HydroUnit S (Malvern Instruments Ltd, Worchestershire, UK).

Dry mass of β-glucan samples was determined by drying to constant mass (*54*). Acidic hydrolysis, using trifluoroacetic acid (TFA) (*55*) or sulphuric acid (*56*), was performed and the obtained hydrolyzates were further used both for analysis of total carbohydrates and determination of β-glucan mass fraction. Hydrolyzates were analyzed by anthrone method (*54*) to obtain total carbohydrates, expressed as glucose equivalents. Glucose concentrations in hydrolyzed samples were determined with high performance liquid chromatography (HPLC) and used to calculate the mass fraction of β-glucan in total carbohydrates. Glucose was determined using HPLC Shimadzu Prominence, equipped with detector Shimadzu RID-10A (Shimadzu, Kyoto, Japan), column Shodex Asahipax NH2P-50 4E and guard column Shodex Asahipax NH2P-506 4A (Showa Denko KK, Munich, Germany). Acetonitrile/water (75:25) was used as a mobile phase at a flow rate of 1 mL/min and column temperature of 40 °C. The time of analysis was 12 min. The mass fraction of β-glucan was calculated from glucose concentrations in hydrolyzates, analyzed by HPLC, and glucose equivalents determined in the same samples.

Proteins were analyzed by Kjeldahl method (*54*). Organic elemental microanalysis (carbon, hydrogen and nitrogen) of lyophilized β-glucan samples was also performed using PerkinElmer 2400 Series II CHNS analyzer (PerkinElmer, Waltham, MA, USA). Infrared spectra were recorded as KBr pastilles with a Bomem MB 100 mid FTIR spectrophotometer (ABB Bomem Inc, Quebec, Canada).

Results and Discussion

Most of the published procedures for isolation of insoluble β-glucan from yeast *Saccharomyces cerevisiae* involve different hot alkaline or acidic treatments or the combination of both (*13,23,42,57–59*). Organic solvent treatment is sometimes used for the removal of residual lipids (*40,43,44*). Most of the mentioned procedures are multi-step processes, where each step (alkaline, acidic and organic solvent treatment) is often repeated.

The goal of our experiments was to compare the composition, structure and sedimentation properties of β-glucans obtained by different isolation and drying procedures, using brewer's yeast as a raw material. At the same time, the intention was to avoid time-consuming procedures and to apply different drying methods to obtain preparations suitable for possible application in different fields of industry.

Chemical composition of isolated β-glucans

Basic chemical composition of β-glucans isolated with AA and AAM procedures and dried using different methods is shown in Table 2. Water content was lowered from around 88 % in wet preparations to the values of (2.8–10.9) % (by mass), depending on the drying method applied.

Yields of AA and AAM procedures, calculated as percentage of dry mass in wet β-glucan preparations and dry mass of spent brewer's yeast, were 11.84 and 8.08 %, respectively (*60*).

Two methods of hydrolysis were previously optimized by Dallies *et al.* (*56*) and Freimund *et al.* (*55*) for analyzing yeast cell wall polysaccharides. Both hydrolysis methods were applied for the analysis of wet β-glucans and the obtained values of total carbohydrates in wet samples were similar (data not shown). The method using trifluoroacetic acid (TFA) (*55*) was chosen for hydrolyzing the dry samples because of its simplicity and shortness. In dried samples, isolated by both AA and AAM procedures, the values of total carbohydrates were higher than 90 % (Table 2). They were in agreement with the values of total carbohydrates in dried samples isolated by procedure A (*53*). Amounts of total carbohydrates in wet samples (data not shown) corresponded well to those in dried samples.

Protein levels were almost equal in all dried samples, regardless of which drying method was used (Table 2), and corresponded well to protein levels of previously isolated preparation A (*53*). The similarity of protein concentrations in all samples can be explained by the well-known affinity between proteins and β-glucans (*61*). Most of the proteins present in our β-glucan preparations are

Table 2. Chemical composition of β-glucans obtained by AA and AAM procedures and dried by three different procedures

Samples	*w*(dry mass) / %	*w*(total carbohydrates)* / %	*w*(proteins)* / %	Elemental analysis* *w*/%		
				C	H	N
AA-AD	89.11±0.373	94.11±3.197	5.52±0.082	n.d.	n.d.	n.d.
AA-L	97.33±0.454	92.85±3.697	5.30±0.186	43.19	7.20	0.87
AA-SD	95.53±0.069	92.24±4.030	5.42±0.135	n.d.	n.d.	n.d.
AAM-AD	95.25±0.446	93.04±3.412	3.90±0.067	n.d.	n.d.	n.d.
AAM-L	97.20±0.355	91.42±3.236	4.08±0.027	44.14	7.51	0.68
AAM-SD	95.44±0.207	92.68±3.017	4.14±0.045	n.d.	n.d.	n.d.

n.d. – not determined

*calculated as percentage of dry mass

intracellular proteins that are not attached to mannan and have high affinity for β-glucan (*61*). Although part of mannoproteins is removed already in alkaline step of isolation (*23*), additional mannoprotein removal is performed after acidic treatment in AAM procedure. Proteins bound to mannan are present just in small percentage in these β-glucan preparations and their removal does not change significantly the amount of proteins. The amounts of nitrogen, determined by organic elemental microanalysis (Table 2), corresponded well with protein content. In comparison with Seeley (*19*), protein amounts in β-glucan preparations shown in this paper were twice lower.

Amounts of total carbohydrates in AA and AAM preparations (Table 2) were in agreement with literature data obtained for other β-glucans isolated from different brewer's yeast strains (*9*,*10*,*23*). Both preparations AA and AAM isolated in this work had similar values of dry mass, proteins and organic elemental microanalysis (Table 2). Elemental analysis performed by Sauter *et al.* (*62*) and Zeković *et al.* (*63*) corresponded to our results.

Alkaline-isolated preparation (A) used for comparison contained (91.12±2.783) % (by mass) of glucan in total carbohydrates (*53*). In this study, no differential characterization between β-1,3- and β-1,6-glucan linkages was done, but it is already known that alkaline-insoluble β-glucan contains predominantly 1,3-β-linkages with a small amount of β-1,6-glucan (*64*). β-Glucan insoluble in alkaline solution and soluble in acid contains mainly β-1,6-linkages and a small percentage of β-1,3-glucan (*65*).

During AA procedure, subsequent acidic treatment resulted in the extraction of β-1,6-glucan with minor amounts of β-1,3-glucosidic branches (*59*), while β-1,3--glucan remained in the insoluble fraction. Therefore, the mass fraction of β-glucan in total carbohydrates in AA sample decreased to 69.73 %. In the preparation AAM, obtained after mannoprotein removal, the mass fraction of β-glucan was increased to 99.12 %.

The influence of drying procedure on β-glucan morphology and particle dimensions

It is known that immunological activity of β-glucan depends on particle dimensions and can be improved by reducing the size of the particles. Agglomerates can be disrupted mechanically (*16*) or using ultrasound (*43*, *44*).

Effects of sonication on the stability of suspensions containing wet β-glucans from AA and AAM procedures were tested according to Hunter *et al.* (*43*). Before ultrasonic treatment was performed, the sedimentation of all three suspended preparations (A, AA, AAM) began immediately after homogenization and was completed almost fully after 5–10 min. Sonicated suspensions of all wet β--glucans were stable even after 60 min and showed no settling tendency. Different isolation procedures made no difference in sedimentation properties of the tested suspensions. Stabilization of β-glucan suspensions, achieved by sonication, is a beneficial characteristic important for potential application in the pharmaceutical preparations. When compared to the aggregated form of β-glucan, the β-glucan microparticles remain in suspension longer, which is important for pharmaceutical applications. Such homogenous suspensions are also more effective in enhancing phagocytosis by peritoneal macrophages following oral administration (*43*).

Hunter *et al.* (*43*) found that air-drying of sonicated β-glucan isolated from baker's yeast leads to reaggregation, while spray-drying of sonicated β-glucan enables the production of fine powder containing a few small aggregates. Similar to the mentioned paper, in our work sonication was applied on isolated wet β-glucans of different purities and combined with spray-drying.

It is known that β-glucan particle dimensions and their structure depend on the selection of drying method, so does its biological activity (*13*). The microstructures of dry preparations isolated by AA procedure are shown in Figs. 2–4 as an example of microscopic morphology which is typical for each drying method. The appearance of the samples corresponded well to those isolated by A and AAM procedures (*53*). Morphology and microstructure of the preparations were not obviously influenced by different isolation procedures. Microscopic structures (Figs. 2–4) were affected by different drying methods.

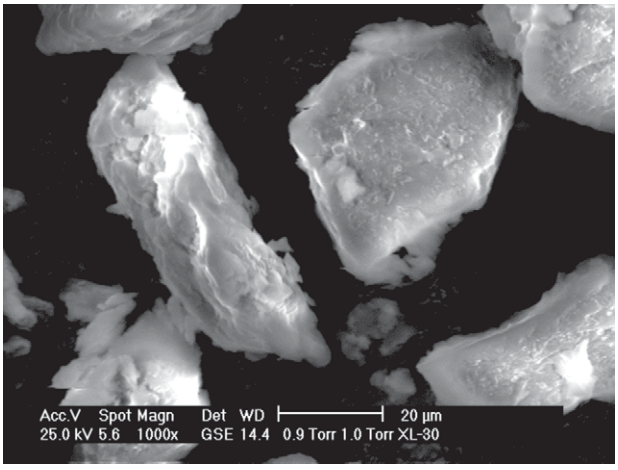

Fig. 2. Microscopic structure of air-dried β-glucan isolated by alkaline-acidic (AA) procedure

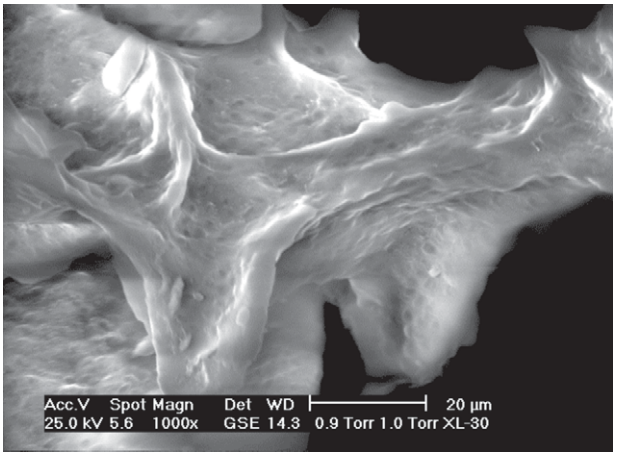

Fig. 3. Microscopic structure of lyophilized β-glucan isolated by alkaline-acidic (AA) procedure

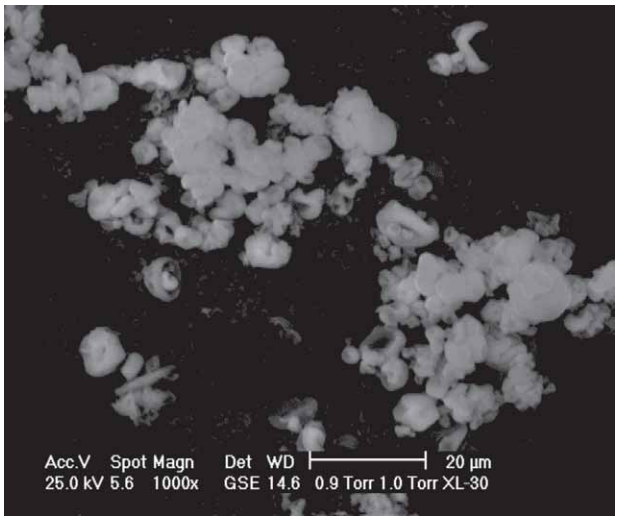

Fig. 4. Microscopic structure of spray-dried β-glucan isolated by alkaline-acidic (AA) procedure

In our experiments, morphology of dried β-glucans isolated from brewer's yeast (Figs. 2–4) was in agreement with the morphology of similar preparations obtained by different isolation procedures from baker's yeast and dried using the corresponding methods (*13*,*39*).

Air-drying resulted in large, granular β-glucan particles (approx. 300 μm) (*53*) that need to be disrupted. They were characterized by irregular shape and sharp edges and consisted of agglomerated residues of yeast cell walls (Fig. 2).

Lyophilized particles were distorted and compressed into sheet-like layers with porous surface and opened structure, but also with a significant degree of agglomeration (Fig. 3).

Spray-drying of sonicated β-glucans preserved the original microstructure of the particles that kept the oval to elliptical shape of the yeast cells (Fig. 4). Those particles showed weak agglomeration tendency and formed fine and non-aggregated powder. Table 3 shows parameters describing particle size distribution.

In the previous work (*53*) the observed average dimensions of native brewer's yeast cells before β-glucan isolation were 6.52×8.8 μm, measured by light microscopy using measuring ocular and object micrometer. Due to the deformations and compression of their original structure, dimensions of single lyophilized particles (Table 3) were a bit diminished in comparison with the dimensions of the yeast cell wall.

Native shape and size of spray-dried particles was conserved, showing agglomeration to some extent (Table 3). The fragile aggregated structures of the clumps formed during lyophilization were disrupted by ultrasound treatment inside of the measuring instrument and therefore lyophilized particles appeared smaller than those dried by spraying. Both microstructures and particle size distributions of the preparations isolated in this work corresponded to those of alkaline-isolated preparations dried by the same methods.

Although it is impossible to define the biological activity of the isolated β-glucans without performing immunological tests, they can be put in relation with other similar products based on the chemical composition, particle dimensions and structure. The obtained spray-dried particles isolated from brewer's yeast were smaller than those of some commercial baker's yeast β-glucans. They also had similar dimensions as spray-dried samples isolated by different methods published in literature (*13*,*62*). In comparison with the results published by Thammakiti *et al.* (*23*), our spray-dried β-glucans after all three isolations had smaller dimensions. Such non-aggregated preparations containing smaller particles could be more favourable for immunostimulation (*43*).

FTIR spectra of dried β-glucan preparations

FTIR spectroscopy was applied to analyze different polysaccharides, including β-glucan (*13*,*42*,*66*) and glucomannan (*66*), both isolated from the cell wall of *Saccharomyces cerevisiae*. Classical 'wet' methods of polysaccharide determination are time-consuming and not favourable for routine application. The application of FTIR analysis could have an advantage when β-glucan is used in food industry (*67*).

FTIR spectra were compared for β-glucans isolated by all three procedures (A, AA and AAM). Due to similar spectra of all three procedures, only FTIR spectra of β-glucan obtained with AA procedure are shown in Fig. 5. Generally, the recorded FTIR spectra of all analyzed preparations were characteristic for β-1,3-D-glucan, which is in agreement with the data published on similar preparations, isolated from yeast (*13*,*42*).

Table 3. Dimensions of β-glucan particles isolated by AA and AAM procedures and dried by lyophilization (L) and spray-drying (SD)

Isolation procedure	Sample designation	Drying procedure	$d(0.1)^a$ / μm	$d(0.5)^b$ / μm	$d(0.9)^c$ / μm	$D[4,3]^d$ / μm
AA	AA-L	lyophilization	2.652	4.306	7.292	5.551
	AA-SD	spray-drying	4.105	8.637	16.194	12.872
AAM	AAM-L	lyophilization	2.609	4.000	6.088	4.207
	AAM-SD	spray-drying	3.105	6.558	13.228	7.525

[a]$d(0.1)$ – 10 % of the particles has dimensions up to the stated value (μm)
[b]$d(0.5)$ – 50 % of the particles has dimensions up to the stated value (μm)
[c]$d(0.9)$ – 90 % of the particles has dimensions up to the stated value (μm)
[d]$D[4,3]$ – diameter of the sphere whose volume is equal to an average particle volume (μm)

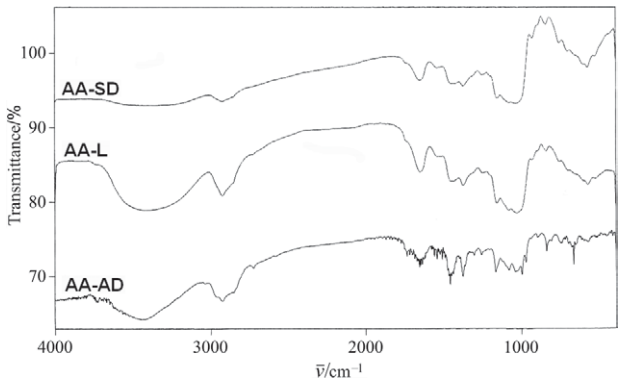

Fig. 5. FTIR spectra of β-glucans isolated by alkaline-acidic procedure (AA) and dried by three different drying methods: a) spray--drying (AA-SD), b) lyophilization (AA-L), c) air-drying (AA-AD)

The presence of hydrogen bonds, formed in the yeast β-glucan during the process of drying from aqueous medium, could be detected by FTIR spectroscopy (*13*). Free hydroxyl groups absorb in the region of 3650–3500 cm^{-1}. The hydroxyl groups, which participate in the formation of hydrogen bond, caused shifting of the band maximum position to lower frequencies, increasing intensity, as well as broadening of the band, but causing its symmetry distortion at the same time.

The values of band maximum were lowered for all dried samples partly due to the formation of hydrogen bonds. Most hydrogen bonds were present in spray-dried samples, because there were the highest shifts of the band maximum and the greatest band broadenings. This could be explained by the presence of helix structure in spray--dried preparations, where the original structure was preserved, stabilized by intra- and intermolecular bonds (*13*). Apart from the appropriate particle dimensions, the preserved helix structure is an additional factor that favours biological activity of spray-dried preparations (*68*). Shift of the band maximum and band broadening were also observed in the lyophilized and air-dried samples, suggesting the formation of some hydrogen bonds during these two drying processes.

The concentration of residual proteins in the β-glucan preparations (3.16–5.52 %) was in agreement with the presence of amide bands at 1650 and 1541 cm^{-1}, similar to the results published by Hromádková *et al.* (*13*). The absorption bands coming from ν(C–C) and ν(C–O–C) showed vibration broadening at 1160 cm^{-1} (*13*). The signal at 891 cm^{-1} (Fig. 5) is in correlation with the presence of β-glycosidic bond, *i.e.* (C_1–H) deformation mode (*13, 64,69,70*). Absorption at 840 cm^{-1} means the presence of α-glycosidic bond (*69,70*) and could be explained by small concentrations of mannan, containing that type of bond.

Conclusions

Two simple procedures for isolation of β-glucan from brewer's yeast were performed. Wet β-glucans showed high agglomeration tendency. Regardless of the isolation procedure, sonication was successfully used to avoid agglomeration of particles and to improve the stability of β-glucan water suspension. The mass fraction of β-glucans in total polysaccharides was affected by isolation procedures, while the shares of total polysaccharides and proteins were not significantly different. FTIR spectra of β-glucan preparations had appearance typical for (1→3)-β--D-glucan.

In this work, microstructures and particle dimensions were significantly affected by the applied drying methods and not by isolation procedures. Dissimilar β-glucan structures, obtained by three different drying procedures, brought to differences in material properties that could be beneficial for various purposes. Different isolation procedures combined with sonication and spray--drying allowed the retention of the original β-glucan microstructure and resulted in powdered non-agglomerated β-glucans that are known to be most suitable for immunostimulation. There were no significant differences in the dimensions of spray-dried preparations isolated by different procedures. Air-dried and lyophilized preparations, due to their properties, would be less favourable for the stimulation of immune system but could find potential application in food industry.

Acknowledgement

We are grateful to BSc Gordan Mršić and his coworkers at Forensic Science Center 'Ivan Vučetić' for providing SEM photographs.

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