

Rheological Properties, Water-Holding and Oil-Binding Capacities of Particulate β -Glucans Isolated from Spent Brewer's Yeast by Three Different Procedures

Vlatka Petravić-Tominac¹, Vesna Zechner-Krpan^{1*}, Katarina Berković¹,
Petra Galović², Zoran Herceg¹, Siniša Srećec³ and Igor Špoljarić⁴

¹Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6,
HR-10000 Zagreb, Croatia

²PIP d.o.o., Bijenik 158, HR-10000 Zagreb, Croatia

³Križevci College of Agriculture, M. Demerca 1, HR-48000 Križevci, Croatia

⁴Forensic Science Centre 'Ivan Vučetić', Ilica 335, HR-10000 Zagreb, Croatia

Received: October 13, 2010

Accepted: February 3, 2011

Summary

Particulate β -glucans were isolated from brewer's yeast using three different procedures – alkaline (A), alkaline-acidic (AA) and alkaline-acidic with mannoprotein removal (AAM) and dried using three different methods – air drying (AD), lyophilization (L) and spray drying (SD). In this work, the obtained β -glucan preparations were tested for their microstructure, rheological properties, swelling, water-holding and oil-binding capacities. According to their rheological properties, suspensions containing 1 and 2 % (by mass) of spray-dried samples belong to the category of dilatant fluids. Among the spray-dried samples, rheological behaviour and water-holding capacity of the preparation AA-SD differed from those obtained by other two procedures (A-SD and AAM-SD). Concerning different drying methods applied, swelling was the lowest in the lyophilized samples and the most pronounced in the air-dried ones. Oil-binding capacity was the highest in the lyophilized preparations and increased proportionally to the number of processing steps applied in the isolation procedure.

Key words: brewer's yeast, oil-binding capacity, particulate β -glucan, rheology, swelling, water-holding capacity

Introduction

It is known that β -glucans have several beneficial properties and potential use in food, chemical and pharmaceutical industries. Spent brewer's yeast is produced in huge amounts as a secondary product in breweries all around the world. Most of it is usually sold after heat inactivation as a cheap feed supplement (1–3). The rest of it ends in the wastewater disposal and pollutes the natural water sources with organic material (4). On the

other hand, spent brewer's yeast could be a good source for isolation of high-value products such as β -glucan (4–7).

As a source of β -glucan, baker's yeast is more often mentioned in literature than brewer's yeast. Seeley (5) described fractionation of baker's yeast in order to isolate proteins, yeast extract and β -glucan intended for use in food industry. Spray-dried yeast β -glucans were shown to be suitable for food production, as food thickeners with neutral flavour, characterized by a smooth and creamy mouthfeel, as fat replacers, dietary fibres (8), emulsifiers

*Corresponding author; Phone: ++385 1 460 5142; Fax: ++385 1 483 6424; E-mail: vzkpran@pbf.hr

and films (5,9,10). Furthermore, yeast glucan has water-holding, fat-binding and oil-binding properties (4,9,11) as well as gelling ability, and can also decrease its viscosity by heating and increase it by cooling. Among others, viscosity of β -glucans depends on the yeast strain used for isolation (4). Yeast β -glucan can be easily dispersed in cold and hot systems (4,5,9,11) and deserves a special attention, because it is safe for oral application and has a GRAS (Generally Recognized As Safe) status. Together with water soluble colour, particulate yeast β -glucan can form water insoluble colouring agent used as food additive (12,13). Bell *et al.* (14) described food additives containing fibres from yeast (β -glucan or glucomannan). Different production procedures were patented for many food products containing yeast β -glucans (15–18).

Recent investigations have shown that β -glucans isolated from the cell walls of brewer's yeast could also find useful applications in immunostimulation (19) and food production (4,20–24).

Insoluble β -glucan preparations have previously been isolated from spent brewer's yeast by three different isolation procedures and dried using three different methods (25,26). In this paper, the obtained β -glucan preparations are tested for their microstructures, rheological properties, swelling, water-holding and oil-binding capacities. The tested properties of these β -glucans are of importance for potential pharmaceutical (27,28) as well as food applications (4,22–24).

Materials and Methods

Samples

Nine samples of insoluble β -glucan investigated in this work as well as their chemical compositions and particle dimensions are listed in Table 1. The samples were previously isolated from spent brewer's yeast by three different isolation procedures and dried using three dif-

ferent methods (25,26). The procedures of isolation and drying are shown in Fig. 1 and will be described briefly.

Spent brewer's yeast used as a starting material for β -glucan isolation was obtained from Zagrebačka pivovara (Zagreb, Croatia). Briefly, after the pretreatment, debittered yeast was autolyzed and yeast cell walls served as raw material for the isolation. Three different procedures were used for isolation of β -glucan – alkaline isolation (A) (25), alkaline-acidic (AA) and alkaline-acidic isolation with mannoprotein removal (AAM) (26). Upon isolations, wet preparations were dried using three different methods (25,26) – air drying (AD), lyophilization (L) and spray drying (SD).

For rheological measurements, only spray-dried samples were used (A-SD, AA-SD, AAM-SD) (Table 1). All samples were tested for swelling, water-holding and oil-binding capacities, except for sample AAM-SD. All dried β -glucan samples were examined using environmental scanning electron microscopy (ESEM).

Rheological measurements

Homogenous suspensions containing 1 and 2 % (by mass per volume) of spray-dried β -glucan preparations were prepared in distilled water from a local supplier, vortexed for a few minutes and used in rheological investigations. Rheological properties of the air-dried and lyophilized samples could not be determined, because their stable homogenous suspensions were not obtained. Rheological measurements were done in fresh suspensions and in suspensions after 24 h of storage at 4 °C and warming up at room temperature before analyses. The measurements were performed at 20 °C using a rotational viscometer (RM-180 Rheometric Scientific Inc., Piscataway, NY, USA) in the range of shear rates between 0 and 1290 s⁻¹ upwards and downwards, *i.e.* shear rate was first increased from the lowest to the highest value (upwards), and then decreased to the lowest value (downwards).

Table 1. Samples of β -glucan analyzed in this work

Sample designation	$w(\beta\text{-glucan})$	$w(\text{total carbohydrates})$	$w(\text{proteins})$	$d(0.5)$
	%	%	%	μm
A-AD	92.00±0.209	91.73±3.352	5.17±0.023	102.360
A-L	96.75±0.364	91.12±4.186	5.13±0.029	4.260
A-SD*	98.24±0.356	91.43±3.949	3.16±0.042	9.609
AA-AD	89.11±0.373	94.11±3.197	5.52±0.082	488.370
AA-L	97.33±0.454	92.85±3.697	5.30±0.186	4.306
AA-SD*	95.53±0.069	92.24±4.030	5.42±0.135	8.637
AAM-AD	95.25±0.446	93.04±3.412	3.90±0.067	265.980
AAM-L	97.20±0.355	91.42±3.236	4.08±0.027	4.000
AAM-SD*°	95.44±0.207	92.68±3.017	4.14±0.045	6.558

Sample designations are composed of two parts: the first one indicating the isolation procedure (A – alkaline isolation, AA – alkaline-acidic isolation, AAM – alkaline-acidic isolation with mannoprotein removal) and the second one indicating the drying method (AD – air drying, L – lyophilization, SD – spray drying)

$d(0.5)$ – 50 % of the particles have dimensions up to the stated value (μm)

*samples used for rheological measurements

°sample omitted from the investigations of swelling, water-holding and oil-binding capacities

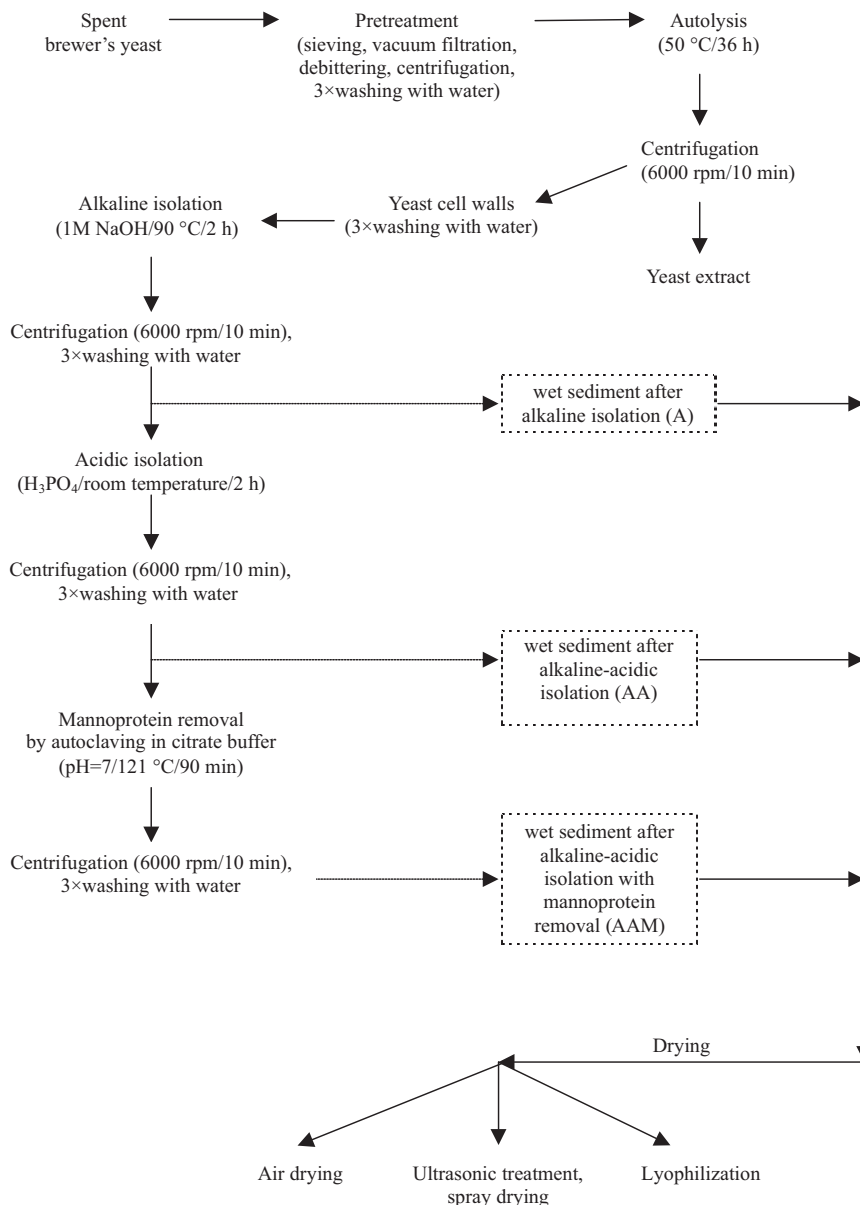


Fig. 1. Scheme of isolation and drying of β -glucan preparations investigated in this work

All rheological measurements were carried out in triplicate and at 2-minute intervals. The results of reported measurements are an average of three measurements.

Shear stress and shear rate values were recalculated for the flow index and consistency coefficient value after Ostwald-de Waele power law model, using a computer program:

$$\tau = k \cdot D^n \quad /1/$$

where τ is shear stress (Pa), k is consistency coefficient (Pa·sⁿ), D is shear rate (s⁻¹) and n is flow index.

Suitability of Ostwald-de Waele power law model for data analysis was determined after the regression analysis according to the least square method.

When $n > 1$, Eq. 1 is satisfying only for a narrow range of shear rates from 0–1290 s⁻¹ and is also applicable to a few dilatant systems, as for the characterization of dilatant flow.

Apparent viscosity at 1290 s⁻¹ was calculated using Newtonian law, as a ratio between the measured value of the shear stress and the corresponding shear rate:

$$\tau = \mu_a \cdot D \quad /2/$$

where τ is shear stress (Pa), D is shear rate (s⁻¹) and μ_a is apparent viscosity (Pa·s).

Water-holding capacity and swelling

Water-holding capacity was determined in triplicate, using the method applied by Thammakiti *et al.* (4). Due to a small amount of sample available for the analysis, the method is modified in this work by decreasing the sample mass and water volume five times. A mass of 500 mg of dried sample was transferred into a graduated glass tube, weighed, and the volume of each sample was recorded. A volume of 3 mL of distilled water was added to the air- and spray-dried samples. Due to high-

er water absorption of lyophilized samples, the amount of added water was increased to 4 mL. After 30 s of intensive mixing using glass rod, samples were completely suspended and the obtained suspensions were allowed to rest for the next 10 min. The mixing was repeated seven times (20 s of mixing and 10 min of resting). Finally, the mixing rod was washed with 1 mL of distilled water in the tube containing sample suspension. Upon centrifugation (3000 rpm at room temperature for 25 min) using centrifuge Rotofix 32 (Hettich AG, Bäch, Switzerland), the supernatants were carefully removed and the tubes were placed downwards at the angle of 15–20° and incubated (50 °C for 25 min) to drain and dry. After cooling, the tubes were weighed and sediment volume was recorded. Water-holding capacity was expressed as the amount of water held by 100 g of sample (4). Sample swelling was calculated as a percent of volume increase, concerning the starting volume of the dry sample. Sample of β -glucan designated as AAM-SD was not used for the determination of water-holding capacity and swelling because of its small amount available.

Oil-binding capacity

Oil-binding capacity was determined in triplicate, using the method applied by Thammakiti *et al.* (4). A mass of 500 mg of sample was put into weighed 12-mL graduated glass tubes. A volume of 30 mL of soybean oil was added to the air-dried and spray-dried samples. Due to higher oil binding of lyophilized samples, the amount of added oil was increased to 4 mL. Upon the addition of oil, samples were stirred for one minute. After 30 min, the tube was centrifuged (3000 rpm at room temperature for 25 min) using centrifuge Rotofix 32 and the volume of free oil was recorded. Oil-binding capacity was expressed as the volume of soybean oil bound by 100 g of sample. The significance of differences in oil binding between different methods was tested by *t*-test for dependent samples.

The samples were statistically treated as dependent because of the same batch of spent brewer's yeast, same time of β -glucan isolation and the same laboratory and personnel. The significant differences in the content of β -glucan, oil-binding and water-holding capacity were obtained by *t*-test for dependent samples at $p < 0.05$.

Electron microscopy

Environmental 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 Genesis v. 5.21 (EDAX Inc., Mahwah, NJ, USA) were used for characterization of the air-dried, lyophilized and spray-dried samples. Photographs were taken at an accelerating voltage of 25.0 kV. The diameter of the observed area was 10 mm.

Data analysis

Differences between the analytical data for water-holding, swelling and oil-binding capacities were checked by *t*-test for independent samples after the ANOVA testing (29).

Results and Discussion

Morphology of dried β -glucan particles

Microscopic structures of β -glucan preparations are important for other properties tested in this work. Microstructures of dried β -glucan preparations, obtained by different drying methods and observed by scanning electron microscope, are shown in Figs. 2a–c.

Morphology of the preparations was dependent on the drying method but the influence of the applied isolation procedure was not observed (25,26). Therefore, only preparations obtained by alkaline isolation are shown as an example of microscopic morphology typical of each drying method (Figs. 2a–c). The appearance of preparations isolated by other two isolation procedures corre-

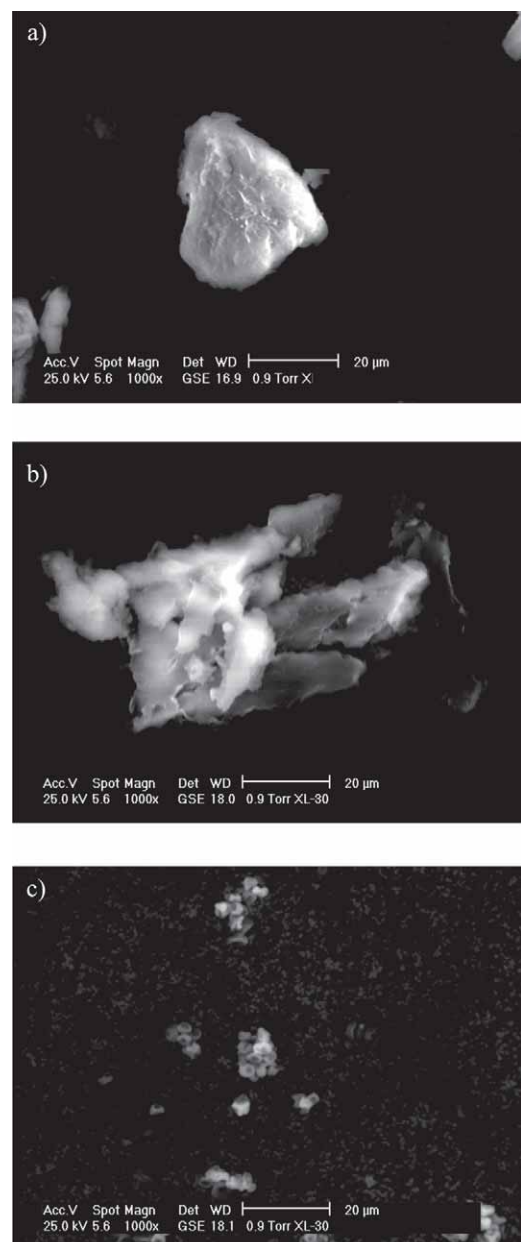


Fig. 2. Microscopic structure of β -glucan isolated by alkaline procedure: a) air-dried and mechanically grounded (A-AD); b) lyophilized (A-L); c) spray-dried (A-SD) (magn. 1000 \times , bar=20 μ m)

sponded well to the shown pictures. The morphologies of the observed insoluble β -glucan preparations obtained by air-drying, lyophilization and spray-drying were in agreement with literature (30–32).

Air-drying was a natural evaporation process at room temperature, which resulted in large, granular β -glucan particles (Fig. 2a). During lyophilization process, water was frozen and removed as solid by sublimation. Therefore, lyophilized particles were distorted and compressed into sheet-like layers with porous surface (Fig. 2b). It is known from the literature that spray-drying combined with sonication gives β -glucan fine powder with few small aggregates (30), and in such preparations the original structure is preserved (31). The preservation of the native microstructure of spray-dried β -glucan particles and retaining of oval to elliptical shape of the yeast cells is shown in Fig. 2c.

Rheological properties of β -glucan suspensions

Different drying methods cause various rheological properties of immunologically active β -glucans, isolated from baker's yeast and suspended in water (32). Additionally, properties of the isolated β -glucans depend on the yeast strain used for the isolation (33).

Rheological properties of β -glucans were investigated using 1 and 2 % suspensions of spray-dried preparations (Table 1). Microscopic structure was one of the factors that affected the ability of preparations to form stable suspensions. Stable homogenous suspensions of lyophilized and air-dried β -glucan samples could not be obtained because the particles of these samples could not be suspended. Their rheological properties were therefore not determined. Figs. 3 and 4 show the dependence of shear stress and shear rate (flow curves) in fresh and 24-hour stored suspensions, containing 1 and 2 % (by mass) of β -glucan, respectively.

The values of apparent viscosities, consistency coefficients and flow indices were calculated from the measurements performed in fresh and 24-hour stored suspensions (Table 2).

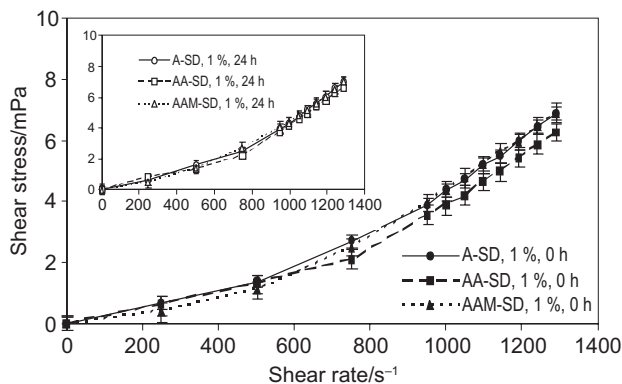


Fig. 3. Flow curves of fresh and 24-hour stored 1 % (by mass) suspensions of spray-dried β -glucans isolated by three different procedures: alkaline isolation (A-SD), alkaline-acidic isolation (AA-SD) and alkaline-acidic isolation with mannoprotein removal (AAM-SD). Bars indicate standard deviations

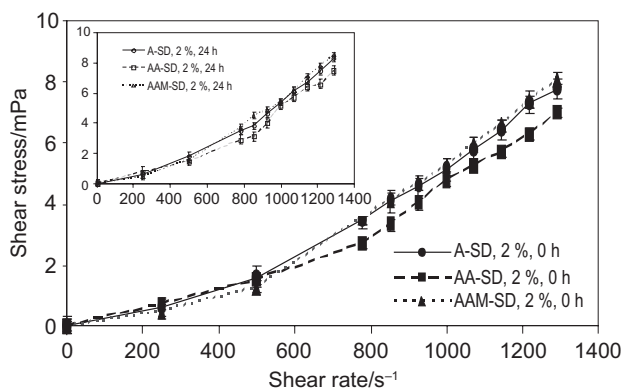


Fig. 4. Flow curves of fresh and 24-hour stored 2 % (by mass) suspensions of spray-dried β -glucans isolated by three different procedures: alkaline isolation (A-SD), alkaline-acidic isolation (AA-SD) and alkaline-acidic isolation with mannoprotein removal (AAM-SD). Bars indicate standard deviations

Table 2. Rheological characteristics of fresh and 24-hour stored suspensions containing 1 and 2 % (by mass) of β -glucan, respectively

Concentration of suspension	Sample	Measuring time	Apparent viscosity				R ²
			at 1290 s ⁻¹	<i>k</i>	<i>n</i>		
%		h	mPa·s	mPa·s ⁿ			
1	A-SD	0	5	1.3807·10 ⁻²	1.8240	0.9981	
		24	5	1.0202·10 ⁻²	1.8770	0.9995	
	AA-SD	0	5	0.9763·10 ⁻²	1.8667	0.9990	
		24	5	1.4193·10 ⁻²	1.8234	0.9983	
	AAM-SD	0	5	1.5491·10 ⁻²	1.8129	0.9989	
		24	6	1.5907·10 ⁻²	1.8173	0.9965	
2	A-SD	0	6	8.4062·10 ⁻²	1.5978	0.9981	
		24	6	2.5159·10 ⁻²	1.7754	0.9947	
	AA-SD	0	5	1.6424·10 ⁻²	1.8103	0.9987	
		24	6	2.4888·10 ⁻²	1.7598	0.9988	
	AAM-SD	0	6	6.4983·10 ⁻²	1.6379	0.9991	
		24	7	6.8375·10 ⁻²	1.6380	0.9925	

Designation of the samples consists of two parts: the first describes the isolation procedure (A – alkaline isolation, AA – alkaline-acidic isolation, AAM – alkaline-acidic isolation with mannoprotein removal) and the second describes the drying method (air drying – AD, lyophilization – L, spray drying – SD)

At maximal applied shear rate, the apparent viscosity value of 1 % suspensions for all samples and all measurements, performed both in fresh and 24-hour stored suspensions, was 5 mPa·s (Table 2). In fresh 2 % suspensions, apparent viscosities were 5–6 mPa·s, and in 24-hour stored suspensions the values were 6–7 mPa·s. The change of rheological properties after the storage of suspensions could be ascribed to swelling of particles.

Values of flow indices were between 1.5978 and 1.8770, and consistency coefficients ranged from $0.9763 \cdot 10^{-2}$ to $6.8375 \cdot 10^{-2}$ mPa·sⁿ (Table 2). There was a good agreement of our data with Ostwald-de Waele power law because regression coefficients are close to 1 in all cases.

All tested suspensions are in the category of dilatant non-Newtonian fluids, because in all cases dilatant curves were obtained (Figs. 3 and 4). In freshly prepared 1 % suspensions (Fig. 3), consistency coefficient values for samples AA-SD were lower in comparison with samples A-SD and AAM-SD at the same shear rate. After 24 h (Fig. 3), consistency coefficient in sample AA-SD was increased in comparison with its original values.

Differences in flow curves of fresh and 24-hour stored A-SD and AAM-SD suspensions (Fig. 4) were visible at a concentration of 2 %, while there was mutual overlapping at a concentration of 1 % (Fig. 3).

At both concentrations, flow curves of sample AA-SD showed the same slopes for fresh and 24-hour stored suspensions (Figs. 3 and 4), while the values of shear stress were increased. Different rheological behaviour of sample AA-SD from the other two tested samples is also observable at both concentrations (Figs. 3 and 4). Generally, dissimilar rheological properties of the tested samples arise from the differences in composition, caused by different isolation procedures (see Materials and Methods section).

It is already known that alkaline-insoluble β -glucan contains predominantly 1,3- β -linkages with a small amount of β -1,6-glucan. 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 (26). According to Thammakiti *et al.* (4), it is not known whether the differences in β -1,3/1,6 ratio affect the functional properties. However, our results suggest that the removal of β -1,6-glucan, which happens in harsh acidic conditions (34), leads to changes of composition and chemical structure of β -glucan molecules and therefore provokes changes of rheological properties of the sample AA-SD. After a subsequent removal of mannoproteins in AAM isolation procedure, β -glucan, located in the inner layer of the cell wall, became swollen and the original compacted conformation was changed (7). The swelling of β -glucan particles caused the increase of consistency coefficient of AAM-SD suspensions compared to suspensions of sample AA-SD. Generally, rheological properties of suspensions are affected by particle dimensions (Table 2) (31).

Consequently, 24 h of storage had an influence on the consistency coefficient of all 1 and 2 % suspensions (Table 2). Consistency coefficients of sample AA-SD in 1 % suspension were most affected by the storage.

All three tested spray-dried preparations (A-SD, AA-SD, AAM-SD) could be used for food thickening. Higher values of shear stress were recorded in all 2 % suspensions, which makes them more convenient for

such purpose. Isolation procedures AA and AAM were more energy- and time-consuming and consequently more expensive than isolation procedure A. Additionally, no significant differences between the shear rates of fresh and 24-hour stored A-SD suspensions were observed (Figs. 3 and 4). If A-SD preparation were used as a thickener, prolonged mixing would not be necessary. Finally, concerning the rheological properties of all spray-dried preparations (Figs. 3 and 4, Table 2) and costs of their isolation procedures, preparation A-SD would be the most profitable for use as food thickener.

The volume occupied by suspended particles corresponds to a certain package of spherical particles or their mutual arrangement in the state of rest, as well in the cases of slow or fast flow. Displacement of tightly located particles is necessary to enable the penetration of fluid from the surface between the particles and also to decrease the flow resistance. The space among particles increases due to the penetration of fluids from the surface into a system, which expands. The higher shear rates lead to further reorientation and formation of a network. The firmly mechanical contact between particles and shear resistance increase significantly. In dilatant systems, cavities or interspaces formed during mechanical impact disappear when particles rest due to gravitation force which packed them tighter. Consequently, the space between particles is smaller and the present fluid is squeezed once more onto the surface (35).

β -Glucan water-holding, swelling and oil-binding capacities

Possible β -glucan application as a food component is already described in literature. Water-holding and oil-binding capacities are beneficial properties for the use of these preparations in food such as sausages and hamburgers. Water-holding capacity of each preparation is important for juiciness of a final product (4).

In this paper, experiments were conducted to compare the influence of different drying methods on swelling, water-holding and oil-binding capacities of β -glucan preparations (Figs. 5 and 6).

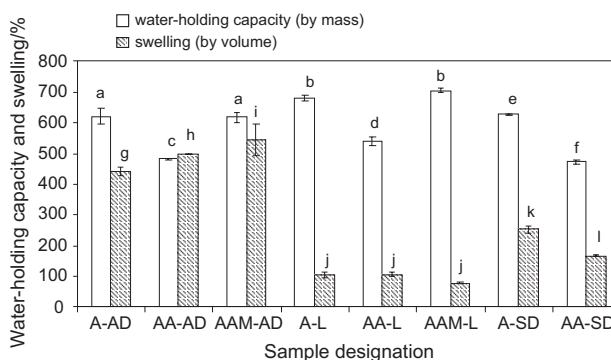


Fig. 5. Water-holding capacities and swelling of β -glucans obtained by different isolation procedures and dried using different methods. Designation of the samples consists of two parts: the first part describes the isolation procedure (A – alkaline isolation, AA – alkaline-acidic isolation, AAM – alkaline-acidic isolation with mannoprotein removal) and the second part describes the drying method (AD – air drying, L – lyophilization, SD – spray drying). Bars with the same letter are not significantly different ($p > 0.05$)

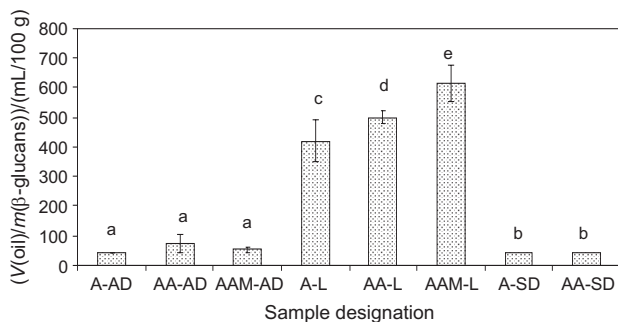


Fig. 6. Oil-binding capacities of β -glucans obtained by different isolation procedures and dried using different methods. Designation of the samples consists of two parts: the first part describes the isolation procedure (A – alkaline isolation, AA – alkaline-acidic isolation, AAM – alkaline-acidic isolation with mannoprotein removal) and the second part describes the drying method (AD – air drying, L – lyophilization, SD – spray drying). Bars with the same letter are not significantly different ($p > 0.05$)

Influence of different drying methods on β -glucan swelling and water-holding capacity

β -Glucan isolated from baker's yeast possesses excellent water-holding properties (4,31). Polysaccharides insoluble in water can absorb and retain water, which leads to their swelling (32).

Fig. 5 shows swelling and water-holding capacities of β -glucan preparations isolated from cell walls of spent brewer's yeast by A, AA or AAM procedures and then air-dried (AD), lyophilized (L) or spray-dried (SD). Sample AAM-SD was not analyzed because of relatively small amount available.

When the results were compared for each drying method separately, the preparation isolated by AA procedure had lower water-holding capacity than the samples obtained by the other two isolations. This was in agreement with differences of rheological properties between alkaline-acidic isolated preparations and other samples. It is already known that interactions between glucan and water molecules affect rheological properties (32).

A polysaccharide network can be formed upon contact of β -glucan with water (32). These structures are influenced by the applied isolation procedures and drying methods, while rehydration is not the reverse of drying. Texture changes, solute migration and loss of volatile compounds can be observed during the process of drying. Additionally, heat coagulates proteins and also reduces the degree of carbohydrate hydration and elasticity of cell walls. Consequently, all these factors reduce water-holding capacity of the preparations (4).

Results obtained for spray-dried samples (A-SD and AA-SD) (Fig. 5) were compared with literature data on β -glucan preparations dried by the same method. While sample AA-SD had similar water-holding capacity as those of Thammakiti *et al.* (4), water-holding ability of sample A-SD was significantly better.

Except AA-SD, other two preparations obtained by alkaline-acidic isolation (AA-L and AA-AD) also had similar water-holding capacities as in the work of Thammakiti *et al.* (4). Compared to this data, lyophilized samples

A-L and AAM-L bound approx. 25–30 % higher amount of water. Air-dried samples A-AD and AAM-AD showed 14–26 and 13–25 % higher ability of water holding, respectively.

Generally, volume increase due to water absorption was the highest in the air-dried samples (Fig. 5). Swelling capacity of the air-dried preparations increased proportionally to the number of processing steps involved in the isolation procedure. Swelling was more pronounced in the spray-dried than in the lyophilized samples. The reason why swelling of the lyophilized samples is significantly lower than swelling of the samples obtained by other two applied drying methods is the voluminosity of lyophilized material (Fig. 2b). Lyophilized samples occupied larger volume than the same mass of the samples dried using other methods. Ratio of the final volume, obtained after water absorption, and the starting volume of dry sample was the lowest for lyophilized samples.

Influence of different drying methods on β -glucan oil-binding capacity

Fig. 6 shows oil-binding capacities of β -glucans isolated and dried by different methods. Only sample AAM-SD was not analyzed because of relatively small amount available. Binding of oil was in connection with the sample morphology and the method used for drying.

Concerning oil-binding capacity, lyophilization provided 10.5–11.5 and 10.5–12.5 times better results than air drying and spray drying, respectively. Compared to air-dried samples, spray drying offered no improvement in oil-binding properties.

To explain the differences in oil-binding capacity, it is necessary to describe the structure of the isolated β -glucan preparations. In this work, alkali-insoluble β -glucan was isolated and further purified. About 80–85 % of *Saccharomyces cerevisiae* β -glucan is alkali-insoluble, with molar mass of 240 000 g/mol, consisting primarily of 1,3-glycosidic bonds with only 3 % of 1,6-glycosidic bonds (36). Most of β -1,3-glucan has single or triple helix conformation (37). It forms a fibrous network visible by scanning electron microscopy of the inner surface of the walls, and also amorphous components. In electron micrographs fibres are 10 to 30 nm in diameter, consistent with lateral associations of multiple chains, each with a diameter of 0.5 to 1 nm (38). While β -1,3-glucan is fibrillar, β -1,6-glucan has amorphous structure (36).

In the lyophilized samples, we observed an increase in the amount of bound oil, proportional to the number of processing steps applied during isolation procedure (Fig. 6). When compared to sample A-L, oil binding increased by 19.01 and 45.97 % in samples AA-L and AAM-L, respectively.

Generally, the preparations with high oil-binding capacity contain numerous non-polar side chains, binding to fats (4). During different β -glucan isolation procedures, degradation of β -glucan native structure and opening of the structure increased proportionally to the number of processing steps involved in the isolation. The resulting insoluble glucan preparations have a certain number of sites available for oil binding. Chemical structure, purity of β -glucans obtained after different isolation steps as well

as drying method had an influence on the water-holding and oil-binding capacities of the preparations. Due to their porous structure (Fig. 2b), lyophilized preparations were able to bind more oil than air-dried and spray-dried samples. Oil-binding capacities of air- and spray-dried samples were not influenced by the number of steps involved in the isolation procedure.

According to the paper published by Seeley (5), oil-binding capacity of β -glucan isolated from baker's yeast is characterized as good, but the author gave no data for comparison.

Except binding much more oil than samples dried using other two drying methods applied in this work (Fig. 6), lyophilized samples A-L, AA-L and AAM-L had also 3.5–5 times higher oil-binding capacity than spray-dried β -glucan samples described by Thammakiti *et al.* (4). Therefore, the original analytical method described in the mentioned paper was modified for lyophilized samples by using a higher oil volume (Materials and Methods section). Alkaline isolation is the cheapest of the three isolation procedures and it is clear from the obtained results (Fig. 6) that alkaline isolation, combined with lyophilization, gave sufficiently good results of oil binding for its application in food industry.

It is evident from the results shown in Figs. 5 and 6 that drying methods had more influence on the oil binding than on the water-holding capacity.

Conclusion

Concerning the published literature and our results, it is reasonable to claim that the isolated β -glucan preparations have potential applications in food production. β -Glucans isolated from spent brewer's yeast by three different procedures possess different chemical structures. Chemical and structural differences caused by different isolation and drying procedures lead to diverse properties of the samples. Suspensions of spray-dried β -glucans, at concentrations of 1 and 2 %, belong to the category of dilatant fluids. At both concentrations, suspensions of spray-dried sample AA-SD had different rheological behaviour from A-SD and AAM-SD samples, which were obtained by two different isolation procedures but dried by the same method. Concerning its rheological properties and simple isolation procedure, sample A-SD showed to be the most promising among the tested spray-dried samples for potential use in food production. Compared to other two isolations, samples from AA isolation had the lowest water-holding capacities. Due to the differences in microstructure of β -glucans dried by three methods, swelling was the most pronounced in the air-dried and the lowest in the lyophilized samples. Lyophilizates had the highest oil-binding capacities, proportional to the number of processing steps during isolation. Microscopic structure was one of the factors that affected swelling, water-holding and oil-binding properties of the preparations, as well as their ability to form stable suspensions.

The tested rheological properties of suspensions containing 1 and 2 % (by mass) of spray-dried β -glucans confirmed their beneficial application in food industry. To put our results of investigation into use, additional

characterization of the obtained β -glucans is the next step to be done.

Acknowledgements

We are grateful to Dr Jelena Filipović-Grčić, full professor, and her coworkers at the Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia, for providing lyophilization and spray drying of wet glucan preparations.

References

1. V. Marić, K. Štefanić, New approach of spent brewer's yeast processing and use as a biological additive in feeding some animals, *Proceedings of the 4th European Congress on Biotechnology*, Vol. 2, Amsterdam, the Netherlands (1987) pp. 498–501.
2. D.G. Hayen, D.S. Pollmann, Animal feeds comprising yeast glucan. *US patent 6214337 B1* (2001).
3. M.T. Cook, P.J. Hayball, W. Hutchison, B.F. Nowak, J.D. Hayball, Administration of a commercial immunostimulant preparation, EcoActiva™ as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Pagrus auratus*, Sparidae (Bloch and Schneider)) in winter, *Fish Shellfish Immunol.* 14 (2003) 333–345.
4. S. Thammakiti, M. Suphantharika, T. Phaesuwan, C. Verduyn, Preparation of spent brewer's yeast β -glucans for potential applications in the food industry, *Int. J. Food Sci. Technol.* 39 (2004) 21–29.
5. R.D. Seeley, Fractionation and utilization of baker's yeast, *MBAA Tech. Quart.* 14 (1977) 35–39.
6. M. Suphantharika, P. Khunrae, P. Thanardkit, C. Verduyn, Preparation of spent brewer's yeast β -glucans with a potential application as an immunostimulant for black tiger shrimp, *Penaeus monodon*, *Bioresour. Technol.* 88 (2003) 55–60.
7. X.Y. Liu, Q. Wang, S.W. Cui, H.Z. Liu, A new isolation method of β -D-glucans from spent yeast *Saccharomyces cerevisiae*, *Food Hydrocolloids*, 22 (2008) 239–247.
8. R.E. Sucher, E.A. Robbins, D.R. Sidoti, E.H. Schuldt Jr., R.D. Seeley, Yeast glucan and process of making same. *US patent 3867554* (1975).
9. G. Reed, T.W. Nagodawithana: Yeast-Derived Products and Food and Feed Yeast. In: *Yeast Technology*, Vol. 2, AVI Books, Van Nostrand Reinhold Co, New York, NY, USA (1991) pp. 369–412.
10. C. Larroche, P.H. Michaud, New developments and properties for β -(1,3)-glucans, *Recent Pat. Biotechnol.* 1 (2007) 59–73.
11. J. Wylie-Rosett, Fat substitutes and health – An advisory from the nutrition committee of the American heart association, *Circulation*, 11 (2002) 2800–2804.
12. J.C. Hobson, R.N. Greenshields, Water insoluble coloring agent. *US patent 5545557* (1996).
13. J.C. Hobson, R.N. Greenshields, Yeast debris products. *US patent 6274370 B1* (2001).
14. S.J. Bell, R. Armour Forse, B.R. Bistran, Dietary supplement and method for lowering risk of heart disease. *US patent 6210686* (2001).
15. F. Lazzari, Product based on polysaccharides from baker's yeast and its use as a technological co-adjutant for bakery products. *US patent 6060089* (2000).
16. T.P. Shukla, G.J. Halpern, Cookies comprising emulsified liquid shortening compositions comprising dietary fiber gel, water and lipid. *US patent 0084584* (2005).

17. T.P. Shukla, G.J. Halpern, Processed meats comprising emulsified liquid shortening compositions comprising dietary fiber gel, water and lipid. *US patent 0084590* (2005).
18. T.P. Shukla, G.J. Halpern, Breads comprising emulsified liquid shortening compositions comprising dietary fiber gel, water and lipid. *US patent 0084585* (2005).
19. V. Petravić-Tominac, V. Zechner-Krpan, S. Srećec, B. Šantek, D. Špoljarić, H. Valpotić, M. Popović, I. Valpotić, Spent brewer's yeast – A raw material for isolation of β -glucan applicable for animal feed production and veterinary medicine, *Veterinarska stanica*, 40 (2009) 281–295 (in Croatian).
20. V. Zechner-Krpan, V. Petravić-Tominac, I. Panjkota-Krbavčić, S. Grba, K. Berković, Potential application of yeast β -glucans in food industry, *Agric. Conspec. Sci.* 74 (2009) 277–282.
21. P. Thanardkit, P. Khunrae, M. Supphantharika, C. Verduyn, Glucan from spent brewer's yeast: Preparation, analysis and application as a potential immune stimulant in shrimp feed, *World J. Microbiol. Biotechnol.* 18 (2002) 527–539.
22. S. Worrasinchai, M. Supphantharika, S. Pinjai, P. Jamnong, β -Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise, *Food Hydrocolloids*, 20 (2006) 68–78.
23. R. Santipanichwong, M. Supphantharika, Carotenoids as colorants in reduced-fat mayonnaise containing spent brewer's yeast β -glucan as a fat replacer, *Food Hydrocolloids*, 21 (2007) 565–574.
24. S. Satrapai, M. Supphantharika, Influence of spent brewer's yeast β -glucan on gelatinization and retrogradation of rice starch, *Carbohydr. Polym.* 67 (2007) 500–510.
25. V. Zechner-Krpan, V. Petravić-Tominac, V. Galović, P. Galović, J. Filipović-Grčić, S. Srećec, Application of different drying methods on β -glucan isolated from spent brewer's yeast using alkaline procedure, *Agric. Conspec. Sci.* 75 (2010) 45–50.
26. V. Zechner-Krpan, V. Petravić-Tominac, I. Gospodarić, L. Sajli, S. Đaković, J. Filipović-Grčić, Characterization of β -glucans isolated from brewer's yeast and dried by different methods, *Food Technol. Biotechnol.* 48 (2010) 189–197.
27. G. Betageri, S. Prabhu: Semisolid Preparations. In: *Encyclopedia of Pharmaceutical Technology*, J. Swarbrick, J.C. Boylan (Eds.), Marcel Dekker, Basel, Switzerland (2002) pp. 2436–2457.
28. H.E. Junginger, M. Thanou, J.C. Verhoef: Mucoadhesive Hydrogels in Drug Delivery. In: *Encyclopedia of Pharmaceutical Technology*, J. Swarbrick, J.C. Boylan (Eds.), Marcel Dekker, Basel, Switzerland (2002) pp. 1848–1863.
29. T. Hill, P. Lewicki: *Statistics Methods and Applications*, StatSoft, Inc., Tulsa, OK, USA (2006).
30. K.W. Hunter, R.A. Gault, M.D. Berner, Preparation of micro-particulate β -glucan from *Saccharomyces cerevisiae* for use in immune potentiation, *Lett. Appl. Microbiol.* 35 (2002) 267–271.
31. S. Jamas, C. Rha, A.J. Sinskey, Morphology of yeast cell wall as affected by genetic manipulation of β -(1 \rightarrow 6) glycosidic linkage, *Biotechnol. Bioeng.* 28 (1986) 769–784.
32. Z. Hromádková, A. Ebringerová, V. Sasinková, J. Šandulá, V. Hříbalová, J. Omelková, Influence of the drying method on the physical properties and immunomodulatory activity of the particulate (1 \rightarrow 3)- β -D-glucan from *Saccharomyces cerevisiae*, *Carbohydr. Polym.* 51 (2003) 9–15.
33. S. Jamas, C. Rha, A.J. Sinskey, Glucan composition and process for preparation thereof. *US patent 4810646* (1989).
34. S. Freimund, M. Sauter, O. Kämpelli, H. Dutler, A new non-degrading isolation process for 1,3- β -glucan of high purity from baker's yeast *Saccharomyces cerevisiae*, *Carbohydr. Polym.* 54 (2003) 159–171.
35. M.A. Rao: *Rheology of Fluid and Semisolid Foods: Principles and Applications*, Springer Science+Business Media, New York, NY, USA (2007).
36. G. Krešić, V. Lelas, A. Režek Jambrak, Z. Herceg, S. Rimac Brnčić, Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins, *J. Food Eng.* 87 (2008) 64–73.
37. F. Kath, W.M. Kulicke, Mild enzymatic isolation of mannan and glucan from yeast *Saccharomyces cerevisiae*, *Angew. Makromol. Chem.* 268 (1999) 59–68.
38. N.P. Lipke, R. Ovalle, Cell wall architecture in yeast: New structure and new challenges, *J. Bacteriol.* 180 (1998) 3735–3740.