Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XVI, Issue 4 - 2017, pag. 190 - 195

RESEARCH ON OBTAINING HIGH B-GLUCANS CONTENT FROM DIFFERENT SOURCES OF YEAST BY HARNESSING THEIR BIOLOGICALLY ACTIVE POTENTIAL

Ionuț AVRĂMIA[1], *Sonia AMARIEI[1]

[1]*Faculty of Food Engineering, Ştefan cel Mare University of Suceava,*
Universitatii 13, RO 720229 Suceava, Romania
*Corresponding author
Received 9th October 2017, accepted 29th November 2017

Abstract: *Isolated polysaccharides from different natural sources have gained a real interest from the scientific community due to biologically active effects on body functions such as: lipid metabolism correction, immune stimulating, glycemic control in type II diabetes, antitumor activity, etc. A category of these polysaccharides is β-glucans, β-D-glucose polymers produced by various organisms such as bacteria, yeasts, algae and plants. This paper presents the experimental results obtained from the analysis of four types of yeast available on the market in order to select the one with the highest content of β-glucan, which can be later exploited in various fields: medical, pharmaceutical, food or cosmetics. According to the experimental data, the highest level of β-glucans content is represented by beer yeast, 21.49% higher than bakery yeast and 36.36% higher than wine yeast.*

Keywords: *β-glucan, yeast, immunity system, food supplement.*

1. Introduction

β-Glucans are widely spread biopolymers of glucose in many prokaryotic and eukaryotic organisms. Although they derived from different sources of yeast, fungi, algae, mold, oats or barley, β-glucans exhibit some biologically active features depending on their origin. Thus, some authors state that β-glucans isolated from yeast *Saccharomyces cerevisiae* have the highest biological effect [1]. Immunomodulation induced by β-glucans has been demonstrated both in vitro and in vivo in numerous studies on animals and humans in a wide range of tumors of breast, lung and gastrointestinal cancers [1]. β-Glucans are formed at several cellular structures and are predominantly found in the cell wall of the yeast. In the *S. cerevisiae* yeast cell wall structure is 15-25% of the dry matter of the cell [2] and 25-50% by volume based on a calculation carried out at the electronic microscope [3]. The cell wall consists of three layers: glucanic, mannanic and lipidic, and the percentage in these compounds varies from one strain to another and depends largely on cultivation conditions including: nutrition, oxygenation, temperature and pH of the nutrient medium [4].

Table 1.
Average cell wall content [8]

Component	Mass (% s.u.)
(1→3)-β-D-glucan	50-55
(1→6)-β-D-glucan	5-10
(1→4)-α-(1→3)-β-D-glucan	3-7
Manoprotein Complex	35-40
Chitin	2

Table 1 shows that glucans represent the major components of the cell wall in the proportion of about 60% of the dry matter, which is favorable in the subsequent isolation processes. From the literature it was found that the three dimensional structure of the glucan is essential for the biologically active effect on the body. Thus, some authors consider the triple-helix conformation to be the most active [1, 5].

β-1,3 glucan forms a microfibrilar mesh, visible through electron microscopy, to the internal surface of the cell wall. It has a molecular weight of 240 kDa and a maximum fiber length of 600 nm [3].

The greatest part of the β-1,3 glucan has a helical conformation on the basis of in vitro studies demonstrated and confirmed by nuclear magnetic resonance of intact solid state yeast cells [3]. Thus, the helical structure consists of three hydrogen chains (triple helix conformation). The chains are separated at a distance of 1.56 nm with a 0.6 nm period between the fibers, as a result defining six units of β-D-glucopyranose each helical structure [6].

β-1,6 glucan is a highly branched polysaccharide that binds each component of the cell wall [3]. In contrast to the microfibrilar structure of β-1,3 glucan, in the *Saccharomyces cerevisiae* yeast cell the polymer is amorphous in structure, bonded in β-1,6 position, shorter and acts as a flexible glue to form crosslinks with β-1,3 glucan, chitin and manoproteins [7].

The main challenge in the process of isolating glucan is to remove impurities such as mannoproteins and lipids without a significant loss of biologically active compounds. The American Food and Drug Administration (FDA) has named S.cerevisiae β-glucans with the "generally recognized as safe" (Food and Drug Administration, 1997) and may be used as ingredients in the food industry but not as additives [6].

The European Food Safety Authority (EFSA) has issued an opinion that yeast β-glucans are a "safe food ingredient" that can be used as a dietary supplement at a concentration of up to 375 mg/day and in foods for "special nutritional use"at doses up to 600 mg/day [8, 9]. However, these do not exclude the possibility of adverse reactions from impurities resulting from isolation processes (in rare cases the accumulated proteins can cause allergies) [1]. Research on glucans focuses primarily on two similar medical domains.

The most recent and important area of research is the stimulation of β-glucans immunity by binding to dectin-1 and toll-like (TLR) receptors [10, 11]. The other field of study is anti-tumor activity mediated by macrophage cells [10].

Specifically, β-glucans do not directly attack infected cells, or agents that cause infections, but they modulate the host's defense system by stimulating the production of precursor cells, which are distributed by the blood stream in various lymphoid organs of the body. Regarding the second field of study, some reports have shown that triple helix conformation plays a decisive role in the antitumor activity and cytokine secretion [10].

Moreover, in vitro studies have shown that the introduction of sulfate groups in the structure of β-glucan increases the inhibitory activity against the proliferation of sarcoma-180 tumor cells [10, 12].

In the two main areas of use (immunomodulation and antitumor activity), β-glucans also find medical applications in: development of new polyvalent vaccines, prevention of infections, reduction of cholesterol, glycemic control, wound healing, potential for healing of digestive ulcers and proved to have a prebiotic effect [8, 13, 14]; and in the food industry as: thickening agents, water retention, oil binders or stabilizers of some emulsions without affecting taste and

smell [15, 16]. Adjustment of glycemic levels is explained by the rheological ability of β-glucans to increase the viscosity of the solutions, molecular weight dependent properties, solubility and concentration of the glucose polymer [17] that reduces glycemic response by complexing with ingested carbohydrates leading to slow release of carbohydrates in the blood [18].

With a strong topical effect, creams containing β-glucans stimulate skin macrophages that are considered to be the first barrier to cellular defense against infectious agents [14]. This property is especially beneficial in treating wounds in people with diabetes who have a condition called diabetic neuropathy as the wounds heal heavily, have a high susceptibility to developing infections and skin ulcers. Beyond preparations for human use, there are also some examples of using glucan as antiviral agents in plants such as: protecting numerous tobacco species against mosaic virus invasion or against the virus that causes the black ring in tomatoes. These plants can be treated by injection or spraying with the glucanic polymer [19].

2. Materials and methods

Materials. Four different types of active yeasts of the species *Saccharomyces cerevisiae* available on the market were used in this study. Of these, two types of baker's yeast (noted as: Sample 1 and Sample 2) were chosen from a native producer and import yeast, a yeast brew species (Sample 3), and a wine yeast strain (Sample 4). All other chemicals used in this research were of analytical grade and freshly prepared.

Methods. Isolation of β-glucans from yeast cells of *S. cerevisiae* was carried out in two steps:

1. Destruction of the cell walls followed by centrifugation to separate the insoluble cytoplasmic compounds;
2. The proper extraction of the cell suspension using the alkaline-acid method patented by James *et al.* (1989) and modified by Usatii and Chiseliţă, (2015) [5, 20]. Determination of β-glucans content was performed gravimetrically.

Induction of autolysis. The destruction of cell walls was accomplished by induced autolysis. Thus, the mixture of 10 g of yeast (30% v/v) and 10 ml of sterile water was subjected to heat treatment at 55° C for 8 hours. The obtained autolysate was centrifuged at 3500 r.p.m. for 10 minutes to separate the suspension component from the yeast extract. It is also known from the bibliographic study that autolysis proceeds under optimal conditions at a temperature of 50° C [21].

Extraction and isolation of β-glucan. The cell disintegration sediment is treated with 50 mL of 1N NaOH for 1 hour on the water bath at 90±5°C. After alkaline treatment, the cell walls are separated and treated with 0.5 N acetic acid in volume 1: 5 at 75±5°C for 1 hour to remove glycogen. The deposit was washed twice with distilled water, centrifuged at 3500 r.p.m. for 10 minutes and dry in the oven at 50 ± 5°C. The resulting product consists of a mixture of β-1,3 and β-1,6 glucans.

3. Results and discussion

For the presentation of glucan content from different yeast brands, a table has been compiled that includes the amount of polysaccharides of bioactive interest - as a percentage and weight of the yeast active biomass. The results are shown in the Table 2.

The results of analyzes of the four types of active yeasts obtained from different producers, Table 2, showed that the maximum percentage of β-glucans content

was identified in the brewery yeast (15.91%) followed by the two bakery yeast samples (12.49%) respectively (12.10%) and finally the lowest concentration (5.78%) was identified in the wine yeast.

The variations in β-glucan content from the four samples can be explained mainly by the diversity of yeast strains subjected to isolation but it should not be neglected and the influence of growth factors on the carbohydrate accumulation in yeast biomass. In order to evaluate the yield of the glucan from the four yeast sources, we have chosen to refer to the highest level of polysaccharides obtained from the extraction. The figure 1 shows the percentage of glucan isolate from samples.

Table 2
Content in β-glucans relative to active Levurian biomass.

Sample Number	Biotech Purpose	Active yeast mass (g)	β-glucans mass (g)	glucan extract (%)
1.	bakery	10	1.2106	12.10
2.	bakery	10	1.2491	12.49
3.	brewery	10	1.5913	15.91
4.	winery	10	0.5786	5.78

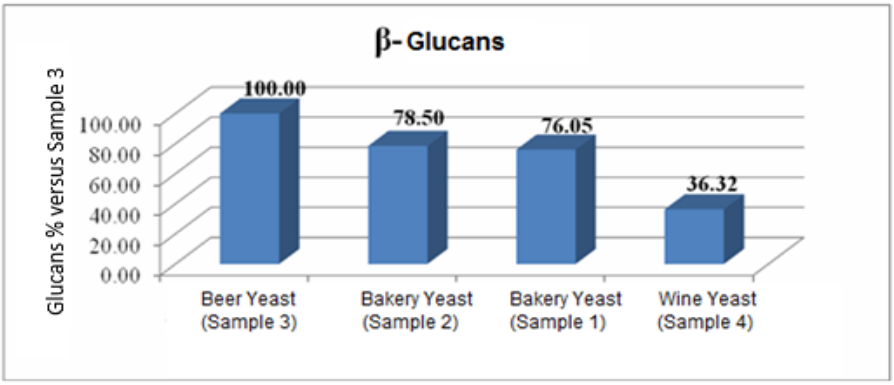

Fig. 1. Influence of yeast strains on β-glucan accumulation.

The results show that 21.5% more glucans are found in the beer yeast than in the baking yeast (Sample 2) and 63.68% more than in the wine yeast (Sample 4). No significant variation in β-glucans content (12.49% versus 12.10%) was observed between the two yeast samples that indicates regularities of analyzed strains.

The present research on the isolation of bioactive compounds from the wall of yeast *Saccharomyces cerevisiae* by the method of alkaline-acid are consistent with other studies carried out by different authors in particular conditions, and by optimizing the culture medium from strains patented as producing β-glucans end to obtain a maximum of up to 29.47% ± 0.52% β-glucans [22]. Similar research on three different types of baking yeast reveals a maximum content of 7.35% β-glucans [23], compared to 12.49% obtained in this paper.

Similar studies in the yeast resulting from the fermentation process by spray drying shows a content of 23.59% ± 0.34% of the total cell mass [15].

The composition of the β-glucans of the yeast study examined in this work is limited to 15.91% that can be explained by replacing the drying process to isolate the glucan or by using other yeast strains. Also, similar results on the level of β-glucans were recorded in the strain *Saccharomyces cerevisiae* CNMN-Y-20 (approx. 20%) using the same autolysis and extraction methods [5].

Maximum values were also obtained in *S. cerevisiae* CNMN-Y-18 (18.54%) and *S. cerevisiae* CNMN-Y-19 (17.61%) [24].
As noted, the literature shows that under normal conditions and using the alkali-acid method, a maximum level of yeast glucan content from yeasts available on the market are up to 15-20% of the active mass.

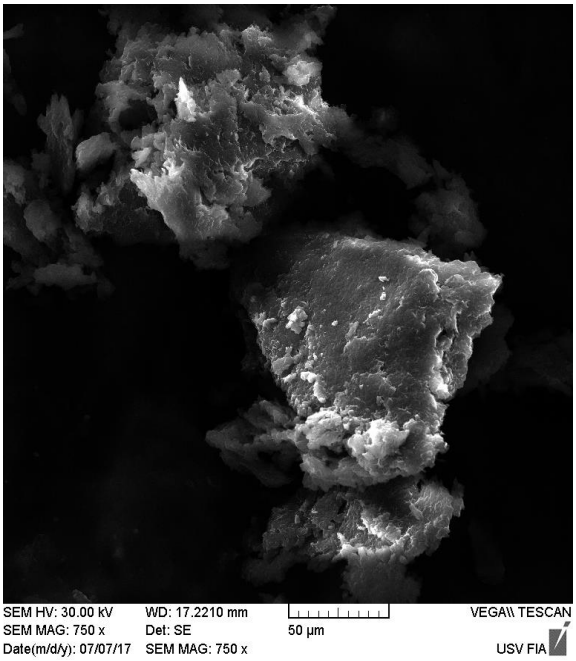

Fig.2. β-glucan mixture from bakery yeast(increased to 1.50kx.)

The SEM microscopic image of the β-1,3 and β-1,6 glucans mixture, Fig. 2, the microfibrilar structure of β-1,3 glucan and the highly branched polymer with binary branching of β-1,6 glucan, with high binding capacity of other components, demonstrates the behavior of this mixture of polymers in liquids and their importance in the rheology of the mixtures in which they are used.

4. Conclusions

The screening of the four available yeast sources revealed a varied level of β-glucans content.
The results confirm the hypothesis of other authors that glucan is found in varying proportions depending on the strain of the species. The study conducted shows that the highest level of β-glucan possesses the beer yeast (15.91%), with 21.49% more than the baker's yeast.
The problem of triple helix conformation (known to produce biologically active effects) and the aggressive chemical method for the extraction of glucan is solved by using the thermal drying process at 55° C with the reconfiguration of the structure. The extraction method seems to be appropriate for the large-scale production of β-glucans from yeast biomass.
Unlike other sources of glucans (oat, barley, rye and wheat), yeast isolates are not toxic in terms of aflatoxin contamination.
Although it is impossible to define the biological activity of β-glucans isolated from four samples without carrying out clinical tests and immunological tests, they can be put into relation with other data presented in the scientific literature.
The yeast selection study with the highest content in bioactive compounds allows for future the development of glucan supplements from levurian compounds resulting in fermentative processes.

5. References

[1]. PETRAVIC-TOMINAC V., ZECHNER-KRPAN V., GRBA S., SRECEC S., PANJKOTA-KRBAVCIC I., VIDOVIC L., Biological Effects of Yeast β-Glucans, *Agriculturae Conspectus Scientificus*, 75 (4): 149-158, (2010).
[2]. WAITES M., MORGAN N., ROCKEY J., HIGTON G., Industrial Microbiology: An Introduction, School of Applied Science, *South Bank University, London, UK*, (2001).
[3]. LIPKE P., OVALLE R., Cell Wall Architecture in Yeast: New Structure and New Challenges, Minireview, *Journal of Bacteriology, 180* (15): 3735-3740, (1998).
[4]. STEWART G.G., RUSSELL I., Brewer's Yeast, *Brewing Science & Technology Series III (1998)*: 28-46, (1998).

[5]. USATÎI A., CHISELIȚA N., Metode de extragere din levuri a β-glucanilor și proprietățile lor fizico-chimice, *Buletinul Academiei de Ştiinţe a Moldovei (*Methods of extraction in yeasts of β-glucans and their physicochemical properties, *Bulletin of the Academy of Sciences of Moldova*), 1 (325): 153-159, (2015).
[6]. ZEKOVIC D.B., KWIATKOWSKI S., VRVIC M. M., JAKOVLIEVIC D., MORAN C., Natural and modified (1→3)-β-D-glucans in health promotion and disease alleviation, *Critical Reviews in Biotechnology*, 25 (4): 205-230, (2005).
[7]. LESSAGE G., BUSSEY H., Cell wall assembly in Saccharomyces cerevisiae, *Microbiology and Molecular Biology Reviews*, 70 (2): 317-343, (2006).
[8]. KWIATKOWSKI S., KWIATKOWSKI S.E., Yeast (*Saccharomyces cerevisiae*) Glucan Polysaccharides – Occurrence, Separation and Application in Food, Feed and Health Industries, *InTech, The Complex World of Polysaccharides*, (2): 47-70, (2012).
[9]. EUROPEAN FOOD SAFETY AUTHORITY, Scientific Opinion on the safety of 'yeast beta-glucans' as a Novel Food ingredient, *EFSA Journal*, 9 (5): 2137, (2011).
[10]. BATBAYAR S., LEE D.H., KIM H.W., Immunomodulation of Fungal b-Glucan in Host Defense Signaling by Dectin-1, *Biomol Ther*, 20 (5), 433-445, (2012).
[11]. STIER H., EBBESKOTTE V., GRUENWALD J., Immune-modulatory effects of dietary Yeast Beta-1,3/1,6-D-glucan, *Nutrition Journal*, 13:38, doi:10.1186/1475-2891-13-38, (2014).
[12]. XIAOHUA W., LINA Z., Physicochemical properties and antitumor activities for sulfated derivatives of lentinan, *Carbohydrate Research*, 344 (16): 2209-2216, (2009).
[13]. RAFAEL O., AFRICA G., MARTIN-LOMAS M., PENADES S., Preparation of multifunctional glyconanoparticles as a platform for potential carbohydrate-based anticancer vaccines, *Carbohydrate Research*, 342 (3-4): 448-459, (2007).
[14]. MASON R., What is Beta Glucan, *Editura Safe Goods, ISBN No. 1-884820-66-2*, (2011).
[15]. THAMMAKITI S., SUPHANTHARIKA M., PHAESUWAN T., VERDUYN C., Preparation of spent brewer's yeast β-glucans for potential application in the food industry, *International Journal of Food Science and Technology*, 39 (1): 21-29, (2004).
[16]. PETRAVIC-TOMINAC V., ZECHNER-KRPAN V., BERKOVIC K., GALOVIC P., HERCEG Z., SRECEC S., SPOLJARIC I., Rheological Properties, Water-Holding and Oil-Binding Capacities of Particulate b-Glucans Isolated from Spent Brewer's Yeast by Three Different Procedures, *Food Technology and Biotechnology*, 49 (1): 56–64, (2011).
[17]. KWONG M., The glycemic response elicited by oat β-glucan solutions and hard gel varying in physiochemical properties and food form, *Master of Science, Department of Nutritional Sciences University of Toronto,* (2012).
[18]. SILVA V.O., LOBATO R.V., ANDRADE E.F., MACEDO C.G., NAPIMOGA J.T.C., NAPIMOGA M.H., *et.al*., β-Glucans (Saccharomyces cereviseae) Reduce Glucose Levels and Attenuate Alveolar Bone Loss in Diabetic Rats with Periodontal Disease, *PLoS ONE*, 10 (8): e0134742, (2015).
[19]. BLAIR P. *et.al*., Biopolymers: Making Materials Nature's Way-Background Paper, *Office of Technology Assessment*, OTA-BP-E-102, (1993).
[20]. JAMES S., RHA C., SINSKEY A., Glucan compositions and process for preparation thereof, *Brevet de invenție US4810646 A (Patent US4810646 A), Massachusetts Institute Of Technology*, (1989).
[21]. JAVMEN A., GRIGISKIS S., GLIEBUTE R., β-glucan extraction from Saccharomyces cerevisiae yeast using Actinomyces rutgersensis 88 yeast lyzing enzymatic complex, *Biologija*, 58 (2): 51-59, (2012).
[22]. CHISELIȚA N., USATÎI A., MOLODOI E., EFREMOVA N., FULGA L., BORISOVA T., Optimizarea matematică a mediului de cultură pentru producerea β-glucanilor la tulpina Saccharomyces cerevisiae CNMN-Y-20, *Revista Științifică a Universității de Stat din Moldova* (Mathematical optimization of the culture medium for the production of β-glucans in the *Saccharomyces cerevisiae* strain CNMN-Y-20, *Scientific Review of the State University of Moldova*), nr.6 (66), (2013).
[23]. MANY J.N., VIZHI K., Analysis of Different Extraction Methods on the Yield and Recovery of β-Glucan from Baker's Yeast (Saccharomyces Cerevisiae), *International Journal of Innovative Science, Engineering & Technology,* 1 (6): 268-271, (2014).
[24]. USATÎI A., MOLODOI E., CHISELIȚA N., CHISELIȚA O., EFREMOVA N., BORISOVA T., FULGA L., Tulpini de drojdii – surse valoroase pentru obţinerea β-glucanilor şi mananilor, *Buletinul Academiei de Ştiinţe a Moldovei* (Yeast strains - valuable sources for obtaining β-glucans and mannan, *Bulletin of the Academy of Sciences of Moldova*), 3 (315): 128-134, (2011).