UDC 664.785.86 https://doi.org/10.15407/biotech16.01.051

ULTRASOUND-ASSISTED AND ENZYMATIC-BASED METHOD FOR ISOLATION OF β-GLUCANS FROM OAT BRAN

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Received 2022/01/11
Revised 2022/02/12
Accepted 2023/02/28

β-Glucans are a group of non-starchy polysaccharides, or (1,3),(1,4)-β-D-glucans, that can be found in the cell walls of several species of bacteria, algae, lichens, fungi, and cereal grains. These carbohydrates are extensively used in food industry, cosmetics, pharmaceuticals and healthcare, therefore optimization of the extraction and isolation of β-glucans from grain sources has an especial importance in various fields of biotechnology, drug design, food science and technology.

The aim of the study was to develop an optimized technological scheme for isolation of β-glucans from oat bran based on ultrasonic and enzymatic processing of raw material.

Materials and methods. β-Glucans were isolated from grinded oat cereals during multi-stage process, which includes extraction of grain fats, hydrobarothermic processing, ultrasonification, enzymatic hydrolysis of concomitant starch and proteins, precipitation of β-glucan fraction by ethanol, centrifugation, and dry-freezing. Yield of β-glucans from raw material and its concentration in the final product were determined after hydrolysis by sulfuric acid or enzymatic cleavage by endo-1,3(4)-β-glucanase.

Results. As shown by acidic hydrolysis of the final product, the yield of β-glucans was 10.8 ± 0.23% and concentration was 79.6 ± 3.89%, while enzymatic hydrolysis gave 8.7 ± 0.82% and 65.1 ± 4.72%, respectively. Thus, the use of hydrobarothermic and ultrasound pre-treatment of raw material in combination with proteolytic digestion of ballast lipids and proteins allowed producing oat β-glucans in amounts comparable with those in case of acid- or alkali-based procedures.

Conclusions. The described technological scheme of β-glucan isolation from oat bran based on sequential hydrobarothermic processing, ultrasonification, and enzymatic removing starch and proteins can be widely used for routine β-glucan production for various purposes in food technology, pharmacological industry, and medicine.

Key words: β-glucan; oat; hydrobarothermic processing; ultrasonification; enzymatic hydrolysis.

β-Glucans are a group of non-starch polysaccharides, in which D-glucose residues are linked by β-(1–4) and β-(1–3) glycosidic bonds. Single β-(1–3) linkages are generally separated by 2 or 3 β-(1–4) linkages but the ratio between β-(1–4) and β-(1–3) linkages differs between various species (Fig. 1) [1].

These polysaccharides are present in the cell walls of many natural sources including bacteria, yeasts, lichens, fungi, algae, edible mushrooms, and cereal grains such as oats, barley, wheat, and rye. β-Glucans are referred to as a type of dietary fibers, which are classically used to boost the immune system and to treat hyperlipidemia [2]. Results of the studies conducted during the past two decades support the suggestion that regular intake of oat β-glucan at daily doses of at least 3 g may reduce plasma total and low-density lipoprotein (LDL) cholesterol levels by 5–10% in normocholesterolemic or hypercholesterolemic subjects. As an effective food supplement with no side effects even at a higher consumption, β-glucans have been reported as generally recognized as safe (GRAS) by U.S. Food and Drug Administration (FDA) [3]. β-Glucans have numerous healthcare properties and have found a variety of applications in human

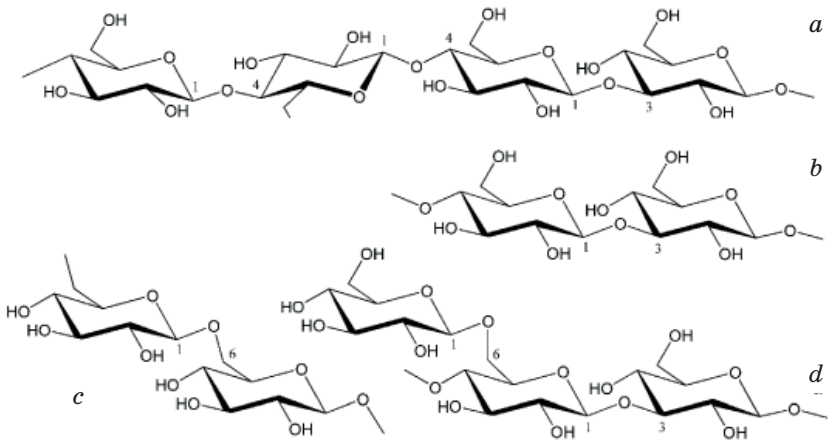

Fig. 1. **Structure of various β-D-glucans:**
a — cereal mixed-linkage (1-3) (1-4)-β-D-glucan, *b* — microbial (1-3)-β-D-glucan,
c — 1-3-β-D-glucan from lichens, *d* — branched fungal or seaweed (1-3) (1-6)-β-D-glucan

and veterinary medicine, pharmaceutical, cosmetic and chemical industries, food and feed production. β-Glucans can be incorporated into various products, such as bread, muffins, pasta, noodles, salad dressings, beverages, soups, and reduced-fat dairy and meat products [4]. Mushroom- and cereal-based foods containing these polysaccharides have been reported to be beneficial for health to display anticarcinogenic, antiviral, anti-inflammatory, prebiotic, antioxidant, neuroprotective, and immunostimulatory properties [5].

The oat (*Avena sativa*) is a well-known cereal and one of the first cultivated plants by humans. This crop is used extensively and currently world oat production is about 22 million tons per year, however less than barley at 170 million tons per year and much less than the 772 million tons of wheat in 2021. Oat groats are rich in protein (usually 13-20% of dry weight), a source of unsaturated fatty acids and contain natural antioxidants such as tocopherols, tocotrienols, sterols, and phenolic acids. Quantitative analysis have shown that β-glucan content in whole oat grains and oat bran products is typically in the range of 2% to 8.5% and from 6% to 12%, respectively [6]. β-Glucans are present in the aleurone cell wall, but their amount is small compared with that in the underlying starchy endosperm, which is the primary storage site of starch, protein, lipid, and β-glucans. β-Glucans are concentrated mainly in bran during the processing of grain crops into flour and cereals [7]. Therefore, oat bran can be used as a raw material for the industrial production of β-glucan concentrates and isolates for food manufacturing, pharmaceutical industry, and cosmetology. This justifies the need for isolating β-glucans in maximally pure form. Thus, studies on the extraction and isolation of β-glucans from oat bran remain of tremendous interest and have outstanding applied significance.

There are different extraction methods for the isolation of β-glucans from grain sources mainly oats and barley [8]. Four classes of extraction methods are described as follows:

i) water extraction,
ii) alkaline extraction,
iii) acidic extraction, and
iv) enzymatic extraction.

As considered, extraction methods for isolating β-glucans involve the use of acid or alkali. Meanwhile, these substances may cause corrosion of equipment, are dangerous in producing process, and toxic to humans. Therefore, the development of technology using softer and more efficient methods of β-glucan extraction is an urgent problem. Further, presence of starch and protein in minor quantities in the final product will not be harmful for human organism as being extracted from edible source. On the contrary, their presence at higher levels may decrease the viscosity of β-glucans and consequently exerts adverse effect on biological activity of these polysaccharides. Hence, it is a challenge for researchers to obtain high yield of the produced component with high purity via removing undesirable impurities preferentially by enzymatic processing of raw material. Thus, the aim of the presents study was to elaborate an optimized method for β-glucan isolation based on ultrasonic and enzymatic processing of the oat bran.

Materials and Methods

Oat bran obtained by processing naked grain oats into flour according to the state

standard (DSTU 4963:2008 Oat Technical Conditions) was used in the study. The bran was preliminarily ground into flour with a particle size of 0.5 mm. For grain fat extraction, oat flour (150 g) was suspended in 50% ethanol (1 l) during 1 h on the water bath at 60 ^{O}C. Then, ethanol extract was separated from defatted bran by centrifugation at 16,000 g for 30 min at 4 ^{O}C. Sedimented bran was resuspended in distilled water (1:5) and heated up to 115 ^{O}C for 1 h. Hydrobarothermic processing of crude material results in gelatinization of oat starch, formation of dextrins, and dissolution of water-soluble proteins of aleurone grains, as well as microbiological sterilization. After cooling, the suspension was sonicated with a density of acoustic energy of 0.5 W/cm^3 for 10 min. Ultrasonification is carried out in a mode that allows generating multi-scale acoustic flows directly in the zone of the mass transfer process, and as a result, a developed system of flows appears in the extractor. It includes such flows from the sizes comparable to the scales of the containing capacity of the extractor to the scales of the hydrodynamic boundary layer (1–10 μm). In this case, ultrasonic action is accompanied by cavitation and, consequently, the appearance of many local impact waves with pressures up to hundreds and thousands of atmospheres. Such an impact on the solid phase leads to a decrease in diffusion resistance inside the solid particle, removes diffusion resistance at the solid phase-liquid interface, and significantly increases the efficiency of the extraction process. During ultrasonic processing, β-glucan is extracted from the cellulose matrix in combination with nutrients, vitamins, microelements and other biologically active compounds. β-Glucan-containing extract forms a gel that prevents the reverse sorption of the extracted substances. As a result, a suspension of cellulose particles with extracted substances is formed.

After hydrobarothermic and sonification procedures, extract of oat bran was incubated with a thermostable α-amylase (EC 3.2.1.1) (Amylase®AG XXL, Novozymes, Sweden, 3,000 Units/ml) for 9 hs at 60 °C, pH 7.0–7.4, and constant agitation for starch degradation (liquefaction). Starch degradation was monitored by measuring glucose concentration with glucose oxidase glucometer until reaching the maximal plateauing concentration of the monosaccharides. Then, fermented mixture was cooled at 40–45 °C and incubated with alkali protease (EC 3.4.21.62) (Protease Subtilisin A, Novozymes, Sweden, 8 Units/mg protein) for 9 hs at pH 8.0 and constant agitation in order to digest plant proteins. Rate of proteolysis was monitored by determining free amino acids by ninhydrin method until saturating concentration [9]. After proteolytic treatment, hydrolysate containing starch and protein monomers and oligomers and cellulose particles was separated from liquid phase by centrifugation at 16,000 g for 30 min at 4 °C. β-Glucan was precipitated from supernatant by ethanol (1:3) during 12 hs at 4 °C, sedimented by centrifugation as mentioned above, and freeze-dried. Determination of β-glucan concentration in the final product was made by acidic hydrolysis with 2 M H_2SO_4 for 30 min followed by measuring reducing carbohydrates as described elsewhere [10]. As an alternative approach, β-glucan concentration was evaluated by enzymatic hydrolysis with non-specific endo-1,3(4)-β-glucanase (EC 3.2.1.6) (E-LICACT, Novozymes, Sweden, 186 Units/mg) for 6 hs at 40 °C, pH 6.5.

The principal technological scheme for β-glucan production from oat bran is presented in Fig. 2.

Procedures of β-glucan isolation were made in triplicate. Quantitative results were expressed as Mean ± SEM (Standard Error of Mean).

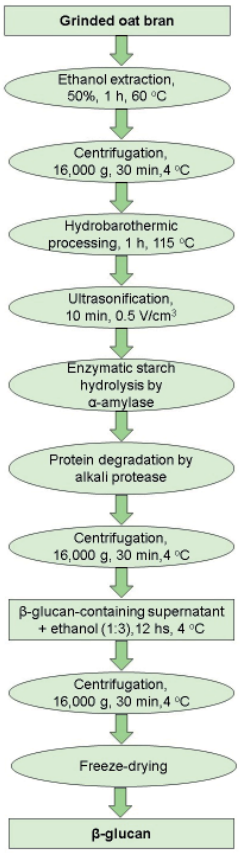

Fig. 2. **Technological scheme for β-glucan isolation from oat bran by ultrasound-assisted and enzymatic processing method**

Results and Discussion

Raw material and final product are depicted in Fig. 3. The yield and concentrations of β-glucans isolated from oat bran are presented in the Table. Some differences in β-glucan concentrations measured after H_2SO_4 hydrolysis and enzymatic degradation can be explained by the fact that acidic hydrolysis could provide total hydrolysis of β-glucans as compared with cleavage by endo-1,3(4)-β-glucanase, which is preferentially is endo-acting enzyme. The content of β-glucan in the final product was obtained as high as ~ 80% that is comparable with β-glucan abundance produced by various methods based on alkalic or acidic treatment of raw material [5, 11]. Moreover, the final product contains less of 5% monosaccharides and trace amount of proteins and amino acids. Thus, combination of hydrobarothermic and ultrasound pre-treatment of raw material followed by its enzymatic processing was shown to be effective strategy for β-glucan extraction and isolation from oat bran. Small amounts of monosaccharides and amino groups make this product potentially usable for many applications.

Summarizing obtained results, it can be assumed that hydrobarothermic treatment of raw material promotes starch gelatinization and dextrinization and contributes to a more complete dissolution of the proteins of the aleurone layer, which makes them more accessible for enzymatic hydrolysis. Ultrasonification step allows extracting β-glucans from cell wall, thus increasing its yield to the final product. In the future experiments, some principal parameters of β-glucans produced by the described methods should be tested because the processing of oats has a crucial impact on the main parameters of β-D-glucans, such as molecular weight (Mw) and viscosity. It has been reported in literature that viscosity of β-glucans in the gut is mainly responsible for its cholesterol lowering effects [7, 12]. Water-solubility and Mw of β-glucans are considered to control availability to immune cells or other biological or biomolecular targets [5, 13].

A *B*

Fig. 3. Oat bran as raw material (*A*) and isolated and freeze-dried β-glucans as a final product (*B*)

Considering the prices of many products, containing β-D-glucans, it should be taken in mind that oats belong to the cheapest grains available in the world. Therefore, oat β-glucans may have extensive industrial applications (foods, medicines, cosmetics, feeds, etc.). Consumption of oat β-glucans has well-documented health benefits [2, 5, 6] (Fig. 4).

β-Glucan is used classically to boost the immune system and to treat hypercholesterolemia [7, 12, 13, 14]. For example, β-glucan can modulate the autoimmune mechanisms directed to pancreatic islets and inhibit the development of diabetes mellitus [15, 16]. It is of interest that in the central nervous system, β-glucans activate microglial cells, which act as scavengers of the brain cell debris and play a protective role in Alzheimer's disease, AIDS, ischemia injury, and multiple sclerosis [5, 16, 17]. Using an atherosclerosis model, Gao et al. [18] have shown that dietary oat fiber had an anti-neuroinflammatory effect and

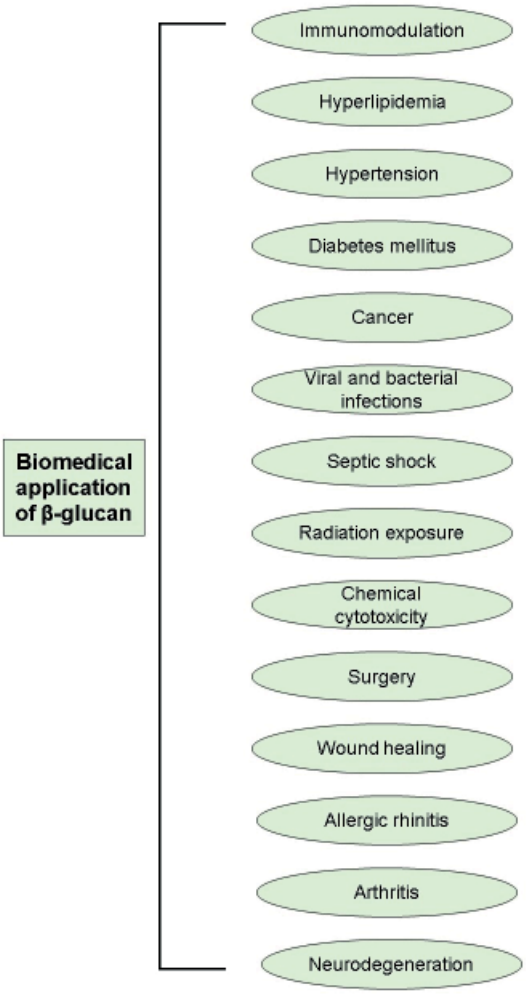

Fig. 4. **Prospective biomedical applications of oat β-glucan**

Evaluation of β-glucan concentration in the final product (n = 3)

Parameter, %	Method for determination of reducing carbohydrates	
	H_2SO_4 hydrolysis	β-glucanase hydrolases
β-glucan yield	10.8 ± 0.23	8.7 ± 0.82
β-glucan concentration in the final product	79.6 ± 3.89	65.1 ± 4.72
Monosaccharide concentration in the final product	3.48 ± 0.55	
Protein/amino acid concentration in the final product	0.24 ± 0.03	

reduced expression of reactive astrocytosis marker, glial fibrillary acidic protein (GFAP), in the cortex and hippocampus of rat supplied by high cholesterol diet. As potent immunomodulators, β-glucans orally administered may be useful as adjuvants, improving the effectiveness of various vaccines currently marketed against SARS-CoV-2 [19]. Purified β-glucan as a substrate for β-1,3-glucanase enzyme assays are widely applied in the research of plant pathology and adversity physiology as well as in routine analysis of microbial β-glucanase activities in their industrial production [20].

Conclusion

Taken together, elaboration of ultrasound-assisted and enzymatic-based method for isolation of β-glucans from oat bran represents highly reproducible and cost-effective approach for β-glucan production. Isolated oat β-**glucans** are free of harmful impurities and suitable for various industrial and biomedical applications.

Conflict of interests. Authors declare no conflict of interest.

Funding. The present research was conducted without any external financial support.

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УЛЬТРАЗВУКОВИЙ ТА ЕНЗИМАТИЧНИЙ МЕТОД ОТРИМАННЯ β-ГЛЮКАНІВ З ВІВСЯНИХ ВИСІВОК

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β-Глюкани — група некрохмалистих полісахаридів, або (1,4),(1,3)-β-D-глюканів, які входять до складу клітинних стінок деяких видів бактерій, водоростей, лишайників, грибів і зерна злакових рослин. Ці вуглеводи широко використовуються в харчовій промисловості, косметиці, фармацевтиці та сфері охорони здоров'я, тому оптимізація методів виділення β-глюканів із зернових має особливе значення для розвитку різних галузей біотехнології, дизайну ліків, харчових технологій.

Метою роботи було розробити оптимізовану технологічну схему виділення β-глюканів з вівсяних висівок із застосуванням ультразвукової та ензиматичної обробки сировини.

Методи. β-Глюкани було виділено з подрібнених вівсяних злаків в ході багатостадійного процесу, який включав екстракцію жирів зерна, гідробаротермічну та ультразвукову обробку, ензиматичний гідроліз супутніх крохмалю та протеїнів, осадження фракції β-глюкану етанолом, центрифугування та ліофільне висушування. Вихід β-глюканів із сировини та його концентрацію в кінцевому продукті визначали після гідролізу сірчаною кислотою або ензиматичного розщеплення ендо4)1,3-)-β-глюканазою.

Результати. Кислотний гідроліз кінцевого продукту дозволив встановити, що вихід β-глюканів склав 10,8 ± 0,23%, а концентрація — 79,6 ± 3,89%, тоді як ензиматичний гідроліз дав відповідні величини 8,7 ± 0,82% і 65,1 ± 4,72%. Таким чином, використання попередньої гідробаротермічної та ультразвукової обробки сировини в комбінації із застосуванням протеолітичниого розщеплення баластних ліпідів і протеїнів дозволило отримувати β-глюкани вівса в кількостях, порівнянних з такими, що отримуються при використанні кислотної або лужної обробки сировини.

Висновки. Описана технологічна схема виділення β-глюкану з висівок вівсяного зерна на основі послідовної гідробаротермічної та ультразвукової обробки, ензиматичного видалення крохмалю та протеїнів може бути широко використана для рутинного виробництва β-глюкану для різних потреб харчової технології, фармакологічної промисловості та медицині.

Ключові слова: β-глюкан; овес; гідробаротермічна обробка; ультразвук; ензиматичний гідроліз