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Review

Insight on Extraction and Preservation of Biological Activity of Cereal β-D-Glucans

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Abstract: β-glucan is a soluble nonstarchy polysaccharide widely found in yeasts, fungi, bacteria, algae, barley, and oats. The cereal β-D-glucans are considered a functional food ingredient due to their numerous health benefits. Its high molecular weight and high viscosity are responsible for its cholesterol-lowering and hypoglycemic properties, which are based on scientific evidence collected in recent decades, both by the FDA and EFSA, which has allowed the reporting of health claims concerning the lowering of cholesterol and the control of the glycemic response exhibited by β-D-glucans from barley and oats. Considering that the biofunctional properties of β-D-glucans are closely linked to their structural and conformational properties, it is of primary importance to implement extraction and processing methods that guarantee these molecules' preservation and the maximum functionality of these molecules.

Keywords: β-D-glucans; extraction; dietary fiber; functional foods; bioactive compounds

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1. Introduction

β-D-glucans is a predominant nonstarch polysaccharide composed of linear chains of β-D-glucose linked by 1,3-, 1,4-, or 1,6-β-glycosidic linkages either in branched or unbranched form. The major sources of β-D-glucan are cereals, microorganisms, mushrooms, lichens, and seaweeds.

The glycosidic linkages in cereal and lichenan β-D-glucans are a combination of 1,3 and 1,4-β-glycosidic linkages; hence, it is called (1→3) (1→4)-β-D-glucan. Lichenan is a linear polymer of predominantly 1,3-β-glycosidic-linked cellotriosyl units. The proportion of cellotriosyl units is higher than in the cereal β-D-glucans. 1,3-β-glycosidic-linked cellopentaosyl units are the second most prevalent feature, unlike the cereal β-D-glucans, where the cellotetraosyl units are the second most common component. There are also regions of 1,3-β-glycosidic-linked cellodextrins with a higher degree of polymerization (DP), similar to the cereal β-D-glucans [1]. Mushroom β-D-glucans consist of a linear 1,3-β-glucan chain with single 1,6-β-glycosidic-linked glucose units attached to the backbone. Yeast β-D-glucans, extracted as large molecules, show a highly branched structure. They contain only side chains linked with 1,6-β-glycosidic linkages to the backbone consisting of 1,3-β-glycosidic linkages. Seaweed β-D-glucans are composed of β-1,3-glucan chains with the presence of β-1,6 branching. Some seaweed β-D-glucans are characterized by the presence of mannitol at the end of the β-1,3-glucan chain. Finally, β-D-glucans extracted from bacteria and *Euglena gracilis* (a single-cell microalgae) consist of linear β-D-1,3-glucan chains without branching [2].

Based on the above molecular structure, β-D-glucan has a high water-binding capacity, resulting in its physicochemical properties, such as solubility, viscosity, and gelation [3]. 1,3-β-bonds interrupt the system of interunit hydrogen bonds in a cellulose-like chain that permits water to solvate glucan macromolecules [4]. For this reason, β-D-glucans are often used as thickeners, stabilizers, and fat substitutes in foods [5]. The constitution of a viscous gel is also an essential aspect at the digestive level, as it incorporates part of the

digestible sugars and fats taken, reducing their assimilation and making nutrients less accessible to human digestive enzymes [6,7]. The β-D-glucans from these different sources are recognized as functional and bioactive food ingredients due to their biological activities, such as, hypocholesterolemic properties, immunomodulation, hypoglycaemic activity, prebiotic properties, antioxidation, and anti-inflammation [8]. The content of β-D-glucans differs greatly between different sources. The β-D-glucans content is higher in barley than in any other cereal, ranging from 5.0 to 11.0%, and it is present in the endospermic and aleuronic layers. The percentage of β-D-glucans in oats varies between 4.5% and 5.5% and is predominantly present in the aleuronic and subaleuronic layers. β-D-glucans content is relatively low in wheat and ranges between 0.2% and 1.2%. Most of the β-D-glucans in cereals are present in cereal brans; so, the byproducts of cereal milling are mainly used as the main source of β-D-glucan extraction [7,9]. The β-D-glucans found in cereal brans, such as barley and oats, are usually produced as an agricultural byproduct. Their extraction and isolation allow to obtain a value-added product [10]. Among other sources, cereal β-D-glucans have been shown to be the most effective for preventing type 2 diabetes and cardiovascular disease. This is likely due to their ability to effectively reduce postprandial glycemic response, as well as to improve long-term blood cholesterol levels, a known risk factor for cardiovascular disease [11]. Based on the scientific evidence gathered over the past decades, both the Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) have allowed the reporting of health claims regarding the cholesterol lowering and glycemic response control exhibited by β-D-glucans from barley and oats. According to the EFSA's opinion, it is scientifically proven that 3 g of oat or barley β-D-glucan per day contributes to maintaining average blood cholesterol concentrations [12,13]. Concerning the hypoglycemic effect, the EFSA has positively evaluated the health claim for β-D-glucans derived from barley or oats; it has been proven that 4 g of β-D-glucans from oats or barley for every 30 g of carbohydrates available in a quantified portion within a meal would contribute to a reduction in the increase in postprandial blood glucose [12]. Similarly, in 1997, the FDA authorized, in the United States, health claims relating to the lowering of serum cholesterol and a reduction in coronary heart disease for β-D-glucans from barley and oats, if containing in a minimum of 0.75 g per serving of reference food for a daily intake of minimum 3 g [14]. In addition, recent studies have indicated cereal β-D-glucans as bioactive carbohydrates with potential prebiotics capable of promoting abundant and stable beneficial microbial flora with high biodiversity. Human digestive enzymes do not hydrolyze dietary fiber, but gut bacteria can act on it by releasing Short-Chain Fatty Acids (SCFAs) and other metabolites [15]. Several studies have also shown that increased SCFA levels are positively correlated with higher insulin sensitivity, reduced serum cholesterol concentrations, weight control, and reduced inflammation, all of which can minimize the risk of developing metabolic diseases [16].

The origin, molecular weight (MW), and structural properties of β-D-glucans all directly impact their functional characteristics, both on the technological and the health side. In the food industry, most extraction, purification, processing, and preservation procedures have a significant impact on the MW and structural/conformational characteristics of cereal β-D-glucans, which results in their lower MW and loss of viscosity [7,10].

Based on these considerations, this review aims to conduct a comparative analysis of existing β-D-glucan extraction methods, indicating how these methods affect the biological and functional structures and properties of cereal β-D-glucans.

2. The Molecular Structure of Cereal β-D-Glucans

Cereal β-D-glucans are linear homopolymers formed from D-glucose residues bound primarily through two or three consecutive 1,4-β-glycosidic linkages (oligomeric cellulose segments) separated by a single 1,3-β-glycosidic linkage. Longer segments with glucose residues bound consecutively by 1,4-β-glycosidic linkages and with a DP between 5 and 28 are less frequent. There is no evidence that two or more adjacent 1,4-β-glycosidic linkages are present in the β-D-glucan chains of cereals [17]. The molecular characteristics of β-D-

glucans generally derive from the analysis of oligomers obtained from the depolymerization of polymers with enzyme lichenase, a (1→3; 1→4)-β-D-glucan-4-glucanohydrolase (EC 3.2.1.73) specifically cleaves the 1,4-β-glycosidic linkages of the three-substituted glucose residues in β-D-glucans, yielding oligomers with different DP.

The major hydrolysis products for the cereal β-D-glucans are 3-O-β-cellobiosyl-β-D-glucose (DP3) and 3-O-β-cellotriosyl-β-D-glucose (DP 4), which account for 90–95% of total oligosaccharides, while longer oligosaccharides (DP ≥ 5) account for only 5–10% of the total [4,11]. The molar ratio is considered as the ratio of cellotriosyl to celloteraosyl units (DP3/DP4) after depolymerization of the β-D-glucan polymer chain [7]. In β-D-glucans, molar ratio is a unique feature of each cereal and is strongly influenced by cultivars and environmental conditions. In major cereals, the molar ratios are approximately as follows: wheat (3.0–4.5), barley (2.3–3.4), rice (2.4–2.7), and oats (1.5–2.3) [7]. The generalized structure of cereal β-D-glucans and their cleavage with lichen is shown in Figure 1.

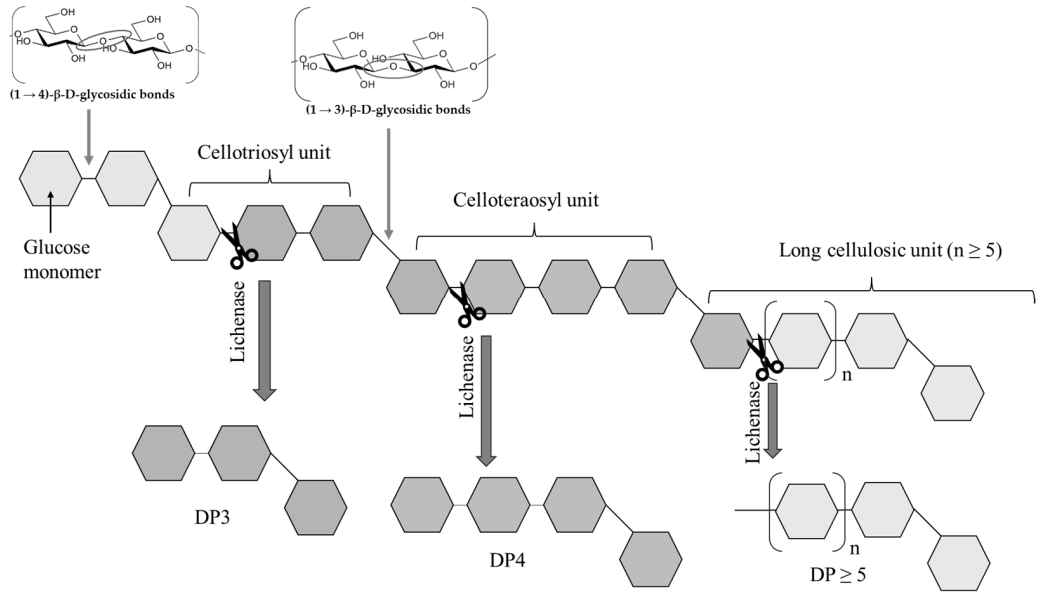

Figure 1. Generalized structure of cereal β-D-glucans and their cleavage with lichenase; scissors indicate the hydrolysis sites of lichen on the polysaccharide chain. Hexagons: glucose monomers; DP3: 3-O-β-cellobiosyl-D-glucose; DP4: 3-O-β-cellotriosyl-d-glucose; DP ≥ 5: cellodextrin-like oligosaccharides containing more than three consecutive glucose residues bound to 4-O.

Despite being classified as soluble fibers, the molecular irregularity of β-D-glucans is reflected in their solubility in water. Although the molecular structure of β-D-glucan allows numerous interactions and connections between its chains and water molecules, a high DP can reduce its solubility. Cellulose-like segments in cereal β-D-glucans could contribute to the rigidity of molecules in a solution; β-D-glucan chains containing adjacent 1,4-β-glycosidic linkages segments may show a tendency for intermolecular aggregation and consequently to lower solubility through hydrogen bonds along these portions. The insertion of 1,3-β-glycosidic linkages leads to the breakage of internal hydrogen bonds in the cellulose-like sequence, resulting in flexible conformation and better solubility in water due to easy solvation with water molecules [4,5,18]. Solubility appears to decrease as the DP3/DP4 ratio along the β-D-glucan chains increases. Moreover, the coexistence of different biopolymers in the cell wall, their spatial organization, and interactions between wall components will likely affect mechanical strength and permeability, thus altering the solubility of the compounds [17]. The highest β-D-glucan content of cereals is found in the outermost layers. Depending on the tissue considered (pericarp, aleuronic layer, and endosperm), their molecular structure changes. Thus, β-D-glucans in the outer layers have a lower solubility and a higher molar ratio in DP3/DP4 than β-glucans located in the endosperm. The β-D-glucans present in pearl byproducts show a higher molar ratio in

DP3/DP4 (~3.3–3.6) than those present in flours or fiber-enriched fractions (~2.8–3) [19]. The high content of consecutive cellotriosyl units along the chain can impose a certain conformational regularity and consequently a greater organization of the polymers in solution capable of decreasing their solubility [4]. The solubility/extractability in water (under comparable conditions of time, temperature, pH, and other extraction conditions) of oat β-D-glucans is generally higher than barley, which in turn is greater than wheat. Therefore, solubility appears to decrease with increasing DP3/DP4 ratio along β-D-glucan chains. Moreover, the coexistence of different biopolymers in the cell wall, their spatial organization, and interactions between wall components will probably affect mechanical strength and permeability, thus impairing the compounds' solubility [17]. In order to improve the applicability of β-D-glucans, some studies have investigated how environmental conditions can facilitate their solubilization in order to have a high extraction yield. As far as temperature is concerned, the best results were reported at 55.7 °C. In fact, at this temperature, there is a greater mobilization of β-D-glucans from cell walls. Higher temperatures are not indicated, as the gelatinization and partial solubilization of starch that would contaminate the extract would be favored [20]. Interestingly, the limited depolymerization of the polysaccharide increases its solubility in water and the stability of the solution. But depolymerization from a low MW (130,000 Da) leads to a reduction in solubility due to an aggregation of low MW units that give rise to aggregates that are no longer in solution [21]. Also, microwave treatment or germination (24 h) before the extraction of β-D-glucans from barley grains improves extraction capabilities, as well as nutraceutical properties. Methodologies are currently being studied to perform controlled depolymerization by acid, hydrogen peroxide, and enzymatic treatment in order to improve the solubilization of β-D-glucans in water [7,8,22]. The MW of β-D-glucans found in barley and oats can vary approximately between 50 and 2000 kDa [12]. These differences are mainly attributed to environmental factors but can also be influenced by extraction, purification, and depolymerization events and the analytical methodologies used to calculate MW [4].

3. Technological and Nutraceutical Value of Cereal β-D-Glucans

In recent years, more and more attention has been paid to the bioactive components of foods and to the continuous research and implementation of functional and nutraceutical foods. The application of cereal β-D-glucans is desirable for a wide range of food products, as they can bring significant advantages both at a technological and health level [23]. In the food industry, cereal β-D-glucans are widely used in the preparation of beverages, sauces, soups, and other foods for their stabilizing, thickening, emulsifying, and gelling properties [15]. However, as extraction, handling, and upstream processing treatments pose a number of challenges, their use, particularly as a health ingredient, is still limited [7]. In fact, the incorporation of cereal β-D-glucans is restricted due to their high viscosity and industrial handling difficulties. Moreover, this problem is particularly evident when they are applied at concentrations sufficient to carry out their beneficial actions on the body [12,13]. Therefore, it is important to consider the characteristics that fiber confers on final products when applied in large concentrations [24]. Just recently, cereal β-D-glucans were applied in different food matrices, such as couscous enriched with β-D-glucans from barley, meat emulsions, yogurt, whole meal, or oat bread, prebiotic sausages, fresh skimmed milk, fermented milks, oat bread and porridge, snacks, cow's milk, chicken breast emulsions, meatballs, and Asian noodles [10]. The addition of β-D-glucans in meat emulsions aims to increase fiber levels in the diet and, at the same time, achieve better technological results [10]. Carrageenan and starch are the hydrocolloids commonly used in meat products due to their technological properties. However, these ingredients have stringent maximum limits dictated by legislation, while the amount of extracted β-D-glucans that can be used in a food application depends on the characteristics of the matrix itself, and there is still no legislative consensus on the maximum and minimum amount of

β-D-glucans that can be added to a given food matrix, nor on the procedures for applying β-D-glucans in each food group or category [10].

In liquid and semiliquid food matrices, such as milk matrices, the incorporation of high-MW oat β-D-glucans, an ingredient that promotes health and obtains low-calorie and cholesterol-lowering dairy products, has been widely studied. However, the thermodynamic incompatibility and phase separation caused by the interaction between milk proteins and β-D-glucans do not allow for the incorporation of an adequate quantity of β-D-glucans to perform health functions [25]. Indeed, mixtures containing a sufficient amount of barley β-D-glucans or oats required for health claims would show an undesirable appearance and texture due to the incompatibility between milk proteins and β-D-glucans [26]. Despite their consumption being associated with many health benefits, β-D-glucans in dairy products are used essentially as thickeners, prebiotic agents in yoghurt, or fat substitutes in reduced-calorie dairy products but not in functionally significant concentrations to fulfil health claims [23]. In addition to potentially being applied to a wide range of food products to improve texture, stability, and viscosity, it has also been shown that cereal β-D-glucans can be used to promote in situ folate synthesis (vit. B_9) by specific yeasts and bacteria naturally present or added to the raw material [27].

In solid matrices, the addition of cereal β-D-glucans has been studied mainly for cereal products (bread, rusks, pasta, biscuits, sweets, etc.). The incorporation of soluble dietary fibers, such as β-D-glucan concentrates from barley or oats, is configured as a successful strategy to lower the glycemic index of these products, as well as determining a greater yield of the dough due to their hydrocolloid nature [5,28–31].

4. Methods of Extraction and Purification of Cereal β-D-Glucans

Cereal β-D-glucans are considered a precious ingredient for different applications. The health properties of cereal β-D-glucans are mainly attributed to the ability of this polymer to form highly viscous solutions capable of increasing viscosity in the intestinal tract [7]. Since the high MW and solubility of cereal β-D-glucans are fundamental physicochemical characteristics for viscosity development, it is essential to preserve them in extracts. Moreover, since the presence of β-D-glucans are naturally reduced in cereals, postextraction concentration is required for their use [32].

Traditionally, there are two main techniques for separating β-D-glucans from cereals, which are called the dry and wet separation techniques. Recovery by dry separation is usually less than 30%, while wet processing results in a recovery of 50–70% [9].

Figure 2 summarizes the different dry and wet processing technologies to obtain concentrated β-D-glucans.

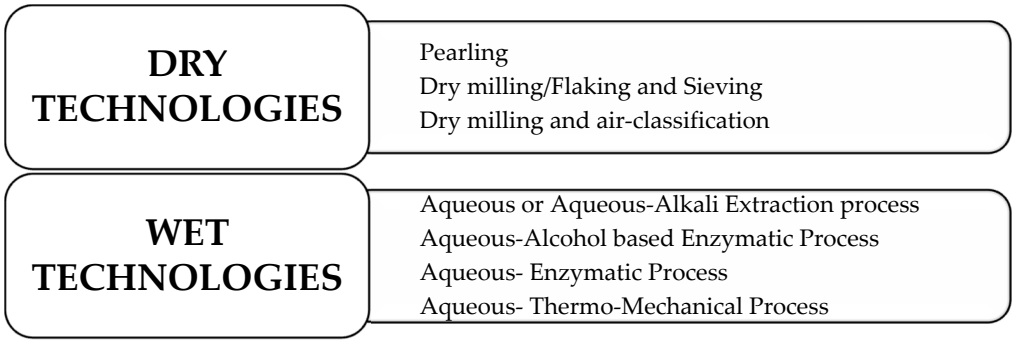

Figure 2. Technologies for the concentration of cereal β-D-glucans, adapted from [33].

4.1. Dry Technologies

Pearling is a dry fractionation process in which the outer layers of the granular tissue are gradually removed through abrasion, without breaking the grain. This technology allows to obtain fractions enriched in β-D-glucans up to a concentration of 25% [34]. Pearl barley and pearl barley flour are commercially available, while the pearling of oat

grains has not been successful due to extensive caryopsis breakdown attributed to oats' relatively high lipid content [33]. In dry grinding and sieving, the separation of β-D-glucans is based on postgrinding particle size. Raw flour is a complex particulate, where each particle, depending on the extent of the size reduction, varies in its chemical composition (starch, proteins, β-D-glucan, hemicellulose, cellulose, lipids, and minerals) and physical characteristics (size, shape, and density). This practice is generally employed in barley; in oats, it is not feasible due to the high lipid content compared with barley, which entails an obstruction of the sieve meshes [33]. Generally, before dry extraction, it is necessary to carry out a degreasing of the sample; in fact, the removal of lipid content from plant material improves the extraction of cereal β-D-glucans [35]. Following various destructive grinding and selective sifting processes, concentrations of β-D-glucans of about 12% from barley and 17% from oats can be obtained. Although researchers have also obtained β-D-glucans from the cell walls of the amyliferous endosperm, these approaches are much less widespread. Generally, β-D-glucans are obtained from bran fractionation, a fraction normally removed to avoid interference during processing and used as animal feed [7]. Separation occurs in dry grinding and air classification based on particle density. Through the optimization of the parameters, it is possible to obtain a concentrate of fibers containing up to 30% β-D-glucan [33]. The principle of operation of the various air classifiers is based on the Stokes equation, thanks to which it is possible to obtain the different speeds of fall of the particles depending on their density compared with that of the medium (i.e., the air), as well as consider their diameter. As reported by Gomez-Caravaca et al. (2015), air classification can be considered a green technology to produce barley coarse fraction with high amounts of β-D-glucans from the whole grain [36].

To date, milling and subsequent sifting are commonly used in combination with wet extraction techniques to obtain sufficiently concentrated and pure β-D-glucan extracts [37]. The industrial relevance of dry extraction is very limited, mainly due to the intensity of the process's costs, low yields, and the need to degrease the sample before extraction. To increase the concentration of β-D-glucans in the extracts produced by dry methods, the possibility of implementing a combined effect of ultrafine grinding and electrostatic separation in defatted oat bran fractions was evaluated, obtaining a concentration in β-D-glucans of 48.4%. This portion, further fractionated by a combination of jet-milling and air classifier, allowed to reach concentrations of β-D-glucan up to 56.2% in the final product. Electrostatic separation, as an emerging technology, could become a much more feasible process, offering an interesting alternative for the extraction of β-D-glucans [35]. Anyway, to date, the most common way to fortify starchy foods products with cereal β-D-glucans is the addition of bran obtained by selective milling. However, the concentrated fractions with this approach are not suitable to be integrated with all foods, such as liquid matrices, because high quantities would be needed to obtain significant health benefits, and this would result in the impairment of the organoleptic qualities of the product or that it does not contain enough β-D-glucans to qualify as a functional food [29].

4.2. Wet Technologies

Wet extraction procedures are more popular than dry extraction techniques, mainly due to the higher yield and purity of β-D-glucan extracts obtainable. As shown in Table 1, most commercially available β-D-glucan extracts have been obtained by wet extraction procedures and may contain up to 75% cereal β-D-glucans [33].

The extraction techniques of β-D-glucans vary depending on the source. The five main methods for solvent extraction are hot water extraction, alkaline extraction, enzyme extraction, solvent extraction, and ultrasonic/microwave-assisted extraction. These techniques can be used alone or in combination. Figure 3 shows the entire wet extraction process, with previous pretreatments and subsequent purification processes to increase yield and purity [38].

Table 1. Extraction processes used by different companies to extract β-D-glucans from barley and oats to obtain the corresponding commercial product [33].

Company	Source	Processes	Registered Name
Natraceutical Edmonton, AB, Canada	Barley and oats	Enzymatic semialcoholic	Viscofiber ® (Up to 65%)
GTC Nutrition, Golden, CO, USA	Oats	Watery	OatVantage ™ (Up to 54%)
Cargill Inc., Minneapolis, MN, USA	Barley	Watery	Bfiber ™ (Up to 70%)
GraceLinc Ltd., Canterbury, New Zealand	Barley	Watery	Glucagel ™ (Up to 75%)
Van Drunen Farms, Momence, IL, USA	Oats	Aqueous-thermomechanical	Nutrim ™ (Up to 6%)
Danisco, Thomson, IL, USA	Oats	Aqueous-enzymatic	Oatrim ™ (Up to 25%)
Nutrition Inc., Boulder, CO, USA	Oats	Air grinding and classification	Oatwell ™ (Up to 22%)

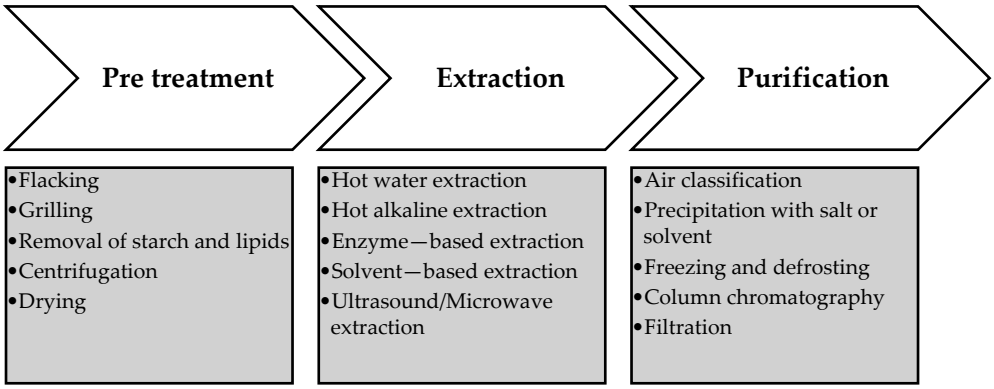

Figure 3. Scheme for the extraction and purification of β-D-glucans, adapted from [38].

The extractability of β-D-glucans depends on particle size, pH, temperature, extraction time, and solvent and solute ratio. Generally, in wet extraction, the β-D-glucans present in barley/oat/wheat flour are solubilized in water, acidified water, or an alkaline solution. As highlighted in the following sections, the most used method involves extraction in an alkaline solution, as it is the best method to preserve the structure of β-D-glucans, as well as allow the removal of starch and other insoluble fibrous fractions. Proteins can be removed by isoelectric precipitation and subsequent centrifugation. Enzymatic treatments with thermostable protease, α-amylase, are generally performed to hydrolyze proteins and starch bound to fibrous particles to maximize the purity of the extract. Subsequently, fibrous particulates rich in starch and protein-deprived β-D-glucans are recovered by precipitation with ethanol and centrifugation. Ethanol also inhibits some indigenous enzymes and removes dissociated sugars, proteins, and some nonpolar compounds [4,9]. Following this crude extraction, β-D-glucans can be purified further using freezing–thawing methods and alcohol in order to remove starch, protein, fat, and other residual unwanted species [9]. During extraction and purification, an additional phase of β-D-glucanase inactivation should be employed. This enzyme is responsible for the hydrolysis of β-D-glucans, which causes an alteration of the structure, MW, and viscosity of β-D-glucans; consequently, their cholesterol-lowering activity and glucose modulation is reduced. Enzymatic inactivation

may be possible by autoclave, HCl, trichloroacetic acid, ethanol precipitation, or heating. With alkaline extraction with NaOH, the fraction of arabinoxylans (AXs) is also coextracted with β-D-glucan. The percentage of AXs and β-D-glucans coextracted by alkaline extraction with NaOH depends on the cereal source and fraction considered [37]. For example, in the starchy endosperm of barley, these two fibers are present in cell walls in these percentages: 70% (1→3, 1→4)-β-D-glucans and 20% AXs. However, in the aleurone cell walls of barley, the situation is the opposite [38]. In contrast, wheat endosperm cell wall polysaccharides comprise about 70% AXs and 20% (1→3, 1→4)-β-D-glucans [39]. Therefore, selecting the fractions and origin of cereal byproducts to extract and isolate β-D-glucans is crucial. Table 2 shows the amount of AXs and glucans (mainly β-D-glucans) in cereal residues and byproducts [38].

Table 2. Amounts (%, dry weight) of arabinoxylans and glucans in cereal residues and byproducts. Table adapted from [39].

Sources		Arabinoxylans	Glucans (β-D-Glucans)
Bran	Barley	19.0–21.4	6.15–7.58
	Oat	3.0	5.4–8.5
	Rice	4.8–5.1	0.04–0.21
	Wheat	18.0–24.0	2.1
	Rye	12.0–18.0	2.9
	Corn	27.2–29.9	0.1
Husk	Barley	19.0–20.0	37–40
	Oat	3.5	37.10
	Rice	8.4–9.2	34.2
	Wheat	NR	38.2
	Soybean	13.1	NR
Straw	Barley	11.0–14.0	33.6
	Oat	NR	32.1
	Rice	11.0–18.3	34.1
	Wheat	NR	30.4
	Rye	NR	33.12
	Corn	27.0–30.0	40.9
Corn cob		12.8	41.6

NR: Not reported.

Studies on possible molecular interactions between AXs and β-D-glucans indicate a spontaneous and intermolecular solid association between unsubstituted regions of xylan chains and cellulose-like 1,4-β-linked fragments from the β-D-glucan chains. These associations are thought to be based on hydrogen bonds. These noncovalent associations, as well as the extent of cross-interactions between β-D-glucans and AXs in plant cell walls, could contribute to the poor water extractability, solubility, and enzymatic indigestibility of these polysaccharides [37,40,41]. Since solubility, which is linked to viscosity, is the main functional and technological characteristic of cereal β-D-glucans, this is a problem. The purity of β-D-glucans extract in alkali can be increased by fractional precipitation and digestion with xylanase [42]. Fractional precipitation can be performed at increasing concentrations of $(NH_4)_2SO_4$ in water, because the solubility of β-D-glucans and AXs in this medium is different. β-D-glucans can be precipitated to a much lower $(NH_4)_2SO_4$ saturation level (20–55%) than AXs (55–95%) [43]. However, the precipitation techniques do not always separate the polysaccharides completely. Another approach is to use more

selective extraction solvents, such as $Ba(OH)_2$ solutions that interact preferably with pentose sugars and then extracting AX and avoiding coextraction with β-D-glucans that remain insoluble in solution [41,42]. The key disadvantage of these wet separation technique is their high cost of production [9].

Extraction with Water

The β-D-glucans obtained by simple aqueous extraction, although leading to a high-purity β-D-glucans extract, showed low viscosity in the solution when reconstituted in water. This is mainly attributed to the depolymerization of β-D-glucans by endogenous enzymes (β-D-glucanases) naturally present in flours. The loss of viscosity due to depolymerization following enzymatic hydrolysis is not desirable, as viscosity is a fundamental parameter for its health effects. Another disadvantage of this type of extraction is the high cost of production, mainly due to the large volume of water required in the initial phase to hydrate and solubilize the β-D-glucans (the β-D-glucan ratio: water should be at least 1:200, w/w) and the consequent high volume of absolute alcohol to dehydrate and precipitate the β-D-glucans from the aqueous solution in the terminal phase (∼50% v/v) [33].

Alkali Extraction

Among the best conventional methods used for the wet extraction of cereal β-D-glucans, the extraction with alkali is the one that allows the greatest solubilization and consequent extraction of cereal β-D-glucans. In addition to the high yield, it also has good purity of extract. The process involves four main steps:

a. Mixing: The ground wheat/flour/oat bran or barley is mixed in an alkaline aqueous solution (pH~10), generally basified using Na_2CO_3 or NaOH. The β-D-glucans and proteins are then solubilized.
b. Centrifugation of the compound: The insoluble solid particles, starch, and insoluble fiber are separated from the liquid phase containing the solubilized β-D-glucans and proteins.
c. Precipitation of proteins once their isoelectric point (pH~4–5) is reached by adding acid. The proteins are then removed from the liquid phase by centrifugation.
d. Recovery of β-D-glucan concentrate from the liquid phase by alcohol precipitation and centrifugation, followed by drying [44].

Concentrated β-D-glucan molecules showed a porous/spongy/fluffy appearance, and, in particular, showed no trace of cell wall structures [33].

Hydro-alcoholic Enzymatic Process Extraction

Cereal flours or bran are kneaded in a hydro-alcoholic solution and then screened to remove other unwanted components, mainly starch and protein, from the filtrate. Fibrous particles rich in β-D-glucans were retained on the screen. This concentrate is kneaded again in alcohol and then treated with enzyme preparations, namely thermostable proteases and α-amylases, to hydrolyze the proteins and starch bound to the fibrous particles. Subsequently, the particulates are recovered using simple screening techniques. With this technology, it is possible to obtain fiber concentrates containing up to 65% β-D-glucans. Scanning electron micrographs of the concentrated fiber showed the structure of the native "honeycomb" cell wall, indicating that β-D-glucans remain intact within the cell wall and are not solubilized [33].

4.3. Emerging Extraction Methods

In addition to conventional extraction methods, some new extraction techniques reported in the literature are ultrasonically assisted extraction (UAE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE).

In the UAE technique, ultrasound can induce specific actions such as fragmentation, erosion, sono-capillary effect, sono-poration, and stress or local detexturation (or combined actions of the above) within the raw material, thus improving the efficiency and yield of extraction [40,41].

Reports on MAE technology, used for the extraction of phytochemicals, reveal that this technique requires a lower volume of solvent and offers a higher yield in shorter extraction times than conventional methods [42].

The ASE technique involves extraction with a pressurized solvent at high temperature. While high-temperature extraction speeds up the process, high pressure helps keep the solvent in a liquid state, thus promising safe and fast extraction with less solvent and less extraction time [40,43].

Among the methods reported, the ASE is configured as the best in the ratio between speed and yield. The conventional solvent extraction method has several drawbacks, such as time- and labor-intensive operations, as well as extended concentration steps that can result in loss and degradation of target analytes. ASE can potentially be used as a cereal β-D-glucan extraction technique at the industrial level. ASE has a wide range of advantages. It uses an eco-friendly extraction system and solvent (water), extraction times are shorter than most other techniques, and extraction discrimination, compared with conventional methods, is reduced [45]. However, further optimization of the β-D-glucans is needed before ASE is established on an industrial scale to extract β-D-glucans in the food and pharmaceutical sectors. Especially, it is of paramount importance to accurately control the extraction time to prevent excessive depolymerization and consequent loss of the functional properties of the polymer [46].

4.4. Considerations on Different Extraction Methods and Purification of β-D-Glucans in Cereals

The methods of wet extraction of cereal β-D-glucans differ mainly in the raw materials used, the ratio of β-D-glucans in the raw material to the solvent used, and the type of solvent used. All these factors affect the contamination of the extract with unwanted residues of starch and protein. After β-D-glucan is extracted, purification processes to remove starch and proteins are not always carried out. In any case, these contaminants in the extract can prevent adequate characterization and create considerable difficulties in applying the application of this polysaccharide to food [47]. Among the different types of wet extraction, those that use alkaline aqueous solutions can provide the highest yield of cereal β-D-glucans extracted [48]. As known, the functionality of soluble fiber lies mainly in its ability to absorb large amounts of water forming viscous solutions at the gastrointestinal level that make it more difficult for nutrients to pass from the lumen to the intestinal mucosa. The viscosity is conditioned by the MW and the concentration (c) of β-D-glucan in the solution. Thus, the parameter MW × c is a key indicator of the potential health-promoting effect of β-D-glucan extract [7,49]. Just as endogenous β-D-glucanases can reduce polymer MW following enzymatic depolymerization, a low pH can also compromise the primary structure of the molecule. A pH 4 can lead to acid hydrolysis of β-glycosidic bonds, thus decreasing the MW and viscosity of the solution [10]. Primary solvent extraction is effective at pH 8, endogenous β-D-glucanases are inactivated, MW is high, and consequently, viscosity is high [20]. Removal of contaminants (proteins and starch) is generally performed using hydrolytic enzymes or selective adsorption. The following precipitation of β-D-glucan is usually carried out with absolved alcohol. Finally, the residue is dried by freeze or spray drying [17]. The main β-D-glucan purification technique is enzymatic treatment with α-amylase and protease [50]. However, to reduce contaminants, it is important to evaluate different enzyme combinations during β-D-glucan extraction [51]. Since enzyme purification is costly, some researchers have begun to investigate alternative purification techniques for food applications. Future investigations should aim to produce β-D-glucan extracts as a convenient nutraceutical ingredient. Finding the optimal conditions to facilitate rapid extraction with minimal impurities will be essential to make the best use of the potential of cereal β-D-glucans as a nutraceutical ingredient.

5. Causes of Degradation of β-Glucans

Many of the functional and health properties related to β-D-glucans are attributed to the viscosity of β-D-glucans, in turn governed by their MW and concentration. Extraction

and processing methods can affect the functional characteristics of β-D-glucans, causing their depolymerization and viscosity reduction. The main cause of β-D-glucans depolymerization is due to the hydrolase activity of endogenous β-D-glucanases when extracts are in an aqueous medium. Especially in the preparation of bakeries, the hydrolysis of β-D-glucans is an important problem. The mixing time, in which the flour is hydrated and processed, as well as the fermentation time, cause a substantial depolymerization of the β-D-glucan chain, resulting in a decrease in MW and viscosity and loss of technological and functional properties [52,53]. It becomes imperative to understand the stability of β-D-glucans and the effect of various extraction and processing conditions on physicochemical characteristics, in particular viscosity and MW. Many studies have reported that various processing methods, such as application of high-pressure-assisted homogenization, ultrasonic treatment, addition of ascorbic acid, extrusion, and irradiation, result in a decrease in the MW of β-D-glucans [7,9,10].

β-D-glucans can also be chemically degraded by the presence of Reactive Oxygen Species (ROS), which can lead to oxidative depolymerization. Iron (Fe^{2+}), even in small quantities, can promote the formation of hydroxyl radicals (OH·) and, consequently, the depolymerization of polysaccharides. Moreover, this degradation process can be increased by the presence of ascorbic acid [54]. High temperatures can also lead to the hydrolysis of β-D-glucan molecules, and there is a synergistic effect with the presence of ROS. For example, the same treatment at 85 °C causes a slow hydrolysis of the β-D-glucan molecule without affecting the glucose monomers in the absence of pro-oxidant species, while in the presence of Fe^{2+} and hydrogen peroxide (H_2O_2), large amounts of OH· are induced, causing a rapid and extensive degradation of β-D-glucan and, at the same time, the generation of new functional groups (lactones, carboxylic acid, aldehydes, and ketones) [55]. The presence of phytic acid, on the other hand, a natural molecule used by plants to accumulate phosphorus inside the seeds and fibrous parts during maturation, can protect against oxidative stress. This is because phytic acid, known as an antinutrient capable of chelating certain mineral cations such as calcium, iron, magnesium, manganese, and zinc into insoluble complexes that reduce their bioavailability, is also a powerful antioxidant, since subtracting iron from the Fenton reaction reduces oxidative stress, protecting against free radicals and delaying cellular aging processes [56]. Moreover, thermal degradation is drastic if the treatments are carried out at high temperatures for prolonged periods (120 °C for 30 min), and it is even more intense in unpurified extracts [57].

The extrusion firing treatment can also decrease β-D-glucan content, especially if humidity and temperature are high [58]. Extrusion can have an impact on both the MW of β-D-glucan and its extractability depending on the parameters applied. Food ingredients undergo many "order–disorder" transitions (starch gelatinization, protein denaturation, and formation of complexes between lipids and amylose), thus altering the physical and chemical properties of the extruded products [59]. High temperatures, high pressures, and mechanical forces applied during extrusion can break and recombine the covalent bonds between monomers, thus modifying the physical structures of macromolecules and leading to a change in their functional properties. Extrusion variables such as screw speed, screw configuration, cylinder temperature, and food humidity can affect the rheological and functional properties of ingredients, including viscosity, solubility, and thermal and colloidal properties [60]. However, a not too intensive extrusion process can improve the functional and technological properties of this extruded fiber that manifests more aggregates, a higher gelatinization temperature, greater solubility, better swelling capacity and solvent retention, and an increase in apparent viscosity and consistency, as well as a decrease in the flow index and greater foam stability [61].

One of the most important unit operations in the development of cereal products with a long shelf life, improved sensory parameters, and preservation of nutritional value is roasting. This heat treatment has no effect on the total content of cereal β-D-glucans—what varies is their solubility. This decrease in solubility can be attributed to the polymerization of β-D-glucans during heat treatment [62]. Since the viscosity conferred by β-D-glucans,

which is functional to its health and technological aspects, is the product between MW and soluble β-D-glucan concentration (MW × c), excessive solubility loss is a problem [21,63].

Preservation by freezing also leads to a reduction in the solubility of β-D-glucans, as well as a reduction in MW [53,64]. Water crystallization reduces the hydration of polysaccharide macromolecules and leads to the cryoconcentration of solubilized β-D-glucans during previous processing and cooking. These concentrated low-MW β-D-glucans will tend to aggregate via intermolecular hydrogen bonds that will reduce their solubility. The subsequent thawing process will allow greater mobility of the accumulated β-D-glucans, thus accelerating the formation of new intermolecular hydrogen bonds between the polymers, ultimately leading to a more stable aggregation. The reduction in the spatial occupation of β-D-glucans accompanied by the decrease in their solubility also reduces the viscosity of the dough [65,66]. Furthermore, subjecting the dough to repeated freeze–thawing cycles progressively reduces the solubility of β-D-glucans [67].

Table 3 summarizes the main causes of degradation in terms of depolymerization and/or a reduction in the solubility and viscosity of cereal β-D-glucans, in relation to different processing practices, and provides indications on how to avoid/reduce such degradation.

Table 3. Main causes of degradation of cereal β-D-glucans during extraction and processing and possible solutions to avoid the loss of their functionality.

Cause of Degradation and Functionality of Cereal β-D-Glucans	How to Avoid Depolymerization/Degradation
Activity of endogenous β-D-glucanases	■ Inactivation of β-D-glucanases by autoclave, HCl, trichloroacetic acid, ethanol precipitation, or heating. ■ Extraction in alkaline environment (pH > 8). ■ Avoiding acid extraction conditions (pH 4 can lead to acid hydrolysis of β-glycosidic bonds, thus decreasing MW and viscosity of the solution). ■ Reduce, as far as possible, mixing and fermentation times of the dough.
Oxidative depolymerization in the presence of ROS	■ Avoid contact with pro-oxidant metals such as Fe2+, Cu2+. ■ The presence of phytic acid can chelate pro-oxidant metals by removing them from the Fenton reaction and protecting β-D-glucans from oxidative depolymerization. ■ Avoid the presence of ascorbic acid. ■ Avoid high temperatures (>85 °C)
Thermal degradation	■ Avoid prolonged high-temperature heat treatment for long periods (120 °C for 30 min). ■ Prefer heat treatments over purified extracts.
Germination	■ Drying and roasting to block the activity of endogenous β-D-glucanases.
Extrusion firing	■ Process food in nonintensive conditions ■ Work in conditions of low humidity (≈20%) and temperatures that are not too high (≈150 °C).
Toasting	■ Prefer lower roasting temperatures and longer warmup times.
Freezing and thawing cycle	■ It is preferable to avoid frozen storage in order to maximize the physiological effects of β-D-glucans. ■ Reduce freeze–thaw cycles to a minimum. ■ Prefer ultrafast freezing to traditional freezing, which is responsible for the formation of macrocrystals and the cryoconcentration of solutes. ■ Freezing with liquid nitrogen is the most effective for maintaining the physicochemical characteristics of β-D-glucans.

6. Conclusions

To better manage and preserve the chemical–physical properties of cereal β-D-glucans, paying close attention to the processes of extraction, processing, handling, and preservation of food will be necessary. The methods that subject this functional polymer to a series of stress factors undermine its characteristics and potential. During the extraction and processing procedures of cereal β-D-glucans, the molecules can be degraded mainly by oxidation reactions, enzymatic depolymerizations, thermal cleavages, freezing, or interaction with other compounds present in food. In addition, processing/extraction conditions significantly influence the concentration, viscosity, MW, and solubility of β-D-glucan. A thorough study should be carried out to understand the behavior of β-D-glucans mixed with other ingredients (synergism or antagonism) and interactions with other compounds. The main challenge in incorporating β-D-glucans into foods is the use of high enough concentrations that allow the application of the associated health claims to the labels of healthy (enriched/fortified) products and the expected health effects. Furthermore, the cereal β-D-glucan content in the final product and at the end of the shelf life should be evaluated to understand the extent of fiber degradation during processing and storage.

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Abbreviations

The following abbreviations are used in this manuscript:

AXs	Arabinoxylans
ASE	Accelerated Solvent Extraction
DP	Degree of Polymerization
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
MAE	Microwave-Assisted Extraction
MW	Molecular Weight
ROS	Reactive Oxygen Species
SCFAs	Short-Chain Fatty Acids
UAE	Ultrasound-Assisted Extraction

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