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REVIEW ARTICLE

β-Glucan, a Promising Polysaccharide for Bio-based Films Developments for Food Contact Materials and Medical Applications

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Abstract: ***Background*:** β-glucans are naturally occurred polysaccharides of glucose units, present in the cell wall of various living organisms such as bacteria, yeast, fungus and plants, in particular cereals (oat and barley). β-glucans are considered as GRAS and are currently used as texturing agents in the food industry. Alternative applications of β-glucan are the development of bio-based by using isolated β-glucan or in combination with other biopolymers.

***Objective*:** The aim of this work is to review all the potentiality that β-glucans present to develop bio-based films for food contact materials and medical applications.

***Results*:** It was very well described that the intake of β-glucan is related to the decrease of plasma cholesterol and stimulation of the immune system, depending on the β-glucan nature. However, during the last decade there has been an impressive grow on the interest on the development and use of bio-based films and packaging materials. Yeast cell wall, that consist of β-1,3-glucan network crosslinked to β-1,6-glucan, mannoprotein, and a small amount of chitin, are an attractive encapsulation matrix and for film forming preparations. In addition, dispersions of β-glucan from oat cultivars demonstrated to be promising films forming hydrogels, with potential to be used as biodegradable edible packaging film. Indeed, some investigations suggested the production of polysaccharide nanocrystals based on β-1,3-glucan from S. cerevisiae by using an esterification method.

***Conclusion*:** β-glucan was found to be a biopolymer with interesting features to be applied in materials science field, food contact and medical applications.

Keywords: β-glucan, bio-based films, yeast cell wall, cereals, biopolymer combinations, food contact materials.

1. INTRODUCTION

Bio-based films consist in a thin matrix performed from a solution or dispersion of biopolymers of long chains such as polysaccharides and protein. To form the film matrix, the solvent is removed from the solution or dispersion by an appropriate method in order to decrease the distance between polymers, favoring their interaction. These interactions allow the formation of a polymer network matrix film [1]. Bio-based films based on polysaccharide of different origin have a broad spectrum of applications in food production, pharmaceutical industry, cosmetics (anti-ageing), and food ingredients [2]. β-Glucans from various natural sources: plants, algae, yeast, fungi and bacteria, are beneficial to human health due to notable physiological effects as biological response modifiers [3], with benefits on wound healing [4]. In 2011, the European Commission included the β-glucan to the list of novel food components of functional food and food of special nutritional designation [5]. β-glucans also present rheological, biocompatibility and biodegradable properties that make them interesting molecules to be used in different fields. In addition to all these particular characteristics, β-glucan have film forming ability that make this biopolymer interesting to be studied in materials science field and food contact applications.

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2. STRUCTURAL POLYSACCHARIDES IN NATURE: β-GLUCAN

Polysaccharides are a very important class of biopolymer, constituted by repeating sugar units. They are structurally characterized by the type of sugar, the chain length, type of glycosidic linkage and branching degree. Polysaccharides are synthesised in nature with structural porpoises, like cellulose in plants, glucan in some microorganism and cereals, chitin in animals or as storage carbohydrates as starch and glycogen.

A special carbohydrate, conformed by β-1,3-linked polyglucoses, known as β-glucan is present in many bacteria, fungi, yeast, mushrooms and higher plants and has called the attention due to its bioactive properties such as immune-stimulation, anti-inflammatory, antimicrobial and wound healing activity; among other properties related to functional activities such as emulsifying, viscosifying and water holding capacity [6, 7]. All these splendid properties along with its non-toxic characteristics make this biopolymer an ideal compound for new developments and applications.

In nature, β-glucans are mainly present in fungi (yeasts and mushrooms) and plants, and they are present in different conformations and linkage depending on the origin as Fig. (**1**) shows. In addition, the bioactivity and properties of this biopolymer change due to this different conformation. Table **1** shows a comparison of the diverse β-glucan from different sources and with different structures and the corresponding bioactivity.

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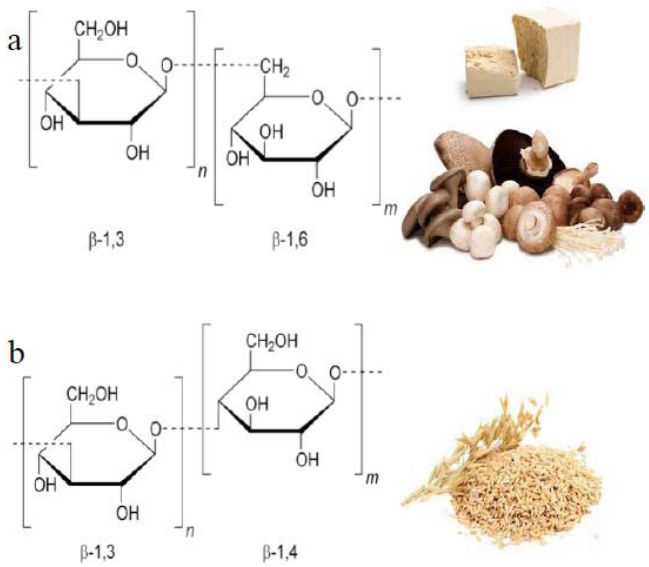

Fig. (1). Types of β-glucans: a) fungal β-(1,3)(1,6)-glucans; b) plant β-(1,3)(1,4)-glucans.

β-1,3-glucans in microorganism, have a structural function like cellulose in plants. Fungal glucans are conformed by a main chain of β-(1,3)-D-glucose residues to which β-(1,6)-D-glucose side groups are attached (branched), which give the molecule interesting properties like its bioactivity. In the cell wall of yeast, β-glucans present a triple helix conformation, unique in the polysaccharide family. β-(1,3)-D-glucose may present molecular weights between 20,000-240,000 Da [16]. *Saccharomyces cerevisiae* is the common food grade yeast for brewery and bakery applications and are also well known for the production of immunopotentiating glucans found in the cell wall. The highly branched glucan obtained from yeast (β-YG) are insoluble but with some chemical modification or enzymatic treatments a soluble glucan could be reached to be applied in food applications. The insoluble β-YG, have shown strong mitogenic activity and enhance the production of cytokines and monocyte phagocytosis [7]. Regarding other fungal glucans, "lentinan" is a glucan elaborated by edible mushrooms *Lentinusedodes* (Shiitake mushrooms), branched in the (1,6) position every third glucose unit with an average molecular weight of 500,000 Da. Then, schizophyllan (also called sizofiran), is a similar polysaccharide produced by the edible mushroom *Schizophyllum commune*, with a molecular weight of 100,000 and 200,000 Da, also present a triple helix conformation. These are the two most studied glucan from mushrooms sources among others [17]. High β-glucan content was found in edible filamentous fungi such as *Paecilomyces variotii* and *Penicillium nalgiovense* with concentrations of the polysaccharide of 24 and 17%, respectively. These values are higher than those found in Basidiomycetes and yeast. These findings provide new non-conventional ingredients [18]. However, yeast β-glucan represents in most of the research work, either in medical application, food application and new applications such as film conformations for food contact materials.

Cereal β-(1,3)(1,4)-glucans occurs in the sub-aleurone and endosperm cell walls of the grains. The content of β-glucan in different cereals are: barley (3-11%), oats (3-7%), rye (1-2%) and wheat (<1%). These glucans are linear homopolysaccharides of D-glucose units linked via a mixture of β-(1,3) and β-(1,4) linkages. Some studies reported that β-(1,3)(1,4)-glucans are unbranched, and that the (1,3) linkage occurred singly, meanwhile the linkage (1,4) occurs in groups of two or three. Molecular weight of these glucans is ranged from 20,000 to 40,000,000 Da. This variation is attributed to the different cell wall structure depending on the origin (cereal source), extraction and isolation methods, aggregation phenomena, depolymerisation events, due to enzymes. Cereal β-D-glucans are able to form gels under certain conditions depending on the origin. Molecular weight has a big influence on the gelling potential.

3. POLYSACCHARIDES AND PROTEINS FOR BIO-BASED FILM DEVELOPMENTS

During the last decade there has been an impressive grow on the interest on the development and use of bio-based packaging materials. Food contact materials developed by natural biopolymers, such as proteins, polysaccharides and lipid, are commonly known as edible films. The utility of edible films and coatings are their capacity to improve food quality, extending shelf life, and possibly improving economic efficient packaging materials [8]. Moreover, edible films and biodegradables films do not contribute to the environment pollution. The main characteristics of edible films are renewable natural sources and their capacity to act as carriers of food ingredients and the potential of use of these films as selective barriers to the transport of gases, vapours and solutes [19]. Generally, to reduce water transmission fats and waxes are the best to be used; polysaccharides may be used to control oxygen and other gases transmission and proteins give mechanical stability. More often, these materials are used individually for film developments, however many applications were tested by using mixtures of them, like whey protein and okra [20], soy protein and pectins [21] or cassava starch blended with soy proteins [22] and in this way is possible to achieve best properties of each biopolymer.

Mainly, protein films are produced from sources like milk, soy, wheat and milk, and they present very good physical stability. We should mention that these proteins are a group of different proteins with different molecular weights; lower molecular weight components are better solubilized but films are more permeable to gases or low mechanical properties. These drawbacks can be solved by different treatments such as cross-linking by using additives or playing with the isoelectric point of proteins [23, 24]. When selecting a protein to be used in edible films, they should present the GRAS (Generally Recognized as Safe) status and one should be aware that a given segment of people is allergic to some proteins, in particular to wheat proteins. As mentioned, proteins major advantage is their structural stability (e.g., sausage casing).

Polysaccharides produce physical stable films and they present the ability to decrease oxygen transmission. However, they do not provide very good barrier against water vapour. This detrimental feature is linked to a good oxygen barrier and though, polysaccharides can be used to protect food from oxidation [25]. Polysaccharide films are also known as "sacrificing agents" instead of being a true barrier, when they are applied on the surface of the food and absorb water vapour, retarding moister loss from the food. Polysaccharides mostly used in film formation are starch, cellulose and its derivates, alginate, carrageenan, pectins, chitosan. Literature related with the use of β-glucan as a source for biodegradable films is scarce; however the potentiality of this biopolymer for these porpoises is getting the interest of many researchers.

3.1. β-Glucans for Bio-Based Film Development

As commented before, cereal β-glucans present gelling and water retention capacity that made this biopolymer interesting to be used as a raw material to form edible films. In this way, Skendi

Table 1. β-glucan properties from different sources.

β-glucan Properties	β-glucan Source					Ref.
	Oat	Barley	Mushrooms	Brewer´s Yeast	Baker´s Yeast	
Structure	β-1,3/1,4 glucan	β-1,3/1,4 glucan	β-1,3/1,6 glucan	β-1,3/1,6 glucan	β-1,3/1,6 glucan	
Reduce serum cholesterol levels	X					[8]
Attenuate blood glucose level	X	X			X	[9, 8]
Improve/stimulate immune function			X	X	X	[10]
Promotes healthy inflammatory response			X		X	[11]
Topical application/skin treatment/wound healing					X	[12]
Mycotoxin adsorption	X	X		X	X	[13]
Fat replacer in food formulations				X	X	[14]
Food thickener, emulsion stabilizer	X	X			X	[2]
Film forming biopolymer	X	X	X	X	X	[8, 15]

et al. [8] used water soluble β-glucans from oat to develop edible films by using sorbitol as plasticizer. They evaluate mechanical properties and how they are influenced by molecular weight, concentration of plasticizer and water content of the films. The authors extracted the soluble β-glucan from milled oat seeds with hot ethanol to remove lipids and inactivate enzymes and dry. Then, an aqueous extraction step was performed at low temperatures and proteins were eliminated by adjusting the pH. Films were obtained by casting of an aqueous solution of 3% w/v and stirred at 60°C, and then sorbitol was added at a fixed concentration of 15% w/w. Films were dried at 50°C during 2 days. Results from this investigation demonstrated that aqueous dispersion of whole oat flours obtained at low temperatures produced low molecular weight glucan with promising film forming solutions to be used as biodegradable edible food contact materials. The films obtained presented good mechanical properties that were influenced by the presence of sorbitol and water.

Regarding yeast β-glucans, they are located in yeast cell wall and usually covalently linked with mannoproteins and chitin to form a macromolecular complex. The cell wall is based on an internal skeletal layer of yeast β-(1,3)-glucans which are directly linked to mannoproteins at the non-reducing end or indirectly through an interconnecting β-(1,3)-glucans. Chitin is also linked to the skeletal very close to the plasma membrane. Literature describe three classes of yeast β-glucans: 1) alkali insoluble-acid insoluble β-(1,3)-glucans, which are involved in maintaining cell wall strength and shape; 2) alkali soluble β-(1,3)-glucans, which confer flexibility of the cell wall; and 3) highly branched β-(1,6)-glucans, which act as interconnector between β-(1,3)-glucans, chitin and mannoproteins. Many processes for isolation and purification of β-glucan have been reported, most of them use hot alkali, acids or a combination of both which solubilize proteins and other polysaccharides. However, these methodologies may degrade the polymer chain to such extent that alternatives for the extraction have been tested as those performed by Freimund *et al.* [26], which described an isolation treatment that combined hot water and organic solvents with enzymatic treatments. The process is perfectly scalable and high yields and high purity glucans were obtained. Isolated β-(1,3)-glucans from yeast cell wall for film forming application were described by Novak *et al.* [27]. These authors isolated the biopolymer from commercial baker's yeast (*S. cerevisiae*) and prepared an aqueous solution of β-glucan at a concentration of 10% w/v and glycerol was used as plasticizer (25 % w/w dry base). They produce biodegradable films by the casting method and the evaluation of physicochemical, morphological and mechanical properties of the obtained films was performed. Results demonstrated that films were not water soluble; the measured soluble matter corresponds to the glycerol added. The enzymatic assay showed that the concentration of β-glucan was around 55% w/w and together chitin and α-glucans do not exceeded 20% w/w. The films were non porous and present no crystallinity measured by X-ray Diffraction (XRD) analysis. Atomic Force Microscopy (AFM) analysis demonstrated that films surface was granular maybe due to the presence of rest of cell wall. Mechanical properties revealed dependence with glycerol and water as expected and values were 712±31 MPa, 17.48 MPa and 14.16±1.11 MPa for Young Modulus, strength and strain at break, respectively.

3.2. β-Glucans Combined with Other Biopolymers for Bio-Based Film Development

Although the raw materials: grains, yeast, fungi, are reasonably inexpensive, the extraction and purification processes of β-glucans usually involve high costs and may degrade the polysaccharide [26]. The high cost may be a drawback for some industrial applications [28]. Major components, both from the grains or yeast cell, such as proteins are removed during the extraction and purification of β-glucan and discarded during the washing steps. Therefore, using purified β-glucan for film food contact materials and edible coating is not economically feasible. For this reason, if the isolation is not forced to high purification, some proteins may remain together with the β-glucan and multicomponent dispersions could be achieved. Interactions between polysaccharides and proteins are well described and it was demonstrated that this interaction produces materials with a complex network with specific properties [29]. In this section, combination of β-glucan with other polymers from the same biomass (vegetal or fungal) or added externally will be described for food contact materials and coating applications.

Polysaccharides and proteins interact strongly with water; therefore films made from these biopolymers are hydrophilic films. Polysaccharides and proteins are generally used for their ability to establish polymer interactions and create a continuous network responsible for the structural and functional properties of the biodegradable films [21, 30]. In general polysaccharides-based films allow the control of permeability of gases while proteins-based films display better mechanical properties [31, 32]. In this way, combination of polysaccharides and proteins may be used to obtain the best properties of each biopolymer. A natural combination of β-glucans and proteins may be obtained from the integral cell biomass of *S. cerevisiae* [15]. Yeasts contain approximately 40-60% of proteins and 35-40 % polysaccharides such as β-glucans and mannans which are basic components of yeast cell walls [33]. Since cells of *S. cerevisiae* can be regarded as food-grade, low cost and abundant food ingredient, they represent an interesting source for the production of films made from the combination of polysaccharides such as β-glucans and proteins [15].

Cells of *S. cerevisiae* demonstrated to be capable to form films when specific treatments of high pressure homogenization and thermal heating, in a certain order, were applied to yeast dispersion [15]. Heating treatment causes the unfold of polypeptide protein chains, while high pressure produces the rupture of cell wall and the release of cytoplasmic components from the cell, allowing the availability of polysaccharides and proteins to interact and form the film. According to these results yeast biomass must be viewed as an alternative and valuable option to traditional sources of biodegradable polymers to generate new ecological-friendly packaging films [15]. This last work demonstrates the importance of the rupture of the yeast cell wall in order to release intracellular materials leading to a better interaction between proteins and polysaccharides. Many investigations based their research on different methods for breaking the cell wall for releasing the cytosol by homogenization in a bed mill, autolysis, autoclaving, sonication and their combinations [34]. The highest release of the cytoplasm was observed when cells were treated in the bed mill homogenization. This investigation also give results for obtaining isolated β-glucan from the cytosol, but interaction between this polysaccharide and proteins from the cell wall still exists if not extra purification is performed. Autolysis combined with bed mill or sonication reached the same results but with longer times. Liu *et al.* also studied ultrasonic disruption of yeast cells for the release of the cytoplasmic material [35].

In order to find new application of yeast cell wall, once it was separated from the cytoplasm, Kasai *et al.* [36] prepared acid-treated yeast cell wall for pharmaceutical applications and they develop a coating based on acidified yeast cell wall (AYC). The AYC films presented a large tensile strength and high barrier to oxygen. In addition, water vapour permeability was sufficient to protect any product from moisture. The authors added also acetaminophen 3% and the release of the drug showed a sigmoidal shape.

Interaction between β-glucan and proteins from the same biomass was also studied for vegetal original biodegradable films. Razzaq *et al.* [37] studied the possibility of using all natural components from barley (β-glucan and proteins) as an inexpensive renewable resource to produce biodegradable films. The authors tested different extraction conditions that gave the best interaction between polysaccharide and proteins. For this, they performed a full characterization of the biodegradable films based of chemical characterization, structural properties, tensile properties, water vapour permeability, resistance to water and thermal stability. Results demonstrated that there was a strong network established through interactions between the polysaccharide and protein polymer chains when the extraction was performed in an alkaline media. Thermal properties were significantly increased due to the interaction between biopolymers and mechanical properties, were improved getting higher elastic modulus, small deformability and strength similar to many commodities films such as LDPE and HDPE. Results from this investigation suggest the potential of the system as novel, sustainable and cost-effective food packaging plastic material. Further combinations of β-glucan and other biopolymers were reported by Sárossy *et al.* [38]. Their investigations were focused on the development of biodegradable films based on agricultural crop by-products by the extraction of rye polysaccharides and combined them with extra β-glucans, from other source. In their work, they extracted water soluble hemicelluloses fractions (they called this fraction WE), other fraction containing approximately 65% arabinoxylans and 20% mixed-linkage β-glucan (called WE-AX). Indeed, they extracted β-glucans from oat and used it as reference and to be added to the WE and WE-AX formulations. When β-glucans were extracted from the isolate fractions, mechanical properties were changed and these films presented a decrease in tensile strength and in the elongation at break values. When β-glucans was added to the WE-AX the elongation at break and tensile strength increased. However, the addition of the β-glucan had no influence on oxygen permeability, while pure WE-AX and β-glucan films presented excellent oxygen barrier properties. On the other hand, water vapour permeability increased with the addition of the β-glucan. Therefore, considering mechanical and oxygen permeability properties, the addition of β-glucan is beneficial when analyzing its application to films based of arabinoxylans. In a different work, two alkali-extracted arabinoxylan fractions from barley fiber with varying β-glucan content were studied [39]. The authors found that the presence of β-glucan had a positive effect on film flexibility, where the combination present twice as high elongation at break values than pure arabinoxylan films.

4. MICROENCAPSULATION AND NANOTECHNOLOGY APPLIED TO β-GLUCAN

Apart from film formation, yeast cells are also used as for the production of low cost-high volume microcapsules to deliver essential oils and flavours. *S. cerevisiae* cells are available in large quantities, have a light colour, bland taste, making all these characteristics an interesting media to encapsulate and protect bioactive compounds. Natural antioxidants, colorants and flavours were encapsulated in *S. cerevisiae* cells. Chlorogenic acid (CGA) is a water soluble and natural antioxidant that present many beneficial properties mainly related to its ability to interact with reactive oxygen species. Shi *et al.* [40] encapsulate this antioxidant in order to protect it during storage against oxidation before use or protect it from the transesterification reaction that this compound is involved during processing. The authors studied the system for functional food developments; therefore the release of the antioxidant was performed in simulated gastric fluid (HCl) where the release was found to be higher than in a buffered solution at pH 7.4 or water. Results agreed with the yeast cell architecture, which is composed by an outer protein layer and plasma membrane, both act as a permeable barrier and can be destroyed by acid or base [41]. Therefore, it is expected that the encapsulated antioxidant be released easily under acidic conditions. Bishop *et al.* [42] suggested that the bilayer of yeast cells could act like a liposome, allowing the stabilization of the encapsulated essential oils, providing stable products. The authors

encapsulated orange peel oil in non-plasmolysed yeast cell. The encapsulation process involves mixing an aqueous suspension of the yeast and essential oils and the oil pass through the membrane by diffusion, remaining passively within the cell. Following this trend, flavour encapsulation in commercial yeast cells and the study of their release performance was studied by Dardelle *et al*. [43]. The yeast cells were emptied with a plasmolyser before staring the encapsulation process. The loading procedure was performed by infusion of 80 g of dry yeasts in 440 g of an aqueous flavour solution at 9.1% w/w. It was observed that not all the molecules penetrate the cell in the same manner, depending on the hydrophobicity of the compound. The remarkable results from this work showed that flavour were very well protected at high temperature due to a decrease in the permeability of the cell, making the system interesting to be used during cooking processing protecting labile compounds. Other authors used yeast cell to microencapsulate curcumin. In this work, the authors were focused on encapsulation parameters such as plasmolysis of yeast, presence of ethanol and ratio between the capsules (yeast) and curcumin [44]. The authors demonstrated that in all the microcapsules prepared, the curcumin was integrated in the plasma membrane but also interact with constituents of the yeast cell wall such as glucans and mannoproteins. Recently, the encapsulation of berberine in yeast cells, a bioactive compound with many positive medicinal properties such as characteristics such as antiplatelet effects and good effect on inflammatory diseases was described [45]. The authors demonstrate that it was possible to load 42% of berberine in the capsules which means that the efficiency was quite good.

Nanomaterials have demonstrated to have many advantages related to their size, high surface-to-volume ratio and unique optical properties. Nowadays, biomolecules are getting most of the attraction for the synthesis of nanoparticles due to their friendliness with the environment [46]. The use of polysaccharides such as starch, cellulose or chitosan for the synthesis of nanoparticles has been reported in recent years [47, 48]. However, the use of β-glucans for nanoparticles production is not very well explored and the investigations related to the use of this polysaccharide with some modifications by covalently attaching functional units to the polysaccharide backbone demonstrated promising results.

It is well known and already commented that β-1,3-glucan has immunostimulating capacity, having a specific receptor on immune cells. It is reported that a type of transmembrane protein receptor binds β-1,3-glucan specifically and may start and regulate the innate immune response [49]. Therefore, since β-glucan is a ligand to dentin receptor, it was suggested that nanoparticles derived from β -glucan may present targeting ability against immune system [50]. Following this trend, some investigations, suggested the production of polysaccharide nanocrystals based on β-1,3-glucan from *S. cerevisiae* by using an esterification method using formate as intermediate and they produce two types of esterified products: β-1,3-glucan hexanoate and β-1,3-glucan acetate [6]. They were able to prepare solid and hollow nanospheres with a diameter ranged between 132 to 487 nm. The authors suggested that this new type of nanoparticles could be potentially used for drug delivery targeting immune cells.

β-glucan nanoparticles were also prepared by isolating the polysaccharide from the cell wall of *Pythium aphanidermatum*, mixed NaOH 2% and stirred at 90 ºC to produce nanoparticles [46]. In order to promote a stable formation of nanoparticles sodium tripolyphosphate (TPP) was added. The nanoparticles obtained were spherical, smooth and homogeneous. The average size of nanoparticle determined by dynamic light scattering by using a NanoZeta Sizer® was about 60 nm and the zeta potential was negative. The authors suggested the application of these nanoparticles to prevent some diseases caused by phytopathogenic fungus, since in their research they found the growth inhibition of *Pythium aphanidermatum* due to β-glucans nanoparticles measured by the disc diffusion agar technique.

CONCLUSION

Since many years ago, the scientific community, called by consumer demands and environmental concerns, are looking for new sustainable and renewable sources for biobased materials development. Throughout this work different applications of β-glucan, coming from different renewable and economical resources were referenced. This polysaccharide is abundant in nature and could be found in fungus, yeast, bacteria and plants. The β-glucan presents, by itself, beneficial properties for health such as antioxidant and immunostimulant activity, wound healing, among other interesting properties. Indeed, it is well known as a food ingredient, mostly used as emulsion stabilizer. In addition to these properties, β-glucan presents good rheological behavior, biocompatibility and biodegradable character, giving to this biopolymer the aptitude to be used in different fields. In this sense, β-glucans are able to form films with very good properties alone or in combination with other biopolymers such as proteins. In addition, it was used to encapsulate flavors and bioactive compounds to develop delivery systems. The synthesis of β-glucan nanoparticles are very well described opening the range of ideas. In the present work, β-glucan is found to be a biopolymer with interesting features to be applied in materials science field, food contact and medical applications.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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