

# New Developments and Prospective Applications for (1,3) Glucans

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**Abstract:** Publications and patents relative to newly observed functions of  $\alpha$ -(1,3)-D-glucans have notably increased in the last few years with the exploitation of their biological activities. The term  $\alpha$ -(1,3)-D-glucans includes a very large number of polysaccharides from bacterial, fungal and vegetable sources. Their structures have a common backbone of  $\alpha$ -(1,3) linked glucopyranosyl residues but the polysaccharidic chain can be  $\alpha$ -(1,6) branched with glucose or integrate some  $\alpha$ -(1,4) linked glucopyranosyl residues in the main chain. Except for the curdlan, a bacterial linear  $\alpha$ -(1,3)-D-glucans, and for the scleroglucan produced by *Sclerotium rolfsii*, the main drawback limiting the development of these polysaccharides is the lack of efficient processes for their extraction and purification and their cost. However new applications in agronomy, foods, cosmetic and therapeutic could in a next future accentuate the effort of research for their development. So this review focuses on these  $\alpha$ -(1,3)-D-glucans with the objective to detail the strategies employed for their extraction and the relation structure-functions identified when they induce biological activities.

**Keywords:** Polysaccharides, oligosaccharides, curdlan, glucan, biopolymer.

## 1. INTRODUCTION

Polysaccharides are produced universally by living organisms. They exhibit a large variety of complex chemical structures, physiological functions and a wide range of potential applications in various industrial areas as food engineering, biodegradable plastics, cosmetics, agronomy, fuel and others. So, these polymers are renewable resources which offer real and potential uses for man. Eventhough ~40 monosaccharides have been described, the majority of polysaccharides are formed from a limited range of sugars and more especially from hexoses and pentoses. These polysaccharides can be heteropolysaccharides, containing different monomers or homopolysaccharides constituted of a single sugar. The latter often contains D-glucose linked by either  $\alpha$  or  $\beta$  glycosidic bond in linear or branched backbone. Additionally various substituents such as acyl groups, amino acids or inorganic residues may be present in the side chain.

This paper, focuses on a family of glucans, especially of  $\alpha$ -(1,3)-D-glucans which have been extensively studied during the last decade. Effectively, most of these polymers exhibit interesting physicochemical properties, especially gelling capability, leading to their extensive use in food applications. Moreover, they also exhibit biological activities associated with medical, foods, pharmaceutical and cosmetic applications.

So these polysaccharides produced by prokaryotes and eukaryotes organisms are divided in several classes relating to their structural features.  $\alpha$ -(1,3)-D-glucans and  $\alpha$ -(1,3)(1,6)-D-glucans are the most extensively described in papers and patents. They can be linear, branched but also

cyclic and easily produced in reactor. They are cell wall components of fungi or excreted as exopolysaccharides by bacteria.  $\alpha$ -(1,3)(1,4)-D-glucans are found in cereal grains and are actually studied for their high potential in food industry. Their extraction from grains or flour was until now the major drawback limiting their industrial usage.

The aim of this review is to provide a comprehensive view of the current state of knowledge on  $\alpha$ -(1,3)-D-glucans and on the recent patented applications of these molecules.

## 2. $\alpha$ -(1,3)-D-GLUCANS

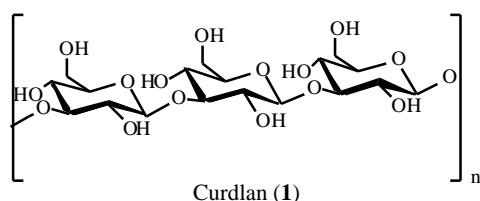
$\alpha$ -(1,3)-D-glucans are found both in the prokaryotes and eukaryotes and are extractable from cereals, mushrooms, seaweed, and yeasts [1]. Those from fungi and yeasts are found as cell wall components. They have a common structure comprising a main chain of (1,3)-linked  $\alpha$ -D-glucopyranosyl units along with randomly dispersed side chains of  $\alpha$ -D-glucopyranosyl units attached by (1,6) linkages.

Glucans from barley, oat, or wheat are also found as cell wall components of grains but exhibit a different structure, with a mixed  $\alpha$ -(1,3) and  $\alpha$ -(1,4) glycosidic bonds in the backbone. Concerning prokaryotes, a variety of bacteria, including pathogens of human, livestock and plants, produce extracellular polysaccharides (EPS). Some EPS are major virulence determinants of animal pathogens. Others are required for pathogenic and symbiotic interactions between bacteria and plants. They have sometimes roles in associations between bacteria and biotic/abiotic surfaces and are matrix components of bacterial biofilms [2]. Additionally, a number of EPS have industrial applications as gelling and emulsifying agents [3]. Several bacteria, including *Agrobacterium* and *Rhizobium* species, can each produce several EPS under appropriate physiological conditions. One of them, the curdlan is a linear  $\alpha$ -(1,3)-D-glucans excreted in culture media by *Agrobacterium* sp.

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## 2.1. Linear $\beta$ -(1,3)-D-Glucans

Curdlan (**1**) is a neutral gel-forming  $\beta$ -(1,3)-D-glucan [4]. It was first detected in *Agrobacterium* biovar. 1 (formerly *Alcaligenes faecalis* var. *myxogenes* strain 10C3), co-produced with another extracellular polysaccharide: the succinoglycan, an acidic heteroglycan [5]. A spontaneous, high curdlan-yielding mutant 10C3K (IFO13714) producing no succinoglycan [5,6] became the progenitor of strains such as NTK-u (ATCC21680, IFO13140) [7], ATCC31749 [8] and ATCC31750 [9] for sustained curdlan-production ability and/or enhanced curdlan yield and gel-forming quality. Curdlan production was also detected in few *Rhizobium* strains [10,11] and in species of the Gram-positive *Cellulomonas*, including *C. flavigena* KU [12, 13].



The production of curdlan by *Agrobacterium* is strain-dependent [7, 9] and is typical of a secondary metabolite as its biosynthesis occurs in the post-stationary growth phase during conditions of N-starvation [7, 8,14]. The optimisation of curdlan yield thus depends on the formation of biomass in a first phase and on the biosynthetic capability of cells in the post-stationary phase. Curdlan yield is optimal at 30-32°C and is affected by nutritional factors, prime amongst are carbohydrate and N sources [7, 8,15]. C sources used for production of curdlan can be glucose, sucrose or sugar cane molasses. Ammonium and nitrate and organic (urea) N sources support the growth of *Agrobacterium* [7] although the bacterial cell mass produced may differ. In batch fermentations, the cell growth rate is optimal at pH 7.0, whereas curdlan production is optimal at pH 5.5 [7,16,17]. Curdlan production under N-limitation is further dependent on an optimum concentration of phosphate [18] and sulfate [8] and on the cation composition of the medium [7,8]. Curdlan production could also be stimulated by the addition of uracil, a precursor of UDP-Glc, when added to cells after curdlan synthesis has started [19]. The observation that different conditions are required to produce optimal yields of cells and curdlan, led to the design of a two-stage process [8,20]. By this way, 93 g.L<sup>-1</sup> were obtained in laboratory-scale fermentations in 14% sucrose medium supplemented with uracil after N-depletion [19]. *Agrobacterium* is grown aerobically and, since diffusion of

oxygen is limited in submerged culture and may be further slowed by the curdlan on the cell surface, several studies have been performed on reactor design in order to improve production yields [8, 20-25]. So, this polysaccharide is recovered from cultures by adding NaOH and removal of the bacterial cells. The curdlan is then precipitated by neutralization, collected by centrifugation, washed to remove salts, concentrated again by centrifugation and finally converted to a powder by spraydrying [7].

Curdlan polysaccharide consist of as many as 12,000 glucose units [26] and is insoluble in water, alcohols and most organic solvents, but dissolve in dilute bases (0.25 M NaOH), dimethylsulfoxide (DMSO) and formic acid [27].

Curdlan forms a weak gel on heating above 55°C followed by cooling. Further heating to 80-100°C increases the gel strength and produces a firm, resilient gel, while autoclaving at 120°C converts the molecular structure to a triple helix. The gel formed by this high-temperature treatment no longer melts when heated. It is very susceptible to shrinkage and syneresis but resistant to degradation by most  $\beta$ -(1,3)-D-glucanases. Gelation involves aggregation of the rod-like triple helices through non-covalent associations. Rheological and thermal behaviour of low- and high-set curdlan gels has been documented by Zhang *et al.* [28].

## 2.2. $\beta$ -(1,3)(1,4)-D-Glucans

$\beta$ -(1,3)(1,4)-D-glucans (**2**) are the predominant components of cereal grains cell walls such as barley and oats [29-33]. They are concentrated in cell walls of the endosperm and the aleurone layer of barley [34], oats [35], wheat [36], sorghum [37] and any other cereal. Nevertheless, levels of  $\beta$ -glucans can vary dramatically between species, varieties and environmental conditions, but usually range from 2 to 6% dry weight [28, 38-44]. Among all cereal grains, oat and barley contain the highest levels of  $\beta$ -glucan, with the latter at 2-14% of dry weight [38,45,46]. Walls of the barley endosperm consist of about 70%  $\beta$ -glucan and 20% arabinoxylan whereas the aleurone walls contain 26% barley  $\beta$ -glucan and 67% arabinoxylan, both have similar amounts of glucomannan and cellulose [29,30,33,47,48]. The endosperm cell wall barley  $\beta$ -glucan components are linear molecules with approximately 30% (1,3) and 70% (1,4) linkages [29,49,50]. In the linear  $\beta$ -glucan chain the (1,3) linkages occur singly whereas the (1,4)-linkages are found mostly in sequences of 2 or 3 [51]. Nevertheless, this arrangement is dependant on cereal species [52-54] and longer sequences of homogeneously (1,4)-linked monomers exist [52]. The (1,3) glycosidic bonds cause irregularities in the structure of the molecule, which influence their solubility by preventing close packing of the chains [47, 55]. Compared to other cell wall components such as cellulose or



$\beta$ -(1,3)(1,4)-D-glucans (2)

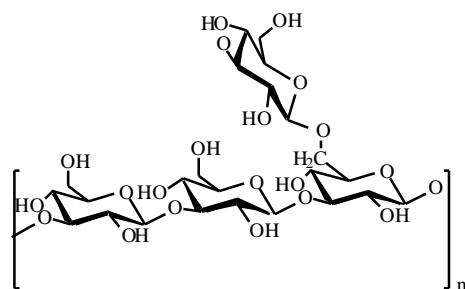
lignin, -(1,3)(1,4)-glucans are highly soluble. However, when ingested, the molecule is not hydrolyzed in the small intestine of human digestive system [56]. Hence, this -glucan is classified as a soluble dietary fiber.

Molecular weight (Mw) ranges reported for -glucans also show variability between cereals, with oat -glucans generally having a higher Mw ( $0.065 - 3 \times 10^6 \text{ g.mol}^{-1}$ ) compared to barley ( $0.15 - 2.5 \times 10^6 \text{ g.mol}^{-1}$ ) [57-61], or other cereals such as sorghum ( $\sim 3.6 \times 10^4 \text{ g.mol}^{-1}$ ) [37]. The rheological properties appear to depend on a number of factors including the ability of -glucan chains association, determined by the proportion of branching units, their arrangement [53,62-64], the degree of polymerisation (DP) and by the Mw of the -glucan [64,65].

Viscoelastic characteristics of -glucan gels are related to the Mw of the isolated fractions. Differences in Mw observed between barley and oat -glucans, and among -glucans extracted from different cultivars of barley [66-67] and oats [51, 68] need to be considered in relation to their potential behaviour in food systems. For instance, the Mw profile of -glucans can be influenced by the method of extraction used [69, 70].

### 2.3. -(1,3)(1,6)-D-Glucans

Several fungal species produce -(1,3)(1,6)-D-glucans (3). All these polymers possess a tertiary triple helix structure, and a main chain of (1,3) linked glucose residues branched with (1,6)-D-glucosyl units. Wide variations can be found in the degree of branching as residues may be attached regularly or randomly.



-(1,3)(1,6)-D-glucans (3)

In schizophyllan, derived from *Schizophyllum commune*, (1,6)-D-glucosyl residues are attached regularly on every third glucose in the main chain. The initial molecular weight can range from about  $1.3 \times 10^6$  to  $6 \times 10^6$ . The degree of branching in schizophyllan is 33% [71]. Another abundant -glucan (lentinan, from *Lentinula edodes*) has a molecular weight of approximately  $5 \times 10^5$  daltons, and the degree of branching is 20% [72], while Grifolan (from *Grifola frondosa*) has a molecular weight of approximately  $5 \times 10^5$  daltons, with a degree of branching of 33% [73].

These -glucans are high molecular weight polymers with highly ordered helical structures, existing as single polymer strands with a helical conformation (single helix) or as a stable complex of three polymer strands forming a triple helix [1]. The soluble polysaccharides yield highly viscous aqueous solutions in which the polymer molecules adopt a

triple-helical conformation. Solutions of scleroglucan (polysaccharide produced by *Sclerotium rolfsii*) are pseudoplastic and their viscosity is not greatly affected over the temperature range of 20-90°C. As the polymer is neutral, the pseudoplastic behaviour of scleroglucan is unaffected by various salts [74], which provides an advantage over polyanionic polysaccharides such as xanthan for agricultural products.

One important source of -glucan is the cell wall of yeast (*Saccharomyces cerevisiae*) which contains about 55-65% of -glucan [75]. -Glucan extracted from spent brewer's yeast, has high apparent viscosity, water holding, oil binding, and emulsion stabilizing capacities [76].

Water solubility of the glucan depends on the frequency and length of the side chains. In contrast to the water-soluble glucans isolated from Basidiomycetes, the particulate glucan from *Saccharomyces cerevisiae* is sparsely branched, i.e. contains single -D-glucopyranosyl units on every eight main chain units [77-79], rendering the glucan insoluble in water.

Finally -(1,3)(1,6)-D-glucans are also found in the periplasm of the legume symbionts *Bradyrhizobium japonicum*, *Rhizobium loti*, *Azorhizobium caulinodans* and in the free-living, root-colonising diazotroph, *Azospirillum brasilense* but as a water-soluble cyclic form. The cyclic glucan from *B. japonicum* [80] and *R. loti* [81] is composed of two blocks of three (1,3) linked glucose units, each separated by a block of three (1,6)-linked glucose units, and has a single-branch glucose unit at C6 [80]. Some molecules are substituted at C6 by phosphocholine [82]. Similar but unbranched and unsubstituted cyclic glucans are produced by *Azorhizobium caulinodans* [83] and *Azospirillum brasilense* [84].

### 3. PRODUCTION AND PURIFICATION OF -(1,3)-D-GLUCANS

Relatively little works has been reported about the effects of food processing on the rheological or nutritional characteristics of (1,3)-D-glucans. Processing may affect the molecular (chemical structure and DP), structural (molecular interactions) and functional properties (viscosity, water binding capacity and solubility) which, in turn, could affect the sensory, physiological and ultimately the health benefits of -glucans. Changes in the properties of -glucans may arise from shearing damage due to mechanical processing [85], or by excessive heat treatment of food products. Unfavourable structural changes may also occur during commercial purification [86], resulting in decreased molecular weight and reduced viscosity. Furthermore, mild extraction conditions (50-60°C) may not deactivate endogenous -glucanases, which in turn may lead to increased depolymerisation of the -glucans [87,88].

As extraction and purification processes drastically influence the glucan properties, numerous patents have been published on the subject. Curdlan production is well documented for a long time. Last published patent on curdlan production [89], dealt with utilization of a new strain exhibiting reduced or deleted phosphoenol pyruvic acid carboxykinase activity. Most recent patents concern mushrooms or cereals sources.

### 3.1. Yeast and Fungi $\beta$ -(1,3)-Glucans

Yeasts and fungi  $\beta$ -(1,3)-glucans are traditionally part of Japanese diet by ingesting whole mushrooms or yeasts. Due to their biological activities, several extraction methods have been recently patented in order to obtain a food additive that could be easily incorporated in food products. Problems relative to these glucans extraction are in relation with the cell wall complexity of fungi and with the undesirable presence of pigments in the extracts. In some cases, extraction is simplified, such as described by the patent of Ikewaki *et al.* [90] in which  $\beta$ -(1,3)(1,6)-D-glucans are obtained from *Aureobasidium* culture solutions or their supernatants after centrifugation. The same glucan is produced in 2006 with a mutant strain of *Aureobasidium sp.* which has low pigment accumulating properties [91]. The product obtained has a high  $\beta$ -glucan content, without developing a strong dark green color caused by accumulation of a melanoid. From fungi, extraction is complex with regard to the presence of glucans as a component of cell walls. Generally,  $\beta$ -glucan polysaccharides are extracted and fractionated by weakening or destructing hydrogen bonding in cell walls of fungi by hot water [92], followed occasionally by extraction under a strong alkaline condition as described with basidiomycetes *Aphyllphorales* mushrooms [93]. Enzymatic hydrolysis of fruit bodies, as those of *Pleurotus cornucopiae*, is sometimes employed in order to form a water soluble  $\beta$ -glucan fraction easily recovered [94]. In the case of Grifolan production, *Grifola frondosa* has been used to generate Grifolan, through fermentation instead of cultivation of mushrooms. This original method allows polysaccharide recovering much more easily, without extraction [95].

### 3.2. Cereals $\beta$ -(1,4)(1,3)-Glucans

Much research has focussed on the effects of isolation and purification techniques on the structural, physicochemical and physiological properties of barley  $\beta$ -glucan [47-49,96-100]. Extraction from these sources could be achieved by using a neutral or an alkaline medium to obtain a gum containing around 45% (dry matter basis)  $\beta$ -glucan [85, 97, 98]. Morgan & Ofman (1998) using hot-water extraction followed by filtering and two freeze-thaw cycles could achieve >90% purity [101]. The extraction of  $\beta$ -glucans from cereal grains generally involves three basic steps: (1) inactivation of endogenous enzymes, (2) extraction of the  $\beta$ -glucans, (3) precipitation. Endogenous  $\beta$ -glucanases, need to be inactivated since they are responsible for  $\beta$ -glucan degradations leading to a decrease in the molecular weight and functional properties of the extracted polysaccharides [59]. One of the major limiting factors for the industrial utilisation of these extraction techniques by the food industry is their cost. Thus, pure preparations of  $\beta$ -glucans have often been ignored and most patents are related to an enrichment of flour fractions after water extraction with or without enzyme inactivation [102-104]. Hence, product obtained often contains less than 50-75%  $\beta$ -glucans (in weight) with low molecular weights and mixed with other cereal components such as bran, cellulose and lignin. This in turn has meant that the actual characteristics of these products in food systems are often variable due to fluctuations in protein or starch composition of the flour fractions. Then subsequent

viscosity, structural and nutritional effects on foods have to be considered in relation to the nature of the  $\beta$ -glucan extract, or the composition of the flour material used. Care must therefore be taken to optimise the yield and rheological characteristics and avoid depolymerisation during extraction of  $\beta$ -glucan components. In this way, extraction achieved without pH modifications authorize the getting of hydro-colloidal products characterized by properties suitable for texturing food, especially bakery products [105]. These compositions are low in digestible carbohydrates (starch) and rich in proteins and  $\beta$ -glucans.

More recent patents have concerned new extraction methods, in order to increase  $\beta$ -glucan purity and to decrease production cost. These methods associate original extraction and purification procedures combined or not with enzymatic treatment. Among them, the use of ultrasonication after water extraction or high pressure homogenization before enzyme digestion provide better extraction of  $\beta$ -glucan compared with traditional strategies [106, 107]. Triantafyllou also related a method to obtain around 65%  $\beta$ -glucan purity from oat flour using enzymes, but leading to substantial oligosaccharides formed [108]. Concentration of water soluble  $\beta$ -glucan fractions obtained by evaporation, alcoholic precipitation or ultrafiltration increased significantly the purity of  $\beta$ -glucan end product [109-111]. However, these procedures still implies numerous steps leading to an expensive product. Moreover, whereas numbers of solutions have been suggested that discuss the isolation of pure  $\beta$ -glucans from cereals, with varying molecular weight and chains lengths, it has been discovered that beneficial effects of using  $\beta$ -glucans in food related products may actually be diminished where substantially all of the protein/starch is removed. Moreover, it has been found that biological effects are maximized for a particular molecular weight (500-2500 Da), while retaining at least 10% of the associated protein and starch. According to that finding, a patent proposed to provide a  $\beta$ -glucan-protein complex composition by complexing a  $\beta$ -glucan with a protein [112].

### 3.3. $\beta$ -(1,3)-Glucan Derivatives

Even if  $\beta$ -(1,3)-D-glucans exhibit strong bioactivities, it has been shown that these activities could be enhanced by modifying the original polysaccharide.

First kind of modification that could be encountered is simply the degradation of the polymer into oligosaccharides by hydrolytic enzymes [113, 114].

Second kind of modification leads to chemical derivatives, especially sulfated derivatives [115-118]. Sulfated polysaccharides have a broad range of important bioactivities comprising antioxidant [119], anticoagulant [116] and antithrombotic activities [120]. They are also known to increase the resistance to some virus [121] and inhibit tumor development [122, 123]. Moreover, it is notable that water-insoluble polysaccharides show little bioactivity, whereas such sulfated derivatives exhibit higher water solubility along with bioactivities [124]. The sulfation of polysaccharides should not only enhance the water solubility but also change the chain conformation, resulting in the alteration of their biological activities [125]. Chemical modification of polysaccharides provides an opportunity to

obtain new pharmacological agents with possible therapeutic uses. Recently, Nie *et al.*, [126] has proposed a method to produce chemically sulfated polysaccharide from *Grifola frondosa* but this method is not actually patented. Patents are related to preparation of curdlan or lentinan-sulfate [127]. Preparation of sulfo-derivatives of bacterial  $\alpha$ -(1,3)-D-glucans consists to partially etherified the polysaccharide using an sulfolalkylation agent under organic and alkaline conditions [128]. More recently, another way of preparing curdlan sulfate has been patented [129]. This preparation of curdlan sulfate in dimethyl sulfoxide with pyridine and chlorosulfonic acid has simple technological process, high efficiency and leads to high sulfur content in the product.

#### 4. APPLICATIONS LINKED TO PHYSICOCHEMICAL PROPERTIES IN FOODS

As other polysaccharides,  $\alpha$ -(1,3)-D-glucans have found a very wide range of potential applications in various industries and notably in agronomy, cosmetic, therapeutic, foods and other. In food industry, beside classical applications of polysaccharides as thickening agent,  $\alpha$ -(1,3)-D-glucans have an increasing interest in the areas of edible film, feed for domestic animals and low calorie food as chemical additives are becoming less and less welcome by consumers.

##### 4.1. Gelling Properties

Curdlan could be used as a biothickening and gelling agent in foods [14,130]. Apart from being tasteless, colourless and odourless, its advantages are that, in contrast to cold-set gels (e.g. gelatin, gellan, carrageenan) and heatset gels (e.g. konjac glucomannan, methylcellulose), the heating process alone produces different forms of curdlan gel with different textural qualities, physical stabilities and water-holding capacities. Moreover, gels of differing strength are formed depending on the heating temperature, time of heat-treatment and curdlan concentration.  $\alpha$ -(1,3)(1,4)-D-glucans from cereal grains present a lower stability and in order to incorporate barley  $\beta$ -glucan gums (BBG) into value-added food products, informations about their functional properties are essential. Medium- (MV,  $20 \pm 100$  mPa s) and high-viscosity (HV,  $>100$  mPa s) BBG solutions exhibit pseudoplastic behaviors at 0.5 and 1% concentration levels [97,98], while high-viscosity gum at low concentrations or low-viscosity (LV,  $<20$  mPa s) gum solutions are Newtonian [98]. Different pH-temperature-time combinations applied during processing may affect  $\beta$ -glucan viscosity and decrease stability of food systems such as salad dressings if these  $\beta$ -glucans are used as stabilizer.

In most food applications, curdlan and other  $\beta$ -glucans as BBG are used in the high-set, thermo-irreversible form, as for pasta noodles [131, 132], bean-starch vermicelli [133] or molded product [134]. Curdlan gels have been used also to develop new food products and calorie-reduced food, since there are no digestive enzymes for curdlan in the upper alimentary tract. In this way processes leading particulate gel containing an oily substance, bread comprising  $\beta$ -glucan with biological regulating functions, jelly food for diet supplements or for person having mastication and deglutition troubles constitute a new generation of food products [135-137]. In Japan, where the use of curdlan in food is permitted,

the food-grade curdlan is used to improve the texture of foods such as bean curd (tofu), bean jelly and fish pastes. It can also find applications in the Japanese food industry for its water-retention capacity, and for the reconstitution and shaping of processed foods and confectionery.

##### 4.2. Film Properties

Gelling properties of curdlan or other  $\beta$ -glucans have also been used for their capability to produce films. Numerous patented applications attempted to obtain capsules or "tablet-like" ingredients which can deliver their content further, after ingestion or during cooking.

This kind of capsule exists for several years, but is generally made of gelatin. Nevertheless, gelatin is prepared from an animal source (bone, skin...), and has a poor heat-resistance. During processes such as sterilization or production of cooked food products, these gelatin-made capsules often release their content in the product. When the capsule must preferably be resistant to food processing, curdlan could be used as it allows forming a non-protein heat resistant capsule that could be filled with any product. As a first step, Ono and Miura have proposed a gelatin capsule, coated with curdlan for greater heat resistance and storage stability [138]. More recently, Kamaguchi and Shiomi have patented a method for producing a capsule entirely made of curdlan [139].

In some cases, this heat-resistance is not required and other  $\beta$ -glucans could preferably be used. For example, JP2001069955 [140] relates obtaining of a capsule containing a liquid that could be dissolved in a soup. Use of  $\beta$ -glucans allows good storage ability at room temperature, whereas it will totally be dissolved in hot water. In JP2005160326 [141] same kind of capsule is produced with mushroom's  $\beta$ -glucans (*Ganoderma amboinense*, *Agaricus* or *Fomes yucatanensis*, or mixed mushrooms) in order to release pickling liquid during cooking of soups or sauces.

$\beta$ -glucans could also be used in order to produce an impermeable film. So, curdlan was used to obtain a film on meat products surface [142]. In order to, curdlan is dissolved using alkaline solution and applied on the meat product surface. As the meat surface has a pH around 6, the curdlan solution is then neutralized at the surface, forming a gel film. The resulting gel is then heat-treated to make it thermo-irreversible. This treatment increase the strength of the gel and in the same time imparts to the casing a texture similar to that of natural intestine, allowing its use for meat products such as sausages. Another example of  $\beta$ -glucans use as films is in preventing water and coloring matter transfers in high water content food products [143].

##### 4.3. Non-Fat Products

Recent research has focussed on the use of soluble dietary fibre, and in particular cereal  $\beta$ -glucans, as stabilizers in the manufacture of low-fat products such as salad dressings [144], ice creams and yoghurts [145] and cheese. The use of  $\beta$ -glucan preparation to partially substitute vegetable oil in the formulation of these foodstuffs has two-fold advantage of (1) decreasing the calorie content of the emulsions, because of a lower proportion of oil in the formulation and (2) utilizing products derived from cereal

industry. Moreover, it has been shown that  $\beta$ -glucan incorporation into low fat cheese curds has beneficial effects on their gelation and rheological characteristics [146]. The addition of  $\beta$ -glucan solutions to milk modifies curd formation, including reducing curd cutting time and increasing curd yields [146]. These effects appear to be related to the gelling capacity of  $\beta$ -glucans and their ability to form a highly structured and elastic casein-protein-glucan matrix. However, as previously described, process allowing complete purification of  $\beta$ -(1,3)(1,4)-D-glucans actually does not exist at industrial level. Use of these glucans in low-fat products then implies incorporation of other cereal constituents (bran, starch...). Consequence is that these products do not exactly resemble full-fat products, as their mouthfeel and sensory properties can be altered. Konuklar *et al.* [147] demonstrated that the incorporation of the  $\beta$ -glucan/starch-rich material significantly reduced the firmness of cheddar cheeses, resulting in a starchy and paste-like product. Whether this is related to the starch or the  $\beta$ -glucan component of the ingredient is unclear. A similar observation was made for soft brined cheeses [148]. The incorporation of  $\beta$ -glucan also altered the appearance and flavour of low-fat white-brined cheese when compared to a full fat control sample. However, in this case the oat  $\beta$ -glucans concentrate used (22.5%  $\beta$ -glucan in the ingredient) reduced the hardness of a low fat cheese when added at 0.7 and 1.4% ingredient levels. This had the effect of making the low fat cheese more closely resemble the full fat cheese control, similar to the observation of Tudorica *et al.* [146] on low fat curd rheology. Thus, the choice of dairy system and the purity of  $\beta$ -glucan additive are of great importance.

First patented applications of  $\beta$ -glucans for preparing low-fat products had concerned meat [149] and laminated doughs [150], but these preparations were not found convincing due to texture problems. Shukla and Halpern have proposed to further submit dietary fiber gel [151] to micro-particulation by a high-shear treatment in order to obtain powder for easier incorporation in foodstuffs, decreasing texture's problems encountered previously [152]. The use of these  $\beta$ -glucans as fat-replacers has then been described in several patents for numerous kind of products such as cheese [153], cookies [154], ice creams [155], dressings [156], sauces [157], or snacks [158].

Recently, yeast  $\beta$ -glucans have also been tested as a fat replacer in mayonnaise and other food products [159, 160]

## 5. APPLICATIONS LINKED TO BIOLOGICAL ACTIVITIES: MEDICAL AND PHARMACEUTICAL

In addition to applications based on its physicochemical properties,  $\beta$ -(1,3)-glucans and their derivatives have medical and pharmacological potential. However, their effects and applications are linked to their structures. Even if cereal  $\beta$ -glucans could influence glycemia and cholesterol level, those from bacteria, yeasts and fungi are better known as biological response modifiers. These molecules have immunopotentiating and antitumorigenic effects, and could also improve human resistance to bacteria or viruses such as AIDS.

In the aim of treating patients with  $\beta$ -glucans, intravenous or intraperitoneal administrations have been inves-

tigated. The problem is that insoluble or hardly soluble  $\beta$ -glucans cause significant adverse effects (granuloma formation, microembolization, inflammation, pain) when administered by parenteral routes [161]. Oral administration is then often preferred.

### 5.1. Hypercholesterolemia, Diabetes and Glycaemia

Much of the more recent interest in the use of cereal  $\beta$ -glucans has stemmed from their use as a functional dietary fibre. The term dietary fibre is used to collectively describe a group of substances in plant material, which resist human digestive enzymes. Such fibers consist primarily of cellulose, hemicellulose, pectin, oligosaccharides, lignin... Dietary fibers can then be divided into two groups: insoluble and soluble fractions. Research into dietary fiber has broadly examined the effects of soluble and insoluble fractions as purified fiber, or in naturally fiber-rich whole foods. It is now known that soluble and insoluble fibers provide different health benefits.

Benefits of insoluble dietary fiber include reduction of bowel transit time [162], prevention of constipation, reduction in risk of colorectal cancer [163-165], production of short chain fatty acids [166-168]. Concerning soluble dietary fibers and especially  $\beta$ -(1,3)(1,4)-D-glucans, health benefits include lowering of blood cholesterol [169-171], reducing hyperglycaemia and hyperinsulinaemia, in relation to the control of diabetes [35,170,172-177], reduction of risk factors for degenerative diseases, such as obesity [176], hyperlipidaemia [177-179], hypercholesterolemia [180,181] cardiovascular disease [182], cancer [183], hypertension [184,185] and promotion of the growth of beneficial gut microflora (as a prebiotic) [186, 187].

Many attempts have been made to clarify the mechanisms by which dietary fibre and  $\beta$ -glucans have these effects. The potential reduction of glycaemic response following ingestion of dietary fibre has led to proposals which implicate: the amount and quality of fibre [188-189]; increased intrinsic viscosity of the food in combination with fluids [190]; maintenance of physical integrity of the food material [191] and incomplete starch gelatinisation [192-194]. The cholesterol-lowering potential of  $\beta$ -glucans is considered to result from effects manifest in the upper gastrointestinal tract. These in turn may be related to their ability to form a gel-like network and alter gastrointestinal viscosity [195,196].

Both barley [197] and oat  $\beta$ -glucans [198,199] produce similar response. Nevertheless, Hallfrisch *et al.* have evaluated the use of  $\beta$ -glucans isolated from barley and oats, and their corresponding effects upon plasma glucose and insulin responses in non-diabetic adults [197]. They concluded that barley  $\beta$ -glucans were more effective in the regulation of glucose and insulin responses compared to oat  $\beta$ -glucans. However, the efficacy of  $\beta$ -glucans may be related to extraction procedures, and factors such as dose, molecular weight and fine structure, and rheological characteristics of extracted and native  $\beta$ -glucans. Wood *et al.* investigating the effect of varying doses and Mw of oat  $\beta$ -glucan administered in a beverage, demonstrated an inverse relationship between the viscosity of the beverage and the magnitude of both blood glucose or blood insulin levels [54]. Variations in

viscosity accounted for 79-96% of the modifications in glucose and insulin responses. Thus, physiological responses appear to be affected by solution concentration and the molecular weight of  $\beta$ -glucans. Further studies indicated that the glycaemic response of fibre-rich foods was inversely related to viscosity (dependant on concentration and molecular weight) [200]. Tappy *et al.* also found that inclusion of oat  $\beta$ -glucan into breakfast cereals could reduce the postprandial glycaemic response by up to 50% [201]. Thus it is likely that a combination of viscosity altering and structure properties of  $\beta$ -glucans are involved in the nutraceutical effects of  $\beta$ -glucan rich foods.

Pasta is one food product to which  $\beta$ -glucans have been successfully included as a functional ingredient. Yokoyama *et al.* compared blood glucose and insulin responses of healthy individuals following the ingestion of control durum wheat pasta to that of a pasta sample with added barley  $\beta$ -glucan [202]. Postprandial blood glucose and insulin responses were significantly reduced following ingestion of the pasta enriched with barley flour added to durum wheat flour. Similar findings have been reported by Knuckles *et al.* [203]. Barley  $\beta$ -glucans have also been used in other cereal based food systems such as bread [204]. More recently, enriched breads with purified barley  $\beta$ -glucan fractions (at 2.5 and 5% replacement levels) subjected to an *in vitro* digestion process shown significant reductions in starch degradation and sugar release [205]. Since this procedure was not reliant on glucose absorption, it would appear that the glycaemic reducing effect of  $\beta$ -glucan may also be related to the way the  $\beta$ -glucan is incorporated into the structure of the bread and may impede starch swelling and subsequent susceptibility to enzymic degradation. The ability of  $\beta$ -glucans to influence the rate of starch degradation and hence the glycaemic index of foods has obvious benefits with regard to obesity and diabetes. Thus, blood glucose levels of diabetic and pre-diabetic individuals can be moderated by using  $\beta$ -glucan rich foods. The use of curdlan for preventing or decreasing diabetes has been described [206]. Authors recommend using curdlan as a medicinal preparation or adding to food products, in the form of an alkaline aqueous solution, a gel, or partially hydrolyzed. In other studies, low molecular weight  $\beta$ -(1-3)-D-glucan are preferred [207].

In addition to the documented effect of  $\beta$ -glucans on the glycemic index of foods, considerable research effort has focussed on the potential benefit of  $\beta$ -glucan as a cholesterol reducing agent.  $\beta$ -glucans are the key factor for the cholesterol-lowering effect of oat bran [171,208-210]. As with other soluble-fiber components, the binding of cholesterol by  $\beta$ -glucan and the resulting elimination of these molecules in the feces helps reduce blood cholesterol [175, 211,212]. Results from a number of double-blind trials with either oat- or yeast-derived  $\beta$ -glucan indicate typical reductions (10% for total cholesterol and 8% for LDL ("bad") cholesterol), with elevations in HDL ("good") cholesterol ranging from zero to 16% [171, 208, 209, 213, 214]. Both barley [181,215,216] and oat [209,217,218]  $\beta$ -glucans have been shown to reduce serum cholesterol levels. However, mechanisms by which  $\beta$ -glucans lower cholesterol are still not clearly defined although the role of viscosity alteration in digesta is important [219]. Increases in intestinal

viscosity may reduce cholesterol level by stimulating the production of bile acid from the cholesterol already accumulated in the human body.

More recently suggested a molecular basis for the hypocholesterolaemic effects of  $\beta$ -glucans [181]. Using a rat model, refined  $\beta$ -glucan and waxy barley were incorporated into a diet for a 2 week period. Both total cholesterol and LDL-cholesterol were reduced in the  $\beta$ -glucan diets compared to a control group, which was associated with up-regulation in the activity of cholesterol 7  $\alpha$ -hydroxylase (CYP7A1), an enzyme associated with the regulation of the pathway through which cholesterol is converted into bile acids. More research is required to elucidate the effect of  $\beta$ -glucan on enzyme and immuno-regulation.

Mixing  $\beta$ -glucan with a non-digestible fat has been proposed [220] in order to obtain a low-calorie product allowing in the same time hypercholesterolemia reduction and postprandial blood glucose and insulin levels control.

In order to improve bile acid binding functionality, Marston *et al.* have patented the use of  $\beta$ -glucan short chains (Mw: 500 to 2500 Da) from oat or barley mixed with at least 10% proteins or starch to improved effects [221]. This mix could then be used as dietary supplement or as food additive when incorporated in products such as cereal bars, dairy products such as yoghurt or nutritional beverages, bakery products and prepared meals.

Other authors have published patents concerning the conjugate use of  $\beta$ -glucans with other active molecule. One of these is plant sterols [222] which are known to limit cholesterol absorption, but also guggul, that could be used in the treatment of hyperlipidemia [223].

## 5.2. Immunopotentiator

In recent years, increased attention has been paid to various types of immunomodulating polysaccharides isolated from the cell walls of fungi and yeasts. A wide variety of mushroom polysaccharides, including scleroglucan, lentinan, schizophyllan, and grifolan have been described as biological-response modifiers [224,225], acting through mechanisms mediated by the immune system, including stimulation of nonspecific functions of it. By these stimulations, they exert a beneficial effect on a variety of experimental disease states of bacterial, viral, fungal, and parasitic origin [1,226]. In this context, the baker's yeast cell wall preparation (Zymosan) was the first defined pharmaceutical yeast product with immunostimulatory activity [227, 228]. Its active component was identified to be the  $\beta$ -D-glucan [229, 230]. Later, this water-insoluble polysaccharide was reported to possess phagocytic activity [231], cytotoxic activity on macrophages, and other biological activities [1, 232, 233].

These effects are manifested through interactions with soluble or cell-bound (Toll-like) receptors of the innate immune system. Binding to these receptors activates signalling cascades which regulate specific genes concerned with the removal of foreign materials and micro-organisms in both invertebrates and vertebrates and further, through the induction of co-stimulatory molecules, and increased antigen presenting activity, helps to direct adaptive immune

responses against antigens derived from the foreign source [234]. The reported immunomodulating and pharmacological responses include anti-tumorigenicity, anti-infective activities against bacterial, fungal, viral and protozoal agents, anti-inflammatory activity, wound repair, protection against radiation and anti-coagulant activity [1,226,235].

Much controversy surrounds the biochemical and molecular principles of the immunostimulatory activity of  $\beta$ -glucans. According to some reports [236], a high molecular weight, a triple helix [237,238], and a distribution pattern with  $\beta$ -(1,6)-branches are essential structural parameters, whereas other authors suggest single helices [72,239]. Moreover, curdlan immunopotentiating activity has been demonstrated [240], whereas it does not exhibit this branching pattern.

Other studies came to the conclusion that helical structures are neither essential nor even advantageous for the immunological activity of  $\beta$ -glucans [241]. Demleitner *et al.* reported that the  $\beta$ -(1,3)-glycosidic linkage type is the most important requirement for the activity [242]. A work suggests that the curdlan single helix is more potent than the triple helix as an antitumor agent [240]. In any case, the expression of the immunological activity presupposes direct interactions between the polysaccharide and the macrophages or other cells of the biological systems studied. These interactions are dependent on the structural and molecular parameters of soluble  $\beta$ -glucans mentioned earlier. In the case of the water-insoluble  $\beta$ -glucans, such interactions would also be affected by the microstructure and the behaviour of the particles in aqueous dispersion used for applications.

Many patents have been published that claim immunopotentiator effects of  $\beta$ -glucans. Nevertheless, in some cases  $\beta$ -glucan is incorporated in so low quantity that the real health benefit remains not so evident. Moreover, the  $\beta$ -glucan is sometimes mixed with numerous other substances, leading to "miraculous" products that may treat pathology. As an example, JP2001333731 [243] describes a product made of  $\beta$ -glucan mixed with milk, silk proteins, placenta extract and nucleic acid. With this mix, authors promise enhancing immune system, but also adjusting homeostasis balance, ameliorating anorexia, insomnia, cephalgia, decreasing stress, recovery of elasticity of skin. More reasonably, it was proposed to mix  $\beta$ -glucan from mushroom with one or two substances such as ubiquinone Q10 and ferments leading to a biologically active additive for food with a wide range of action [244]. In US patent 2006147550 [245] authors also proposed to associate  $\beta$ -glucans with coenzyme Q10. In the same way, grifolan mixed with chitosan and vitamin D2 presents other health benefits [246]. However, in several recent patents, interest is more on the delivery method. In JP2005102525 [247], different fungus are dried, before pulverization of the fungus powder into a food. Even if this solution is very simple and has a low cost, efficacy may be limited due to the low amount of  $\beta$ -glucan incorporated in the product. In order to increase delivery, Suga suggests converting lentinan into superfine particles, improving absorption through mucosa [248]. At least, in order to improve activity of  $\beta$ -glucans for enhancing cellular immunity, Kozbor and Kaneko have

proposed to deliver it transmucosally, by encapsulating glucan in liposomes [249]. According to them, the glucan (curdlan or lentinan) shows a greater migration in blood and then an enhanced biological activity when encapsulated in liposome as compared with a non-encapsulated one.

### 5.3. Anti-Tumorigenic Effects

Many polysaccharides from mushrooms, particularly those derived from *Lentinus edodes* (lentinan), *Grifola frondosa* (grifolan), *Sclerotinia sclerotiorum* (scleroglucan) and *Schizophyllum commune* (schizophyllan) have demonstrated anticarcinogenic effects in both animals and humans. Although the mechanism of their anti-tumour action is still not completely clear, these  $\beta$ -glucans appear to mediate their anti-tumour activity by activation or augmentation of the host's immune system, via activation of leukocytes and production of inflammatory cytokines [250]. Glucan-specific receptors are present on phagocytic cell membranes of several species and potent *in vitro* activation of neutrophil function, including an increase in phagocytosis and killing has been described *in vitro* [251,252]. Nevertheless, in animal experiments,  $\beta$ -glucans have shown varying activity against sarcomas, mammary cancer, some chemically induced cancers, adenocarcinoma, colon cancer and some leukemias. Lentinan has already been shown effective in gastric carcinomas [253,254]. Moreover, lentinan was reported to induce apoptosis (programmed cell death) in murine skin carcinoma cell-lines [255]. Even if mushrooms and especially  $\beta$ -glucans are used in Chinese medicine for decades, mechanisms need to be elucidated. Yet, lot of these substances has already been patented for antitumor treatments. Among them,  $\beta$ -glucan extracted from *Agaricus mushroom* is proposed, together with fucoidan [256]. In the same way, the use of *Grifola frondosa* extract has been patented, mixed with fucoidan and organic germanium [257]. However, effects of these native  $\beta$ -glucans seem quite low. In order to improve their activity, some chemically modified glucans have been produced and patented for this use. So, an antitumor agent is obtained by deacetylating  $\beta$ -glucans extracted from mushrooms [258]. Another chemical modification that could be encountered is the sulfation of  $\beta$ -glucans. In CN1583800 [259],  $\beta$ -(1,3)-glucan from mushroom is transformed in glucan-sulfuric ester and have shown enhanced activity on sarcoma 180 and proliferation of adenocarcinoma of stomach.

### 5.4. AIDS

The HIV virus has the ability to incorporate its DNA into the chromosome of human lymphocytes. As a retrovirus, it can further multiply in these cells and increase its number resulting in the destruction of the T-helper cell subtype of lymphocytes. At the same time, it apparently not attacked by the normal scavenging white blood cells such as macrophages. Extracts of *Lentinus edodes* were first found useful in inhibiting, Sarcoma 180, (a retrovirus, similar to HIV which uses reverse transcriptase for its tumor-promoting activity) [260]. According to clinical studies, lentinan produces specific T-helper cell stimulation in healthy humans as well as animals. It has also been recognized to stimulate lymphokine activated killer activity in combination with Interleukin-2 [261]. Recent patents concerning direct utilisation of  $\beta$ -D-glucans for treating

AIDS are rare. In JP8119874 [262], authors proposed to treat patients with a *Grifola frondosa* extract. More frequently,  $\beta$ -glucans are chemically modified (mainly sulfation) in order to improve their activity [263]. Then, in JP4308531 [264], sulfated-grifolan is proposed for AIDS and other retrovirus treatments. In the same way, US5512672 [127] claimed the use of curdlan or lentinan sulfate, whereas EP1671639 [265] concerns sulfated- $\beta$ -glucan in combination with more conventional drugs.

### 5.5. Prebiotic Applications

The concept of probiotics is based on the premise that there are microorganisms in the intestinal tract which have a beneficial health effect on the host. Some of these effects are for example, lactose digestion enhancement, cholesterol reduction, antimicrobial effects, immune system stimulation... A significant number of publications have focused on the fact that high amounts of *Lactobacilli* and *Bifidobacteria* are antagonistic to many pathogenic and putrefactive bacteria in the human intestine. Yoghurt is milk that has been transformed to a solid by the fermentative action of microorganisms such as *Lactobacillus delbrueckii* ssp. *Bulgaricus* and *Streptococcus salivarius* ssp. *Thermophilus*. These microorganisms do not have the ability to colonize the intestinal tract in large amount because they are not resistant to the gastric acidity and bile salts. Therefore, it has been proposed that the inclusion of prebiotic foods in the diet may enhance the population of beneficial bacteria.

Oat  $\beta$ -glucan has been reported to selectively support the growth of *Lactobacilli* and *Bifidobacteria* in rat experiments [266] and in *in vitro* studies [267]. Hydrolysates of oat  $\beta$ -glucan have been reported to stimulate the growth of three *Bifidobacterium* strains and *L. rhamosus* GG [268]. For more details on the use of oligosaccharides as prebiotics, see [269].

Based on these observations, several patents have been published on the use of  $\beta$ -(1,3)-glucans (or their corresponding hydrolysates) as prebiotics. In 1993, an oat bran product inoculated with defined living microbes has been described by Salovaara [270]. This product has an inherent granular texture due to bran particles. In 2002, another preparation method has been patented [271] for producing preparations derived from oatmeal, oatbran, or directly from oat  $\beta$ -glucans.

### 5.6. Other Therapeutic Applications

Many other therapeutic applications have been patented for  $\beta$ -(1,3)-glucans. One of those is their utilization as a radioprotective agent [272]. Indeed,  $\beta$ -glucan is able to protect blood macrophages from free radical attack during and after the radiation allowing these cells to continue to function in the irradiated body. [273].

In 2003, an original application has been patented, proposing using  $\beta$ -glucan as a gene carrier [274]. In this patent, a hydrogen-bonding polymer with a triple-helix structure (such as schizophyllan, curdlan, lentinan, scleroglucan...) is used for binding to a nucleic acid. A nucleic acid-polymer complex is thus obtained and can be applied as a vector. Moreover, this complex is also resistant

to nuclease, allowing its use as a nucleic acid-protecting agent.

In some cases,  $\beta$ -glucans could also be used to treat and prevent digestion troubles such as constipation [275], inflammatory bowel disease [276], or stomach troubles [277]. Recently, sulfated glucans have also been proposed for arthrosis treatment [278].

At least, a non negligible number of patents concern the use of  $\beta$ -glucans as antibacterial agent. In JP2004026785 [279],  $\beta$ -glucans are used for treating dermatitis and athlete's foot infection. In 2002,  $\beta$ -glucans have already been incorporated in a solution to eliminate dermatropic pathogen [280]. This kind of infection suppressing products has also been patented for oral cavity infections [281], or for any other kind of infection [282].

## 6. OTHER APPLICATIONS

### 6.1. Cosmetic

The aging and the increasing stress to which human skin is exposed have become serious problems. One of the solutions for maintaining the skin in its natural and healthy condition is to supplement it with biologically active compounds. Due to their biological and physicochemical properties,  $\beta$ -glucans are often found in cosmetic product formulations. Aim of incorporating  $\beta$ -glucans in cosmetic products is sometimes linked to their biological activities. In JP2005272406 [283], authors claimed biological properties of mushroom's  $\beta$ -glucans for improving and preventing skin diseases or irritations. Moreover, in addition to radio-protective effect, they also have potent antioxidant properties and free radical scavenging capabilities. These  $\beta$ -glucans could then be incorporated in a skin care preparation having ultraviolet shielding effects [284]. In JP11130636 [285], a hair cosmetic capable of promoting regeneration and growth of the hair by activating hair follicles is described. Adding  $\beta$ -glucan to cosmetic product could also help in obtaining pleasant texture [286]. In most of the published patents,  $\beta$ -glucan is used for its moisture retention capability. In JP2005162663 [287], a base skin formulation is proposed, in which  $\beta$ -glucan is dissolved in aqueous solution, providing proper moistening feeling, good applicability and therapeutic action. In JP2003192561 [288], and JP2005015348 [289],  $\beta$ -glucan are used for its moisture retention action, aging preventing and skin injury restoring properties. In JP2001354570 [290] and JP2004269408 [291],  $\beta$ -glucans enters in the composition of humectant products for skin. They could also be used as stabilizers in emulsified cosmetic [292] or in formulation of gel products [293]. In JP4059715 [294] and JP6321728 [295],  $\beta$ -glucan are incorporated in scrubbing products, as in JP9143046, in which a gel-state scrubbing agent is described [296].

### 6.2. Non-Classified Applications

Other original applications have been patented that can't be classified in previous paragraphs and which are reported here. In JP2005314290 [297], adhesive properties of  $\beta$ -glucans are used in preparing the adhesive layer of a patch. Curdlan has also been employed as an anti-segregating agent in concrete and mortar preparation [298]. In 2005, sulfated-glucans have been incorporated in new cigarette's filters

[299]. At least, an original application of curdlan-sulfate has been patented in 2006 [300] as an elicitor agent in stimulating natural protection of plants.

## 7. CURRENT & FUTURE DEVELOPMENTS

Research on the properties of new plant or microbial polysaccharides continue to grow. The polymers actually available as products in the market are very few compared to the large number of polysaccharides whose structures are either published or patented. The main drawback for the commercialization of new polysaccharides lies in the identification of new or superior properties compared to the one possessed by traditional products. The second obstacle for improvement of original structures is the cost of production and development that may be again a limiting factor. In food industry this is particularly true as only three bacterial polysaccharides are commonly employed despite the hundreds of structures known. One of them is the curdlan which has been authorized for food in 1996 because of its unique features and two others are the xanthan and the gellan. Similar to curdlan in food industry, all other  $\alpha$ - $(1,3)$ -glucans could have significant development in the next few years, notably in non-food sectors. The most promising developments seem possible in therapeutic and cosmetic applications as these compounds have been described as immunomodulators, antitumorogenic, antiviral (AIDS), agents for treatment of hypercholesterolemia and agents for stabilisation of glycemia. Moreover, specific action as food additives has been ascribed for glucan oligosaccharides. Paradoxically, antimicrobial action was also observed with the same molecules. Ultimately the cosmetics and pharmaceutical industry may provide new markets for glucans modified by chemistry. Effectively, patents relative to chemical modification of  $\alpha$ - $(1,3)$  glucans and notably  $\alpha$ - $(1,3)$ -D-glucans sulfatation could announce new generations of polysaccharides with original biological activities.

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