Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2022, 13, 298-315
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

A Review on the Impact of the Plant Bioactive Compound β-Glucans as Phytochemicals for Boosting Human and Animals' Immune Response—β-Glucans as Immunostimulants

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How to cite this paper: Menkovska, M. and Ibrahim, O. (2022) A Review on the Impact of the Plant Bioactive Compound β-Glucans as Phytochemicals for Boosting Human and Animals' Immune Response—β-Glucans as Immunostimulants. *Advances in Bioscience and Biotechnology*, **13**, 298-315.
https://doi.org/10.4236/abb.2022.137019

Received: March 16, 2022
Accepted: July 26, 2022
Published: July 29, 2022

Abstract

The influence of food and nutrition on health and immunity for both human and animals are being demonstrated. Phytochemicals are natural compounds present in cereals, beans, fruits, vegetable, and other plants, believed to enhance immune response for both human and animals. One of these phytochemicals is β-Glucans that are heterogeneous polysaccharides consisted of branched long chains of D-Glucose units present in cereals such as barley, wheat, and oats, and also present in microbial cell walls for yeasts, fungi, bacteria, and algae. β-Glucans extracted from cereals and mushroom were investigated for their positive impact on immunomodulation for both human and animal health. These β-Glucans proved to enhance immune system for innate response as the first defense against microbial infection, toxins, and self-tumor cells, and also, enhance adaptive immune that also referred to specific immunity as the second defense in response to antigen-specific lymphocytes against microbial infection, toxins, and self-tumor cells.

Keywords

β-Glucans, Source, Structure, Properties, Immunomodulation

1. Introduction

Immune system for both human and animals is a network of biological processes that protects the body from foreign particles that enter the body such as living microorganisms of bacteria, viruses, fungi, or parasites, and nonliving substances

such as toxins. The immune system recognizes and responds to the antigen of these foreign particles; that are proteins in nature (antigens) expressed on the surface on these foreign particles of microorganisms, and nonliving toxins, or self-tumor cells. There are two subsystems for immune response, innate immunity [1] and adaptive immunity [2]. Innate immunity is a natural nonspecific immunity as the host first line of defense. This innate immune system includes physical barriers such as skin, gastrointestinal tract, respiratory tract, and body hair. This self-line of defense also includes physiological barriers such as the secretion of soluble proteins (mucous), gastric acids, tears, saliva, and sweat. Adaptive immunity is developed as the host second line of defense, builds against specific antigens expressed on the surface of invade foreign particles to the host such as microbials infection, non-living foreign substances such as toxins secreted from microbial infection, or self-tumor cells due to host normal cell mutation. White blood cells refer to lymphocytes are generated from the host bone marrow and are responsible for adaptive immunity [3]. There are two major types of lymphocytes for this adaptive immunity, B-cells and T-cells. This specific adaptive immune system can be divided into humoral response [4] and cell-mediated immune response [5]. Humoral response, involves the interaction of B-cells with antigens expressed on the surface of the foreign particles resulted in the proliferate and differentiate of B-cells into plasma cells that produce and secrete antibodies in blood circulation against the invaded foreign particles (**Figure 1**). Secreted antibodies from plasma cells [5] bind to antigens of foreign particles surface and lyse the microbial or viral infection and neutralize toxins secreted by microbial infection or ingested from contaminated food/feeds protecting the host from the harmful effect of these invaded foreign particles. T-cells are the one that plays an important function in cell-mediated immune response to foreign particles (**Figure 2**), and there are two types T-cells, T-helper cells (T_h) and cytotoxic T-cells (also refer to CTLs or Ts). These T-cells are activated in the presence of antigens on the surface of foreign particles and serve as cell mediated

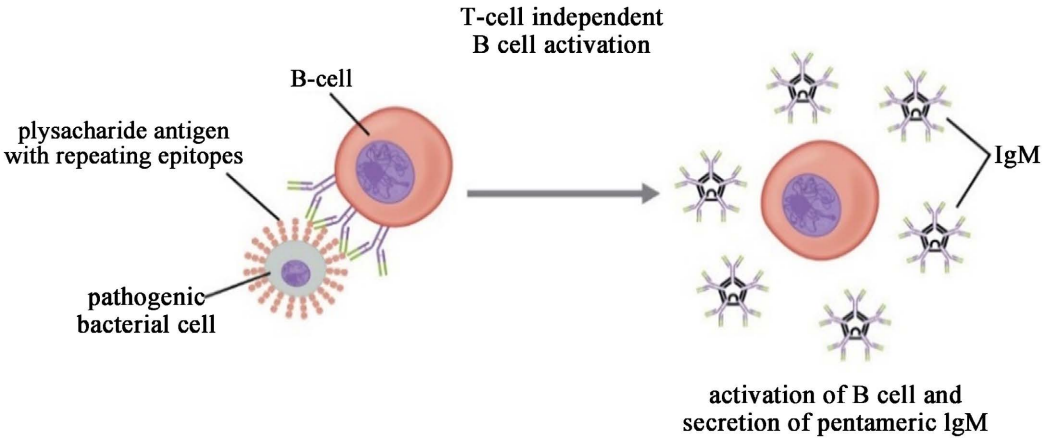

Figure 1. Adaptive immunities (humoral response). Antigens on microbial cells activate B-cells without the involvement of T-cells. Once B-cells are activated they proliferates and differentiates into plasma cells to secrete antibody that neutralize the microbial infection (the image is from Lumen-Microbiology).

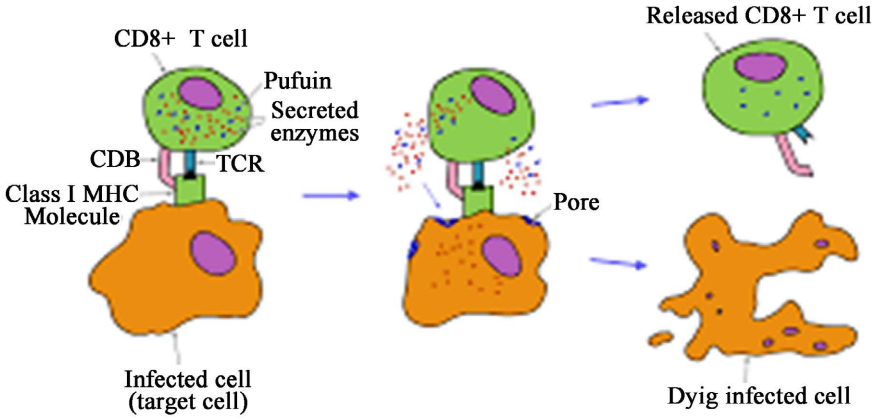

Figure 2. T-cell mediated cytotoxicity (CTL). Cytotoxic T-cells (CTLs), also known by the name CD8+ T-cells, express T-cell receptors (TCRs) accompanied by the CD8. The tight binding of CTLs and tunor cells through the CD8/TCR complex and Tumor cells antigen presented by major histocompatibility complex (MHC), class I causing T-cells to proliferate and secrete enzymes that lyse tumor cells (the image is from Westburg).

immune reactions for the secretion of cytokines responsible to regulate immune response against foreign particles.

T-helper cells express on its surface protein known by the name (cluster of differentiation 4 or $CD4^{+}$) are lymphocytes does not neutralize infections but rather trigger the body's response to infections, by the activation of various phagocytic cells of macrophages, neutrophils, monocytes, and dendritic cells that are having the ability to ingest, and sometimes digest foreign particles that invade the host [6]. Cytotoxic T-cells express on its surface protein known by (cluster of differentiation 8 or CD $8^{\pm}$) are lymphocytes that play an important function in neutralizing and destroying foreign particles as viruses, self-tumor cells or foreign tissue [7].

T-helper cells ($CD4^{+}$ T_h) are activated following antigen recognition only when antigen fragments are displayed together with MHC molecules (major histocompatibility complex) on the surface of specialized cells called antigen presenting cells (APCs). These APCs are macrophage, dendritic cells, and B-cells, are specialized in the internalize of large foreign molecule and express small fragment as antigens together with major histocompatibility complex (MHC) molecule on the APCs surface for T-helper cells ($CD4^{+}$ T_h) to recognize and secret cytotoxins [8]. Secreted cytokines by T-helper cells ($CD4^{+}$ T_h) cells activate B-calls for proliferation and differentiation into plasma sell. Plasma cells secrete antibodies to neutralize the infected foreign particles including self-tumor cells (**Figure 3**).

This major histocompatibility complex (MHC) function in cell mediated immunity is to bind small fragments of digested foreign particle and display these fragments (peptides) on the cell surface of antigen presenting cells (APCs) for the recognition by the T-helper cells ($CD4^{+}$ T_h) and or also by cytotoxic T-cells (CD $8^{\pm}$ T-cells). It is important to highlight that there are two classes of major histocompatibility complex (MHC), these are MHC class II, and MHC class I.

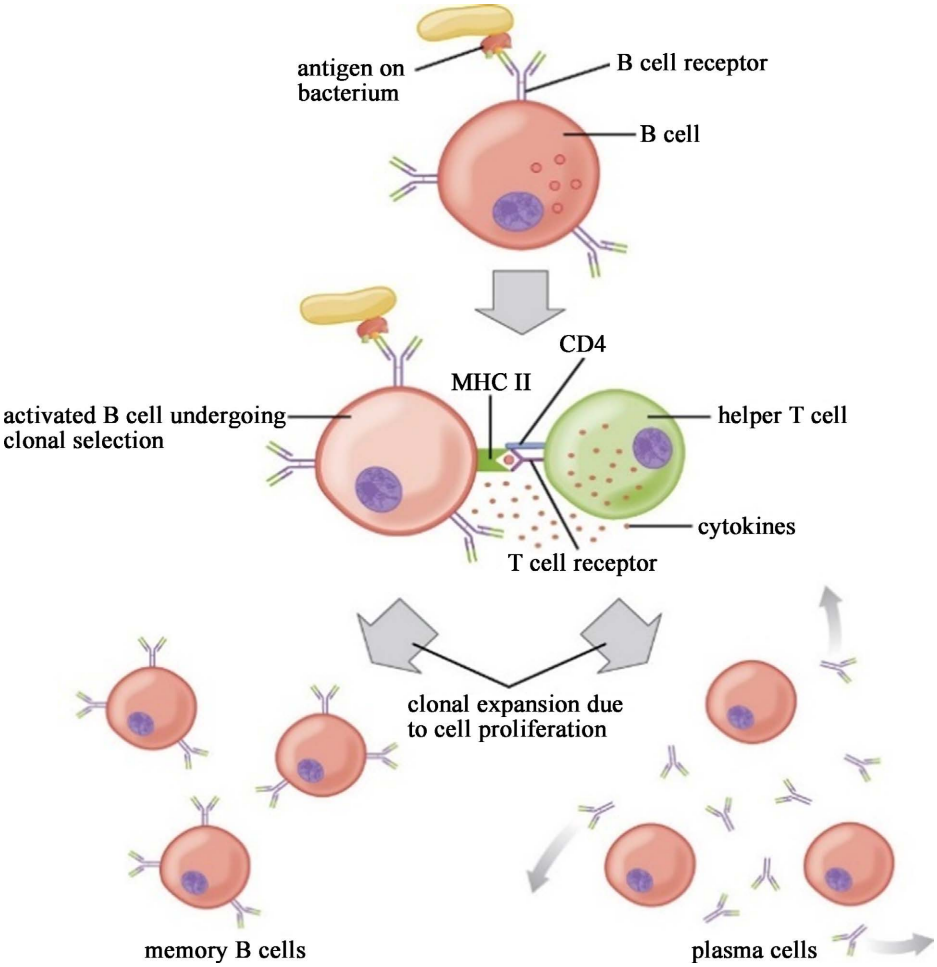

Figure 3. Cell mediated immunity (T cell-dependent activation of B-cells). B-cell or macrophage recognize and internalize foreign particles and present fragments (antigens) to T-helper cells (Th) that are specific to the same antigen. T-helper cells interacts with the antigen presented by antigen presenting cells (APCs). T-helper cells are activated and stimulated to release cytokines that activates the B-cells. Activated B-cells proliferate and differentiate into plasma cells that secrete antibody (IgM & IgG) (the image is from Lumen-Microbiology).

MHC class II molecules are associated with T-helper cells (CD $4^{\pm}$ T-cells), and MHC class I molecules are associated with cytotoxic T-cells (CD $8^{\pm}$ T-cells) (**Figure 4**).

2. β-Glucans and Immunomodulation

Immune stimulation properties of mushrooms to the host (human or animals) are well known in Eastern countries for thousands of years [9]. Investigators demonstrated that mushrooms cell wall contain biologically active polysaccharides known by the name of β-Glucans [10]. These polysaccharides of β-glucans stimulate and increase host immune defense by activating host cell immunity system of lymphocytes including the enhancement of monocytes, macrophages, dendritic cells, natural killer and neutrophils cells activities [11]. β-glucans immunomodulation function on these lymphocytes' stimulation is on *Beta*-(1,3)-Glucan

membrane receptors [12], which a protein known by names Dectin-1, CR3, or TLR-2/6) expressed on the surfaces of these lymphocytes of monocytes, macrophages, dendritic cells, natural killer and neutrophils cells [13]. This immune stimulation functions of β-Glucans involve both innate immune response and adaptive immune response (Figure 5), and also direct anticancer effect (Figure 5). In addition, β-Glucan enhance both opsonic and non-opsonic functions [14]. Opsonic function is immune process using antibodies to tag foreign substances for the elimination by phagocytes. Non-opsonic function is immune system without antibodies for the elimination of foreign particles by phagocytosis, and trigger the secretion of cytokines such as tumor necrosis factor (TNF-α) and interleukins (ILs). These cytokines are small proteins secreted by immune cells and responsible to control the growth and activity of immune system lymphocytes and blood cells. These secreted cytokines are also a signal of triggering immune

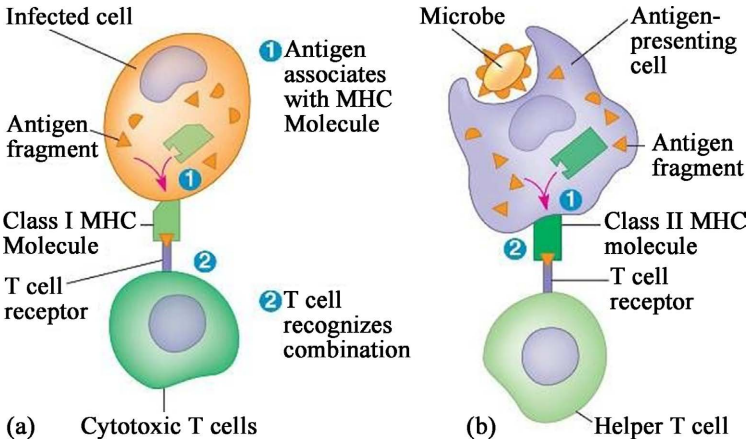

Figure 4. Major Histocompatibility Complex (MHC) class I and Class II. Major histocompatibility complex (MHC) Class I displays endogenous antigens in reproduced from infected or cancerous cells to cytotoxic T cell (CTLs) that are CD8/T-cells (left image). Major histocompatibility complex (MHC) Class II—display exogenous antigens to T-helper cells (Th) that are CD4/T-cells (right image). Source: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, (2012).

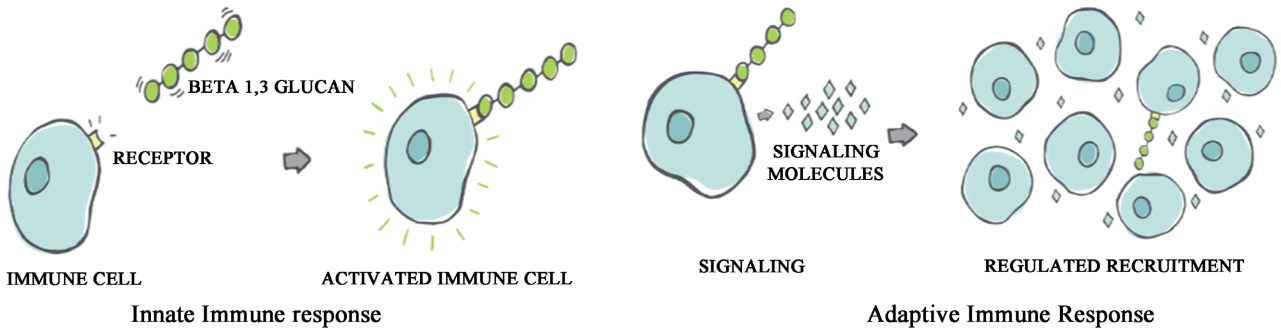

Figure 5. Innate Immune response Adaptive Immune Response. Ingested β-glucan, is passed from intestine and transported into the lymphatic system where interact with host immune cells via Beta 1,3-glucan receptor; "This interact increase phagocytic abilities of immune cells to microbial infection. This phagocytosis is the innate" immune response (left image). In the case of adaptive immune response, immune cells release signals (cytokines) upon it interact with β-glucan via Beta 1,3-glucan receptor, releasing cytokines activate and proliferate other immune cells as second line of defense (right image).

response, and directing anticancer effects against self-tumor cells [15] (**Figure 6**).

Other health benefits from β-Glucans includes lowering blood cholesterol level, lowering glycemic response, and prebiotic function in enhancing the growth of beneficial bacteria in the colon. It was demonstrated that the intake of three grams of oat fiber β-Glucan per day is enough to decrease the bad cholesterol (LDL) in the blood, and reduce the risk of cardiovascular diseases [16].

3. Chemical Structure, and Properties of β-Glucans

β-Glucans are naturally occurred in the cell wall of cereals, mushrooms, yeasts, bacteria, fungi, algae, and bacteria [17]. β-Glucans are heterogeneous polysaccharides with chemical structure consists of D-Glucose units in leaner long or short chain backbone linked together with β-1,3 or β-1,4 glycosidic bonds. D-Glucose polymer with β-1,3 or β-1,4 glycosidic bonds are varied in distribution and length with mostly branched side chains of D-Glucose linked to D-Glucose backbone with β-1,6 glycosidic bonds [18]. In general, chemical structure of extracted β-glucans from different sources are varied in D-Glucose glycosidic bonds and in molecular weight [19]. For examples, β-Glucans extracted from mushrooms have side chain of short β-1,3 D-Glucose units linked to D-Glucose backbone with β-1,6 glycosidic bonds [20], whereas β-Glucans extracted from yeast have D-Glucose side chain with β-1,6 glycosidic bonds branched from D-Glucose backbone and additional D-Glucose regions linked with β-1,3 glycosidic bonds. Cereal β-glucans are linear homopolysaccharides consisted of D-Glucose units linked via mixture of β-1,3 and β-1,4 glycosidic bonds. This β-Glucans structure from cereals features the presence of consecutive D-Glucose units with β-1,4 glycosidic bond linked in blocks oligomeric cellulose segments that are separated by single β-1,3 glycosidic bonds [21]. Most of the

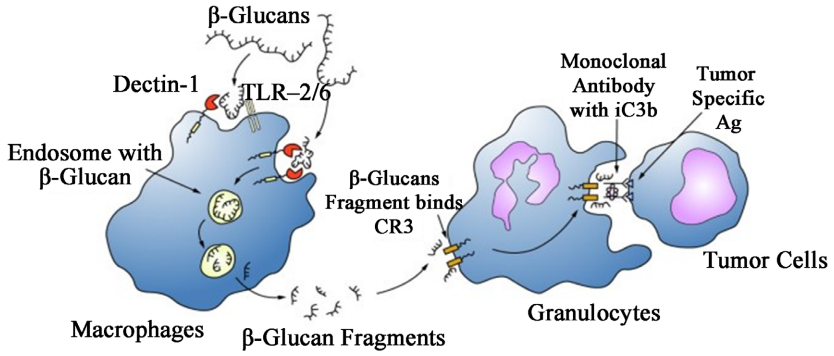

Figure 6. The actions of β-Glucan on immune cells against tumor cells. β-Glucans are captured by the macrophages via β (1,3) receptor (Dectin-1 or TLR2/6) expressed on the surface of macrophages cells. β-Glucans are phagocytosing (taken) by the macrophage and process into small β-Glucan fragments, and subsequently released. Then these small β-Glucan fragments are taken up by the circulating granulocytes, via the complement receptor (CR-3) and trigger the immune response (antibody) against tumor cells. Source: Chan, G. C-F., Chan, W. K., Sze, D.M-Y (2009). The Effects of β-Glucan on Human Immune and Cancer Cells. *Journal of Hematology & Oncology,* 2 (25).

cellulose segments in β-glucans from cereals are trimers or tetramers, and in some case these cellulosic oligosaccharides are longer present in the polymeric chains (Figure 7).

These variability in β-Glucans chemical structures from different sources in addition to this chemical structure of β-glucans could form secondary structures and leads into differences in immunomodulating activities [22], physical properties, binding affinity to receptors, and other biological functions. Physical properties include water solubility, rheological, and viscosity properties. It appears that the active side in β-glucans immune response activity is β-1,3 glycosidic bonds in D-glucose chain for all β-glucans with variable chemical structures. Some evidences suggest that the immunomodulating activities of β-Glucans are related to their molecular weight (MW). High MW of β-Glucans have high effect on the immune system, while low MW (<10,000) with short side chain is commonly regarded as inactive [23]. In general, β-Glucans immune responses are still not well understood and sometimes are quite confusing.

4. Extraction Methods of β-Glucans from Natural Sources

β-Glucans can be extracted from cereals such as oat, barley, and wheat, also can be extracted from yeasts and mushrooms. Before extraction of β-glucans from cereals, grains must be milled into small particle size, grind, and washed with

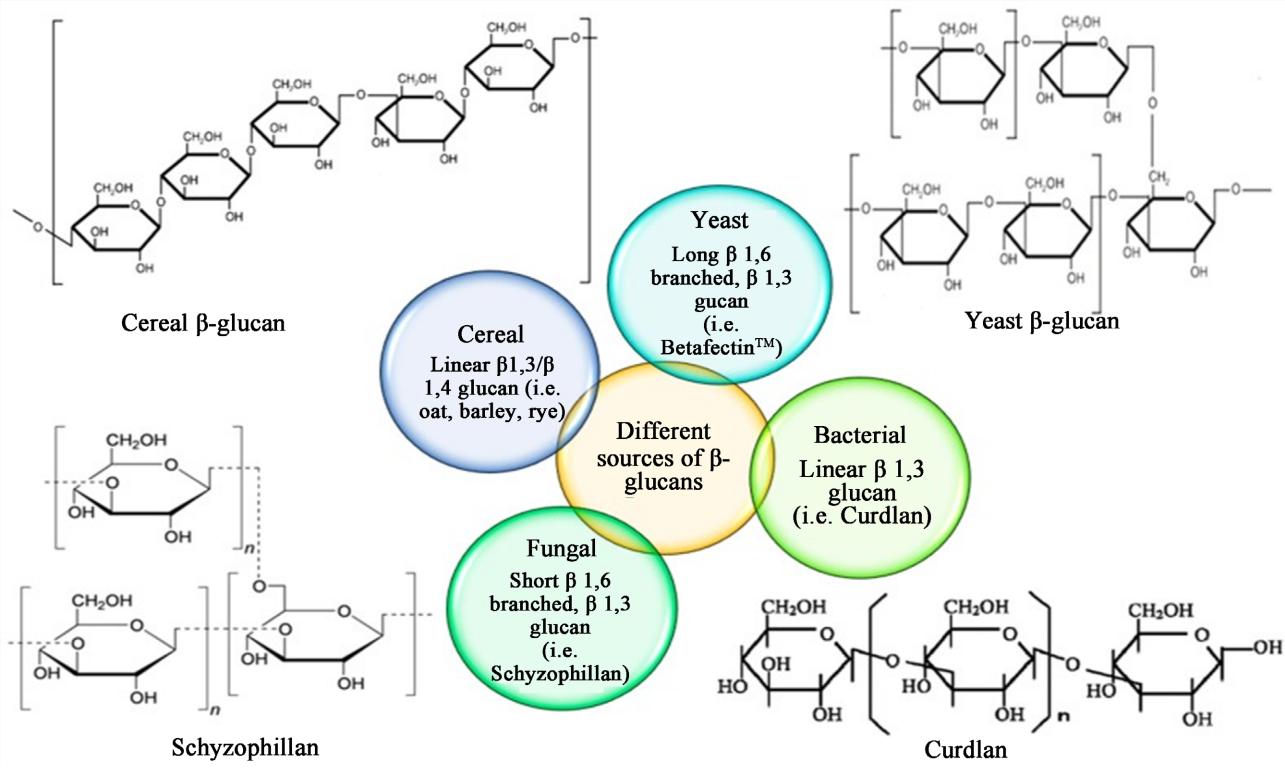

Figure 7. Structure and branching degree of β-Glucan from different sources-ceral, yeast, fungal and bacterial. Structures are presented of D-Glucose polymer of β (1,3 or 1,4) glycosidic bond with variable branching degree of β (1,6) glycosidic bonds. Source: Du, B., Meenu, M., Liu, H., Xu, B. (2019) A Concise Review on the Molecular Structure and Function Relationship of β-Glucan. *Int J Mol Sci.,* 20(16), 4032. https://doi.org/10.3390/ijms20164032.

ethanol. Ethanol washing is important pre-extraction step to deactivate the enzyme β-Glucanase and to remove most of the lipids in the grain. β-Glucanase is endogenous enzyme in grains that breakdowns β-Glucans [19].

Extraction methods from cereals or from microbial sources are by hot water, alkaline solutions, acid solutions, or enzymes. Extraction method and optimum conditions are important factors for β-Glucans yield, and the biological activity of extracted β-Glucans. It was reported that hot water extraction with no chemical used is the best method for β-Glucans on large scale production [24].

4.1. Hot Water Extraction

The optimum temperature for this method is in the range of 47˚C to 50˚C. This temperature prevents starch solubility and gelatinization, plus plays important factor in the molecular weight (MW) of the extracted β-Glucans [25]. Water extraction at higher temperature leads to the decrease in β-Glucans yield and molecular weight (MW), this is due to the significant increase in the rate the of β-Glucans degradation. High molecular weight (MW) of β-Glucans exhibits great influence on its biological activities and physiological properties [26].

4.2. Alkaline Extraction

Sodium hydroxide at one normal concentration (1N NaOH) is used for β-Glucans extraction from cereals or microbial cells. The ratio of alkaline solution to sample, extraction temperature, and extraction time are important factors for β-Glucans yield and the inactivation of the enzyme β-Glucanase. The optimum extraction temperature that is required for alkaline extraction method is 45˚C. This optimum temperature is good enough to prevent starch gelatinization, and to inactivate the enzyme β-Glucanaze. Higher extraction temperature and longer time in alkaline extraction method could lead to poor β-Glucans yield with lower molecular weight (MW). β-Glucans with low MW have poor biological activity [27].

4.3. Acid Extraction

β-Glucans extraction from cereals or microbial cells by acid method is not popular due to the lower yield comparing to other methods. Acid used in this method is perchloric acid ($HClO_4$) at the concentration of 50 mM to extract both β-Glucans and starch from grains. In this method both β-Glucans and starch are solubilized in single acid extraction, and it was reported that second acid extraction to grains yielded no β-Glucans or starch. The extracted starch can be removed from β-Glucans by using amylase enzyme following by dialysis [28].

4.4. Enzyme's Extraction

Both amylase and protease enzymes are used in this method. Small particle size of grains is previously washed with ethanol and dried, treated first with α-amylase enzyme at 40˚C for three hours and centrifuged. The supernatant is treated with

protease enzyme at 37˚C for three hours and centrifuged. β-Glucans in the supernatant are precepted by using 80% ethanol for 20 minutes, then centrifuged to remove the gummy mass from the end product of β-Glucans [29].

β-Glucans extracted by these four methods are a mixture of water soluble and insoluble, the separation of soluble β-Glucans from insoluble can be applied by water and filtration. The soluble fraction of β-Glucans can be dried by conventional methods under vacuum or by freeze drying. For the production of highly purified soluble β-Glucans for pharmaceutical applications, dialysis or column separation methods must be applied on soluble β-Glucans before drying process [30].

5. β-Glucans Assay Methods

There are different methods for β-Glucans determination during processing and end product. The common method is based on complete hydrolysis of D-Glucose units using acids or enzymes hydrolysis [31]. Released D-Glucose units are measured by colorimetric method. Hydrochloric acid (HCl) or sulphonic acid (H_2SO_4) is used in acid hydrolysis method, and is a simple method. However, this acid hydrolysis method is nonspecific and gives higher error in β-Glucans value due to the hydrolysis of other glucose-containing polysaccharide present as contaminant in β-Glucans sample such as starch and cellulose from grains extraction or glycogen from yeasts extraction. Enzyme assay method is more specific and gives more accurate result. In this enzymatic method, two enzymes are used that are specific in converting β-Glucans into D-Glucose [32]. These two enzymes are (1,3, & 1,4)-β-D-Glucan 4-Glucanohydrolase (Lichenase), and β-Glucosidase (**Figure 8**). First, samples prior β-Glucans assay is treated with alkaline such as potassium hydroxide (KOH) for the gelatinization (swelling) of

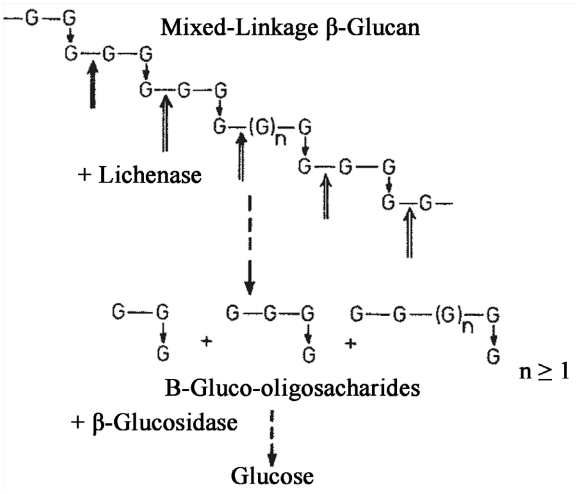

Figure 8. β-Glucans assay method. Two specific enzymes are used in enzymatic methods to covert β-Glucans into D-Glucose, which can be measured by colorimetric by enzymatic assay. Source: McCleary, B. V. (2000) Importance of Enzyme Purity and Activity in the Measurement of Total Dietary Fiber and Dietary Fiber Components. *Journal of AOAC International*, 83(4), 997-1005.

polysaccharide structure of β-Glucans. This alkaline treatment is assisting the acid or the enzymes to penetrate and breakdown the polysaccharide of β-Glucans into D-Glucose units [33]. Released D-Glucose from both assay methods is measured by a calorimetric method. There are commercially available methods for D-Glucose assay. One of these D-Glucose assay method is by using the two enzymes of glucose oxidase, and peroxidase [34]. This method is preferred for its sensitivity, and is based on the enzyme glucose oxidase that oxidize D-Glucose into gluconic acid and hydrogen peroxide (H_2O_2). Released hydrogen peroxide is measured calorimetrically after it reacts with chromogen such as O-dianisidine. O-dianisidine in the presence of peroxidase enzyme to form a color that is corelated to D-Glucose amount (**Figure 9**). β-Glucan's value in the sample is measured using D-Glucose standard curve. β-Glucans's value is calculated by dividing the amount of measured glucose by the amount of original sample used for testing.

6. Global β-Glucan's Market

β-Glucans are marketed by the source (cereal, mushroom, yeast, etc.), and by the category (soluble and non-soluble). Water soluble β-Glucans from oat and barley are gaining high market due to its slow food transit in the intestines for longer food digestion [35] [36].

This longer food digestion reduces sugar absorption into blood circulation and lowering blood sugar level. Plus, this indigestible property of β-Glucans assists in taking out cholesterol in ingested foods to the colon, lowering blood cholesterol level. In general, β-Glucan's properties such are molecular weight, solubilization in the gastrointestinal tract, in addition to the intake dose are important factors for achieving health benefits.

β-Glucans are approved as generally recognized as safe (GRAS) by food and drug administration (FDA) in United States and by other worldwide health

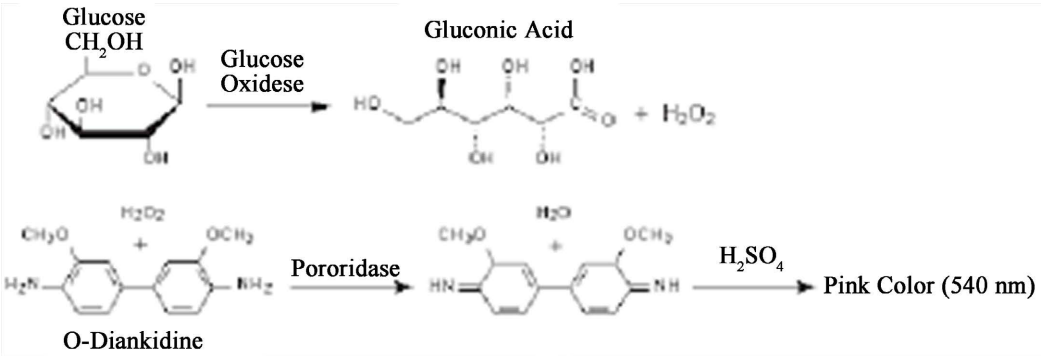

Figure 9. Enzymatic method for D-Glucose assay using glucose oxidase. Glucose is oxidized D-Glucose into gluconic acid and hydrogen peroxide. Hydrogen peroxide reacts with ortho-dianisidine in the presence of peroxidase enzyme to form a color that can be measured. Source: De Toledo, V. de A. A., Ruvolo-Takasusuki, M. C.C., de Oliveira, A. J. B., Chambó, E.D., Lopes, Sh.M.S. (2012) Spectrophotometry as a Tool for Dosage Sugars in Nectar of Crops Pollinated by Honeybees. In: Macro to nano spectroscopy (Chapter 14*) Spectrophotometry as a Tool for Dosage Sugars in Nectar of Crops Pollinated by Honeybees, P*ublisher. InTech Eds., Uddin, J.

organizations. This β-Glucans acceptance as safe is due to its extraction methods from cereals, and natural microorganisms that are recognized as safe. β-Glucans from different sources as food ingredient and additive [21] [36] are currently incorporated in various food products such as backed goods, salad dressing, dairy products, processed meats, pasta, noodles, and other food products. In addition, is its applications in cosmetics products [37] and pharmaceutical drugs [38].

All marketed β-Glucans are having the same standard specifications (**Table 1**). The global market for β-Glucans is estimated in the range of $ 404 million for the year 2020, and is expected to grow at Compound Annual Growth Rate (CAGR) of 7.6% to reach $628 million by the year 2026 [39].

7. Discussion

β-Glucans are naturally occurring in heterogenous polysaccharides structures, with health benefits including enhancing immune response. These heterogenous polysaccharides of β-Glucans are roughly consisted of over 250,000 D-Glucose

Table 1. Specifications of marketed β-Glucans.

Tests	Specification
Physical Parameters	
Appearance	Yellowish to yellow brown powder
Odor	Characteristic
Assay (beta-D Glucan) (UV)	Min 70%
Protein	Max 5%
Sieve analysis	98% pass 80 mesh
Ash (g/100g)	Max 5.0%
Loss on drying	Max 8.0%
Heavy Metal Content	
Heavy Metals	Max 10 ppm
Lead (PPM)	Max 2.0 ppm
Arsenic (PPM)	Max 1.0 ppm
Cadmium (PPM)	Max 1.0 ppm
Mercury (PPM)	Max 0.2 ppm
Microbiological Quantity	
Total Plate Count (cfu/g)	Max 1000 cfu/g
Total yeast & mold	Max 100 cfu/g
E. Coli	Negative
Salmonella	Negative
Staphylococcus	Negative

units. D-Glucose unites in β-Glucans extracted from yeasts and mushrooms are arranged in a leaner backbone of β-1,3 glycosidic bonds, and having short side chains of D-Glucose unites with β-1,6 glycosidic bonds branched from the linear backbone of D-Glucose chain with β-1,3 glycosidic bonds. In the case of cereals, β-Glucan's structure extracted from oats and barley is a leaner D-Glucose chain of β-1,4 glycosidic bonds linkages separated by shorter D-Glucose chain of β-1,3 glycosidic bonds.

These heterogenous structures of β-Glucans are due to the variability of β-Glucan's biosynthesis from different sources, with involving of different classes of enzymes. The biosynthesis of β-1,3 glycosidic bonds as linkages for D-Glucose units is by the enzyme 1,3-β-Glucan synthase, while the biosynthesis of β-1,4 glycosidic bonds as linkages for D-Glucose units is by the enzyme synthase. In the case of the biosynthesis of β-1,6 glycosidic bonds it is still not well understood.

Oats, barley, yeasts, and mushrooms, contain the highest concentration of β-Glucans and are the major source for β-Glucans manufacturing. β-Glucans in oats are located in the sub-aleurone layer, while in barley β-Glucans are located in the endosperm. In the case of microbial sources such as yeasts and mushrooms β-Glucans are located in cell walls. Extraction process of β-Glucans from whole grains of oats, barley, or wheat involves first pre-extraction process of dry or wet milling, and the separation of the bran from the rest of the grain. Extraction process from microbial sources does not require these grains pre-extraction process.

There are four extraction methods for β-Glucans from cereals or microbial sources. These extraction methods are hot water extraction, alkaline extraction, acids extraction and enzymes extraction. Optimization of these extraction methods is very important to avoid the damage or alter β-Glucans molecular weight and structure. The damage or alter β-Glucans molecular weight and structure could result in the loss of β-Glucans physiological activities such immunomodulation and other activities for the applications in food, feeds, pharmaceuticals, and cosmetics. These optimum extraction conditions are namely PH, temperature, extraction time, centrifugation gravity, and purification steps. In addition to these optimum extraction conditions, other factors that have effects on β-Glucan's structure and physiological properties are β-Glucan's source for extraction. After β-Glucan's extraction, there are multiple methods that are used for isolation and purification of β-Glucans such as isoelectric precipitation, ammonium sulphate precipitation, ethanol precipitation, and affinity chromatography separation. Optimization of these isolation purification methods are also important factor on the yield and physiological properties of the end product of β-Glucans.

In general, β-Glucans from different sources, differ in chemical structure, conformation, physical properties, binding affinity to receptors, and biological functions. Due to these heterogenicity of β-Glucans structures and function, analyti-

cal assay methods to confirm the biological activity of manufactured β-Glucans before marketing is very important step. There are several methods for β-Glucan's assay and the most widely used method is the enzymatic method, which is the official by American Association of Cereal Chemists (AACC), Association of Analytical Chemists (AOAC), International Association for Cereal Science and Technology (ICC) and Intraclass Correlation Coefficients (ICC). This enzymatic method is based on sequential enzymatic hydrolysis using the enzyme (1,3, & 1,4)-β-D-Glucan 4-glucanohydrolase (Lichenase), followed by the enzyme β-Glucosidase for complete β-Glucan's hydrolysis into D-glucose. The released D-Glucose units are measured enzymatically using the enzyme glucose oxidase generating hydrogen peroxide (H_2O_2) that is detected in the presence of horse radish peroxidase (HRP), and chromogenic substrate.

Applications of β-Glucans are in foods, dietary supplements, pharmaceuticals, cosmetics, feeds and others with multiple health benefits including claims to immunomodulation functions to consumers when is taken by mouth. These immunomodulation functions claims are such as, enhance and increase host immune defense by activating complement system, and enhancing macrophages and natural killer cell function. Immunomodulation functions research studies claims that β-Glucans activate number of cellular immunity cells such as T-cells, cytotoxic T cells (CTLs) that fight, kill or inhibit tumor growth in promotion stage, plus protect human or animals from potent genotoxic carcinogens substances. Immunostimulant activity of these cellular immunity cells showed to express glucans receptors (dectin-1 and TLR) on cell surface, and upon binding β-Glucan to these receptors induce signaling cascade that activate immune cells and triggering immune response [40].

Other β-Glucan's health benefits are lower blood cholesterol resulted in heart health, regulate blood sugar levels, lower risk of obesity, decrease inflammation, improve gut health, and might fight cancer [41] [42] [43]. Due to the recent pandemic of COVID-19 illness with severe respiratory tract infection by coronavirus SARS-CoV-2 some publications claimed that the combination of β-Glucans as a prophylactic with a healthy diet rich including vitamins C and D could provide natural synergistic immune system to the body against virus's infection such as SARS-CoV-2 virus infection [44]. It was also reported that the combination of two beta-glucans produced by Aureobasidium Pullulans (AFO-202 and N-163) strains was shown to reduce inflammatory biomarkers in COVID-19 patients, compared to the standard treatment [45]. These are not reliable scientific information due to that there are not yet enough clinical trials that were performed to confirm such claims.

β-Glucans are not digested by human or animal digestive systems, but its viscosity property slow food transit in the intestine causing slow carbohydrates absorption that maintain steady normal blood sugar level, plus lower total and bad (LDL) cholesterol absorption into blood circulation from high fat diet. β-Glucans also showed improving in host gut heath by increasing the population of

beneficial bacteria in intestinal tracts, and reduce the population of non-beneficial bacteria. Finally, it is important to highlight that in pharmaceutical applications some health care provider authorized given by IV β-Glucans at a dose between 0.5 - 2 mg/kg before and after surgery to help prevent infections. This pharmaceutical β-Glucans for injection must be highly purified and meet United State Pharmacopeia (USP) standard.

Food and Drugs Administration (FDA) in United States was the first to approve health claims for β-Glucans in 1997. Currently β-Glucans are authorized worldwide as safe with health benefits from Europe, Australia, New Zealand, Brazil, Malaysia, Singapore, Indonesia and South Korea. It was reported by U.S. Food and Drug Administration and by European Food Safety Authority (EFSA) that consuming three grams of β-Glucans per day can help reduce cholesterol levels by about 5 to 8 percent. They were also reported that higher doses up to ten grams per day curb hunger leading to reduce calorie intake and obesity. β-Glucan's market size was $404 million in the year 2020 and estimated to reach $561 million by the year 2028, exhibiting CAGR of 7.6%. This high β-Glucan's market size is due to rapid expansion of global dietary supplements consumption and educated consumers for β-Glucan's health benefits.

8. Conclusion

β-Glucans are natural biologically active heterogenous polysaccharides of glucose polymers extracted from the cell wall of cereals and microbial cells. β-Glucans are recognized as safe for both human and animal consumption and are gaining worldwide strong attention not only as food or feeds, but also as dietary supplements, nutraceuticals, immunostimulants, potential anticancer with applications for both pharmaceuticals and cosmetics. Such claims that β-Glucans have immunostimulant, and potential anticancer function required more clinical trials to support *in vitro* testing results. The application of β-Glucans in everyday consumption in human and animal nutrition should increase for wellbeing of humans and animals, through the natural food and various dietary supplements offered by the industry.

Acknowledgements

We would like to thank ABB Managing Editor Joy Deng and Catherine Hopkins from SCIRP for their assistance with technical support of this paper. Dr. Osama Ibrahimis also thanked for his contribution in writing and technical support of the immunological part of thepaper.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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