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Mushroom chitosan: a promising biopolymer in the food industry and agriculture

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Abstract

Mushrooms are recognized not only as a valuable source of nutrients but also as a treasure of bioactive components that have found application in obtaining dietary supplements and functional food. In recent times, the classification of products from fruiting bodies and mycelium of mushrooms into the category of "new food" is an increasing challenge. Chitin, a polymer present in the cell wall of fungi, as well as his derivate chitosan, has an increased application in various branches of industry, pharmacy, and biomedicine. Also, they are recognized as a potential new food. Due to the low solubility of chitin and chitosan at physiological pH, often, there is a need for further chitosan modification to obtain derivatives and smaller molecules with extensive application. This review aims to highlight the advantages of using mushrooms in obtaining chitosan, a safe, biodegradable polysaccharide with pronounced antimicrobial activity and numerous benefits for human and animal health. We draw attention to the physicochemical properties of chitin and chitosan, the presence in the cell wall of mushrooms, as well as methods of extraction and purification of chitosan. The literature data on their application in the food industry, in the making of chitosan-based composites with wide application in the food industry, and especially for edible and active film production, is discussed. Finally, application in agriculture is also considered.

Keywords: *chitosan; mushrooms; biotechnology; food; biological activity.*

INTRODUCTION

Since ancient times, mushrooms have been used in human nutrition as a functional food with many-sided biological effects [1,2]. Moreover, mushrooms are recognized for their nutritional importance such as high protein content rich in all essential amino acids, low fat content, and low glycemic index [2,3]. They are rich in many essential minerals such as iron, zinc, magnesium, phosphorous, copper, potassium, and others, as well as in vitamins and their precursors like riboflavin, thiamine, niacin, folate, choline, pantothenic acid, ergothioneine, ergosterol carotenoids and ascorbic acid [2,3,4]. They also contain bioactive secondary metabolites which can improve cellular longevity and lifespan such as terpenoids, organic acids, alkaloids, polyphenols, lactones, sterols, nucleotide analogs, and also polysaccharides primarily β-glucans [3,5,6,7,8,9].

In the past decade, mushrooms have also attracted attention as a rich source of natural polymer chitin, precursor of chitosan [3,10]. Compared to waste from the shellfish industry, which is the main source of industrial chitin and chitosan worldwide, mushrooms as a source of these polymers have significant benefits [8,10]. While supplies of crab waste are seasonally limited, commercial mushroom production is a convenient process that has no geographic or seasonal limitations; mushrooms have a lower level of inorganic materials, and therefore no demineralization treatment is required to remove minerals during the isolation process; mushroom chitosan has relatively consistent properties due to the possibility of controlled production; finally mushroom chitosan is more effective in stimulating an immune response because it is covalently linked to β-glucans [10,11].

The popularity of chitosan is contributed by its versatile and eco-friendly nature linked to renewability, biodegradability, and non-toxicity [11,12,13]. The PubMed search with "chitosan" as keyword resulted in 4932 publications (on 16 October 2024) and this underlines the great interest of researchers in chitosan [14]. It can be applied in various forms – from gels and liquids to powders, tablets, edible films, and nanoparticles [13,15]. Chitosan has also found applications in bioactive compounds and drug encapsulation with extended delivery [15]. Furthermore, as a fibrous molecule, chitosan can cooperate with weight management thanks to its potential to connect upon ingestion with phospholipids, cholesterol, bile acid, and gut toxins, and has been shown to lower low-density lipoprotein (LDL) cholesterol, systolic and diastolic blood pressure [13,15]. As a dietary fiber, chitosan can be degraded by intestinal microbiota that produces short-chain fatty acids and therefore modulate good bacteria with a positive effect on colonic health [13,15]. Moreover, chitosan and its composites have significantly influenced a key area of human physiology by replacing damaged parts, for example as components of artificial bones and skin [3,13]. Finally, chitosan fits into green technologies through its application in identification and elimination of pollutants for environmental remediation [3,13].

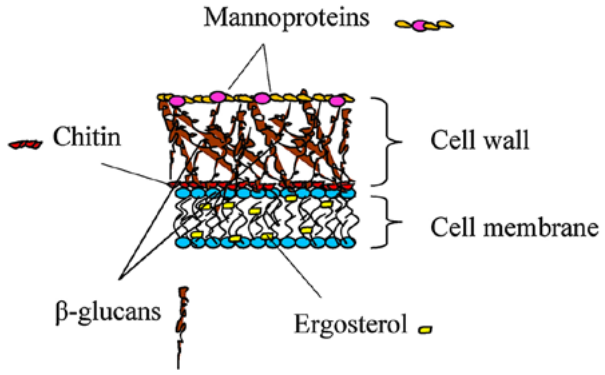


Figure 1. Schematic overview of mushrooms cell wall composition.

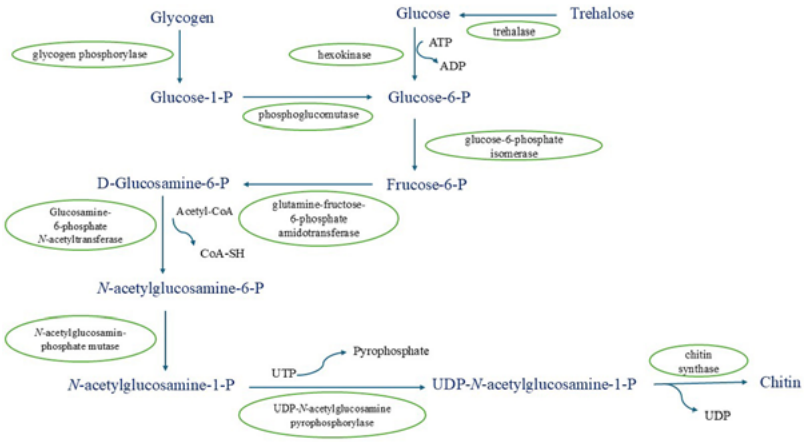


Figure 2. Biosynthetic pathway of chitin Abbreviations: acetyl CoA, acetyl coenzyme A; ADP, adenosine diphosphate; CoASH, coenzyme A; Gln, L-glutamine; Glu, L-glutamate; UDP, uridine diphosphate; UTP, uridine triphosphate.

This review aims to draw attention to the perspectives of mushrooms as a natural source of chitosan and promote its application in the food industry and modern biotechnology. Chitosan extraction can offer a profitable solution for mushroom growers and the biotech industry.

CHEMICAL STRUCTURE AND CHARACTERISTICS OF MUSHROOM CHITOSAN

Chitosan is a binary heteropolysaccharide composed of 2-acetamido-2-deoxy-*β*-D-glucopyranose and 2-amino-2-deoxy-*β*-D-glucopyranose linked by *β*-(1,4) glycosidic bonds [13,16]. In essence, chitosan is a deacetylated derivative of chitin, defined by the degree of deacetylation (DD). Chitin is a structural component of the mushroom cell wall and exists as a complex with *β*-glucans [17]. Its fibers are located close to the cell plasmalemma, and next to *β*-glucan fibers and mannoprotein, **Figure 1**.

The chitin biosynthetic pathway in mushrooms uses glucose and other carbohydrates, such as trehalose and glycogen, to synthesize N-acetylglucosamine, **Figure 2**. Finally, chitin is synthesized by the removal of N-acetylglucosamine from uridine diphosphate-N-acetylglucosamine (UDPGlcNAc) into a growing filament that is transported across the cell membrane by chitin synthase [18]. Enzymes such as glutamine-fructose-6-phosphate amidotransferase, UDP-N-acetylglucosamine pyrophosphorylase, and chitin synthase are the main regulatory enzymes and limit the rate of chitin production.

In the mushroom cell wall, chitin can be organized as microfibrils in three isomeric forms: *α*-chitin, *β*-chitin, and *γ*-chitin. The differences between forms are due to how the microfibrils are arranged. In the *α* form, microfibrils are arranged only in an antiparallel mode, while in the *β* form, they are in parallel mode. In the *γ* form, sets of two parallel sequences are replaced by one antiparallel sequence [18]. It is speculated that the α-chitin is the most prevalent and the most resistant form in mushrooms. Furthermore, the predominance of *α*-chitin has been reported in the mushroom *Agaricus bisporus* (champignons), *Boletus bovinus,* and *Laccaria laccata* [19,20]. In contrast to these findings, the presence of *α*-chitin and *γ*-chitin was confirmed in the mushroom *Ganoderma lucidum* [21].

Physico-chemical characteristics of mushroom chitosan

The physicochemical properties of chitosan are crucial for their application. Degree of deacetylation (DD) and average molecular weight (MW) are the most frequently investigated properties [13,16]. Other commonly analyzed features are crystallinity, thermal stability, solubility, and viscosity. Besides presence of β-glucans and protein residues are important parameters in determining its use [17].

Degree of deacetylation (DD) and molecular weight (MW)

DD presents the percentage of β-1,4-D-glucosamine, i.e. the quantity of free amino groups in the biopolymer [13,16]. DD of commercially available chitosans is usually higher than 60% [13]. Generally, a high DD was reported for mushroom chitosan, for instance, 66.35% for *A. bisporus,* 90% for *G. lucidum*, 70% for *B. bovinus,* and 74% for *L. laccaria* [20]. Considering that the free amino groups of chitosan are involved in the complexation mechanism, the ability to complex metal ions increases with higher DD. As polycations, chitosans can interact electrostatically with oppositely charged ions, such as metal ions, anionic surfactants and polyanions such as carboxymethylcellulose, pectin, heparin, xanthan, carrageenan, hyaluronan, dextran sulfate, chondroitin sulfate, and sodium alginate building the so-called polyelectrolyte complexes [13].

Based on the molecular weight, chitosans are divided into small (< 150 kDa), medium (150-700 kDa), and large molecular chitosan masses (700–1000 kDa) [11]. Mostly, mushroom chitosan is characterized by low MW and medium MW [16]. For example, a MW of 5–5.8 kDa was reported for chitosan extracted from *B. bovinus* and *L. laccata* [20]. Contrary to these findings chitosan extracted from *Lentinula edodes* stipe, without dialysis treatment had MW, of 382–437 kDa, and chitosan from the same source subjected to dialysis treatment of 54.23–60.93 kDa [22]. In the last years, the interest of researchers in short-chain chitosans, which are called oligochitosans (< 50 kDa), has been growing [16]. They are obtained by the hydrolysis process of high-molecular chitosan chains [13].

Crystallinity, solubility, viscosity, and thermal stability

Crystallinity refers to the degree to which a polymer exhibits a highly ordered, crystalline structure, affecting its mechanical, thermal, and optical properties. At room temperature, chitosans are semicrystalline polymers that show polymorphism [16]. Like most carbohydrates that have the ability to form hydrogen bonds, they are degraded by heating before they start to melt. The results of various studies have shown that mushroom chitosan has low crystallinity [13]. Low crystallinity is related to some functional characteristics of chitosan. For example, the sorption capacity, the availability of the main free amino groups of chitosan, and its solubility in acidic solutions are associated with reduced crystallinity [23]. The primary role of low-crystallinity chitosan is the ability to eliminate metal compounds by providing low diffusion resistance.

The solubility of chitosan in water depends on their MW and DD. Oligochitosans with a low MW (2-4 kDa) are easily soluble in water even at a neutral pH value, while chitosans of larger MW, whose pKa value usually ranges from 6.2 to 7.0 are practically insoluble in physiological conditions (pH 7.4) and higher pH values [16]. With the addition of acids, the solubility of chitosan increases due to the protonation of free amino groups at the position of C-2 glucosamine units. DD indirectly affects the solubility of chitosan. Chitosans with a DD of about 40% are soluble in aqueous media with a pH value of up to 9.0, while chitosans with a DD of about 85% dissolve in media with a pH value of up to 6.5 [16, 24]. The presence of electrolytes also has a great influence on the solubility of chitosan. In the absence of an electrolyte, chitosan chains are maximally elongated due to the repulsion of positively charged groups of monomer units, while in the presence of an electrolyte, the repulsion decreases, the chitosan chains twist and the effect of salting out, i.e. deposition of chitosan, occurs [24]. The low solubility of chitosan at pH values above pKa is the main factor limiting their application, especially when they are used to improve the resorption/absorption of medicinal substances through mucous membranes [16].

The viscosity of chitosan solutions is another property that must be considered during their biomedical application. The viscosity of the chitosan solution is affected by concentration, MW of chitosan, DD, ionic strength, pH value, and temperature. As the MW of chitosan increases, the viscosity of their solutions increases. So in addition to good solubility in water, low viscosity is another advantage of oligochitosan compared to high MW chitosans [16]. The viscosity of the chitosan solution also increases with the increase in the DD i.e. stretching of polymer chains due to the repulsion of protonated amino groups. On the contrary, an increase in temperature and pH results in a decrease in the viscosity of the chitosan solution [24].

Thermal stability of chitosan refers to the ability to withstand changes in structure and performance in different temperatures. It was reported that chitosan extracted from mushrooms *B. bovines* and *L. laccata* has lower thermal stability, with the maximum degradation temperature (MDT) of 317 °C and 309 °C, than chitin from crustaceans (349–385 °C) and chitosan from insects (325 °C) [20]. In another study, a value of 310 °C was recorded for the MDT of chitosan from *A. bisporus*

[25]. Furthermore, Yen and Mau [22], in their study on the characterization of chitosan from *L. edodes*, reported that MDT of chitosan was a function of its MW. They concluded that precipitated chitosan of high MW has higher thermal stability (283.58–289.76 °C) than soluble chitosan of lower MW (247.64–257.56 °C).

Presence of β-glucans and protein residues

A concern in the commercial production of chitosan may be the presence of protein in crustacean shells as well as the cell walls of mushrooms. It may present potential complications in chitosan biological activity. For example, in crustaceans, chitin molecules are directly linked to proteins and together form a complex network [12,26]. Incomplete deproteinization of crustacean chitin and chitosan using chemical methods can lead to the presence of residual heat-stable allergenic proteins, such as tropomyosin, myosin light chain, and arginine kinase in the final product [12,13,27]. Moreover, tropomyosin as the main allergenic protein in seafood can cause frequent allergic reactions in sensitized individuals. Unlike crustaceans, mushrooms can be considered an alternative ecological source of chitin and chitosan due to the absence of these allergenic proteins [13,27]. Also as a vegan-friendly alternative, the isolation of chitosan from mushrooms is currently gaining importance.

In the mushroom cell wall, chitin is directly associated with *β*-glucans and is embedded in an amorphous matrix, **Figure 1**. The linkages between chitin and glucan in mushrooms have been proven to be covalent [11]. Most commonly, *β*-glucan exhibiting (1-3) backbone and (1-6) branching is associated with chitin. *β*-glucans act primarily as immunostimulators [3]. They can prevent immune disorders and maintain a quality of life, especially in immunodeficient conditions. Accordingly, mushroom chitosan is apparently more effective in inducing an immune response than crustacean chitosan because it is covalently linked to *β*-glucans [11].

EXTRACTION OF CHITOSAN FROM MUSHROOMS

The extraction process of chitosan from mushrooms is similar to that used industrially to extract chitosan from marine sources, except that no demineralization treatment is required [11,12,13]. Generally, the extraction procedure includes alkaline treatment to remove protein, lipids, and alkali-soluble polysaccharides and to deacetylate chitin to yield chitosan; acid treatment to separate chitosan from chitin residues; and finally alkaline precipitation of chitosan [12], **Figure 3**.

Deproteinization and deacetylation of chitin with alkali is most often performed with 1M NaOH at 95 °C for 2 to 10 h [12]. Separation of chitosan by acid treatment is commonly performed with 2 to 10% acetic acid at 95 °C for 6 to 14 h. The extracted chitosan must be purified to be suitable for proper use. Purification is often achieved by the removal of insoluble materials, reprecipitating chitosan with 1 M NaOH, and removing traces of metals with sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic acid (EDTA) of retrieved chitosan [12].

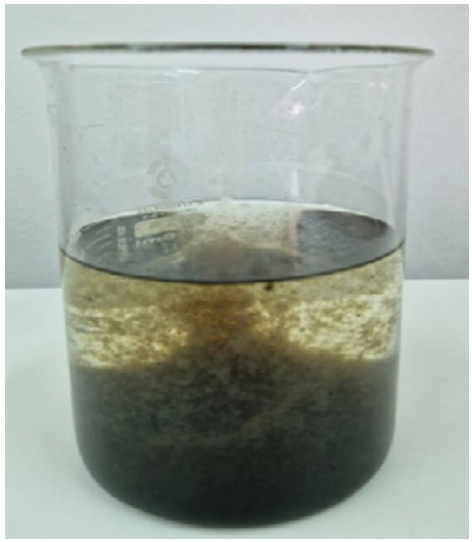


Figure 3. Precipitation under alkaline conditions of chitosan extracted from *A. bisporus* fruiting bodies at the Laboratory of Industrial Microbiology, Faculty of Agriculture, University of Belgrade.

Due to the detrimental effect of acid and alkali on the quality of chitin and chitosan, many studies have been conducted to investigate more environmentally friendly approaches in extraction procedures. In addition, deproteinization and deacelation by chemicals at high temperatures can take a long time and affect the quality of chitosan, i.e. the MW and DD [13]. In an attempt to solve this problem, green extraction techniques have been reported including microwave-assisted extraction, ultrasound-assisted extraction, subcritical water extraction, ionic solvents, deep eutectic solvents, alkali/urea aqueous solution, and enzymatic extraction methods [13,28].

Enzymatic extraction of chitosan using proteases for deproteinization and chitin deacetylase for deacetylation of chitin has been reported to offer more advantages, such as obtaining pure chitosan of higher MW with controlled DD [28].

BIOLOGICAL ACTIVITY OF DIETARY CHITOSAN

Chitosan is known for numerous biological properties of which most important for application in the food industry and biotechnology are biocompatibility, antibacterial, antifungal, and antiviral activities, biodegradability, antioxidant activity, hypocholesterolemic activity, antiulcer and antidiabetic activities and non-toxicity [16,29].

Antimicrobial activity

Microorganism growth on food surfaces is a major cause of food spoilage and foodborne illness. Since chitosan possesses antioxidant and antimicrobial properties, it is recognized as a potential natural ingredient for the development of packaging films and coats, or to be used as a natural preservative [30,31,32]. The ability of chitosan and its derivates to inhibit a wide range of bacteria, molds, yeasts, and viruses has been published in numerous review papers [24,33,34,35]. Factors that influence the activity [31] are classified into three groups (**Figure 4**). Chitosans of different molecular weights show different solubility and as a consequence different activity. While larger molecules show activity on the microbial cell surface, chitosan with a smaller molecular weight (below 10 kDa) shows better activity passing through the cytoplasmic membrane [36]. Additionally, pure chitosan shows different activity compared to its derivatives and oligomers.

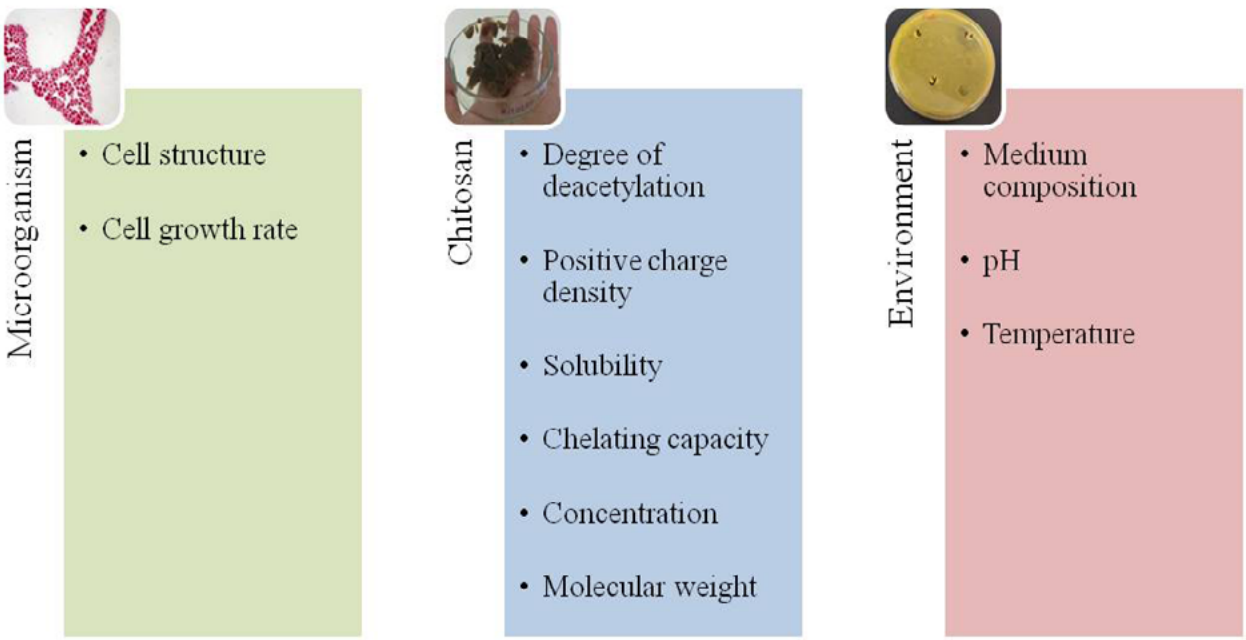


Figure 4. Factors influencing the antimicrobial activity of chitosan.

Considering the solubility of chitosan in weak organic acids, the application of chitosan as an antimicrobial agent in the food industry is possible in the form of liquid, paste, coating, or the form of powder. Application of chitosan in food is reduced since it is more soluble and has better antimicrobial activity below pH 6 [37]. Combining chitosan with other antimicrobial compounds of natural origin, the activity of chitosan in a neutral environment can be enhanced. The potential application of chitosan formulations with soy proteins for use in foods from biscuits, burgers, coffee, and snacks to lactic-acid products, and with benzoates and EDTA in various fruit juices were published [30]. However, some food components such as starch, proteins, and lipids, have the role of protectors, thereby reducing the antimicrobial activity of chitosan, which leads to the conclusion that the activity of chitosan will not be equal in all food products.

Chitosan acts stronger against fungi and algae compared with bacteria [34]. The antifungal effect of chitosan is based on preventing the process of spore formation and germination [38]. It additionally shows different activity on Gram-positive and Gram-negative bacteria, due to the different cell wall composition.

The exact mechanism of antimicrobial activity is unknown, but several mechanisms have been proposed. The most common is the reaction between positively charged chitosan and negatively charged groups of structural molecules located on the surface of microorganism cells (lipopolysaccharides and proteins), which causes damage to the cytoplasmic membrane, and release of intracellular compounds [35]. It is assumed that chitosan competes with Ca^{2+} ions for electronegative sites on the membrane surface. The proposed mechanism is valid for chitosan with large molecular masses. At dense accumulation, chitosan will form a coating over the cell. In the nucleus of eukaryotic cells, oligochitosan of small molecular masses obstructs transcription of RNA and synthesis of protein. Another proposed mechanism of the antibacterial activity of chitosan and its derivatives is the chelating ability, as well as induction of the formation of reactive oxygen species (ROS), which leads to oxidative stress in cells, damage to DNA molecules, and death [33,34].

Antioxidative activity

The replacement of synthetic antioxidants with natural ones has been in the sphere of interest, recently. Summarizing research data, chitosan can be considered a natural antioxidant agent. Still, to meet the standard of primary antioxidants, chitosan of low molecular weight (below 30 kDa) with a high degree of deacetylation (over 90%) should be used. Chitosan with a higher molecular weight and a lower degree of deacetylation can be considered a secondary antioxidant [39]. Its possible usage in food production refers to its addition to food and beverages to slow down oxidative damage of fresh food [40]. Chitosan shows the ability to bind free radicals that cause oxidative damage [41]. A greater degree of deacylation of chitosan means a greater number of cationic groups that bind free radicals. Chitosan has also been proven to have the ability to chelate various metal ions, stopping their catalytic activity [24,37]. This property can be applied to remove metal ions in industry, or for prevention of lipid peroxidation in the food.

Biodegradability

The biodegradation of chitosan is of great importance for the formation of packaging films and coating materials in the food industry. The property depends on the molecular weight of the polymer, viscosity, and arrangement of $-NH_2$ groups. Chitosan is a solid material, but if necessary, it can be converted into oligomers using different methods. The most preferred method is the application of different enzymes under controlled conditions. The enzyme chitosanase (chitosan N-acetyl-glucosaminohydrolases, EC 3.2.1.132) catalyzes endohydrolysis of β-(1,4)- glycosidic linkages between GlcN residues in partially acetylated chitosan. Chitosinase activity has been proven in various bacteria, fungi, plants and some viruses [42].

Antiulcer activity

Ulceration in the stomach leads to abdominal pain, bloating, nausea, loss of appetite, and others. It is caused by an imbalance of pepsin due to increased acid, or the presence of bacteria *Helicobacter pylori*. Due to the ability to dissolve in an acidic environment, low molecular weight chitosan and chitin have a protective effect. As a result of antioxidant activity in the intestine, chitosan binds H^+ ions and neutralizes acid [43], which is important from the point of functional food additive application.

Antidiabetic and hypocholesterolemic activity

Miura *et al* [44] in their study conducted on mice showed that chitosan in the amount of 5% as a food supplement maintains blood glucose levels and lowers the lipid content in the intestine, which is especially important for diabetics and application of chitosan in functional food products. It is assumed that chitosan is transformed into oligosaccharides before being absorbed. In a study of the hypocholesterolemic effect of chitosan conducted on adult men who consumed biscuits with chitosan (3 g/day during the first week, 6 g/day during the second week) a decrease in total serum cholesterol, compared to the control period during which the subjects consumed biscuits without chitosan, was measured [16]. According to the EFSA health statement, a daily intake of 3 g of chitosan per day maintains a normal level of LDL cholesterol [45]. This activity is conditioned by chitosan molecular weight, degree of deacetylation, and viscosity.

Non-Toxicity

The advantage of chitosan used in the food industry is its low toxicity or absence of toxicity compared to other polysaccharides. The usage of chitosan as a dietary additive has been approved in many European, American, and Asian countries (USA, Finland, Italy, South Korea, Japan, etc.) [16]. The first time it was approved for human use by the EU was in 1997. Most often, approved dietary supplements with chitosan are dose for body weight reduction, although there are no official health statements about this. Chitosan and chitin still don't have GRAS status. Obtaining status is hampered by its structural diversity (degree of deacetylation and molecular weight) which may affect its biodistribution and toxicity in the body [46]. For the above reasons, toxicity studies must include detailed data on the chitosan structure. Despite this, in some food products, the addition of chitosan is allowed. Chitosan is allowed to be used as an auxiliary agent in the production of fruit juices and nectars [47]. Chitosan isolated from *Agaricus bisporus* mushroom is allowed to be used as a natural food and beverage preservative, by USA FDA in 2022 (GRAS Notice No. GRN 000997).

It is believed that the toxicity of chitosan depends on the purity of the polymer and the method of application. Reported side effects in moderate form are nausea, skin and throat irritation [43]. Additional caution should be on the possible influence of chitosan on the intestinal microflora, reduction of fat-soluble vitamins, reduced absorption of minerals, and some medications [48].

APPLICATION OF CHITOSAN IN THE FOOD INDUSTRY

Chitosan, with the mentioned biological characteristics (especially antimicrobial activity), has potentially wide applications in biomedicine, biotechnology, pharmacy, cosmetics, and textile industry [49]. Considering the limited solubility of pure chitosan in wa-

ter, publications indicate the significant application of oligochitosan, cationic derivatives, and chitosan composites prepared using different natural or synthetic polymers. In recent years, active biomolecules such as chitosan and its derivatives have been recognized in the food industry. Potential applications include being a natural preservative to extend the shelf life of foods [43], biodegradable edible packaging material, or as a sanitizer. Due to its gelling properties, chitosan can be used for enzymes or probiotics immobilization, as a dietary supplement with a hypocholesterolemic effect, or in combination with dietary fiber for functional food production [30].

Packaging films and coating

Food product quality changes during storage due to the influence of a large number of physical, chemical, and microbiological factors. In order to maintain quality and extend product shelf life, packaging of fresh and processed foods is essential. The global focus on "green consumption" promotes the use of biopolymers obtained from natural sources such as chitosan, collagen, cellulose, gelatin, and corn zein that do not adversely affect the environment. Research results indicate the potential application of chitosan for the production of biodegradable, edible films that can be consumed together with the packed product [40,41,50,51]. Chitosan contributes to the extension of the shelf life of coated products through controlled transfer of moisture between the food and the environment, controlled oxygen leakage, impermeability to certain food components (fats and oils), the presence of bioactive compounds (antimicrobial and antioxidative activities) and others. Coats based on pure chitosan exhibit an antibacterial effect during the storage of shrimp, cod, fillets in the refrigerator, vegetables, fruits, and meat [31,52]. Since chitosan possesses antifungal activity, the application of chitosan-based packaging for the storage of grains, fruits, and vegetables in order to prevent spoilage on the surface of raw materials after harvesting is of great potential, and a far safer approach than the application of fungicides [33].

However, due to the hydrophobic nature of chitosan, thermal stability, and poor mechanical properties, films prepared only from chitosan cannot exhibit the aforementioned mechanisms during long-term storage. For an improvement of mechanical characteristics, as a plasticizer, glycerol is most often applied [53]. A new trend is the development of active chitosan films/coatings in combination with natural antimicrobial agents in the form of composite films with various bioactive compounds, nanoparticles, microcapsules, and nanotubes, with the application of modern preparation technology including lyophilization, spray drying, solvent casting, wet spinning, electrospinning, and layer-by-layer assembly [31,54].

Blending chitosan with other polymers is a very attractive approach for the application of chitosan in various industries. The choice of copolymer depends on the application of the composite, its solubility, biodegradability, biocompatibility, and other mechanical and physical properties. A large number of papers indicate the antimicrobial activity of composite films of chitosan and other polysaccharides, such as pectin and cellulosic materials [33]. Nanoparticles of zinc oxide or titanium dioxide improve the antimicrobial activity of chitosan-based films, which extend the shelf life of fresh-cut fruit, chicken fillets, and cheeses [31]. Composite films based on chitosan and silver nanoparticles can extend the shelf life of chicken breast fillets, or white crucian carp, considering that silver nanoparticles have a known antimicrobial activity. Yazdi *et al* [52] recognized the advantages of active packaging film for bread based on bacterial cellulose with the addition of chitosan and silver nanoparticles. The analysis indicated improved thermal and structural properties of the film, as well as an extension of the product's freshness and durability.

Plant materials, such as essential oils of thyme, basil, oregano, anise, cloves [51,55], plant extracts of sage, rosemary, green tea, fruit, chokeberry pomace [56], bioactive components in the form of anthocyanin, phenolic compounds, and flavonoids are well known for their biological potential and application in the food industry. A large number of publications indicate the potential application of composite films of chitosan and the mentioned plant materials as natural, biodegradable, and non-toxic packaging materials. Additionally, the combination of chitosan with plant extracts can improve the sensory properties of the product. The addition of natural pigments, such as anthocyanins, can lead to the formation of active packaging that changes color due to product spoilage [57]. Active films/coatings based on chitosan could be applied in various food products, especially fruits, fresh products, and meat for the controlled release of antimicrobial and antioxidant components [30].

Functional food additives and stabilizers

On the global market, chitosan is currently present as a food additive, dietary supplement, or food quality enhancer. It fulfills some of the dietary fiber criteria, such as indigestibility in the upper part of the intestinal tract, polymeric nature, and water-binding capacity. The gastric acid promotes the solubility of chitosan. Chitosan reacts with fatty acids and binds lipids due to hydrophobic interactions, and a large part of bound lipids is excreted [58]. In the intestines, chitosan is deposited in the form of complexes formed by ionic interactions between positively charged amino groups and negatively charged fatty acid groups. Based on those facts, chitosan in combination with dietary fibers has

found applications for obtaining functional food, and supplements for lowering the level of bad cholesterol in the blood [30]. According to the inhibitory activity of chitosan against bacteria, a potential application is also for the production of functional chewing gums with an increase in salivary secretion and the growth of oral bacteria suppression [59].

Chitosan has the properties of product stabilization, emulsification, and humectants due to which it can be used as an additive to improve the sensory and rheological properties of the product [32]. The properties have been demonstrated in water-oil systems. The composite of chitosan and fatty acids could have a significant application in the food industry. Positively charged chitosan has also found a potential application for clarifying cloudy fruit juices, with reduced use, or without the use of pectinase [60]. Chitosan shows the properties of binding mycotoxins, heavy metals, pesticides, and other chemicals in food, which makes them suitable for the detoxification of food and feed [32,61].

Immobilization of enzymes and probiotics

Due to the large number of free functional groups and its polycationic nature, chitosan shows an affinity towards proteins, which can be used for the immobilization of enzymes in the food industry [30]. The successful immobilization of enzymes such as amylase, catalase, glucosidase, invertase, proteinase, etc., has been proven for application in the food industry in order to hydrolyze starch, remove peroxide from food, produce wine and juices, produce cheese, and more [62].

Probiotic microorganisms are used in the production of functional food and pharmaceuticals products. It is necessary to preserve their viability in the conditions of the acidic environment of the stomach and thus keep their health benefits. Alginate microcapsules coated with chitosan have a positive effect on the survival rate of various probiotics in *in vitro* gastrointestinal conditions and further the storage stability of various types of enriched food products (such as yogurt) [63].

Food and hand sanitizer

In food processing, one of the most common sources of contamination is the poor hygiene of food handlers. Many pathogenic microorganisms that often cause intestinal disease can be found on the hands, especially if workers come into contact with food of animal origin. Since the spread of COVID-19 in 2020, awareness of the need to maintain hand hygiene has increased. Considering its antimicrobial potential, chitosan has found application in the production of new gels for hand disinfection [64] as non-alcoholic, with the addition of silver nanoparticles or with a reduced alcohol content that dries the skin. In addition, the treatment of leafy vegetables, fish, or poultry and livestock after processing, as contaminants of equipment, usually involves the use of synthetic compounds, such as chlorine, in order to reduce pathogenic microorganisms on the surface. Exposure to chlorine can lead to irritation or even damage to the respiratory tract. Organic sanitizers based on chitosan could be an excellent substitute for synthetic disinfectants [65].

APPLICATION OF CHITOSAN IN AGRICULTURE

Healthy vegetables, fruits, or crops are important for high-quality and safe food production. Additionally, nowadays organic food and sustainable agriculture are recognized which include the use of organic fertilizers and soil without pollutants. For this purpose, chitosan and its oligosaccharide known as chito-oligosaccharide, or amino-oligosaccharide found application. It is a positively charged oligosaccharide, obtained by enzymatic modification of chitosan. These molecules in plants stimulate the biosynthesis of phyzoalexins, defense substances, production of reactive oxygenase systems and cause structural changes in the cell walls of plants [33]. When the plant develops resistance, due to the attack of the pathogen, the cells of the microorganism are destroyed at the place of occurrence of the threat, and the biochemical response of the plant in the surrounding tissues grows. According to that, chitosan can be considered a substitute for synthetic pesticides and represents a type of plant growth stimulant with increased yield and improved seed germination. Increased resistance to unfavorable environmental conditions was recorded in cereals. However, the efficiency of using chitosan for these purposes depends on the plant, the characteristics of chitosan, and the prevailing environmental factors. The specificity of oligosaccharides for certain types of plants has been observed.

Since chitosan is a biodegradable natural polymer, it is desirable for use in bioremediation as environmentally friendly. It reduces the number of pathogens in the soil, and binds heavy metals, thereby improving the quality of the soil and promoting the growth of beneficial microorganisms [66]. To obtain a satisfactory quality of fertilizer, mixing chitosan with other nutrients is often used. Copolymers of chitosan with different materials have potential applications for the absorption of heavy metals from soil and aquatic media, such as wastewater, as well as for fuel desulfurization. Wastewater from food, and other industries is a carrier of toxic organic and inorganic pollutants, a great danger to ecology, especially the aquatic environment. It has been proven that chitosan has the property of a coagulant for removing compounds from water such as pesticides or microplastics, it binds heavy metals, re-

duces COD, BOD, and color in wastewater, and can be used in surface water treatments [67]. Nano-biocomposites have shown good application in this direction.

CONCLUSION

As a result of the polycationic nature of chitosan, its biodegradability, the ability to form composites with numerous polymers, as well as the ability to be translated into smaller molecules and derivatives, chitosan attracts great attention in biotechnology, biomedicine, and other fields. Opposite to the multiple advantages of using chitosan from mushrooms compared to crustaceans, the application of chitosan from mushrooms is still insufficient. The production of chitosan from mushrooms can be profitable by using waste material from industry and nature for mushroom growing. The mushroom processing industry often discards stipes as waste, which can be used to extract the chitosan-glucan complex. This is an important part of sustainable production development. Several companies on the market offer chitosan from mushrooms for widespread use, such as Chibio Biotech (https://www.chibiotech.com; accessed on 17 October 2024), Ihumico (https://www.ihumico.com; accessed on 17 October 2024), ChitoLytic (https://chitolytic.com; accessed on 17 October 2024). Standardization of the biosynthesis process of chitosan from mushrooms is needed to achieve equal quality and bioavailability. For food industry applications, and especially for biomedicine applications, proof of non-toxicity and permits for biomedical use by regulatory bodies must be provided. Finding the composition of active films for food shelf life prolongation that match the functionalities and prices and does not affect the sensory properties and composition of the product is a challenge for researchers in the future.

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Hitozan iz pečuraka: biopolimer sa potencijalnom primenom u prehrambenoj industriji i poljoprivredi

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Kratak sadržaj

Pečurke su prepoznate kao vredan izvor hranljivih materija, ali i bioaktivnih komponenti koje su našle primenu u dobijanju dijetetskih suplemenata i funkcionalne hrane. Sve je veći izazov svrstavanje proizvoda iz plodonosnih tela i micelijuma pečuraka u kategoriju „nove hrane". Hitin, polimer prisutan u ćelijskom zidu pečuraka, kao i njegov derivat hitozan, imaju povećanu primenu u različitim granama industrije, farmaciji i biomedicini. Takođe, prepoznaju se kao potencijalna nova hrana. Obzirom na slabu rastvorljivost hitina i hitozana u fiziološkoj pH često postoji potreba za daljom modifikacijom hitozana u cilju dobijanja lako rastvorljivih derivata i manjih molekula sa širokom primenom. Ovaj pregledni rad ima za cilj da ukaže na prednosti korišćenja pečuraka u dobijanju hitozana, bezbednog, biorazgradivog polisaharida sa izraženim antimikrobnim delovanjem i brojnim benefitima po zdravlje ljudi i životinja. Skreće se pažnja na fizičko-hemijska svojstva hitina i hitozana, prisustvo u ćelijskom zidu pečuraka, kao i na metode ekstrakcije i prečišćavanja hitozana. Razmatraju se literaturni podaci o njihovoj primeni u prehrambenoj industriji, u izradi kompozita na bazi hitozana sa širokom primenom u prehrambenoj industriji, a posebno za proizvodnju jestivih i aktivnih filmova. Konačno, razmatra se i primena hitozana u poljoprivredi.

Ključne reči: *hitozan; pečurke; biotehnologija; hrana; biološka aktivnost.*