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Extraction and Use of Insect-Derived Chitin and Chitosan

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ABSTRACT : Chitin is the second most widespread polysaccharide in nature after cellulose and is mainly found in the world of invertebrate animals. Its function is protective and structural and it is present in the exoskeleton of arthropods, insects, while it is absent in vertebrates. Chitin is also found in the plant kingdom, as a cellulose substituent, in the cell membrane of many fungi, lichens and bacteria. It is insoluble in water, dilute acids and alkalis; it can be partially deacetylated, transforming into its derivative, chitosan, with promising application properties.
In insects, chitin is a material that acts as a scaffold, supporting the cuticles of the epidermis and trachea, as well as the peritrophic matrices that line the intestinal epithelium.
The growth and morphogenesis of insects are closely dependent on the ability to remodel chitin-containing structures, and to achieve this, insects continuously produce different enzymes in various tissues. During their development and growth, it is important to coordinate the processes of chitin synthesis and degradation under the control of the enzymes involved in the process.
Little research has been performed on the extraction and characterization of chitin present in insects, as previous studies have focused on chitin and chitosan present in crustaceans. Currently, many properties of these substances have been highlighted and research has been increased for application in various sectors such as food, medical, cosmetics and biomaterials engineering.
KEYWORDS: Insects, chitin, chitosan,extraction methods, applications

I. INTRODUCTION

Insects are animals that are part of the group of arthropods, which have a chitinous exoskeleton: a segmented body divided into three parts, head, thorax and abdomen, from which three pairs of articulated legs depart and in many specimens also the wings; on the head, compound eyes and two antennae are evident.

Insects are the only winged invertebrates, they are cold-blooded and undergo metamorphosis in order to adapt to seasonal variations, reproduce quickly and form very large populations. Their respiratory systems are equipped with stand air and vacuum pressure, high-altitude flight, and radiation, and also with an effective orientation system.

Arthropods are the largest group of invertebrates and are widespread in all environments. The word arthropods means "with articulated limbs": in reality, not only the legs, but the whole body of arthropods is made up of various segments, covered with a more or less rigid coating called exoskeleton that supports the body and protects the animals from predators. The insect exoskeleton is an apparatus that performs both the functions of the skeleton and the integument systems of vertebrates and is composed of a layered structure with three layers:

- an inert tissue, the cuticle, formed by the structural organization of macromolecular organic compounds that give the exoskeleton rigidity, elasticity and chemical inertia. It is devoid of cellular elements, therefore it is an inert tissue, crossed by nerve fibers: its complex functionality derives from the presence of different layers in which both the chemical composition and the organization of the organism vary.
- an epithelial tissue, the epidermis, formed by a single layer of cells that are cubic or cylindrical in shape in the juvenile stages and flattened in the adult insect that mainly presides over the construction of the cuticle.
- a connective tissue, the basement membrane, formed by collagen fibers that connects the integumentary system with the internal cavity of the body.

The cuticle, in turn, is made up of three layers:
1. Epicuticle is the outermost layer, a few microns thick, which performs functions of isolation and protection of the insect's body from the external environment; it is an inert and impermeable membrane, of a waxy-lipoprotein-tannic nature that contains small amounts of chitin, responsible for its hardness.
2. Exocuticle is thicker than the epicuticle and performs protective functions as it provides the exoskeleton with rigidity. It is made up of chitin

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fibrils immersed in a tannized lipoprotein matrix: its structure therefore has both flexibility and mechanical strength due to the presence of chitin, and rigidity conferred by the tannized lipoprotein matrix.

3. Endocuticle is the thickest layer of the entire cuticle and is made up of chitin, lamellar, multi-layered fibrils, immersed in a non-tannized lipoprotein matrix that gives the exoskeleton considerable elasticity.

Exocuticles and endocuticles make up the procuticle .

Chitin is the substance present in the highest concentrations in the cuticle: while in the endocuticle it is present in its pure form and gives elasticity and flexibility to the layer, in the exocuticles it is combined with mineral substances, such as calcium carbonate and is responsible for the hardness and rigidity of the cuticle.

Insects have a soft body as adults or in the larval state due to a higher percentage of endocuticle in their exoskeleton. The legs of insects are very robust because they are covered with a mineralized cuticle, while the joints of the legs and segments of the body are made up of the flexible cuticle that allows them to expand. Chitin is essential for the integrity of the procuticle and performs the task of connecting the cuticle to the epidermal cells, thus maintaining the epidermal morphology. Chitin deficiency causes abnormal embryos, cuticular structural defects, and stunted growth [1].

Each insect produces its own chitinous skeleton from the epidermal layer, which protects it during the evolutionary process and reduces the danger of drying out. The hardness of this exoskeleton, however, limits the size of the body, so it must be eliminated and rebuilt during the various evolutionary stages that characterize the life of the insects themselves. The process of elimination of the exoskeleton is known as molting/ecdysis and is triggered by steroid hormones, ecdysteroids, with the contribution of juvenile hormones, sesquiterpenes in nature, and neuropeptides. [2-4].

The rigidity of the exoskeleton prevents continuous linear growth and a developing insect changes its size only during molting. In this phase, the insect develops a new cuticle in place of the old one: in the interval between two moults, the insect increases in weight but not in size. On the other hand, in correspondence with the moulting, there is an increase in body size.

Chitin functions as a scaffold supporting both the cuticles of the epidermis and trachea, as well as the peritrophic matrices that line the intestinal epithelium.

The growth and morphogenesis of insects depend on the ability to renew and shape chitin-containing structures and therefore chitin production is continuous and is regulated by chitin synthase and chitinolytic enzymes. [5].

Moulting is controlled by a hormone, ecdysone, produced by the ventral protoracic glands, which stimulates the activity of epidermal cells by inducing moulting. The process takes place gradually in several stages: detachment of the old cuticle; emission of exuvial fluid, containing lytic enzymes that dissolve a large part of the endocuticle; reprocessing of substances produced by the dissolution of the old cuticle; laceration of the old cuticle and abandonment of the exuvia. During moulting, the new cuticle remains soft. Then the oxidation of polyphenolic substances occurs with the formation of quinones, responsible for the tannization and hardening of the outer layers of the new cuticle [6].

II. EXTRACTION OF CHITIN FROM INSECTS

Chitin is a polymer consisting of D–N–acetyl–glucosamine units linked together by alpha 1-4 glycosidic bonds. Ledderhose in 1878 determined the structure of chitin and demonstrated that it could be synthesized from molecules of glucosamine and acetic acid. In chitin, the various units form long chains that aggregate into laminae through hydrogen bonding: the various ways in which these laminae can aggregate together determine the three known crystalline forms of chitin called α, β and γ with different orientations of the microfibrils.

The α form is made up of antiparallel chains and is the most abundant in nature, present in crustacean shells, in the shells and skeletons of molluscs and krill, insects, etc. The β form, has parallel chains and is rare in nature, it occurs in squid, pogonophores, and some diatoms. The γ form is formed by a mixture of antiparallel and parallel chains and is found in insect cocoons [7,8] .

In its pure form, chitin is translucent, flexible, resistant and rather hard, but in most arthropods it is often present in aggregate with other substances, such as in sclerotin, a tanned protein matrix, which forms a large part of the exoskeleton of insects. Chitin has recently attracted

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the attention of researchers as many applications have been highlighted in different industrial sectors and studies to extract chitin from insects have been increased.

The extraction methods generally used are mainly of a chemical and biological nature and have been applied to crustaceans. In recent times, as the interest in chitin and its main derivative chitosan, present in insects, has increased, researchers have turned their attention to experiment with a valid process of extraction and characterization of these substances from a different matrix, insects [9].

In one of the first studies with spectroscopic methods, differences in chitin extracted from different sexes of grasshoppers were highlighted: the chitin present was characterized as a form□ □ □ for both sexes, but the content was higher in males than in females.

Elemental analysis, thermal properties, and crystal index values were also similar in males and females.

Observing under the scanning electron microscope, the females' chitins were rather uniform, while the males' were characterized by nanopores and nanofibers. These differences suggest that chitin samples obtained from the female and male could be used in a wide variety of fields, depending on gender.

Chitin is extracted from insects using various methods. The chemical treatment involves demineralization using HCl at different concentrations to remove the mineral substances associated with the basic structure of the exoskeleton followed by deproteinization with KOH at different concentrations to remove the proteins bound together with all the constituents. The parameters for acid treatment such as concentration, temperature, time and solution-to-solid ratio used for chemical extraction from insects are moderate compared to the requirements for chitin isolation of crustaceans. Parameter optimization is crucial in order to minimize chitin degradation and reduce impurity levels to a satisfactory level in all types of applications [10]. Insects, in fact, have a lower level of inorganic material than crustacean shells.

The biological method involves the use of enzymes such as proteases and microorganisms such as Lactobacillus Pseudomonas aeruginosa K-187 and Bacillus subtilis [11-13]..

Chitin bioextraction is emerging as a greener, cleaner, environmentally friendly and cost-effective process: microorganism-mediated fermentation processes are highly desirable due to ease of handling, simplicity, speed, controllability through the optimization of process parameters, such as ambient temperature and negligible solvent consumption, thus reducing environmental impact and costs [14].

In these biological processes, demineralization and deproteinization occur simultaneously but incompletely, however green extraction methods are gaining ground due to their environmentally friendly nature [15].

In addition to chemical and biological treatments, a physical method has also been proposed in which the main treatment of acid demineralization and basic deproteinization is complemented by an ultrafast microwave treatment [16] and an ultrasonic treatment [17].

Chitin was isolated from the cuticle of the beetle larva and the pupa of the silkworm, Bombyx mori using the traditional shrimp method and was characterized as α form. [18,19] Various analytical methods have shown some differences between chitin from insects and chitin from crustaceans. A significant change in the degree of crystallinity has been noted for insect chitin samples derived from silkworm pupae and cuticles of beetle larvae [20]. This difference has been attributed to the catecholic compounds present in the insect cuticle, which are necessary for its hardness, which affect the solid structure, the semicrystalline morphology, the size of the crystalline lamella of the chitin extracted from them, which is also more easily attacked by HCl.

From the cuticle of the honey bee, Apis mellifera, Nemtsev et al. have isolated chitin using a biological process with different enzymes, to obtain water-soluble chitosan with low molecular weight [21]. The chitinolytic enzyme complex was obtained from Streptomyces kurssanovii associated with a hydrolase obtained from the Trichoderma reesei fungal strain.

This combination was necessary to simultaneously hydrolyze the components of the bee cuticle, chitin, muscle and cuticle proteins, and melanin. A 3% hydrogen peroxide solution was used to completely remove the melanin and obtain perfectly white chitin and chitosan. To optimize this process for large-scale production, the hydrolysis time required for chitosan to reach a stable weight was also evaluated. After 180 minutes chitosan reached a weight of 20 kDa, which remained constant even after further hydrolysis, as a result of the loss of hydrolytic activity by the enzyme complex.

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Chitin was isolated from Holotrichia parallela Motschulsky, adult beetle, belonging to the Scarabaeoidea family, by chemical treatment followed by decolorization with potassium permanganate.

In China these insects are pests of agricultural crops and are also used as food and in traditional medicinal products in China and East Asia. The low ash levels and nitrogen content indicated the effectiveness of the chitin extraction method. The surface morphology of the chitin examined with the scanning electron microscope was rough and thick, similar to a commercial chitin sample obtained from shrimps and therefore Holotrichia parallela adulta can be considered an alternative source of chitin [22].

The black soldier's fly (Hermetia illucens Linnaeus 1758) is known to be a good source of nutrients such as proteins, lipids, minerals. The larvae/prepupae, due to their high protein content, have been proposed as feed for fish, chickens and pigs and as food for pets; the large amount of fat is used for the production of biodiesel, but above all they represent an important source of chitin [23].

The extraction process was very difficult due to the presence of abundant organic material, in particular for the chitin-protein separation. Wanting to separate lipids, proteins and chitin at the same time, different extraction methods were employed based on total chemical extractions or enzymatic assisted extraction, optimized to obtain the highest level of purity for all three compounds. It was noted that the chemical process was advantageous in terms of yield and purity, comparing the extracted chitin with a commercial sample, while it was not satisfactory for the protein yield resulting partly denatured [24].

III. COMPARISON OF CHITIN AND CHITOSAN EXTRACTIONS FROM DIFFERENT MATRICES

Kim et al. studied the extraction of chitin and chitosan from the two-spotted field cricket, Gryllus bimaculatus, by following the chemical process with a strong acid and alkali. Chitin and chitosan obtained from crickets were subjected to physicochemical analysis, moisture, ash, elemental analysis, and the data were compared with the respective commercial shrimp products showing favorable similarities. The results indicated that adult cricket exoskeletons could be used as a source of chitin and chitosan which are useful as functional additives in industrial animal feed [25,26]

Some studies have also been carried out on spiders, showing that chitin extracted from different species showed morphological differences in the surface structure, especially with regard to porosity, while no significant differences in the values of the degree of acetylation were noted. During their moulting cycle, spiders lose large amounts of tube-shaped, chitin containing cuticles, from which it has been possible to extract a tubular chitin with a high potential for application in technology and biomedicine.

The moulting cuticle of Caribena versicolor, a species of tarantula that lives in the forests of the French Caribbean islands, looks like a tubular scaffolding in the shape of a spider, soft and flexible due to the absence of mineral elements, but very resistant, due to the presence of chitin-protein bonds that support it.

The extraction of chitin, lacking mineral substances, is carried out in two phases: deproteinization with alkaline solution assisted by microwave irradiation and decolorization with hydrogen peroxide. A transparent exoskeleton was obtained which underwent hydrolysis by chitinase to break the chitin-glucosamine bond.

Chitin has undergone extensive analysis to determine its structure and composition and has been found to be a unique tubular chitin form suitable for providing tubular and porous scaffolds that are almost naturally ready for potential applications in tissue engineering and regenerative medicine. [27,28].

In one study it was reported that the origin of chitin influences not only its crystallinity and purity, but also the arrangement of its polymer chain, and consequently its properties [29].

The parameters that define the properties of a polymer are the molecular weight, the degree of deacetylation, the polydispersion index and the crystallinity that is consequent to the degree of purity.

Knowing these parameters is essential for the choice of various applications both related to human consumption such as food and medical applications, and in other industrial sectors.

The growing interest in green technology has driven research into bio-based polymers that exhibit good physical properties, numerous biological activities, and are biodegradable, unlike synthetic polymers.

Chitin is made up of a chain of acetylglucosamine groups and the removal of a certain amount of acetyl groups, through the deacetylation process, gives rise to chitosan, which

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has a high concentration of reactive amino groups and, compared to chitin, has solubility in dilute acid solutions.

IV. PHYSICO-CHEMICAL PROPERTIES OF CHITOSAN

The solubility factor is very important, because not only does it condition the antimicrobial and antifungal activity of chitosan, but it allows the creation of films for food protection, also with the addition of other substances to enhance its antimicrobial activity.

Chitosan-based films exhibit modest oxygen barrier properties, good carbon dioxide barrier properties, high water vapor permeability. To increase the water barrier property, hydrophobic compounds, such as essential oils, are usually incorporated into chitosan films. The in vitro efficacy of essential oils against food borne pathogens and their mechanism of action have been widely studied and among the oils that have demonstrated antimicrobial properties against altering microorganisms and the main ones are: coriander oil, oregano oil, rosemary oil, sage oil, cloves and thyme oil.

The anti-chemical and antifungal activity of chitosan can be understood both as an interaction between the amino group of positively charged chitosan and a negatively charged foreign group which, by modifying permeability, would prevent the entry of essential materials into the cells or the pouring of fundamental solutes to the outside; and as a binding of chitosan protonated amino groups to cellular DNA, leading to the blockage of microbial RNA synthesis [30].

There are numerous applications of chitosan solutions to extend the storage time of plants after harvesting. In fact, during the storage period, fruits and vegetables are exposed both to bacterial attack and to a process of deterioration promoted by enzymatic reactions.

The efficacy of chitosan against Fusarium concentricum was evaluated by immersing an asparagus sample in both high and low molecular weight chitosan solutions and a carboxymethyl chitosan solution for 10 minutes [31]. After treatment, the asparagus was dried for two hours at room temperature and stored at 2°C with relative humidity of 95% for 35 days. Both high and low molecular weight chitosan solutions at a concentration of 0.05mg/mL effectively inhibited the growth of Fusarium concentricum and blocked spore germination. The treated asparagus showed no signs of phyto-toxicity and kept well for up to 28 days without loss of turgor and external quality.

Tissue engineering is an emerging discipline that using biomaterials individually or in combination, aims to restore, maintain or improve the function of biological tissues. The principle of this discipline consists in the isolation of healthy cells from an individual, followed by their expansion in vitro; subsequently, these expanded cells are seeded on a three-dimensional scaffold composed of biomaterial that acts both as a support and as a growth factor and slowly degrades over time to be replaced by the tissue formed by the seeded cells.

The materials used for these applications are of different types; natural polymers such as chitosan, collagen, gelatin, hyaluronic acid; synthetic polymers: such as lactic acid derivatives, polyurethanes, polyvinyl ethylene acetate, polycaprolactides that are reduced to nanofibers by means of the electrospinning technique to form scaffolds of different degrees of porosity.

The mechanical and biological properties of chitosan, in fact, can be improved by mixing it with collagen or gel polymers, such as gelatin, which has free carboxylic groups that can interact with the cation groups of chitosan, resulting in the formation of a network stabilized by the hydrogen bond. These chitosan-gelatin mixtures provide a structural scaffold for embryonic stem cell growth, bone tissue reconstruction, or constitute tissues for wound bandaging or other industrial applications [32-34].

Recent studies have highlighted the activity of chitosan and its derivatives as antimicrobial agents against fungi, bacteria and viruses and as biochemical activators of the plant defense system [35-37].

According to some hypotheses, the antibacterial activity of chitosan would depend on its structure. In fact, the cationic nature of chitosan, due to the presence of ammoniacal nitrogen in the glucosamine molecule, would represent a fundamental factor in its interaction with the negatively charged microbial cell surface, inducing the blockade of vital bacterial activity [38,39].

V. APPLICATION OF CHITOSAN IN THE PHARMACEUTICAL INDUSTRY

Chitosan-based materials have been extensively studied for oral drug delivery and topical administration. It has inhibitory effects on tumor cell growth, tumor-induced angiogenesis and

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metastases, by the administration of targeted drugs that act directly on the affected organ thus showing good antitumor activity [40].

Several strategies have been proposed to maintain the therapeutic levels and concentration of a drug for a long time in the body for the treatment of diseases, promoting greater adherence, increasing dosing intervals and thereby decreasing side effects with the involvement of knowledge from many disciplines [41].

For this purpose, solid lipid nanoparticles based on chitosan were prepared and the drugs were administered with targeted sensitivity and fewer side effects. It has been found that chitosan is very effective as a system of delivery through the nasal mucosa of large hydrophilic molecules, such as salmon calcitonin and insulin, of which it facilitates the passage through the mucosa by increasing their bioavailability while preventing these substances from being metabolized by the liver.

Improved absorption by chitosan of peptides and proteins and vaccines through the nasal and also intestinal epithelia has been demonstrated which is based on an ionic interaction between the muco- adhesive properties of this positively charged polymer and the negatively charged cell membrane. [42-44].

Chitosan is used in the pharmaceutical industry as an excipient to be used directly in the preparation of tablets, as a disruptor, to improve drug dissolution, for the production of solid forms of controlled-release dosage. The antimicrobial activity of chitosan suggests its use in a variety of different formulations, for example dressing tapes, toothpastes or artificial tears; chitosan nanoparticles are targeted drug vehicles in neoplastic diseases or central nervous system pathologies [45-47].

One of the most well-known applications of chitosan is in the dietary field, due to its ability to act as a non-digestible dietary fiber, intervening in the absorption of ingested fatty substances, before they are metabolized. In this sense, it prevents the absorption and accumulation of excess calories and is then eliminated from the body along with lipid substances. The fiber of chitosan swells in contact with water and forms a jelly in the stomach that gives a sense of fullness by reducing the stimulus of hunger.

The beneficial effects, in addition to a weight loss due to an increase in the sense of satiety, are evidenced by the decrease in cholesterol and triglyceride levels in the blood [48,49]. The safety of use of chitosan administered at dietary dosages in humans has confirmed that fiber is well tolerated and also demonstrated the absence of deficiencies related to fat-soluble vitamins and minerals. The cholesterol-lowering action of chitosan is attributable to a lower gastrointestinal absorption of exogenous cholesterol and the concentration of 2g/day, present in dietary supplements, it can also be used for a long period of time without causing damage to the body.

Chitosan in the form of soluble fiber has shown a particular action, such as that of acting as a prebiotic and restoring and increasing the development of native intestinal microflora, especially if damaged as a result of inflammatory diseases of the intestinal tract or by the intake of certain drugs such as antibiotics.

An experiment conducted on rats showed a bifidogenic effect of chitosan and chito-oligosaccharides, with an increase in bifidobacteria and lactobacilli and a simultaneous reduction in the concentration of Enterococcus and enterobacteria.

The effects of chitosan-alginate microparticles on the survival of Lactobacillus bulgaricus KFRI 673 in simulated gastric juices and intestinal fluid and on their stability during storage were investigated. These coating materials are effective at protecting bacteria from harsh environments such as acidic pH. [50,51].

Chitin and chitosan are used as components of various cosmetics, toothpastes, creams for hands,for
the body, and as hair care products. Chitosan has a moisturizing effect on the skin, depending on its molecular weight and degree of deacetylation, improving its appearance following the activation of fibroplasts.

High molecular weight chitosan increases the water resistance of protective emulsions against solar radiation, improving its ability to form a protective film on the skin; due to its antiseptic properties, the formation of the film also protects the skin from possible microbial infections [52,53].

Chitosan is used in dentistry for its remineralizing property that hardens the tissues of the tooth, and therefore its role as a desensitizer used in mouthwashes and toothpastes where it acts by reducing the permeability of dentin for the formation of a gel that also has an antimicrobial action. The action of chitosan is especially useful in the treatment of pyorrhea where it significantly improves the health of the gums and oral mucosa [54-56].

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VI. CONCLUSION

Chitin is widely distributed in nature in all realms of life and performs a variety of functions. The ability to synthesize chitin, which is found in diverse organisms including fungi, sponges, annelids, and arthropods, has been critical in the evolution of arthropods, as it allows survival in different aquatic and terrestrial environments. Chitin and its main derivative, chitosan, have been the subject of great interest by researchers as they are polymers of natural origin, free of toxicity, which present a high variability in their chemical and physical properties linked not only to the origin of the raw materials from which they are extracted, but also to the various extraction methods.

The differences do not only concern the polymer yield which is higher for crustaceans than for insects, but above all morphological such as crystalline, granular, fibrous appearance, or even as a pre-built scaffold in the case of arachnids.

A lot of research has been directed to obtain a well-defined product, of maximum purity to be used in various industrial sectors. In-depth research of physicochemical properties is essential to identify specific applications for chitin and chitosan such as drug delivery, tissue engineering, functional foods, food preservatives, without neglecting the biological activities present such as bioavailability, mucus adhesion, anti cholesterolemic, antimicrobial and antitumor activity.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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