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Original Research Paper

Bio-Medical

ASPERGILLUS NIGER PRODUCTION OF CHITOSAN WITH LOW MOLECULAR WEIGHT

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KEYWORDS :

INTRODUCTION

Biotechnology is science of using Midgets vita material in order to produce different products like Citric acid. Biotechnology is a science that discusses the use of living things or their metabolites in order to produce many different food products, medicinal products, chemical products and etc, through industrial scale. Biotechnology developing process has been formed through thousands of years, but modern technology was born in the middle of 70s.

Chitin and Chitosan

Definition

Chitin is a hard white material with crystal structure that exist in big amounts in the outer crust of hard shells and insects and fungus cell walls, chitin bio polymer is well adjusting material with living fibers and it is non toxic and disintegrable material in the environment. Yet its solubility is low and its reactivity is weak. Chitin after cellulose is the second common polymer in the nature that is extracted from the shell [crust] of fauna like cancer and shrimps, insects and some kinds of funguses. The main source of chitin industrial producing in the world, comes from shell waste produced from cancer and shrimp industrial productive and countries like India, Japan, Australia and Austria are the main producers of this bio polymer.

Chitin is the primary material for producing chitosan. By trepan removing chitin, one of the most plenty polymer in nature, chitosan is to be obtained.

Mostly chitosan is known as chitin material with acetylation percentage above 50 per cent. Chitosan is a kind of poly carbohydrate that is formed of amine glucose units and amine glucose N acetyl by beta 1and 4 connections. Chitosan structure is shown in figure 1

Figure 1. Chitin and Chitosan Structure

[Figure not included in the supplied manuscript.]

Feature

Chitin is a polymer got from D-Glucosamine. Chitin polymer formula is [N-Acetyl-β-D-Glucosamin]. Chitin bio polymer has the role of consistency in the cell wall. Chitosan turn into biological polymer with scientific name, β-D (1-4) –2-amino-2-deoxy- α- glucan, that is got from deacetylated out of chitin shell of sea hard shells, and its features is defined by the degree of deacetylation, and by deleting the acetyl group and making amino group, become alkalinized and is considered as a separated polymer with different physical and chemical features.

Chitin is indissoluble in water, alkali and acid, now that chitosan for having free dual groups of nvklyvfylyk in amino groups and because of low amounts of hydrogen bands, is almost solvent in acid solutions, this solubility is related with ph range of 2 till 6.5. This two polymers are natural azetic polysaccharides that in each chemical structure of them there is more than 5000 monomer glucose amino.

Chitosan cell weight is less than 50 k Daltone is known for chitosan oligosaccharide solvable in water, has biological adjustability, non toxic and anti microbe features, admixture ability with heavy metals ions.

Commercial chitosan generally have acetylation percentage above 70 per cent. Chitosan at PH below 6 is in form of poly cationic and easily react with negative load admixtures like proteins, ion polysaccharides, fatty acids and phospholipids.

This matter can affect the structure and texture and rheology features of product that chitosan is used in their production, because of amino groups in the structure of chitosan, in acidic environment has a better solubility. The degree of deacetylation that shows the proportions of amino glucose acetyl groups toward amino groups in chitosan structure is important factor at solubility scale and other chitosan features. The important features of chitosan are anti-microbe and anti-oxidant impact that noticeable researches about this features and its usage in food industry has been done. Many researches about anti microbe and preservative abilities of chitin and chitosan had been done, it is fix that chitosan is a non-toxic, biological dissoluble, biological adaptable with anti-microbe feature material. Chitosan anti microbe feature comes from positive load amino groups, this groups reacts with embryonic layer with negative load, this reaction lead to leakage of protein ingredients and other inner cell ingredients.

Extract Sources

Till now more than 300 different sources for extracting chitin and chitosan from kinds of invertebrates and outer shell of sea zoo planktons including corals and gel fishes, sea plants, alges, bacteria, skin shell hard shells like cancer shrimp and sea cancers of alga cell wall, fungus like aspergillus and mucor and yeast and sea diatomaceous 2 and outer skin of insects like butterflies and ladybirds are under observation for extracting this materials. (Dodane and Vilivalam,1998; Kumar, 2000; Khor and Lim, 2003; Krajewska004; , Sinha et al., 2004; Qin et al., 2006).

Today widely and commercially chitosan is got from residuum procreation of commercial sea food factories like cancer and shrimps and sea cancers and also biomas fungus residuum like aspergillus niger in citric acid factories.

Shrimp and Cancer Crust

Today widely and commercially chitosan is produced from cancer and shrimp residuum molecular weight between 105 and 106 dalton and viscosity about 1500 cp.

Residuum got through the proses cancer, shrimp an shells include 75 per cent of the hard shells weight. For now low amounts of shell residuum is used for animal food or extracting chitin [1].

Fungus

One of old fungus kind name, aspergillus comes from a name micheli in 1729. This name is influenced by a part religious religion named asperges.[Ainsworth, 1976]

Thom and church in 1926, first published aspergillus kind monogram. Aspergillus is the most known kind of fungus and most studies been done on it. The high accession of them in natural environments and easy laboratory cultivation and multi part economic importance have convinced many mycologists and industrial microbiologists to study them.

Aspergillus mostly grow as saprophyte on musty plants. Most kinds of it are adopted for destruction of plants polymer complexes yet mostly they use many different substrates. (Polacheck et al 1989).

They have the ability to move around the places by winds and they can grow anywhere they get food and water and that means they are every where existing [ubiquitous].

Figure2; Aspergillus Niger

[Figure not included in the supplied manuscript.]

Aspergillus niger one of most important kind of aspergillus, is a fungus that produce black mould in food and plants, niger like other kind of aspergillus is not capable of many human illnesses, but in high intensity can cause pulmonary illnesses and aspergillus flavus among gardeners. Aspergillus kinds like aspergillus niger are the reason in food corruption. Among this fungus aspergillus flavus is counted as important source of fumigatus aflatoxin and by world wide spread infect food material. Acratoxin is produced by some kind of aspergillus and penicillium specially aspergillus acratoxin in tropical environment and penicillium is produced in depots. Low number of aspergillus niger mould isolated can produce acratoxin.

Chitosan Production

The outer shell of hard sells is mainly constructed of protein, inorganic salts, chitin and fat. So extracting chitin and chitosan require step by step chemical filtration methods.

Oligosaccharide chitosan occur by [chemical methods] acid hydraulis or alkalify [physical method] high temperature [enzyme method] chitosanas, chitinas, lysozyme, cellulase, pectinase, pepsine, papaein.

This mater happens in diverse situation of temperature [80 to 140 'c] and time up to 10 hours by using heavy sodium hydride and potassium solutions [30 to 60 percent]. Density of alkali and time and process temperature must be controlled strictly because of its affect on deacetylation degree, molecular degree and molecular weight distribution plus deacetylation units distribution along the poly saccharide chain.

At first stage, chitin production depends on food industry cancer cannery. At second stage, producing chitosan was dependent on formentation proses like making citric acid of aspergillus niger and mucor rouxii and Streptomyces that is produced with chitosan alkali care. Briefly hard shells crust and fungus cell walls by solubilizing in acid hydrochloric and steering in certain temperature and under controlled, become mineralization and decalcify. Detergent protein of the left over material happen with care and solubilize in sodium hydride water buffer and this way the pollution of produced chitin with protein was stopped. The resultant chitin deacetylated in 40 - 45% sodium hydroxide at 120 'C for 1 to 3 hours in non oxygen terms and followed by purification process and chitosan by normal form of cationic was in hand.

Alkali protein delete and chitin deacetylation happens at the same time. By using different alkali density, some glycan get deleted from work surface.

In deacetylation proses, some acetyl groups delete from chitin molecule. This draw of chitin molecule eventually give remaining polymer with hole amino groups named chitosan. This care produce chitosan with 70 per cent deacetylation. (Kumar 2000; Khan, 2001; Krajewska,2004; Kim and Rajapakse, 2005).

Basic methods based on alkali cares for reaching N-deacetylation were used because deleting N-acetyl groups with acidic reactors is not possible like alkali care. Any way parts of deacetylation runs under hard alkali care terms. (Muzzarelli, 1973)

Based on kumar 2000 studies, to produce 1kg deacetylation chitosan 70 per cent from shrimp crust, 6.3 kg hydrochloric acid and 1.8 kg sodium hydride an also 1.4 tones water and nitrogen is needed. Commercial and usable form of chitosan is in shape of small chunks, solutions and fine powders. (Duarte et al., 2002; Sinha et al, 2004)

Chitin hydraulise with concentrated acids in chronic terms produce pure D-glucosamine. [kumar 2000]

In India, Carla fishery technology center, researches about chitin and chitosan been started. They have learned in the researches that shrimp and squilla dried residuum have by order 23 and 13 per cent chitin.right now chitin and chitosan are commercially produced in India, Japan, Austria, Norway and Australia. [kumar 2000]

In future by biotechnology innovation the possibility of chitin and chitosan sources appear, exist, specially when the focus is on medical usage. [Khor and Lim 2003]

Therefore the produce and use of chitin and chitosan form an interesting economic activity when cancer shell residuum get widely available in the world.

Mahmoud and co workers [2007] organic acids [lactic and aseteak] got from cheese water fermentation is used for inorganic detergent, protein detergent shrimp shell with microbe method. Acid kind affects, inorganic detergent terms, time and shells and acid percentage were study by them, the results show that the use affect of acid lactic or aseteak for shrimp shell inorganic detergent is comparable with using hydrochloric acid [8]. [Shahidi and Synowiecki 1991] separated and measured a cancer and shrimp kind secondary product. In this deliberation the chitin percentage out coming from cancer and shrimp shells based on dried weight between 17 and 32.2 per cent and the amount of produced carotenoid pigments between 3.4 to 14.7 mg in 100 g of shells. Chitin and chitosan get efficiency by order was 86 and 74 per cent. [9] Khanfari and co workers [2008] collected chitin and chitosan from semisulcatus Penaeus shrimp clutches from a shrimp center in Persian gulf in south Iran by chemical and microbe methods was extracted.

Chitin and chitosan were extracted by acid-alkali care and the outputs by order were 510 and 410 mg/g. the chemical extract method included use of sodium hydride solution and aseteac acid. [10] Al Saghir and co workers [2009], extracted chitin in form of β and α from some kinds of Persian gulf hard shells. In this study different external skeleton contents and inorganic salt percentage [including different elements], was defined as protein and chitin. Chitin different product Deacetylation used to be done by traditional thermal heating and microwave. The got chitin and chitosan were studied by elemental analyze, fourier transform IR specteroscopy [FTIR] and NMR, X-ray powder diffractometry [XRD] and elemental weight measure [11]. In another study done by Iranian researchers, Tajik and co workers [2008] declared that chitosan has a stronger antibacterial affect on G+ bacteria than G- bacteria [2].

Transforming chitin to chitosan is done by two chemical and enzyme methods and the results of them been observed.

In biological method for protein detergent proteolytic bacillus subtilis bacteria and used for acetyl detergent from aspergillus niger fungus. Researchers words chitin and chitosan quality got from biological production compared with chemical method product is a lot better. The main aim of replacing biological method [enzyme] with chemical method is to increase product quality and delete sodium hydride in the production proses.

Chitosan History

Chitin, chitosan preformation, for the first time was got by a French professor in 1811 from a fungus kind colony named Henri Braconnot.

Chitosan history and time return to two centuries before it got discovered in 1850 and chitosan deacetylation form time in 1859. (Hennen, 1996; Dodane and Vilivalam, 1998; Kim and Rajapakse, 2005).

Researches about chitin and chitosan uses grew in 1930 and about 1940, but the development of synthetic fibers, is similar to synthetic drug growth on natural product, out grew them. The benefits of natural product like chitin and chitosan were beneficial life renewal and after 1970 their use grew up (Hennen,. 1996; Kim and Rajapakse, 2005).

Therefor in mid 1960s notable researches on chitin an chitosan in Asia been performed. Japan has without debate leading in this matter, but other counties of Asia like Corea, Singapore, Taiwan and Thailand have notable share too. Lately china has joined this group and a main development and increase in research sources on chitin and chitosan been done. At the time the focus was on better understanding of this materials and also reports had full coverage on products and better purity of chemical derivatives and thousands other usages. (Khor and Lim, 2003).

Studies

Rodricos and ramus [2003] studied chitosan anti fungus feature when bio polymers were used as a food layer in food making, this action was against fungus destruction that was considered as main reason in food corruption. It was observed that using chitosan in acid aseteac as food cover (m2 8.6 g.) stop the growth of Alternaria, penicillium and cladosporium kinds. This operation is similar to preservative feature of calcium propionate [0.15 per cent wt] and potassium sorbate [0.05 wt]. but chitosan showed lower sensibility on aspergillus kind. Covering fruit surface like strawberry and grapes with chitosan caused decrease in glotomat , from chitin derivatives in laboratory and apple juice environment against 15 barmy and food mould been studied.

Grondin and Lehoux studied the affect of chitosan on liver, based on the results adding 5 per cent chitosan to rats food decreased plasma cholesterol to 54 per cent and liver cholesterol to 64 per cent. Also chitosan [in higher amounts has bigger affect on plasma cholesterol] and co workers done a study about the affects of chitosan in blockage of hyper cholesterol and arteriosclerosis in affected rats. Blood cholesterol at the end lack of apo lipo protein at the time in group E 64 per cent decrease than the other group. Arteriosclerosis plates production shoed decrease in the group from 42 per cent till 50 per cent[16].

Gallaher and co workers studied the supplementary affect of fiber glucomonan and chitosan on cholesterol plasma decrease and cholesterol ejectment increase in heavy weight people with normal cholesterol plasma level. Results and blood cholesterol showed LDL and HDL lower than first stage yet the Triglyceride serum level was without change [17]. For that it seems that considered drugs like persica and chitosan and realizing their affects is an affective step for discovering drug cure for Hyprklstrvklmyk patients [17,12].

Most accepted mechanisms showed that oligosaccharide chitosan by abatement molecule cell wall biological function prevent articles to enter the cells and eventually destroy it for lack of food articles [10,12]. This mechanism was offered by Kim and Seyfarth and co workers study in 2005 [19,15].

S.Roller an co workers in 1992 chitosan glutamate affect on 15 food corruption microorganism, from the 7 fungus kind being study with chitosan with agar level 10g density per litter, three kinds (A.) flavus, (C.cladosporioiedes) and P. aurantigriseum showed no certain sensibility but against chitosan glotamat 5 and 10s per litter and 25 'C prevented (M.rasmoses) and three kinds of Byssochlamys from growing.

In a study the affect of chitosan glutomate [5 and 10g per litter], acid glutamic [0,42g per litter] in [18 'C and 25 'C] in two PH on mould showed that acid glutamic alone can actuate the growth [16]. But the anti microbe feature in lower temperature and PH and more density of chitosan showed a better affect in growth decrease up to 75 per cent[18]. Shahidi and co workers in 1999 anti microbe affect of water chitosan solution and chitosan film on different kinds like bacillus cereus, Proteus vulgaris and Escherichia coli showed that eatable film chitosan is as anti bacterial factor for better quality and long preservation ability for food articles.

In here some of studies about chitosan and its affect on food industry will be implied;

Roller and Covill in 2000 showed that 3g per litter chitosan glutamate in mayonnaise sauce and by being preserved in Salmonella enteritidis 5

Will prevent microorganism growth like Zygosaccharomyces bailli , although the anti microbe feature of the mentioned article in 25 'C was reported weaker. The result of their work was that chitosan glutamate can be used as natural preservative for mayonnaise in fridge temperature [16]. Banjakol and co workers [2000] studied the affect of chitosan on a type of jell got from a kind of fish and they come out with that chitosan can be used instead of starch for more consistency and firmness jell [3]. Cammil and co workers [2000] studied the anti oxidant affect of chitosan over the cooked meet of a fish kind and compare it with anti oxidant articles and reported a suitable anti oxidant ability of chitosan to BHT 17 and BHA 18, they come out with that chitosan can be used as natural anti oxidant in fat food [22]. Sung and co workers [2001] blended chitosan and lysozyme by Maillard reaction, as affect of this reaction chitosan emulsion affect and emulsion consistency produced by it increased also anti microbe affect was increased. At the end researchers discovered that chitosan-lysozyme blend can be used as anti bacterial and amulsifire article [19]. Babiker [2002] by blending chitosan and peptides coming from gluten analyzation by maillard reaction, increased chitosan solubility in acidic and androgyny environments, and caused increase in chitosan emulsion and anti microbe features.

Usages

Because of different chemical structure, features and functions till now 3000 different inventions of chitin and chitosan and derivations is recorded [3,1]. Because of chitosan unique features its use in different industries like biology technology[6], medical and medicinal[7], wastewater improvement[8], ornamental[9] and food industry[10]. Chitosan films, fibers, micro ingredient and ato ingredient considered by fiber engineering to be useful in, medicinal transfer, vaccination and agriculture DNA transfer, vegetable productions, water filtration, forgery, heavy metal filtration, animal feeding and loom [11,5]. chitin universal need assessment is 150000 tonne per year when practical production amount of it is 3000 tonne [5]. Disability of more production is because of limited sources [2,4], accessibility and seasonal hard shells fishing situation [4,2]. Right now 45 companies in the world work on producing and trading it [3], universal trade of it is million dollar trade and Japan is the biggest consumer and America is the biggest producer [4], considering the importance and use of these two natural copolymer finding more sources and modern methods of exploitation is under consideration by many countries [7].

For now time, chitosan is noticed for medical industry and food industry for its anti-bacteria [12,13], anti-tumor [14], hypo cholestromyc function [15] features. Now days chitosan anti bacteria and anti fungus feature against kinds of bacteria and fungus is recorded [16,20]. Naturally, because of chitosan low solution in water and acid solubility, have limitation in use, and in recent years studies about producing water soluble chitosan with anti-bacteria features been performed. Hydroxy ethyl acrylate chitosan, ethylamine hydroxy ethyl chitosan, carboxymethyl chitosan, hydroxy propyl chitosan is used but studies about water soluble chitosan production is continued [21,24]. About this mater using hydrogen peroxide, showed positive results.

Microbe growth on the surfaces is one of food corruption main reason. Now days many progresses in usable polymer article in food packing has occurred.

Growing demand of consumer for better quality and food freshness caused progress and use of chow films. Chow films in chow coverage in food packing, are like an attached net that can be made of proteins, poly saccharides and lipids. Some of them contain anti microbe articles therefore increase food preserving period. [seragosa and Dycson 1992]. This type of films, the minute they attach food surfaces stop microbe growth. Among different materials, biopolymers have high importance. Using chow films out of articles like chitosan helps to preserve product quality, adding sensitive features, improving product safety and increasing lasting period of ready to use food products [4]. Chitosan ability for making film without any added articles, has the ability of being used for packing and coverage. Chitosan film has lower oxygen and carbon dioxide penetrance than poly ethylene films and has good mechanical features that are measurable with many commercial semi strong polymers [5,6,7].

Chitosan use in japan in 1983 and in corea in 1995 at some food articles was authorized [6,24].

Chitosan biologic activities such as anti tumor affects, blood cholesterol decrease factor and anti bacterial affects are known [4]. Chitosan is non digestive therefore is non absorbance, therefore has no nutrition value and like fat sponge connects to fats up to 4 or 5 times of it size, stops fat absorption [5]. Different studies showed hypocholesterolemic affects of it in rats [6,10]

Tissue engineering had interests of many specialists in engineering and medical sciences for being a science of developing biological adaptable articles and biologic destroyable articles with improving natural tissue function point of view. The main goal of tissue engineering is to use suitable biological replacement for injured tissue recovery and natural activity revival [3]. Mean wile, Nano science and technology appearance and development caused affective methods for making three dimensional scaffolds with nanocomposite basis, bio destructive polymers/bio adaptable to be made. The reason for using scaffold is to create environment convenient and similar to Exteracellular matrix [ECM] that because of it physical support and chemical actuation needed for cell growth is accreted.

First step in designing useful scaffolds in neuro tissue engineering, is choosing a right combination. For this matter wide range of natural and synthetic polymers can be used. Because of amino groups in chitosan, this polymer has unique features that include biological capability, non toxic and adaptable with cells and tissues. Nonetheless for some physical inconvenient features of this polymer including elasticity and elasticity [tensility], using chitosan in some tissue engineering aspects and bio medical uses is limited [4]. For this reason researchers thought about using a combination of chitosan and a synthetic polymer for making nanofibers with optimized features.

Nanofiber structure chitosan poly vinyl alcohol [CS/PVA] because of convenient mechanic features and eligible bio adaptability are considered as suitable combination for nanocomposite production [9,7]. Till today polymer combination [CS/PVA] with crosslinking is used in different studies like checking monkeys liver cellular duplication amount [VERO] [12], fibroblast cells [PC] [7], skin and ulcerous recovery [13,14].

Polyvinyl alcohol [PVA] for being non toxic and non carcinogenic is used to blend with chitosan.

Chitosan oligo saccharide [COS] [low molecular weight chitosan] has diverse biologic activities like anti fungus, anti bacterial, anti carcinogenic and high protective activities. Different chitosan oligo saccharide features such, deacetylation degree, charge spread and its chemical aspect cause high use of it. Unlike high weight chitosan, chitosan oligo saccharide easily enters blood circulation through intestine and cause biological affects on organs. Also in food industry have high affect on anti microbe factors, anti oxidant and food quality increasers. Different factors like barmy, fungus and spore bacteria and non spore bacteria cause food quality corruption [13,14]. Now days using anti microbe preservatives including chitosan for food preservation is recommended.

Now days cultivable scaffolds is used in curing many diseases related to central nervous system [CNS] including Parkinson's disease, Alzheimer's disease and Traumatic Brain Injury [TBI] [1]. Common cure for this diseases is, using healthy tissue around injured tissue for recovery [2,3] but because of their side effects among disease spread because of after surgery infections, many researchers thought of using neuro tissue engineering science to produce convenient bio replacements. With the high progress in making bio adaptable scaffolds to different kind cells of different tissues duplication, yet making scaffold that can have all natural Extracellular matrix [ECM] qualifications artificially, is facing a lot of problems.

Bio alicitors; alicitors are combinations with biologic and non biologic sources that by projecting defensive answers cause bio synthesis and secondary metabolites accumulation [6], bio alicitors include poly saccharides, proteins, glycol protein or fungus cell wall pieces, plants, cellulose, pectyne and micro organism, chitin and gluecannes [21]. Bio alicitors might have certain combinations such chitin and chitosan or like fungus and barmy extract a combination of bio blends. In general alicitor use in plants metabolite bio technology studies follows two main goals,

1. Information about bio synthesis paths that cause secondary metabolite formation and arrangement
2. Increase in secondary metabolites commercial functions

Different methods are used in secondary metabolite production plant cell cultivation that include the use of alicitors, bases increase, cultivation environment optimization, munin roots planting and metabolite engineering. Using alicitores is of important methods for secondary metabolite production. Alicitores are chemicals or different bio factors that can suggest (phytoalexin) as physiologic changes and phitoalexin gathering [6]. Bio alicitores atr used efficiently in secondary metabolites production increase in many medicinal plants plantation.

In the meantime, fungus alicitores widely used for secondary metabolite production in medicinal plants plantation and are efficient in many secondary metabolites production stimulation like Trepenoid, Cumarin derivatives, Alkaloid, Flavonoids [23,24]. Blend similar to chitin without acetyl structures is of main blends in many fungus cell walls.

Chitosan is a poly catyon poly saccharide, as an efficient bio alcitore for improving secondary metabolite production in medicinal plants plantation [25].

Among bio alicitores, fungus alicitores and many fungus kinds cell wall main admixture such chitin and chitosan widely used for secondary metabolite production in plants plantation [32,33].

Also, biggest amount of Lignans is seen in chitin and chitosan density 100 per mgl [36].

Fungus such Ustilaginodia vernes and Aspergillus niger increases catarantine production. In fungus cell walls there is many alicitore blends. Proteins, oligo saccharides, golicoproteins, peptides, chitin and chitosan are among this blends [42]. It seems that this blends have a role in secondary metabolite induction. Unique and diverse affects of fungus alicitores is suitable to plants cells so every plant cell response is matched with every fungus [43].

A matter is dealt with in health centers and hospitals is, increase is bacteria anti biotical resistance [25]. Been diagnosed that right terms for infection growth in hospitalized patients is available in way that an infection of different parts like surgery, burnt section, birth section can cause big infections in this places [26]. Now days finding new anti microbe articles because of microbe resistance change for pathogen microbes is under study [27].