UNIT 7 CHITIN, CHITOSAN AND GLUCOSAMINE

Structure

7.0 OBJECTIVES

After reading this unit, you will be able to:

- identify the sources of chitin;
- describe the production of chitin and related products;
- summarise the different properties of chitin and chitosan; and
- discuss the uses of chitin and related products.

7.1 INTRODUCTION

Chitin is a naturally occurring carbohydrate containing nitrogen and distributed in arthropods, bacteria and fungi. In abundance, it is second to cellulose among natural polymers. It was first isolated by Braconnot in 1811 from mushroom and was named fungine. In 1859, chitosan, the deacetylated chitin was discovered by Raughet. Chitin is a homopolysaccharide of N acetyl D glucosamine residues (Fig.7.1) joined by β (1-4) glycosidic linkages. It differs from cellulose due to a substituent at the C_2 carbon, which is an acetylated amino group in place of hydroxyl group. It occurs in three polymorphic forms *viz.* α, β and γ chitin, which differ in the arrangement of their molecular chain. α, chitin is the most crystalline polymorphic form. It exists in living organism in the form of microfibrils. Deacetylation of chitin gives chitosan, a high molecular weight linear polymer of amino D glucose.

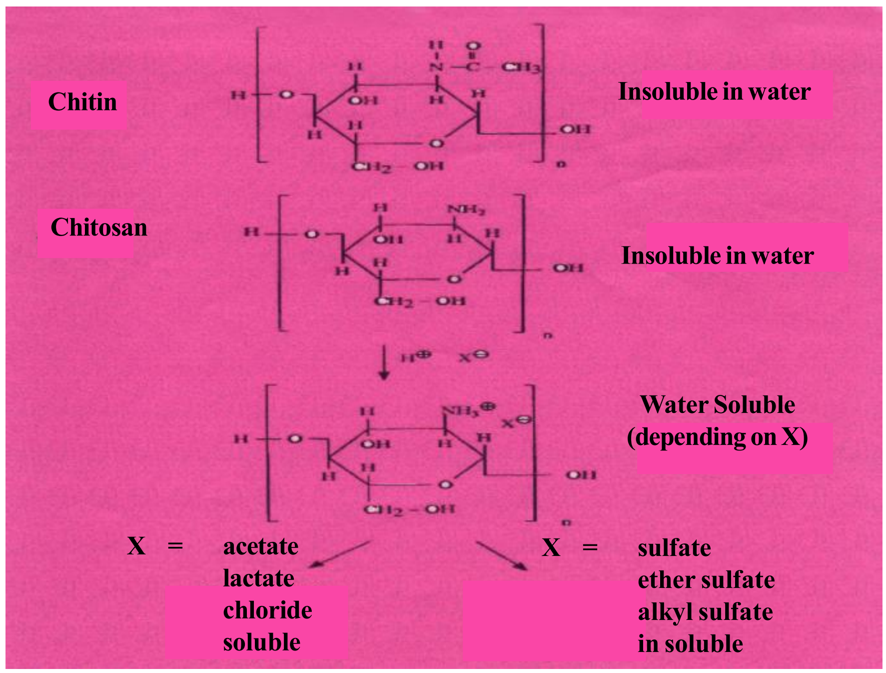

Fig. 7.1: Structure of chitin and chitosan

7.2 SOURCES OF CHITIN

The major sources of chitin are the shell of prawn (Fig.7.2), crab (Fig.7.4), Antartic krill, squilla, insects, clams, oyster, squid pen (Fig.7.3) and fungi. The shell waste thrown out from shrimp processing plants are the major raw material for the extraction of chitin. At present, prawn shells and crab shells are used for the production of chitin and chitosan in industrial level. Proximate composition of prawn shells, crabs shells and squilla are shown in Table 7. 1.

Fig. 7.2: Prawn shell

Fig. 7.3: Squid pen

Fig. 7.4: Crab shells

Table 7 .1: Proximate Composition of Prawn Shell, Crab Shell and Squilla

Parameters	Prawn Shell	Crab Shell	Squilla
Moisture	75-80%	70%	75-80%
Ash (dry basis)	30-35%	45-50%	33-37%
Protein (dry basis)	35-40%	30-35%	40-45%
Chitin (dry basis)	15-20%	13-15%	12-16%
Fat (dry basis)	3-5%	1.0-1.5%	2-3%

Chitin represents 14-27% and 13-15% of the dry weight of shrimp and crab shell waste, respectively. Research findings have proved that dry prawn waste contained 23% and dry squilla contained 15% chitin. Annual availability of shrimp shell (wet basis) in India is 1,00,000-1,25,000 tonnes which is sufficient to make 5,000 tonnes of chitin. Presently, the commercial source of chitin is the shrimp and crab waste from fish processing plants and squilla caught during trawling for shrimp. Though, clam and oyster shell contain chitin they may not be processed for this polymer. Very high quality chitin can be obtained from squid, cuttle fish and diatoms in very small quantities.

7.3 PRODUCTION OF CHITIN/CHITOSAN

Chitin is present in its sources as chitin protein complex along with minerals mainly calcium carbonate. The process for chitin production comprises deproteinisation using dilute alkali or proteolytic enzymes and demineralization using dilute acids. The deacetylation of chitin to produce chitosan is effected by treatment with concentrated alkali (Madhavan and Ramachandran, 1974).

Chitin and chitosan (Fig.7.5 a & b) are now produced commercially in Japan, USA, India, Poland, Norway and Australia. India produces about 1,000 tonnes of chitin/ chitosan per year.

A schematic representation of chitin/chitosan production is given below:

Prawn or Crab Shell
↓
Treat with HCl (dilute)
(Demineralisation)
↓
Washing with water
↓
Boil in Sodium hydroxide (dilute NaOH)
(de-proteinization)
↓
Washing with water
↓
Drying
↓
Chitin
↓
Treat with strong alkali
(de-acetylation)
↓
Repeated washing with water
↓
Drying
↓
Chitosan
↓
Milling
↓
Packed in bags

Fig. 7.5(a) : Mass production of Chitin and chitosan

Fig. 7.5 (b) : Chitin and chitosan

Check Your Progress 1

Note: a) Use the space given below for your answers.

b) Check your answers with those given at the end of the unit.

1) Who is the inventor of chitosan?

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2) Chitin was first isolated by ....

3) Name some of the major sources of chitin.

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....

7.4 PROPERTIES OF CHITIN/CHITOSAN

Chitosan is highly hydrophobic (not able to absorb water) and is insoluble in water and most organic solvents. It is soluble in hexafluro isoproponol, hexafluro acetone, and chloro alcohols in conjuction with aqueous solutions of mineral acids and dimethyl acetamide containing 5% lithium chloride. On hydrolysis of chitin with concentrated acids, under drastic conditions, gives pure amino sugar, D glucosamine. Chitin on deacetylation with strong alkali yields the monomer, chitosan. Chitosan is insoluble in water but soluble in dilute acids. Pure chitosan is not hydrolysed by lysozyme while chitin or partially deacetylated chitin is hydrolysed.

7.4.1 Important Derivatives of Chitin and Chitosan

The following are the derivatives of chitin and chitosan (Fig. 7.6):

1) Glucosamine hydrochloride (Fig.7.7)
2) Carboxy methyl chitin
3) Carboxy methyl chitosan
4) Chitosan Sulphate
5) Chitosan chloride

Fig.7.6: Chitin and chitosan derivatives

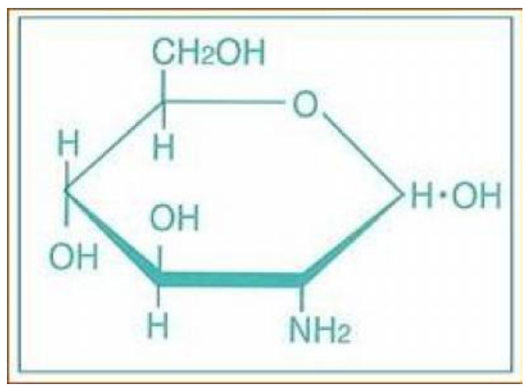

Fig. 7.7: Structure of Glucosamine

The quality parameters of chitosan are given in Table 7.2.

Table 7.2: The Quality Parameters of Chitosan

Sl. No.	Properties	Requirements	Methods of Determination
1.	Appearance	Loose powder with grains of various sizes and colour ranging from light grayish cream or pale pink.	Visual
2.	Grain size /mesh size	Based on the use	Sieving
3.	Clarity of chitosan	Clear to light oplacent	Visual examination of solution (1% chitosan and in 1% acetic acid)
4.	Moisture content	Not greater than 10%	AOAC methods
5.	Ashes	Not more than 1.0%	AOAC method

6.	Degree of acetylation	Greater than 55% (based on application)	Distillation method or spectro-photometric method.
7.	Toxic metal content (Maximum) Hg (Mercury) Cu (Copper) Zn (Zinc) Pb (Lead) Cd (Cadmium)	 0.1ppm 50 ppm 50.0 ppm 5.0 ppm 0.10 ppm	 Atomic absorption spectrophotometer
8.	Viscosity (1% solution of 1% acetic acid)	Very low	Viscosity of standard solution (1% chitosan in 1% HCA measured with Brook field viscometer Sprindle:30, revolutionary

7.4.2 Cationic Properties of Chitosan

Uses of chitosan depend upon its cationic (negatively charged) nature. Some of them are as follows:

- Linear poly electrotyte
- High charge density
- Excellent flocculent
- Adheres to negatively charged surfaces
- Substantive to hair, skin
- Chelates metal ions - Fe, Cu, Toxic metals (cd, Hg, Pb, Cr, Ni)
- Radonucleides (Pu, u)

Chitosan is a linear polyelectrolyte at acidic pH. It has a high charge density, one charge per glucosamine unit. Since many materials carry negative charges, e.g. Protein, anionic polysaccharides, nucleic acids etc., positive charges of chitosan interact strongly with negative surfaces to give electric neutrality. Chitosan is an excellent flocculent due to its vast numbers of NH_3 (ammonia) groups that can interact with negatively charged colloids. Chitosan adheres readily to natural polymers such as hair and skins which are composed of negatively charged mucopolysaccharides and proteins.

7.4.3 Biological Properties

You will be surprised to know that chitosan has immense uses in pharmaceutical field. Many of chitosan's bio-medical application depend on its non-toxic and biodegradable properties.

Chitosan has been shown to facilitate wound healing and reduce serum cholesterol levels, and stimulate the immune system.

Biological properties of chitosan are given below:

1) Biocompatible

- Non-toxic

- Biodegradable
- Natural polymer

2) **Bioactivity**

- Wound healing accelerator
- Reduce blood cholesterol levels
- Immune system stimulant

7.4.4 Chemical Properties

As chitosan is a high molecular weight polymer, it is a linear polyamine whose amino groups are readily available for chemical reactions and salt formation with acids. Since it can be viewed as a cellulose derivative, the primary (C6) and secondary (C3) hydroxyl groups can be used to make derivatives.

Chitosan is a versatile biomaterial available in nature. Large varieties and forms are available for chitosan in different shapes and size. Following description gives the different forms of chitosan:

It is available in powder, films, fibre, shaped objects, gel beads, paste/lotions and solutions. Several other derivatives can be prepared from chitin by appropriate reagents; with alkali chitin, chitin swollen further by dimethyl sulfoxide, hydroxy ethyl, and carboxy methyl and ethyl chitin. They are all known to be enzymatically degradable by lysozyme. Chitosan nitrate and chitosan sulphate can also be prepared by nitration and sulphation of chitosan.

Check Your Progress 2

Note: a) Use the space given below for your answers.

b) Check your answers with those given at the end of the unit.

1) What is the appearance of chitosan?

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2) Give two cationic properties of chitosan?

....

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3) Give two biomedical uses of chitosan?

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7.5 APPLICATIONS OF CHITIN

The application of chitin and chitosan can be classified in the following heads:

- Clarification and purification of water and beverages
- Chromatography
- Paper and textiles

- Photography
- Food and Nutrition
- Agriculture
- Pharmaceutical preparation
- Biodegradable membranes and Biotechnology
- Cosmetics

7.5.1 Clarification and Purification of Water and Beverages

The property of long chain molecules dissolved in chitosan helps it to wrap the solid particles suspended in liquids and to bring them together making it suitable on a coagulant aid. It can remove heavy metals, vegetable matter, proteins and suspended solids from vegetable processing waste and activated sludge and clarify beverages. The largest single use of chitosan at present is in the clarification of wastewater. The non-food related clarification and purification are in the treatment of sewage effluents, metal finishing, electroplating waste, paper mill and radioactive waste. The important food related applications are in purifying drinking water, recovering protein for animal feeds, clarifying wastes from vegetables, poultry, egg and fish processing industries, fruit juices and recovery of micro algae.

According to the U.S. Environmental Protection Agency, chitosan is acceptable for potable water applications when used within the stated ratios, as a bed of chitosan is used on a filter medium. Chitosan is a better clarifying agent than potash alum for contaminated water.

7.5.2 Chromatography

Chitosan has been used in thin layer chromatography for separation of nucleic acids. The ability of chitin layer to separate mixtures of phenols, amino acids, nucleic acids or inorganic ions was almost equal to that of crystalline cellulose, silica gel and polyamide gel.

Novel composite materials like chitosan Cupric chloride ($Cucl_2$) dilute membranes were prepared and a method was developed for qualitative detection of ions by FTIR spectra using membrane.

7.5.3 Paper and Textiles

Chitosan improves the bursting strength, puncture resistance, waterproofness, tensile strength and water vapour transmission rate of Kraft paper. Chitin and chitosan can be used for production of fibres and films. Some researchers prepared a chitosan sodium dodecyl sulphate fibre composed of hydrophobic micro domains which can solubilise water insoluble dyes. This chitosan – SDS three dimensional network may be used in controlled release application. There are also reports of the preparation of chitosan based antimicrobial packaging films.

Because of its chelating ability, adhesive property and ionic bond forming characteristics, chitosan finds application in textiles. Fabrics sized with chitosan have good stiffness, improved dye uptake, added luster and improved laundering resistance. Sizing with chitosan is permanent and coating formed is insoluble in water.

7.5.4 Photography

Due to its optical characteristics, film forming ability and reactions with silver complexes, chitosan finds applications in photography. Silver complexes are not appreciably retained by chitosan and therefore can easily penetrate from one layer to another by diffusion.

7.5.5 Food and Nutrition

The presence of chitin in marine invertebrates, insects, fungi, yeast and cell walls of certain plants and of chitosan in various fungi indicates that chitin and chitosan are already part of our food supply. Toxicity studies showed that LD_{50} value of chitosan is greater than 16g/kg body weight and so it is as safe as salt or sugar. Chitin is used as an ingredient in domestic animal feeds and develops no abnormal symptoms in animals fed on chitin containing feeds. It is a good growth promoter in broiler chicken, pigs and rabbits. Chitosan is a thickening and stabilizing agent in foods and enhances the storage life of meat. Emulsifying properties of chitin, its potential as a non-absorbable carrier food dye and as a protein coagulant and to recover protein from food processing waste were reported by many researchers.

Chitosan has medicinal properties (Fig.7.8 and 7.9). Chitosan is a hypolipidemic and hypocholesterolemic agent (ability to reduce lipid and cholesterol levels). Serum cholesterol, triglycerides and free fatty acids decreased in domestic animals when fed on high fat containing 1.2 to 1.4g chitin/kg of body weight. Glucosamine is ineffective where as chitosan is effective in the hypocholesterolemic action, showing that certain degree of polymerization is required for lowering cholesterol level.

Fig.7.8: Chitosan powder

Fig. 7.9: Chitosan capsules

7.5.6 Agriculture

Chitin and derivatives control nematodes during germination and culture of seeds. It enhances protection against pathogenic organism in plants and suppress them in soil and induce chitinase activity, it finds application to encapsulate fertilizers and for controlled release of herbicides. Chitinous materials are efficient to control parasitic nematodes in ornamental plants, cucumber and tomato. Coating with chitosan delayed ripening process in tomato, bell pepper and strawberry and maintained the quality attributes. Chitosan was found to stimulate the plant and improve the yields of fruits.

7.5.7 Medical and Pharmaceutical Uses

Chitin and derivatives can be employed as bacteriostatic agents, hypocholesterolemic agent, drug delivery vehicle, supermicide, enzyme immobilizer, membrane for dialysis and contact lens, surgical glove powder, hydrozel ointment base, anti-sore compositions, wound dressing treatment of stomatitis, prothetica mycotica and leg ulcers.

Chitin fibre (Fig.7.10) has all the characteristics of an ideal surgical suture such as biodegradability, resistance to alkali and digestive system softness, knot reliability, wound healing acceleration and tissue adaptation. Glucosamine (Fig.7.11) is expected to work as a novel anti-platelet agent for prevention and treatment of thrombotic disorders.

Fig.7.10: Chitin coated sutures

Fig.7.11:Glucosamine

7.5.8 Cosmetics and Personal Care Uses

Chitosan coated liposomes retains water at a higher rate than liposomes. The efficacy of Hydagen CMF, a 1% solution of special grade chitosan in 0.4% glycolic acid was evaluated in different formulae in both skin and hair care applications (Fig.7.12). It improved skin sensation and reduced irritation potential. Hydagen CMF film is homogenous and highly flexible and shows no cracks or scaling in the dry state.

Fig. 7.12: Cosmetic products with chitosan

7.6 CHITOSAN IN BIOTECHNOLOGY

A number of enzymes are immobilized with chitosan. It is entraped and absorbed in the chitosan. In Biochemistry, it is used as a support for enzymes. The most common method of fixing enzyme to chitosan is by cross-linking with dialdehydes like glycerol and glutraldehyde. A list of enzymes immobilized by using chitosan is given in Table 7.3.

Table 7.3: List of Enzyme Immobilized

Enzyme	**Proposed Use**
AMP diaminase	AMP to IMP
Amylase diatase and glucoanalase	Starch and glycogen to d glucose
β-D galactosidase	Hydrolysis of lactose
D glucose isomerase	D glucose to D fructose and preparation of D glucomic acid
β-D glucosidase	Hydrolysis of cellobiose
Lysozyme	Preparation of pharmaceuticals
Urease	Urea to ammonia and carbon dioxide
E. coli cells	Synthesis of L tryptophan.

7.7 CHITOSAN FIBRES, FILMS AND MEMBRANES

Chitosan films are clear, tough, flexible and good oxygen carriers. Chitosan based coatings can protect foods from fungal decay. Edible and biodegradable polymer films offer alternative environmental friendly package.

Membrane of chitin/chitosan is produced by casting their solutions either alone or with suitable polymer and other reagents to give desired properties. Albumin blended chitosan membrane can be used in haemodialysis, artificial skin and drug targeting. A classical polyelectrolyte compex is formed between the anionic sites of collagen and cationic sites of chitosan when chitosan interacts with collagen.

Since chitin yields pyrazines on thermal degradation, there is a potential for developing natural flavour extenders from chitin or chitin containing waste products, mushroom cuttings or yeast, cell walls and by-products of SCP production.

Increased knowledge on the properties of chitin led to the development of its various derivatives having many high value applications, some of which are in areas like medicine, agriculture and cosmetics.

? Check Your Progress 3

Note: a) Use the space given below for your answers.

b) Check your answers with those given at the end of the unit.

1) Name some of the applications of chitosan?

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....

2) Give one use of chitosan in agriculture?

....

....

3) Why chitin fibre is an ideal surgical suture?

....

....

4) Give the uses of chitosan films?

....

....

Activity 1

Visit a local drug store. Ask the pharmacist to give you a list of the drugs available in the shop wherein chitin/chitosan is used. Make a note of the uses of the drugs emphasizing the role of chitin / chitosan.

....

....

7.8 LET US SUM UP

Chitinous polymers are abundant waste products with an unused combination of properties. These products are already part of our food supply. However, there are limitations for their application. The application of chitin in broiler chicks as a feed additive, in surgical sutures, wound healing, encapsulation of medicine and cosmetics have already reached a stage of commercial exploitation. Some other areas with good prospects are in dentistry and biodegradable films are to be popularized. The wasted shell material which caused problems to the fish processing industry due to the environmental pollution has become raw materials for very valuable products with versatile application.

7.9 GLOSSARY

Abundant : Plenty.

Acetylated : Adding acetl group.

Agglomerate : Combined together.

Aqueous : Water.

Bacteriostatic : Related to bacteria.

Biodegradable : Capable of being decomposed by bacteria or other living organisms.

Cationic : Negatively charged.

Cellulose : A polymer present in nature.

Chelating : Organometalic compound containing a ligand which bonds to a central atomat two or more points.

Crystalline : Crystal shaped.

Deacetylation : Removal of acetyl group.

Demineralization : Removal of minerals.

Dialysis : Separate by means of dialysis.

Elicitors : Draw out, evoke.

Emulsifying : Convert into an emulsion.

Flocculent : Precipitate or loosely massed.

Glucosamine : Amino glucose.

Glutraldehye : A chemical.

Hypocholesterolemic : Reduces cholesterol.

Hypolipidemic : Reduces lipid.

Mucopolysaccharides : Compound of protein and carbohydrate.

Nematodes : Parasite or free living worm.

Pathogenic : An agent causes diseaes.

Platelet : A small colourless disc shaped cell fragment without a nucleus, found in large numbers in blood involved in clotting.

Proteolytic : Protein breaking.

Ripening : Matured and starts to ripen.

Scaling : Measuring.

Squilla : A prawn like organism.

Surgical Suture : Threads used for surgery.

7.10 SUGGESTED FURTHER READING

Madhavan, P. and Ramachandran, N.K.G.1974. Utilization of Prawn Waste. Isolation of chitin and it conversation to Chitosan. *Fish Technol*. **11**:50-56.

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7.12 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

1) Raughet was the inventor of chitosan.

2) Braconnot.

3) Major sources of chitin are prawn shell, crab shell, antarcic krill, squilla etc.

Check Your Progress 2

1) It looks a loose powder with varied grain sizes.

2) Excellent flocculent and chelates metal ions.

3) Wound healing accelerator, Reduce blood cholesterol levels.

Check Your Progress 3

1) Clarification, purification, paper and textiles, photography, food and nutrition, etc., are some of the applications of chitin and chitosan.

2) Chitin and derivatives control nematodes during germination and culture of seeds.

3) Chitin fibre has all the characteristics of an ideal surgical suture such as biodegradability, resistance to alkali and digestive system softness, knot reliability, wound healing acceleration and tissue adaptation.

4) Chitosan films are clear, tough, flexible and good oxygen carriers. Chitosan based coatings can protect foods from fungal decay.