

Chitin and its Derivatives

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Chitin is a natural polymer and secures the second position in terms of its abundance only next to cellulose. Cellulose accounts to 35-45% of biomass composition available on the earth. Chitin, a Greek word for ‘envelop’, was discovered in 1811 as a substance occurring in mushrooms. Chitin are synthesized by crustaceans, molluscs, insects, and fungi to the extent of about 100 billion tonnes every year. In spite of its greater abundance, among the natural polymer, chitin is the most underexploited one.

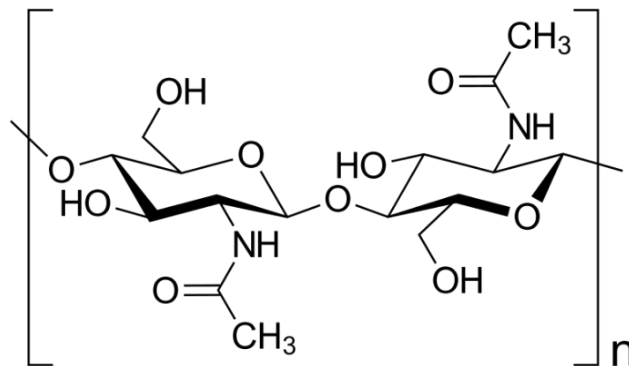


Fig. 1 Chemical structure of chitin

1.0 Biosynthesis pathway of chitin

The pathway of chitin synthesis has been confirmed in insects and fungi. It is a complex process with a number of sequential bioprocess which varies with the organisms. Chitin synthesis follows hexosamine pathway (HP). The steps involved along with the initial compounds, metabolic enzymes involved and final products are presented in Table 1 and the schematic pathway is presented in Figure 2 as presented by Hou et al. (2021). The chitin polymeric chains synthesized are extruded through the cell membrane into the extra-cellular space wherein the polymer chains assemble to form chitin nanofibrils.

Table 1. Sequence of reaction in hexosamine pathway involved in chitin synthesis

| Reaction steps | Initial compound (s) | Enzyme involved | Final product |
|-----------------------|---|--|------------------------------------|
| 1 | Sugars such as glucose, glycogen or trehalose | Phosphorylase kinase | Glucose-1-phosphate |
| 2 | Glucose-1-phosphate | Hexokinase | Glucose-6-phosphate |
| 3 | Glucose-6-phosphate | glucose-6-phosphate isomerase | fructose-6-phosphate |
| 4 | Fructose-6-phosphate | Glutamine fructose-6-phosphate amino transferase | Glucosamine 6-phosphate |
| 5 | Glucosamine 6-phosphate | Glucosamine-6-phosphate N-acetyl transferase | N-acetylglucosamine-6-phosphate. |
| 6 | N-acetyl- glucosamine-6-phosphate | Phosphoacetylglucosamine mutase | N-acetyl- glucosamine-1-phosphate. |
| 7 | N-acetylglucosamine-1-phosphate +UTP | UDP-N-acetylglucosamine pyrophosphorylase | UDP-N-acetylglucosamine |
| 8 | UDP-N-acetylglucosamine | Chitin synthase | Poly- N-acetylglucosamine |

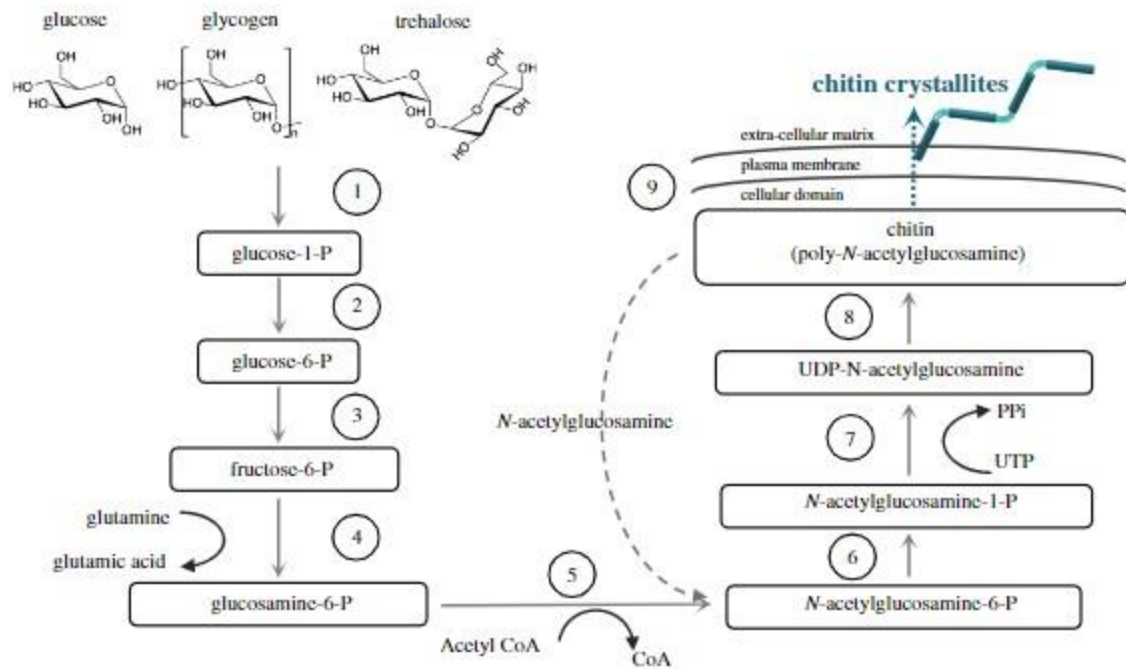


Fig. 2. Steps involved in biosynthesis path way of chitin in fungi and insects (Ref: Hou J,Aydemir BE,Dumanli AG. 2021)

2.0 Structure of chitin

Chitin: Chemically it is a linear aminopolysaccharide linked by glycosidic bond (β ,1-4 linkage). Chitin has got structural similarity with cellulose. The hydroxy group at position C2 of cellulose is replaced by an acetamido group. Based on the fibre/chain orientation, chitin is found in three different polymorphic forms namely α -chitin, β -chitin and γ -chitin.

- α -chitin (most common form) – Chains are parallel and adjacent polymer chains are always in the opposite direction. A strong network dominated by intrachain hydrogen bonds between the group off $C=O\dots NH$ and $C=O\dots OH$ within a distance of 0.47 nm. Additional Interchain hydrogen bonds bind the hydroxymethyl group.
- β -chitin - all chains are parallel and in the same direction. The network is strong and dominated by intrachain hydrogen bonds. No additional inter-chain hydrogen bonds found in this conformation.

- γ -chitin – Two adjacent chains are parallel, and unidirectional while third one is in opposite direction.

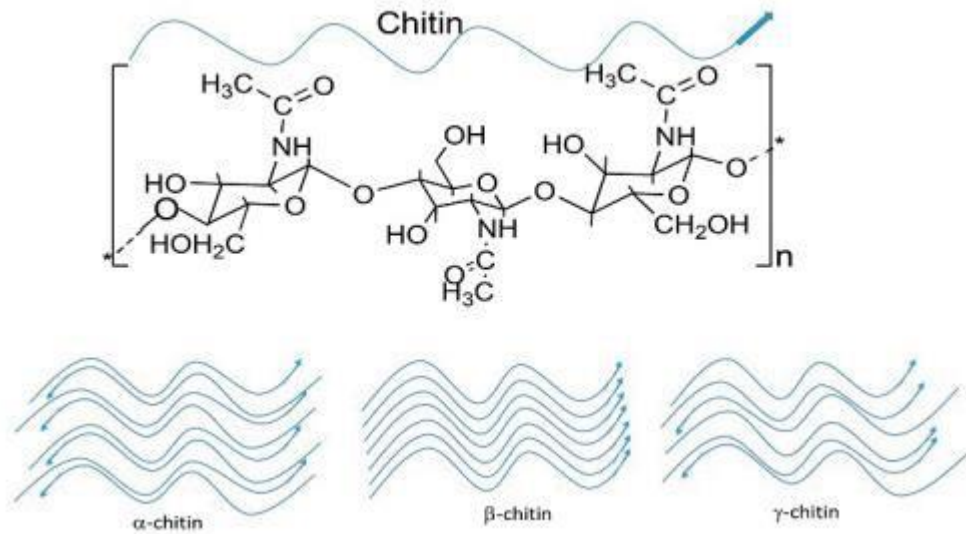


Fig. 3. Polymorphic forms of Chitin (Roy et al., 2017)

3.0 Major sources of chitin of aquatic origin

- Shrimp shell waste
- Crab shell waste
- Lobster shell waste
- Acetus
- Squid pen
- Cuttle bone

Shrimp processing shell waste serve as a major source of industrial chitin production in countries like India where the shrimp is major processed seafood for export market.

4.0 Composition of shell waste

The shell waste contains water, protein, minerals, chitin, lipids and other minor compounds including pigments. In terms of quantity, aforementioned components tend to vary due to various intrinsic and extrinsic factors. To generalize the composition, the following table can be considered

Table 2. Proximate composition of shell waste

| Constituents | Quantity |
|----------------|----------|
| Water | 65-70% |
| Protein | 9-12% |
| Fat | 0.5-3% |
| Minerals (Ash) | 8-13% |
| Chitin | 3-5% |

5.0 Shell structure – Inter linkage of the components

Shrimp shell is a multi-layered natural composite containing epicuticle, endocuticle and exocuticle. In between these three layers, there is a membranous layer. Crustacean shell established to have twisted plywood or Bouligand pattern. Chitin is found mainly in the inner layer of the skeleton, surrounded by a layer of protein. The middle layer consists of chitin and minerals, while the upper layer consists of proteins and minerals.

Epicuticle:

- Outermost layer which is thin and waxy
- Consists of long chain hydrocarbons, esters of fatty acids, and alcohols

Exo and endocuticle:

- Multilayered composite tissue
- Consisting mainly of chitin with various proteins
- Chitin and protein polymers are linked through covalent bond.
- Chitin-protein fibrils are biomineralized with calcium carbonate
- Spacing between the fibers is filled up with proteins and biominerals

Long-chain chitin molecules are bound into fibrils (3 nm in diameter and 300 nm in length) at the molecular level, which are always embedded in a matrix of proteins by covalent bonds, hydrogen bonds, and molecular interactions, to form fibers (about 60 nm in diameter) that further assemble in parallel into bundles to form horizontal planes.[22] These planes are stacked in a helicoid fashion to construct different layers.

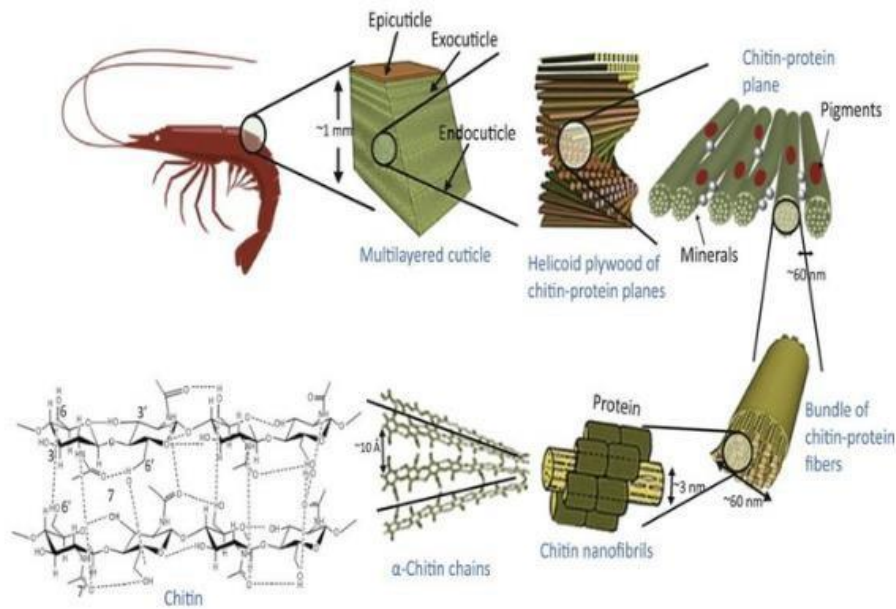
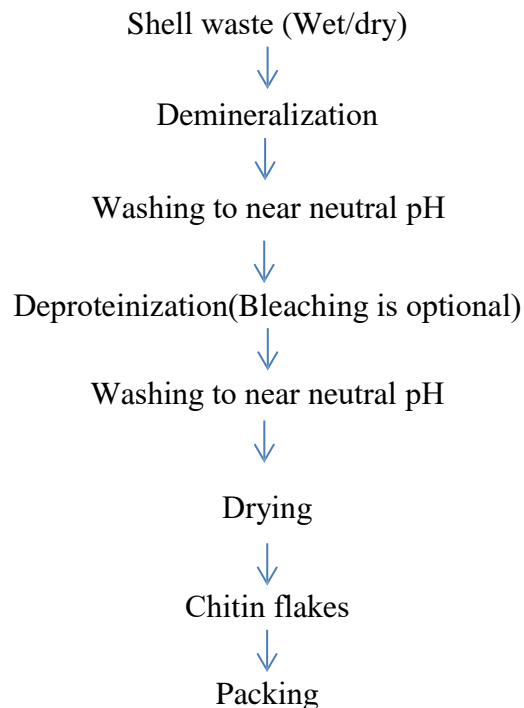


Fig.4 Shell structure and chemical composition (Adapted from Bradic, 2020)

6.0 Chitin process

The process for chitin production basically aims to eliminate other chemical constituents like proteins and minerals. For removing these constituents, conventionally chemical process is employed using diluted acid and alkali for demineralization and deproteinisation, respectively. The general process flow is presented in the flow diagram.



Major unit operations in the chitin process

1. Raw materials

The quality chitin is influenced by the type of raw material, size and part of the raw material, delay in processing or state of raw material, size of the particle (if it is ground) and any other pretreatment given to the raw material in order to preserve when it is abundant or under certain circumference for example drying the shrimp shell waste and later using it for chitin production.

2. Demineralization

Shell waste contains huge amount of minerals particularly as calcium carbonate. During deminarlization carbon-di oxide is liberated. In the commercial production of chitin, demineralization is practiced as first step as it softens the material and makes further operations like handling easier. The extent of demineralization is affected by type of acid, strength of acid, raw material to solvent ratio and duration of demineralization. Generally, hydrochloric acid is used in industries as it is relatively cheaper than other acids. However, depends on the properties and applications aimed, the aforementioned process variables can be modified. Normally this unit operation is performed without any heat processing at room temperature. The completion of demineralization is ensured by testing the formation of effervescence from few pieces of shells in diluted acid solution (HCl).

3. Deproteinisation

Deproteinisation from demineralized shells is carried out using diluted alkali. As mentioned in the demineralization, the strength of alkali, type of alkali, alkali to raw material ratio, duration of deproteinisation influence the extent of deproteinisation. Generally, sodium hydroxide is the most preferred and cost effective in deproteinisation. Both thermal and room temperature process can be employed. Heat assisted process is shorter than the cold process. However, the polymer quality is relatively better in room temperature process.

4. Washing

Use of water in chitin and chitosan production is enormous. In between the demineralization and deproteinisation process many number of washing cycle is required to achieve the near neutral pH. The requirement of water depends on the initial quality of water indirectly the source of water. More alkaline water like bore-well is required in high quantity. Seawater

also can be used for washing the demineralized and deproteinised shell. Washing can also be performed after neutralization. However, one should take care to remove the salt formed by giving wash using fresh water to keep the residual mineral in the chitin and chitosan low

5. Drying of chitin

Wet chitin i.e demineralized and deproteinised shell is subjected to drying under sun in open concrete drying yards or poly-house tent dryer. In 5-6 h the drying is completed. However, other drying methods can be employed. Compare to all the drying methods, open sun drying improves the color of chitin because photo degradation of pigments present in the wet chitin. Drying under sun required large area of land. Hence, alternative effective drying technologies with lesser space occupation need to be developed.

7.0 Chitin derivatives

Chitin is an intermediate product used for producing many derivatives having wide applications. Among them, glucosamine hydrochloride is the most demanded one because of its use in arthritic supplement formulations. Second most important derivative which has received greater attention is chitosan. Similarly there are salt derivatives like chitosan sulphate, chitosan lactate, chitosan-HCl, Chitosan acetate etc. Water soluble chitosan is an another important derivative has received attention. As there are functional group in the structure of chitin like hydroxyl, aminoacetyl as well as free amino group in chitosan, many number of derivetives can be manufactured through various chemical reactions.

Other products like chitosan sponges, chitosan hydrogel, electrospun nanofibres are all receiving interest for their medical uses. Recently chitooligosaccharides is an another derivative of chitosan produced through chemical hydrolysis or using specific as well as non-specific enzymes has found uses as nutraceutical as well as for agricultural applications like immunostimulants

Suggested Readings:

- Hou J, Aydemir BE, Dumanli AG. 2021) Understanding the structural diversity of chitins as a versatile biomaterial. *Phil.Trans.R.Soc.A379: 20200331* (Doi:10.1098/rsta.2020.0331).
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