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Different Aspects of Chemical and Biochemical Methods for Chitin Production a Short Review

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Abstract

The importance of chitin and its derivatives has grown significantly over the last two decades due to its renewable and biodegradable, biocompatible source, and also because of the increase in the knowledge of its functionality in the technological and biomedical applications.The present short review the biopolymer chitin and its derivatives as versatile biomaterials for many applications such pharmaceutical, food, chemistry, agribusiness and medical area, also shows the main limitations of the research groups and the industry to work with this biomaterial. This short review tries to compare many aspects of the production with chemical and biochemical process using different bio catalyser to produce different characteristics of the chitin and its derivatives. In the final considerations, the chitin and its derivatives have a great potential to design new biomaterials for many applications, depends the different characteristics of the process such, chemicals, concentration, time, temperature, bio catalyser, that could affect in the structure of the material reflecting directly with different properties, reducing the costs of the production, less time and making this biopolymer more attractive for the industry.

Introduction

In addition to being the second most abundant natural polymer in nature, a renown has a great potential as a biomaterial in the area of biotechnology, because it is biocompatible, bioreactive and biodegradable [1]. Such characteristics, diverse applications in areas such as agriculture, food, environmental, and as two areas with greater focus: pharmaceutical and health [2,3]. Its structure consists of N-acetyl-d-glucosamine units with β- (1,4) bonds, having as main characteristic the insolubility in water and some organic acids [4]. Chitin belongs to the group of structural polysaccharides, together with cellulose, the second polymer being more abundant in the biosphere [5-6]. Due to its structural nature, a product release system was not found in any of the arthropod exoskeleton, in the structures of Molluscs [7], in the cell wall of fungi [7-8], protozoa and bacteria, egg shells of nematodes [8], the shrimp fishery residue being the most widely used source [9]. Throughout the decades of research and handling of this polymer, many methods of extraction have been developed, being the chemical method, most found in the literature, being also used in the means of production of industrial chitin. The USA, Japan, India, Canada, China, South Korea, Russia and Norway generally use the reject of crustacean fishing for production. The use of strong acids and bases in the chitin extraction process generates critical points to the process, such as: high cost of the materials involved, generation of chemical effluent and final product with low levels of purity [5,10]. Biological processes become more attractive because they have an affordable cost of production, do not generate high risk effluent (such as the chemical process) and high quality final product [1,10]. All the processes found in the literature are an objective: to obtain chitin by separating the proteins and minerals from the raw material used [2].

Chitin, besides having great biotechnological value, generates by-products (such as chitosan) that also have added value and even more relevant properties. In this paper we discuss the already known processes of obtaining chitin known and registered in the literature of 2010 up to the present moment: Chemical, enzymatic and biological processes relating the different methods of obtaining and with the objective to identify the particularities of each process

regarding the industrial viability and economically balancing them so that the reader concludes the best process for their research, also the possibility of executing quality improvements in these processes. We will also discuss the polymorphic structures of α- and β-chitin and the different methods of obtaining each, since different processes are required in each of them due to their structures, properties and reactivity. The main objective of this review is to be able to relate the different processes of obtaining chitin with the most suitable applications for the method, based on such relation in aspects such as degree of purity and economic applicability.

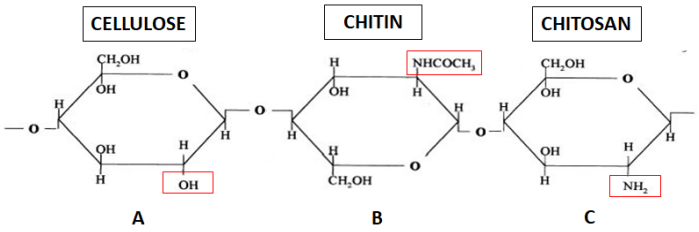
Chitin

Economic Aspect

The main industrial source used for the extraction of the biopolymer is the reject of the fishing industry, reusing mainly the shell of prawns, crabs and lobsters [11-12]. According to Ioelovich (2014), the production of chitin and its derivatives is estimated at 100 billion tons per year [13]. Gortari & Hours (2013) states that the bark discarded by the fishing trade can reach 70% of the total weight of the material [14]. According to Ameh et al. (2014), these carapaces have about 20-30% chitin, varying according to species and season, since chitin is naturally produced on a scale of 10^{10} to 10^{11} tons each year in a study conducted by Ifuku, et al. (2015) [5,15]. Jaganathan, et al. (2016) estimates that chitin costs $ 220 per kilogram on the world market [11].

Chemical Aspect

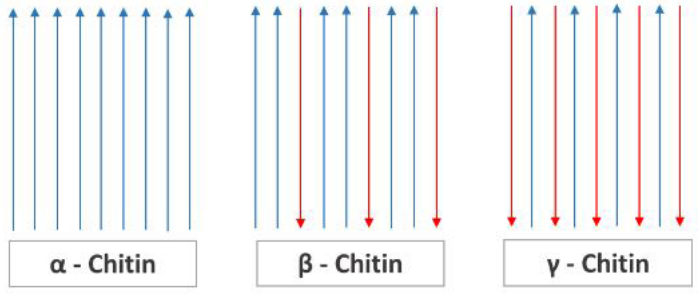
Chitin is a natural polymer composed of units of β-D-glucosamine molecules and N-acetyl groups, forming monomers that will be bound by beta (1 → 4) bonds [2]. Its Degree of Acetylation (DA) must be above 50% to be characterized as chitin [16]. Thus, it is called β (1 → 4) -N-Acetyl-D-glucosamine (Figure 1) [5,17]. The polymer should have acetylation degree greater than 50% to be called chitin Due to the beta bonds between carbons 1 and 4, chitin becomes an extremely stable polymer [18]. Its polymeric extension renders it insoluble in water and practically all organic acids due to the intense hydrogen bonds [19-21]. Chitin contains, on average, 6.5% of nitrogen and its main derivative, chitosan, can reach up to 9.5% of hydrogen content. In obtaining the chitosan, the polymer undergoes a deacetylation process, eliminating about 80% of the acetyl groups to obtain the amino group [22].



Molecular representation of (**A**) cellulose, (**B**) chitin and (**C**) chitosan.

Figure 1: Difference between cellulose, chitin and chitosan.

Chitin has statistically ordered allomorphic configurations. These arrangements, or microfibres, can be characterized in: α-chitin, β-chitin and γ-chitin (Figure 2) [8]. A-Chitin has an antiparallel arrangement of its microfibers that favor hydrogen bonds and give rise to a highly compact structure, resulting in high crystallinity and hardness. It is also known as the most abundant form in nature [18]. B-chitin has parallel chains, being less crystalline and with less packaging, being more flexible and more reactive. Γ-chitin is a mixed composition of forms α and β [6,10]. A-chitin can be transformed into β-chitin, but not the reverse [23]. The three allomorphs can be observed by X-ray diffraction and Nuclear Magnetic Resonance (NMR). The degradability of the polymer occurs through the action of the enzyme chitinase, present in nature [24]. Of course, chitin is found in the macrofibrillar form, allowing the production of nanofibers by mechanical or chemical processes, according to Azuma, K. et al. (2014) [25].



Chitin three configurations: α- chitin, β-chitin and γ-chitin

Image 2: Chitin allomorphs.

Because it is a natural polymer, chitin has characteristics characteristic of polysaccharides, such as: biocompatibility, biodegradability and non-toxicity. It still has bioactivity,

antibacterial activities [26] and antimicrobial [25], anticancer activity, anticoagulant [27] and molecular adsorption property [17-18]. Elieh Ali Komi, Sharma and Dela Cruz (2017) highlights the immunogenic property of chitin and chitinase against pathogens in both insects and mammals as well as plants [8]. It is physically versatile, can be processed in the form of powder, fibers, membranes, sponges, hydrogels [22], scaffolds [21,28]. According to Anitha et al. (2014), the structure of sponges is very favorable for health, with emphasis on tissue engineering, as it has a hygroscopic potential and complexing capacity of other substances to the structure [29].

Applications

Amidst all its unique properties, chitin and its derivatives are an excellent biomaterial with unlimited application possibilities. From the chemical industry [30-31] and agrochemicals [2] to the textile industry [1,5,26,31-34,] and paper industry [13,26,35,] chitin and derivatives are used as biomaterials potential. Most of the studies found in the literature are directed to the health area, as noted below in Table 1. Kandra, Challa and Kalangi Padma Jyothi (2012) presents a vast study on the performance of chitin and chitinase in immunology [22]. Biocomposites of chitin and mainly chitosan is widely studied and developed [7,13,20]. Chitosan, because of its greater bioactivity and adhesiveness, becomes more interesting as scaffolds for areas of medicine and pharmacy and for tissue engineering [29].

Area	Applications	References
Agriculture	Soil modifier; fungicide [6]; defensive mechanism in plants; seed coating; time release of fertilizers [17]; agro-bioscience [49].	[1,5,13,34]
Biocatalysis	Scaffolds as a support for metals in order to produce catalysts [29]	[35]
Bioengineering	Reduced susceptibility to echinocandin with elevated chitin levels in *C. albicans* cells [50]	[29, 50]
Biomedical	Enzyme immobilization and purification chelator [8-9]; emulsifier; flocculen [6]; blood cholesterol control [23,26]; Lectin affinity chromatography; biosensor [48] immobilization of antibody in the presence of alginate [8]; haemostatic agents [29].	[23,45,49]
Biotechnological products	Packaging [32]; manufacture of film and sponge sheet materials [26]; hydrogels [28].	[14,18]
Cosmetic	Lotions; hair additives; body creams [6].	[5,31-34,51]
Energy production	*Clostridium paraputrificum* M-21 to produce hydrogen gas as potential source of alternative energy [22]	[22]
Food industries	Stabilizing agent [9]; dietary supplements [1]; antioxidant [6]; emulsifying agent [35]; food preservation such as for edible films [27]; weight loss agent; food and feed additives [26].	[31-34,49,51]
Medical	Fibers; membranes; artificial organs [6] and skin; surgical sutures [29]; bone and cartilage regeneration [27]; wound healing and dressings; cancer diagnosis [8,24]; aid in cataract surgery; periodontal disease treatment [26]; collagen synthesis [28]; contact lenses [29]; tumor therapy; stem cell tecnology [24].	[21,27-28,52]
Pharmaceutical	Manganese supplement complex [3]; drug release [6,18]; gene delivery [24].	[2,9,27,45,53-54]
Pollutants removal	Copper removal capability to obtain more stable diesel oil.	[22]
Water treatment	Dye removal [17]; absorbent for heavy and radioactive metals [26].	[13,26,33]

Table 1: Applications of chitin and its derivatives as a biomaterial.

The Major Limitation of Chitin Production

The application of chitin and its derivatives depends upon the useful form of the copolymer for different places to use and the quality of the structural and amorphous part of the polymers involved.

The major limitation in the use the chitin and chitosan for design applications are related with: [36-37].

- The collection of the raw material; different characteristics of the initial structure such Mw, ash content and the selection of Alpha or Beta chitin;
- Difficult to obtain reproducible products with different raw materials;
- The cost of production still high;
- The absence of validated process and products of biopolymer manufacture;
- No standardization of product quality and product assay methods for chitin and chitosan.

Extraction and Characterization of Chitin

For decades, chitin has been studied in order to optimize its biomaterial production methods and to better understand its properties and biomaterial potential. Currently, there are several methods of obtaining them in the literature, being the biological processes, with the use of microorganisms and enzymes [1,5,10-11], chemical [6,38] and even a combination of both to achieve an even more process efficient, as published by Younes et al. 2012 [30]. Regardless of the process adopted, the goal is the same: eliminate proteins, minerals, lipids and pigments until only the material of interest is obtained. Today, the most common method in industrial production is the chemical method, due to its productivity and practicality, however, all forms of obtaining chitin are relatively accessible and quite practical [39].

Chemical Process of Obtaining

This is currently the most widely used method in both industrial and laboratory production and is also the most frequently cited in the literature [40]. As mentioned earlier, the purpose of any extraction process is to eliminate all organic and mineral content of the raw material. For this, there are two primordial steps to obtain chitin: deproteinization and demineralization [38]. The order of these steps in a process can be changed according to the purpose and chemicals used. In addition to these two main steps, the production can receive a step of depigmentation and deodorization, if necessary [41].

Firstly, the raw material receives a preliminary treatment to remove impurities and coarse organic waste. At this stage, baths are carried out with deionized water and sodium hypochlorite is sometimes applied [14]. The use of temperature is eventually employed to speed up the cleaning process. The material is still dry or not to follow the milling step, where it will provide a better reaction with subsequent steps [41]. All types of pretreatment are adopted according to the need and condition of the waste used, as well as the species of selected raw material [18].

Deproteinization is the elimination of the protein content present in the raw material. For this, alkaline solutions are used, for example NaOH and KOH [6]. The most used and adopted for industrial production is NaOH solution [42]. A large variation in production aspects is found in the literature. Gortari and Hours, 2013 shows that temperatures in the average of 95°C are used in the deproteinization for commercial chitin, but also indicates that in this range can cause the depolymerization of the material and change some characteristics such as viscosity [14].

Demineralization involves the removal of the inorganic filler (calcium carbonate and calcium phosphate) from the raw material [43]. For this, inorganic acids, such as HCl, HNO_3 and H_2SO_4, and also strong organic acids are used, for example: HCOOH and CH_3COOH [7]. The most commonly used acid in the production of commercial chitin is HCl, due to its high efficiency in the removal of the minerals present. The staining is a strong indicator of the presence of impurities in the material. For this, the depigmentation step is carried out using acetone, sodium hypochlorite, hydrogen peroxide or potassium permanganate [7,27]. Table 2 shows a comparison between some chemical processes found in the literature.

Source	Deproteinization	Condition	DP %	Demineralization	Condition	DM %	Ref
Chicken feet	1:10 (w/v) at 1 N NaOH solution	24h, 90°C	NI	1:18 (w/v) at 1.5N HCl solution	36h, RT	NI	[26]
Crab Shells	-	-	-	1:10 (w/v) at 0,55M HCl solution	15 min, 4 baths, RT	99	[06]
Crustacean waste[1]	1:60 (w/v)² at 1.25 M NaOH solution	2h, 90°C, stirring	~78	1:10 (w/v) at 1M HCl solution	60 min, RT, 800 rpm	98	[34]
	1:60 (w/v)² at 1.25 M NaOH solution (seawater with 35% salinity as base)	2h, 90°C, stirring	~76	1:10 (w/v) at 1M HCl solution (seawater with 35% salinity as base)	60 min, RT, 800 rpm	89	

Cuttlefish	-	-	-	1:10 (w/v) at 0,55M HCl solution	15 min, 4 baths, RT	98	[06]
Fish Scales	n/c (w/v) at 2N NaOH solution	36h, RT	NI	n/c (w/v) at 1% HCl solution	36h, RT	NI	[38]
Housefly pupa Shells	1:11 (g/mL) at 1.25 N NaOH solution	3h, 95˚C	NI	1:11 (g/mL) at 2N HCl solution	3h, RT	NI	[55]
Mushroom	n/c (w/v) at 2M NaOH solution	24h, 85˚C	NI	1:12.5 (w/v) at 2M HCl solution	15h, 60°C	NI	[42]
Shrimp	-	-	-	1:8 (w/v) at 0.2M $C_3H_6O_3$ solution	20 min, RT, Stirred	65	[05]
				1:8 (w/v) at 1M $C_3H_6O_3$ solution	20 min, RT, Stirred	97	
Shrimp	-	-	-	1:10 (w/v) at 0,55M HCl solution	15 min, 4 baths, RT	99	[06]
Shrimp	1:25 (g/mL) at 0,5M NaOH solution	2h, RT	71[3]	1:25 (g/mL) at 0,5M HCl solution	2h, RT	80[3]	[17]
Shrimp	-	-	-	1:10 (w/v) at 2M HCl solution	90 min, 50˚C, 30 rpm, 3 baths	100	[39]
				1:10 (w/v) at 0.5M HCl solution	90 min, 4˚C, 30 rpm, 3 baths		
Shrimp	-	-	-	1:10 (w/v) at 1,5M HCl Solution	6h, 50˚C 150 rpm	98	[30]
Shrimp	-	-	-	n/c (w/v) at 1.1 M $C_3H_6O_3$ solution	20 min, 25˚C	99	[40]
Shrimp	-	-	-	1:10 (w/v) at 1.1M HCl solution	6h, 20˚C, 300 rpm	99	[51]
				1:10 (w/v) at 1.5M CH_2O_2 solution	6h, 20˚C, 300 rpm	99	
				1:10 (w/v) at 2.35M CH_3COOH solution	6h, 20˚C, 300 rpm	99	
				1:10 (w/v) at 1M $C_6H_8O_7$ solution	6h, 20˚C, 300 rpm	99	
				1:10 (w/v) at 1.5M H_3PO_4 solution	6h, 20˚C, 300 rpm	99	

NI: Not informed

RT: Room temperature

n/c: No concentration informed

([1]): Crustacean waste was composed of a variety of crustacean's shells

([2]): Weight of demineralized shells

([3]): Chemical treatment realyzed after mild DP and DM by fermentation

(-): Processes in which the chemical deproteinization step was not carried out

Table 2: Chemical process parameters.

Biotechnological Process of Obtaining

The biotechnological process is the combination of the chemical process with the use of biological methods, with application of microorganisms to the system [43]. This technique shows to be favorable in comparison with the traditional chemical process, where large amounts of highly reactive chemical inputs are used, which can affect the final quality of the material, besides the serious effluent generated in the process. Methods involving biological pathways have also been shown to be more assertive when achieving higher purity states of chitin, with considerably lower molecular weight loss than the conventional chemical process [33-34]. Chemical processes still prove to be more efficient industrially, however the use of biotechnological methods presents a new sustainable vision and new parameter of quality, offering a more suitable biomaterial for health areas [10].

Demineralization can be performed using microorganisms. The material is deposited along with a microbial culture and a sugar source, which will provide the necessary nutrients. As a result of this fermentation process, there are the production of organic acids that react with the minerals and turn them into salts and precipitate. At the end of the process, the above can be removed with a simple washing process [1,27]. According to Aranday-García et al. (2017), lactic acid from the activity of lacto bacteria, provides better results and greater efficiency in demineralization [44]. The inoculation takes place according to each treated species in a culture medium with the necessary nutrients. This medium is basically composed of sugars and fats; amino acids; sources of calcium, iron and magnesium; among other specific compounds. Agar, commercial culture media, are the most used for presenting balanced composition, such as MRS (Man Rogosa Sharpe) [45].

For the elimination of proteins, fermentative and enzymatic processes can be applied. During fermentation, digestive and microbial enzymes are produced and consume organic material [44]. Hydrolytic enzymes (proteases) are very efficient in deproteinization and may result in the production of hydrolyzed proteins as a by-product of high added value [32,46]. Proteases from lacto bacteria are the most commonly used [39]. Carotenoids are pigments found in lipids present in crustaceans and can be isolated from fermentation and enzymatic activity [47]. Astaxanthin belongs to these carotenoids present and has great commercial value in the food area [32].

The main disadvantage of processes involving microorganisms are the time released for fermentation and high cost of some enzymes [40,48]. Often the biological process is insufficient, requiring the application of acids and alkaline solutions to confirm the reaction, but presenting a better deproteinization yield, as described in Table 3, consequently obtaining a material of significantly higher quality due to low molecular weight loss [45].

Microrganism	Process				Substrate	Efficiency			Ref
		Temp (°C)	Stirring (RPM)	Time (h)		DM %	DP %	Chitin %	
Lactobacillus sp. B2	Fermentation (DM and DP occur simultaneously at an orbital shaker)	30	200	120		88	56	34	[01]
B. mojavensis A21	Enzymatic DP under alkaline solution (4% NaOH)	50	-	3		-	89[1], 94[2] and 70[3]	37[1], 27[2] and 6[3]	[06]
P. aeruginosa	Fermentation aiming production of organic acid in orbital shaker	30	180	168	D-Glucose 10% (w/v)	~91	-	From 11,56 to 44,25	[11]
S. marcescens	Fermentation aiming DP in orbital shaker	30	180	168		-	NI		
L. plantarum PTCC 1058	Fermentation in incubator shaker (DM and DP occur simultaneously)	30	180	144	Date syrup	54	45	NI	[17]

B. cereus SV1	Fermentation (DM and DP occur simultaneously)	37	200	120	Glucose 5% (w/v)	77,3	88,6	-	[12]
B. subtilis A26						79,9	91,25	-	
B. mojavensis A21						78,7	88	-	
B. pumilus A1						75,3	91,2	-	
B. lichenformis RP1						55,55	90,8	-	
B. amyloliquefaciens An6						66,05	90,8	-	
B. mojavensis A21	Enzymatic DP	50	NI	3	NI	-	77	NI	[39]
B. subtilis A26		40				-	75		
V. metschnikovii J1		40				-	75		
B. lichenformis MP1		50				-	75		
B. lichenformis NH1		50				-	65		
A. clavatus ES1		40				-	59		
Scorpaena scrofa (scorpion fish) enzyme		45				-	80		

B. mojavensis A21	Enzymatic DP	50	NI	NI	No substract	-	76	NI	[30]
V. metschnikovii J1		40				-			
B. lichenformis MP1		50				-			
B. subtilis A26		40				-			
B. lichenformis NH1		50				-	65		
A. clavatus ES1		40				-	59		
Alcalase (Commercial enzyme)		50				-	54		
Bromelain (Commercial enzyme)		50				-	67		
S. griseus	Enzymatic DP	37	0	5	KH_2PO_4 (0,5M/L)	-	91	NI	[40]
S. thermophilus	Fermentation in incubator (DM and DP occur simultaneously)	42	0	72	Glucose 15% (w/v)	92	94	NI	[33]
B. subtilis	Fermentation in rotary shaker (DM and DP occur simultaneously)	60	150	24	Glucose 1% (w/v)	94	84	NI	[34]
B. licheniformis and	Fermentation in incubator shaker (DM and DP occur simultaneously)	30	180	60	No substract	93.5	87	NI	[45]
G. oxydans				36	Glucose 5% (w/v)				
L. brevis	Fermentation (DM and DP occur simultaneously)	30	0	192	Glucose 10% (w/v) with *Rhizopus*	67,3	96	NI	[44]
Portunus segnis (blue crab) viscera enzyme, Purafect (R 2000E) and Neutrase (P1236)	Enzymatic DP	50	0	3	No substract	-	91	22,2	[31]

P saliphilus	Enzymatic DP	50	0	168		-	85.6	NI	[46]
A. niger	Enzymatic DP under acid solution	20	300	6	No substract	-	95	NI	[51]

NI: Not informed	[4] Yield varies according to crustacean source	□: Substrates added after 120h of fermentation
[1]: Yield from shrimp shells	[5]: Medium using seawater	
[2]: Yield from crab shells	[6]: DP attained after further chemical treatment	
[3]: Yield from cuttlefish bones	[7]: *B. licheniformis* followed by *G. oxydans*	

Table 3: Biological process parameters.

Final Considerations

The mastery of the techniques and study of the kinetics of each process is fundamental for the optimization of the same. The analysis of the different chitin obtaining studies by chemical means found in the literature suggests a higher efficiency in the use of consecutive and short baths instead of a long one for deproteinization as well as for the demineralization, thus dispensing with the use of solutions with large concentrations of reagents, which in turn avoids the loss of the quality of the chitin obtained in the process. Obtaining by biotechnological methods presents better results in most literary studies, however, it lacks further development, since the average time in the process is often greater than by the chemical method, leaving the employment of bacteria and enzymes less attractive for the chitin production at industrial levels. Of these bacteria, a family that proves very promising is *Lactobacillus*, because in the process this produces lactic acid, simultaneously promoting the demineralization of the material.

Chitin and its derivatives, as previously mentioned, have several applications in the most varied areas, but the current trend of research seems to be more focused on the biomedical area, with the development of treatments and methods that promote the regeneration of wounds and / or nervous tissues. For the application of chitin in these areas it becomes more necessary to use a material with a higher degree of purity, so it is advisable to use biotechnological methods in these cases. The fact that the degree of purity is lower in chemical processes does not deprive the application of the chitin obtained by them in other areas that do not demand such purity, these areas may be more attractive for investments, taking advantage of the faster production and greater amount provided by such methods.

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