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Feature Article

Different routes to turn chitin into stunning nano-objects

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ABSTRACT

Due to its intractable structure and inherent insoluble nature, chitin was for a long time an underutilized resource. The increasing interest in the use of chitin as a source of nanostructured materials is quite recent. This review provides the latest advances in different ways to isolate or fabricate chitin nano-objects – chitin nanocrystals (CHNC) and chitin nanofibers (CHNF) – from different chitin sources. It also summarizes the chronology of some important scientific advances on chitin research during its 200 years of history. Additionally, engineered composite materials based on chitin nano-objects are reviewed.
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Contents

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1. Introduction

In 1811, the French botanist H. Braconnot isolated from mushrooms a polysaccharide of major importance: *chitin* (by this time identified as *Fungine*) [1,2]. A point of difference from other polysaccharides is the presence of nitrogen. Years later, in 1823, another French scientist, A. Odier identified chitin in demineralised crab carapace and suggested that it is the basic material of the exoskeleton of all insects. The term chitin is derived from the Greek word χιτων, which means 'tunic' or 'covering' [1]. In fact, chitin is biosynthesized by a vast number of living organisms. It is commonly found in the crustacean shells, insect cuticles and in the cell walls of fungi, yeast and green algae [1,3,4]. Chitin is a crystalline high-molecular-weight linear polymer composed of *N*-acetyl-2-amido-2-deoxy-D-glucose units linked by β(1 → 4) bonds (Fig. 1). Isolated chitin is a highly ordered copolymer of *N*-acetyl-D-glucosamine as the major component and D-glucosamine as a minor constituent (Fig. 1). These residual monomers are present in the native chitin or are formed through hydrolysis of some acetamido groups during the isolation and purification processes [1].

Chitin was for a long time considered as an untreatable polymer because of its inherent insoluble nature (in almost all common solvents) and intractable molecular structure [4–6]. In 1920s and 1930s numerous methods were developed to obtain chitin fibers for the production of artificial silk. However, the interest in chitin decreased quickly with the discovery of nylon and production of synthetic fibers [7].

Only in the late 1970s did chemists look at chitin with scientific interest, and immediately recognized it as an abundant source of chitosan – the unique cationic polysaccharide [1,6]. Chitosan, the major and simplest chitin derivative, is also a high-molecular-weight linear polymer obtained by deacetylation of chitin (cleavage of the *N*-acetyl group at C-2 position) and is therefore composed of 2-amino-2-deoxy-D-glucose units linked through β(1 → 4) bonds (Fig. 1) [1,3,4,8–10].

Nonetheless, the increasing interest in the use of chitin and chitosan in several applications only started at the beginning of the 1980s because of environmental requirements due to the organic solid wastes and by-products generated by the food industry (in particular, shellfish industries and fish farms in Japan and USA). Part of the massive amount of biowaste accumulated (up to 250 billion tons/year) by marine-capture fisheries can be industrially transformed in pure chitin and its derivatives. It is estimated that at least 10 gigatons of chitin is biosynthesized and degraded each year – chitin is considered to be the second most abundant biopolymer on earth after cellulose [8,9].

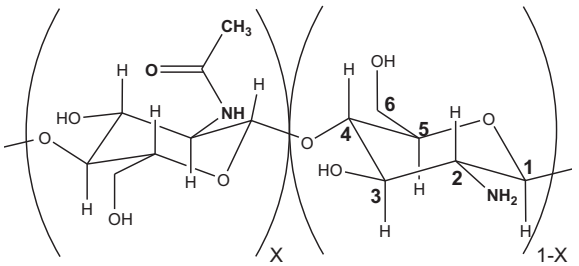

Fig. 1. Illustration of the chemical structure of copolymers chitin (X ≫ 1 − X) and chitosan (1 − X ≫ X)) of *N*-acetyl-D-glucosamine (molar fraction = X) and D-glucosamine units (molar fraction = 1 − X). Conventionally, chitin X > 0.50 and chitosan X < 0.50.

Despite being one of the most available natural polymers, chitin was for a long time an underutilized resource, when compared to the other polysaccharides (including chitosan), due to its insoluble character. It is a quite recent trend that pristine chitin has gained importance as a promising source of new materials – as a nanostructured material in the form of chitin whiskers and/or nanocrystals (CHNC) and chitin nanofibers (CHNF) (altogether called here as chitin nano-objects). One of the first works describing the use of chitin whiskers as new environmentally friendly reinforcing agents in thermoplastic nanocomposites was reported in 2001 by Paillet and Dufresne [11]. Following, new domains of exploitation of chitin have emerged for using it over a broad range of applications including nanocomposite materials, electronics and medical devices and cosmetics [12–16].

Unquestionably, the key consideration of the growing scientific interest in chitin nano-objects is the atypical combination of physicochemical and mechanical properties as well as biological properties. Chitin is widely abundant, biodegradable and biocompatible with low cytotoxicity, present antimicrobial activity and low immunogenicity. Moreover, chitin nano-objects have high aspect ratio, high surface area, low density and reactive surface (–OH and $–NHCOCH_3$ groups, and residual $–NH_2$ groups) that facilitates surface functionalization [3,4,8,9,12,13,17–19].

In this context, recently several methods have been developed to isolate chitin nano-objects from chitin source materials [11,14,20–32]. This review describes and discusses the advances on the research work on different routes to turn chitin in stunning nano-objects.

2. Supporting material in living systems

Why can chitin be turned into nano-objects? In nature, chitin occurs as a highly-organized micro- and nano-fibril structure, whose role is that of providing support and protection to living systems, mainly to crustaceans, insects and fungi, as reinforcing and functional elements [10,12,14,33–35]. Chitin forms part of a well organized hierarchical structure (Fig. 2), in the exoskeleton of many invertebrates – such as crabs, shrimps, lobsters and krills – increasing from the nanometer to the millimeter scale. As shown in Fig. 2, at the molecular level, there are long chains of chitin that form highly crystalline fibrils on the nanometer level (length (L) ~ 30 nm, width (d) ~ 3 nm). Within these fibrils, chitin chains are packed together forming highly crystalline regions that are accompanied by disordered (amorphous) regions, making chitin semicrystalline. These fibrils are enveloped with proteins and assemble into nanofibers (d ~ 60 nm), which further

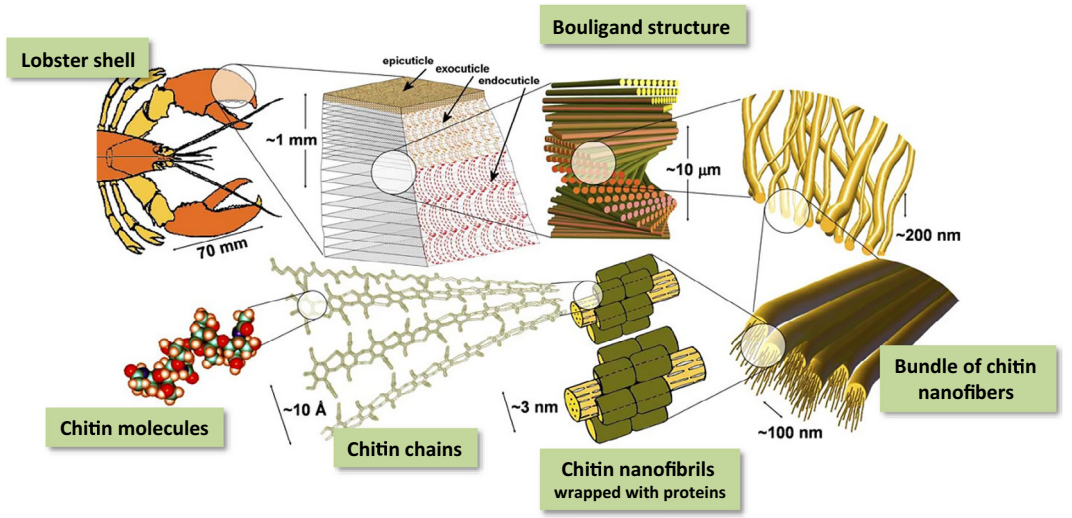

Fig. 2. Scheme of the hierarchical organization in arthropod exoskeleton (*H. americanus*, American lobster), which reveals different structural levels. Reprinted with permission from Ref. [36] Copyright 2005 Elsevier.

assemble into bundles of chitin nanofibers. At the micrometer level, a network of bundles is formed, creating a twisted plywood structure (so-called 'Bouligand structure'), which is embedded in proteins and calcium carbonate [33–40]. This hierarchical structure and Bouligand pattern was also studied in other arthropods specimens like insects [40,41]. Finally, this structure repeats to form the endocuticle, exocuticle and epicuticle at the macroscopic level.

Chitin nano-objects can be obtained by two approaches: top-down and bottom-up. As chitin fibrils are composed of two regions *i.e.* crystalline and amorphous, chitin can be turned in nanocrystals and nanofibers *via* top-down method. This approach breaks down the chitin fibrils from native chitin into nanofibrils. Acid hydrolysis [40,41], 2,2,6,6-tetramethyl-piperidinyl-1-oxyl (TEMPO)-mediated oxidation [40,41], grinding [40,41] and high-pressure homogenizing [32,40,41] are some representative techniques of this approach. On the other hand, self-assembled chitin nano-objects have been produced by regeneration from chitin solutions or gels using appropriate methods (*e.g.* electrospinning [40–42]) *via* the bottom-up approach. Being considered as nanomaterials of great innovative potential, the research related to the isolation and production, characterization and exploitation of chitin nano-objects has achieved new heights over the past decade.

3. Isolation of chitin nano-objects

The isolation of chitin nano-objects from chitin raw material occurs in two steps.

The first step is the purification of the source material, which is dependent of the chitin source material. Up to now, most of the chitin source materials used in industrial applications and scientific research comes from shellfish processing industry wastes; being shrimp, crab and lobster the most popular sources [43]. Considering that crustacean shell waste consists of a mixture of proteins (~30–40%), inorganic salts (~30–60%), chitin (~20–30%) and lipids (~0–14%) [3,7], chitin isolation involves different processes. Conventional chemical approach includes three basic operations: (i) deproteinization – removal of residual proteins by chemical (NaOH) or enzymatic hydrolysis; (ii) demineralization – removal of mineral salts by acid treatment; and finally (iii) removal of lipids and pigments by typical bleaching treatments [1,3,4,44,45]. In some cases, when the raw material is rich in minerals, it is preferable that the demineralization operation precedes the deproteinization process [10]. After it, purified chitin could be: (i) dried and cracked into powders or small flakes; or (ii) keep wet in suspension. This chitin can be used in preparation of chitin nano-objects using different approaches described below. Fig. 3 shows a schematic illustration of the conventional process of the chitin isolation from shell wastes. Fig. 4 displays scanning electron microscopy (SEM) images (at different magnifications) of dry chitin from yellow lobster shell after removing minerals, proteins and pigments. It can be observed that purified chitin is made up of regularly structured fibrils with a variety of thicknesses. The thicker fibers are made of bundles of thinner nanofibers.

The second step involves the separation of purified chitin into crystalline or fibrillar nano-objects. There are several approaches to isolate chitin nano-objects, although the most widespread is the acid hydrolysis, usually with hydrochloric acid (HCl). The separation approaches (acid hydrolysis, mechanical treatment, ultrasonication process, etc.) can be employed isolated or combined to obtain the desired chitin nanomaterials.

The diversity of chitin nano-objects (size, shape, crystallinity, aspect ratio and morphology) results from: (i) the biosynthesis of the crystalline chitin fibrils – dependent on chitin source; and (ii) the isolation process of the chitin nano-objects – dependent of type and harshness of

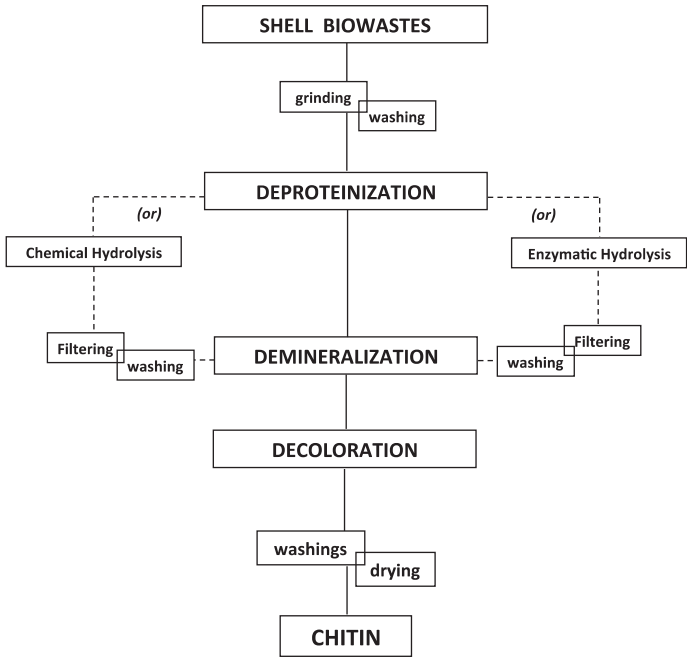

Fig. 3. Illustrative scheme of the extraction process of chitin from shell wastes.

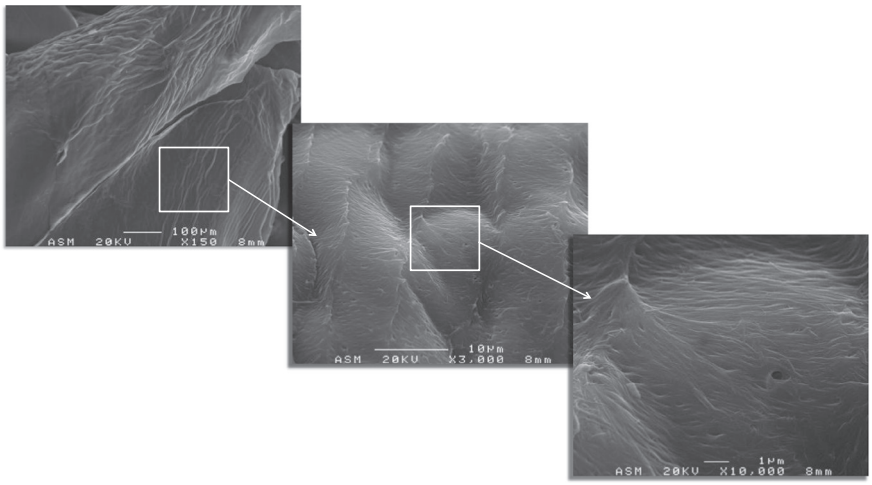

Fig. 4. SEM micrographs of purified chitin flakes from lobster shells at different magnifications (150×, 3000× and 10,000×).

the extraction treatment. The next sections describe the different approaches used for the isolation and production of chitin nanocrystals and chitin nanofibers.

3.1. Chitin nanocrystals (CHNC)

Chitin nanocrystals (also called as chitin whiskers, chitin nanowhiskers and chitin nanoparticles) are rod-like or whiskers shaped nanomaterials (Fig. 5), reminiscent of the crystalline regions within the chitin fibrils, generally obtained after a chemical treatment of chitin (Fig. 5 and Table 1). CHNC are therefore highly crystalline (57–93%) and have very small sizes (6–60 nm in width and 100–800 nm in length) and high aspect ratio (Table 1). As already mentioned, with respect to CHNC isolation, acid hydrolysis is the most used approach. Nonetheless, other new methods, such as 2,2,6,6-tetramethylpiperidinooxy (TEMPO) mediated oxidation, partial deacetylation and ionic liquids are also used. These methods will be described in detail in the next sub-sections and are summarized in Table 1. The typical CHNC morphologies are listed in Fig. 5.

3.1.1. Acid hydrolysis

Acid hydrolysis has been used to isolate the chitin nanocrystals (Fig. 5a) from a variety of chitin source

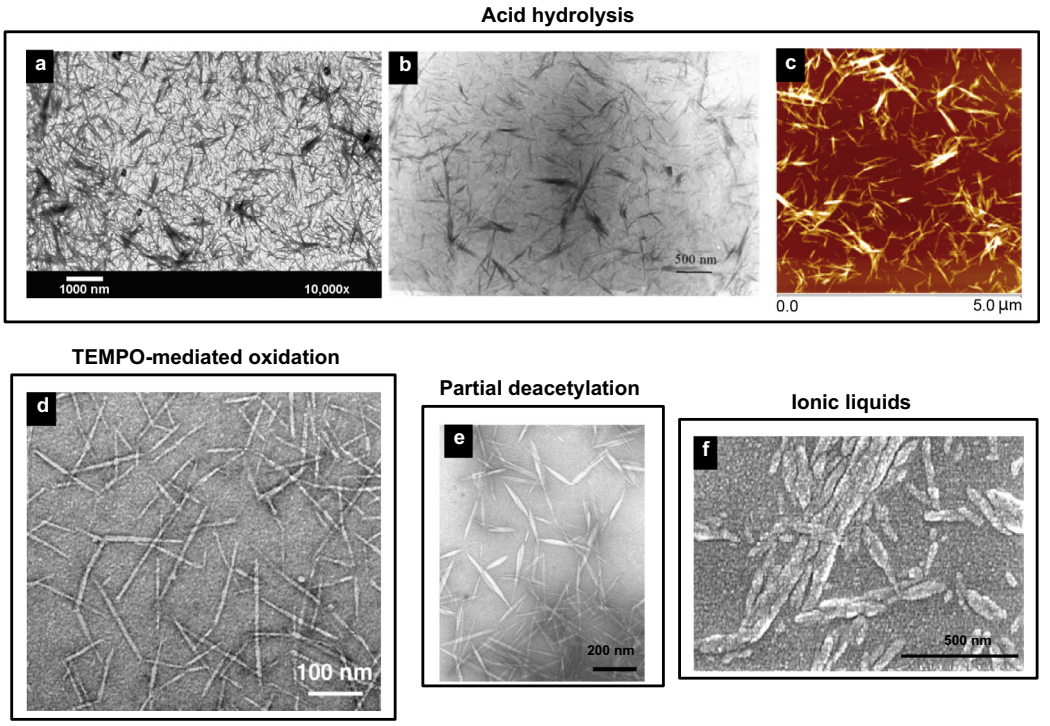

Fig. 5. Images of chitin nanocrystals isolated by: (a) acid hydrolysis from shrimp shells (Transmission Electron Microscopic (TEM) image [57]); (b) acid hydrolysis + mechanical treatment from shrimp shells (TEM image [64]); (c) acid hydrolysis + mechanical treatment from lobster shells (Atomic Force Microscopic (AFM) topography image) (d) TEMPO-mediated oxidation from crab shells (TEM image [21]); (e) partial deacetylation + mechanical treatment from crab shells (TEM image [23]); and (f) ionic liquids method (using AMIMBr) from crab shells (SEM image [24]). Reprinted with permission, (a) from Ref. [57]; (b) from Ref. [64]; (e) from Ref. [23]; (f) from Ref. [24] Copyright 2014, 2005, 2010 and 2014 Elsevier; and (d) Reproduced from Ref. [21] Copyright 2008 American Chemical Society.

materials like crab [46–53], shrimp [20,54–60] and lobster [61] shells, squid pen [11] and *riftia* tubes [62,63] (Table 1). The mechanism of acid hydrolysis removes (hydrolyze) the disordered and amorphous domains within the chitin fibrils, while the high crystalline domains remains intact [12].

In general, this method consists in suspending purified chitin in a strong acid aqueous solution, typically hydrochloric acid (e.g., 3 M HCl). After reacting at boiling point under vigorous stirring for a given time (1.5–6 h), the suspension was diluted with deionized water to stop the reaction. Then, this suspension suffers a series of separation (centrifugation and/or filtration) and washing steps [11,20,46–57,59–64]. To ensure that there was no residual acid, the suspension was transferred to dialysis membranes and dialyzed against deionized water for several days [46,57,61,65]. Depending on the chitin source material the variables like reaction time and temperature can change. For instance, if chitin is from shrimp shells, the reaction time must be increased in order to obtain a good colloidal suspension [55,57,59].

Ultrasonication or homogenization treatments can be used after acid hydrolysis to facilitate dispersion of the chitin nanocrystals (Fig. 5b) in the suspension [11,20, 46–48,59–64]. Several authors have reported the use of low pH during these treatments. The low pH protonates chitin fibrils, by charging positively the residual amino groups ($-NH_3^+$) on their surface, that combined with physical or mechanical treatments facilitates the isolation of chitin fibril into nanocrystals [11,46,59,64].

Among the different chitin source materials, CHNC obtained from shrimp shells present the longer dimensions, reaching up to 800 nm in length [20,54,56]. Concerning the width, most of CHNC obtained by the different chitin origins presented small diameters, in general between 10 and 20 nm. However, CHNC isolated from lobster shells exhibit higher diameters, *i.e.* 60 nm (Fig. 5c) [61].

3.1.2. TEMPO-mediated oxidation

Chitin nanocrystals [21] and nanofibers [30] (see Section 3.2.2) can be prepared by a method similar to the preparation of cellulose nanofibers through 2,2,6,6-tetramethyl-piperidinyl-1-oxyl (TEMPO)-mediated oxidation [66,67]. α-Chitin nanocrystals with 10–15 nm in width and 270 nm in length were obtained from crab shells by TEMPO-mediated oxidation [21]. To achieve this, α-chitin was suspended in water containing TEMPO and sodium bromide (NaBr). The oxidation of chitin started by adding NaClO as co-oxidant. The pH of the suspension was maintained to be 10 at room temperature by continuous addition of NaOH solution [21]. During the oxidation, the amorphous domains were dissolved forming polyuronic acid and some of the hydroxyl groups of C-6 in the crystalline α-chitin surfaces (insoluble parts) were converted

Table 1
Chitin source materials, isolation methods and conditions, and chitin nanocrystals characteristics.

Source materials	Method	Temp. (°C)	Time (h)	Chitin nanocrystals characteristics				Ref.
				Width (nm)	Length (nm)	Aspect ratio	C.I. (%)	
Crab shell	Acid hydrolysis – 3 M HCl	100	3	6–8	100–200	–	–	[50]
		100	1.5	20	300	–	–	[51]
		90	3	20	300	–	–	[52]
		80	1.5	10–30	400	–	–	[49]
		95	1.5	–	–	–	–	[53]
Shrimp shell	Acid hydrolysis – 3 M HCl	104	6	7.3	>400	>55	–	[57]
		105	3	–	–	16	–	[55]
		105	1.5	18–40	200–560	18	–	[54]
		80–90	–	10–50	150–800	–	–	[56]
Crab shell	Acid hydrolysis – 3 M HCl + 5′ US (pH4)	90	1.5	15	240	16	–	[46]
	Acid hydrolysis – 3 M HCl + US (pH4)	100	1.5	50	500	10	–	[47]
	Acid hydrolysis – 3 M HCl + 5′ US (pH3)	104	1.5	16	214	13	86	[48]
Shrimp shell	Acid hydrolysis – 3 M HCl + homogenization	90	1.5	10–15	200–500	–	84	[20]
	Acid hydrolysis – 3 M HCl + 5′ US (pH 2.5)	104	1.5	33	417	10	–	[64]
	Acid hydrolysis – 3 M HCl + 10′ US	104	6	43	427	10	–	[59]
	Acid hydrolysis – 3 M HCl + 5′ US	100	1.5	15–20	150–400	–	–	[60]
Lobster shell	Acid hydrolysis – 3 M HCl + 10′ US	100	1.5	60	300	5	89	[61]
Squid pen	Acid hydrolysis – 3 M HCl + 2.5′ US (pH 3.5)	–	1.5	10	150	15	–	[11]
Riftia tubes	Acid hydrolysis – 3 M HCl + 2′ US	–	1.5	18	–	120	–	[62]
	Acid hydrolysis – 3 M HCl + 20′ US	–	6	20	300	15	–	[63]
Crab shell	TEMPO mediated oxidation + 1′ US	–	–	10–15	270	–	93	[21]
Crab shell	Partial deacetylation + 5 days stirring + 1′ US	90	1–4	6.2	250	–	57	[23]
Crab shell	IL + 5′ US	100	48	20–60	>100	–	–	[24]
	IL + 10′ US	100	24	20–60	>100	–	–	[72]

US: ultrasonication; IL: ionic liquids; C.I.: crystallinity index.

to carboxylated or carboxyl groups by TEMPO-mediated oxidation. Afterwards, the α-chitin suspension was subjected to an ultrasonic treatment leading to the individualization of the α-chitin nanocrystals (Fig. 5d). The existence of carboxylate groups at the surface of α-chitin nanocrystals inhibits their agglomeration forming a stable colloidal suspension.

In comparison with acid hydrolysis, TEMPO-mediated oxidation is more controllable by the amount of NaClO, and the yield of chitin nanocrystals (insoluble part) can reach 90%. Another advantage of TEMPO-mediated oxidation isolation is that the *N*-deacetylation of chitin does not take place during the reaction.

3.1.3. Partial deacetylation

In 2010, Fan et al. [23] reported another method to obtain chitin nanocrystals, that consists in submitting partially deacetylated chitin fibrils to a mechanical treatment at low pH. The protonation of the amino groups provides a superior number of positive charges at the surface of the partially deacetylated chitin fibrils, which increase the repulsion effect between the fibrils. This repulsion facilitates the disintegration of chitin fibrils during the mechanical processing resulting in chitin nanocrystals. The treatment proposed by Fan et al. [23] was carried out in three main steps: (i) first, partial deacetylation of chitin – a chitin suspension in 33% (w/w) NaOH solution (containing $NaBh_4$ to avoid alkaline depolymerization and weight loss) was heated at 90 °C for 1–4 h under stirring. Then, the partially deacetylated chitin suspension was washed with deionized water and centrifuged until neutralization; and (ii) submitted to mechanical disintegration – carried out at pH 3–4 (adjusted by adding acetic acid) using a magnetic stirring at 1200 rpm for 5 days; finally, (iii) the suspension was subjected to sonication – 1 min using an ultrasonic generator. Fig. 5e shows the morphology of individual nanocrystals with average width and length 6.2 ± 1.1 and 250 ± 140 nm, respectively.

The major driver for individualization of CHNC using the two latter approaches is the charge-induced electrical repulsion. The difference is that partial deacetylation provides CHNC with cationic charges and the TEMPO-mediated oxidation gives CHNC with anionic charges.

3.1.4. Ionic liquids

Ionic liquids (ILs) are commonly defined as salts which melt below 100 °C. These compounds have been proposed as an alternative for the dissolution and swelling of biomass where organic and aqueous solvents are not effective.

Similar to cellulose [68–70], chitin can be swelled to form gel like-materials (ion gels) by ionic liquids [71]. In 2009, Prasad et al. reported the use of 1-allyl-3-methylimidazolium bromide (AMIMBr) for the formation of weak gels of chitin. In their study, the gel formation was obtained by heating chitin with AMIMBr at 100 °C for

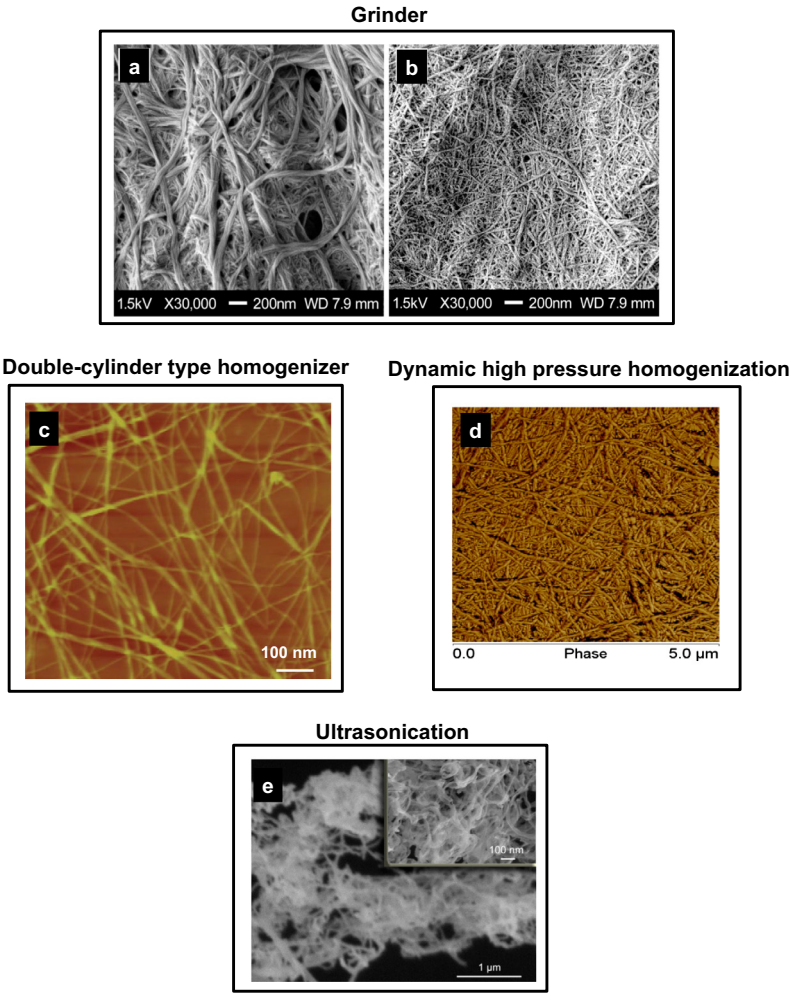

Fig. 6. Several chitin nanofibers isolated by: (a) grinder treatment under neutral conditions using chitin from crab shells (SEM image [26]); (b) grinder treatment under acidic conditions (pH 3) from crab shells (SEM image [26]); (c) dilution/double-cylinder type homogenizer treatments with chitin from squid pen (TEM image [31]); (d) dynamic high pressure homogenization using chitin from lobster shells (AFM phase-contrast image [32]; and (e) ultrasonication after 30 min with chitin from prawn shells (SEM micrograph [82]). Reproduced with permission, (a and b) from Ref. [26] Copyright 2009 American Chemical Society; and Reprinted with permission, (c) from Ref. [31]; (d) from Ref. [32]; and (e) from Ref. [82] Copyright 2012, 2014 and 2013 Elsevier.

48 h. The authors showed that the viscosity of the gel was dependent of the mixture concentration *i.e.* a mixture of 7% (w/w) chitin with IL resulted in a more viscous material than that prepared with 5% (w/w) chitin with IL [71]. More recently, the same approach was used to produce chitin nanocrystals [24,72]. The gelation of chitin with AMIMBr was followed by the regeneration with methanol. Briefly, the resulting gel was soaked in methanol and subsequent sonicated to produce a chitin nanocrystals suspension. In this work, the chitin nanocrystals suspension was used to prepare a film by filtration [24,72]. Fig. 5f shows the formation of chitin nanocrystals (with ca. 20–60 nm in width and several hundred nanometers in length) using this method.

Recent developments in the fabrication of nano- and microstructured chitin materials focusing on procedures by gelation with suitable dispersion media were recently reviewed by Kadokawa [74].

ILs were also used to prepare chitin solution for the fabrication of chitin nanofibers by electrospinning (see Section 3.2.4) [73].

3.2. Chitin nanofibers

Chitin nanofibers are thin chitin fibrils (Fig. 6), reminiscent of elementary fibrils usually obtained when specific mechanical (grinder, blender, homogenizer) or physical (ultrasonic) techniques are used to facilitate fibrillation. Other approaches including TEMPO-mediated oxidization and electrospinning have been developed to produce chitin nanofibers. These methods are describe in detail in the next sub-sections and summarized in Table 2. CHNF have very high aspect ratio (10–100 nm in width and several micrometers in length) and are crystalline (but less than nanocrystals). The typical CHNF morphologies are given in Fig. 6.

Table 2
Chitin source materials, isolation methods and conditions, and chitin nanofibers characteristics.

Source materials	Method	Chitin nanofibers characteristics			Ref.
		Width (nm)	Length (μm)	C.I. (%)	
Prawn shell	Grinder (pH 3)	10	–		[75]
Mushrooms	Grinder (pH 3)	20–30	–		[25]
Crab shell	Blender (pH 3) + grinder	10–20	–		[26]
Prawn shell	Blender (pH 3) + grinder	10–20	–		[76]
Lobster shell	Blender + microfluidizer	3.6–3.9	1–1.5	89–90	[28]
Lobster shell	5′ UT + 5′ US + high pressure homogenizer	80–100	>1	85	[32]
Crab shell	Grinder + US + homogenizer	30–50	>10		[78]
	Grinder + US	30–50	–		[79]
Squid pen	US(acid condition)	3–4	>1	51	[29]
	US (pH 3–4)	3–10	>1		[80]
	US (pH 3–4)	3–10	>1	54	[83]
Prawn shell	US	20	–	66	[82]
–	Homogenizer	50	>1	68	[77]
Shrimp shell	Electrospinning (ILs)	–	–		[73]
–	Irradiation Co^{60} + electrospinning (HFIP)	163	–		[42]
–	Irradiation Co^{60} + electrospinning	110	–		[85]
Crab shell	Microwave irradiation + electrospinning	8	–		[84]
Tuberworm	TEMPO-mediated oxidation	20–50	>1	51	[30]

US: ultrasonication; UT: ultra-turrax; C.I.: crystallinity index.

3.2.1. Mechanical approaches

Mechanical techniques, such as grinder [25–27,75,76], blender [26,27,76], high-pressure homogenizers [32,77] or combination of these or other techniques with high intensity ultrasonic treatments (see Section 3.2.2) [28,32,78,79] have been used to isolate chitin nanofibers from prawn, crab, shrimp and lobster shells, mushrooms and squid pen. Generally, these processes produce high shear that origins transverse cleavage along the longitudinal axis of the chitin microfibrillar structure, resulting in the isolation of long chitin fibrils, known as chitin nanofibers. Typically, the size of the chitin materials becomes smaller and thinner with the number of the passes in the specific mechanical equipment. In the same manner as chitin nanocrystals isolation, the use of acidic conditions (pH 3–4) is very typical with the procedure to facilitate the chitin fibrils disruption by an increase of repulsive charges on its surface [25–27,29,75,76,80].

Grinder treatment have been used to isolate chitin nanofibers from purified chitin of the exoskeleton of crabs and prawns and the cell wall of mushrooms [25–27,75,76]. This grinding treatment was employed in never-dried state [26] and dried state of purified chitin under neutral conditions [25] and acidic conditions [27]. In brief, the purified chitin is dispersed in water or acidic solution (acetic acid) at 1 wt.%. First, chitin is roughly crushed with a blender, and then the slurry passes through a grinder. The obtained chitin nanofibers from crab and prawn shells formed a fine nanofiber network with a uniform width of approximately 10–20 nm and a high aspect ratio (Fig. 6a and b). In the case of the nanofibers from mushrooms, the diameter varied from 20 to 28 nm [25].

Fan et al. [31] used a succession of dilution/homogenizer (a double-cylinder type homogenizer) treatments of a chitin/water dispersion to obtain chitin nanofibers from squid pen. After the dilution/homogenizer treatment, the chitin/water dispersion is subjected to sonication using an ultrasonic generator. Squid pen chitin nanofibers consist of mostly individualized nano-elements, and present similar and uniform widths of 4 nm with high aspect ratios (Fig. 6c).

Recently, Mushi et al. [28] developed a 'mild' method to isolate chitin nanofibers from lobster using also a combination of processes. A colloidal suspension (1 wt.%) of chitin is blended in acidic conditions (pH 3) and passed several times through a microfluidizer. The ensuing nanofibers presented very small diameters (3.6–3.9 nm in width) and very long lengths (1.0–1.5 μm in length).

Salaberria et al. [32] exploited a simple and environmentally friendly technique – dynamic high pressure homogenization – to obtain chitin nanofibers from yellow lobster. This method was based on the passage of a suspension (1 wt.% in water) of purified chitin at very high pressure through a homogenizing valve, which is able to downsize chitin particles into chitin nanofibers (Fig. 7). Individualized chitin nanofibers with diameters below 100 nm and several micrometers in length were obtained by this simple concept (Fig. 6d).

The disadvantage of mechanical approach is the high energy consumption associated with the processes.

3.2.2. Ultrasonic technique

The effect of ultrasound, caused by acoustic cavitation, generates localized hotspots with very high temperatures,

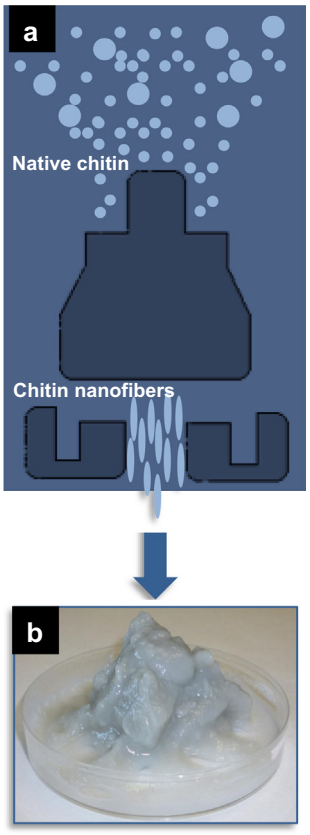

Fig. 7. (a) Illustration of the high pressure homogenization approach to isolate chitin nanofibers; and (b) image of the obtained gel-like aqueous matter of chitin nanofibers (B). Reprinted with permission from Ref. [32] Copyright 2014 Elsevier.

pressures and heating/cooling rates [81]. Such extreme environments provoke the break of the strong chitin interfibrillar hydrogen bonding, allowing gradual disintegration of the nanofibers.

Successful disintegration of purified chitin from different sources can be also achieved by high-intensity ultrasonication treatment [29,31,76,80,82,83]. For instance, Lu et al. [82] isolated chitin nanofibers with dried prawn shells *via* a simple high-intensity ultrasonic approach under neutral conditions (60 kHz, 300 W, 7 pH). The diameter of the obtained chitin nanofibers could be controlled within 20–200 nm by simply adjusting the ultrasonication time. With 30 min of sonication, the authors obtained high-aspect-ratio nanofibers with a uniform width of 19.4 nm (Fig. 6e).

3.2.3. TEMPO-mediated oxidation

Following the same procedure to obtain α-chitin nanocrystals from crab shells [21], Fan et al. [30] obtained individualized β-chitin nanofibers by TEMPO-mediated oxidation from tuberworms. Fig. 8a shows TEM image of mostly individual β-chitin nanofibers from tuberworms presenting a flat and ribbon-like morphology and dimensions of 20–50 nm in width and several microns in length. This was achieved by controlling the amount of NaClO used as co-oxidant [30]. In the same work, the authors also tried to obtain β-chitin nanofibers from squid pen. Although, the water-insoluble fractions were obtained from the TEMPO-oxidized products, the oxidized products were not converted to individual nanofibers under any condition.

3.2.4. Electrospinning method

Electrospinning method allows obtaining very long nanofibers with a uniform width. In electrospinning a high voltage is applied between a small orifice (where a polymer melt or solution passes through) and a conductive plate (which produces a non-woven mat of fibers). This process produces micrometer- to nanometre-scale fibers after the coagulation of the polymer in a solution or drying atmosphere.

A number of studies have been developed in the fabrication of chitin nanofibers by electrospinning technique. Nonetheless, chitin presents some limitations namely: (i) poor solubility; and (ii) high molecular weight of some chitin sources. Therefore, chitin nanofibers have been electrospun, after dissolution in highly toxic solvents (*e.g.* 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)) and strong acids (*e.g.* methanesulfonic acid (MSA)); and depolymerization [42]. Different methods have been employed to decrease the molecular weight: (i) radiation by Co^{60} gamma ray [42]; (ii) microwave radiation [84]; and (iii) ultrasonication [84]. In general, the resulting chitin nanofibers produced by electrospinning showed uniforms widths between 100–163 nm [42,85] (Fig. 8b). The nanofibers obtained by a combination of microwave irradiation and

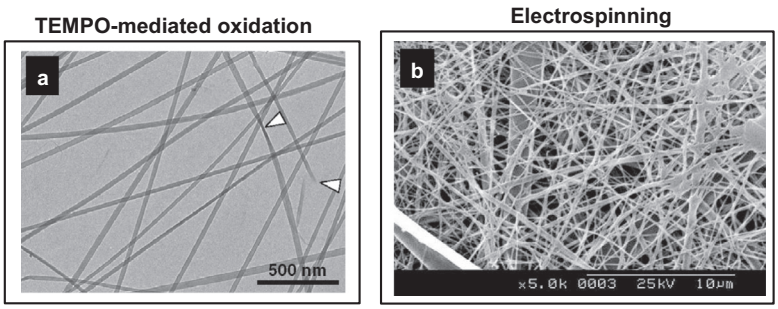

Fig. 8. Chitin nanofibers prepared by: (a) TEMPO-mediated oxidation using tubeworm (TEM image [30]); and (b) electrospinning (SEM image [85]). Reprinted with permission, (a) from Ref. [30]; and (b) from Ref. [85] Copyright 2009, 2004 Elsevier.

electrospinning [84] present very small width (8 nm) (Table 2).

Most recently, for the first time, chitin nanofibers were produced by electrospinning in a one-pot process directly from a 1-ethyl-3-methylimidazolium acetate (ionic liquid, IL) solution of chitin extracted from dried shrimp shells [73]. Summarizing, high molecular weight chitin from shrimp shells was extracted with IL *via* microwave dissolution followed by centrifugation to remove any insoluble materials. Finally, the supernatant was removed and loaded into a syringe for electrospinning. The obtained data suggest that this method provided the optimal viscosity, concentration, and necessary entanglement density required for the electrospinning of smooth, continuous chitin nanofibers. This can be attributed to the ability of 1-ethyl-3-methylimidazolium acetate to extract high molecular weight chitin from shrimp shells [73].

Compared with the previous approaches, this method presents some advantages: (i) eliminates the need of problematical chemicals; and (ii) reduces the energy and time needed to fabricate chitin nanofibers.

4. Engineered composite materials based on chitin nano-objects

Since the 2000s there has been increased research of chitin nano-objects as structural and functional agents in engineered composite materials. Their unique properties, including renewable and biodegradable character, small size, low density, large surface, chemical reactivity, biological activity, and non cytotoxicity make chitin nano-objects unique candidates for use in widespread range of medical applications [13,60,86], nanocomposite fields [8,12,13,58,60–62,64,65,78,86,87], food packaging [14,31,82,89–91], adsorption treatments [56,82], among others.

The use of chitin nano-objects in reinforcing thermoplastic nanocomposites was reported by Paillet and Dufresne in 2001 [11]. In this study chitin whiskers were employed as nano-size fillers in poly(styrene-co-butyl acrylate) matrix. Since then, various studies have been done using chitin nano-objects in different polymeric matrices, including poly(caprolactone) [62], natural rubber [46,88,92], soy protein isolate [47], poly(vinylalcohol) [64,80,93,94], chitosan [49–52,89,90,95,96], starch [61,97,98] and thermoplastic polyurethane [99]. The ensuing nanocomposite materials could be in the form of thin films, membranes, gels, foam and fibers. The great interest in chitin nano-objects as filler for polymer matrices come from their high stiffness combined with biological activity. Chitin nanofillers have shown to improve the mechanical performance of the resulting nanocomposites. For instance, Morin and Dufresne [62] have verified that chitin whiskers from *Riftia* tubes form a rigid network in a latex of poly(-caprolactone) matrix governed by a percolation mechanism. Moreover, the formation of this network allows the thermal stabilization of the material for temperatures higher than the melting temperature of the poly(caprolactone). Recently, Salaberria et al. [61] have designed and compared thermoplastic starch bionanocomposites based on chitin nanocrystals or nanofibers prepared *via* melt-mixing approach. The authors have demonstrated that the mechanical properties of the final bionanocomposites can be regulated as a function of the nano-size fillers load and morphology. The materials prepared with chitin nanofibers displayed better mechanical properties than those prepared with chitin nanocrystals because of their high aspect ratio and entangled nano-size fibrils. Chitin whiskers have also shown to improve the mechanical properties of chitosan-based nanocomposites. Chitin whiskers were used as an interlayer to fabricate the multilayered chitosan membrane through layer-by-layer method [51]. The tensile strength of the multilayered chitosan membranes reaches up to 122.8 MPa, which was about 2.5 times than that of neat chitosan membrane (*i.e.* 49.5 MPa). Nevertheless, the authors of these studies failed to evaluate the antimicrobial properties of their nanocomposite materials. Very little work has been done reporting the antimicrobial activity of chitin nano-objects. Nanocomposites with bactericidal activity against *Escherichia coli* and mechanical robustness were successfully prepared by the introduction of partially deacetylated chitin nanocrystals into bacterial cellulose networks. This green chemistry approach opens new application fields for chitin nanocrystals and a new generation of cellulosic nanocomposite materials with antibacterial activity [100]. Both chitin nanocrystals and nanofibers have showed antifungal activity against different fungi [88,96]. Ifuku et al. have verified that surface-deacetylated chitin nanofiber and the chitosan-based nano-composite films showed antifungal activity against *Alternaria alternate*. Additionally, the nanofibers worked effectively as reinforcement filler to improve the mechanical properties [88]. Salaberria and co-authors [96] have evaluated and compared the role of different nano-chitin morphologies (nanocrystals and nanofibers) on the structural and functional properties of thermoplastic starch-based films prepared by casting/evaporating approach. Their data demonstrated that the nanocomposite films elaborated with chitin nano-objects showed superior mechanical, thermal and barrier properties and antifungal activity against *A. niger* compared to unfilled thermoplastic starch films. These findings highlight the potential use of such chitin nano-objects in functional nanocomposite materials for packaging and medical applications.

Other interesting applications of chitin nano-objects are their use: (i) in the production of chitosan nano-scaffolds through the alkali treatment of chitin nanocrystals with 40% w/v NaOH solution at 150 °C for 7 h. The final chitosan nanoscaffolds showed porous structure [54,55,101]; (ii) in the stabilization of oil-in-water emulsions for creaming. Oil-in-water emulsions were generating by homogenizing chitin nanocrystals with corn oil. Due to the stabilizing ability of chitin nanocrystals (colloidal rod-like particles), the coalescence emulsions were stable for one month [53]; and (iii) in special textiles as antimicrobial agents [15].

5. Conclusion/outlook

This review opens up the different ways to transform chitin into spectacular nano-objects. These nano-objects are promising functional and structural agents for

exploring novel and unusual applications; they combine the unique properties of native chitin and the intrinsic characteristics of the nano-size materials (*i.e.* low density, large surface).

We expect that this review will provides insights on the isolation/production of these exploitable and stunning chitin nano-objects and contributes to a major breakthrough in chitin research and applications.

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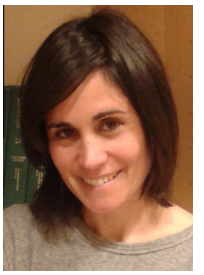

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