We are IntechOpen, the world's leading publisher of Open Access books

Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Quantitative Analysis by IR: Determination of Chitin/ Chitosan DD

Boukhlifi Fatima

Abstract

FTIR spectroscopy has been widely used to quantitatively study the parameters of the chitin deacetylation. A new research on a Canadian chitin has shown that a degree of deacetylation (DD) of 90% has been reached with a base concentration of 12.5 M, a reaction time of 120 min, and a temperature of 110°C. In parallel, our study on Moroccan chitin allowed to reach 75%. A degree of deacetylation of 75% was obtained at T = 120°C and at C_{NaOH} = 12 N in a single step for 6 hours. Another study followed by IR prepared the chitosan under pressure or under irradiation. Firstly, the compression method was used for preparing 100% deacetylated chitosan with less environmental pollution. The 100% fully deacetylated chitosan is produced in low-concentration alkali and high-pressure conditions under 0.11–0.12 MPa for 120 min. Secondly, microwave deacetylation showed that a degree of deacetylation of 95.19% was achieved after irradiating chitin at 60 meshes with 50% NaOH solution in a microwave for 10 min at 1400-W power. To find these results, the authors used different formulas to calculate DD by FTIRM, but the most used and reliable formula is that which calculates DD of chitosan by the report of absorbance of amide at 1655 cm^{-1} that measures the acetyl group and absorbance at 3430 cm^{-1} relating to the hydroxyl group.

Keywords: chitin, chitosan, DD, FTIR, optimization, synthesis

1. Introduction

Very commonly used in quantitative analysis, infrared spectrometry FTIR has been exploited in many fields. It has been used in mineralogy in addition to chemical analyzes to determine the structure of a rock and to know the bonds between the atoms, in pharmaceutical for the quantitative measurement of the constituents and the thickness of the coating of the tablets, in dairy industries to determine the moisture of milk powders and butters, in the textile industry for fiber sizing and maturity of cottons, in the chemical industry, in agribusiness, and in several other industries. When a specific absorption of a chemical function of a complex molecule is sufficiently isolated in its spectrum, it is always possible to carry out quantitative measurements. There are several major fields of application for infrared spectrophotometry. First, we can quote functional analysis is probably the main application of infrared spectrometry, at least in industry. Then, the structural analysis, infrared spectrometry allows to obtain even more fine information, concerning the

"construction of the molecular edifice." In organic compounds, it allows for example to differentiate the isomers of position (ortho-, meta-, and para-) aromatic hydrocarbons, as well as the cis- and transisomers of olefins. For mineral compounds, the infrared spectrum depends on the symmetry of molecules; it often allows finding the system in which a chemical compound is crystallized. It is also possible, but only in the case of small molecules, to calculate geometric parameters such as moments of inertia. The analysis is done by comparing with reference spectra of which there are several files. Also, FTIR spectroscopy is the method most used for calculating the degree of DD of chitosan. This polymer was discovered in 1859 by C. Rouget by treating chitin with concentrated KOH at elevated temperature. But it was not until 1894 that Hoppe-Seyler gave the "modified chitin" the name chitosan [1]. Chitosan has some advantageous properties, such as biocompatibility, biodegradable polymer of high molecular weight, nontoxic, and antimicrobial activity, that encourage its applications in many fields including agriculture [2, 3], paper industry, food and textile industries, pharmaceutics [4, 5], biochemistry, biotechnology, cosmetics, biomedical applications [6–8], environment, and water treatment [9–13]. The properties of chitin and chitosan depend considerably on the degree of deacetylation (DD), a parameter defined as the mole fraction of deacetylated units in the polymer chain [14, 15]. Therefore, the determination of DD has been one of the interesting parameters to study chitosan preparations. The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a complete amino group ($-NH_2$), and chitosan properties are very linked on this high degree of chemical reactive amino groups. Since the degree of deacetylation (DD) depends mainly on the method of purification and reaction conditions, it is therefore essential to characterize chitosan by determining its DD prior to its use. The main parameters involved in the process are temperature, time of reactions, and the concentration of reagents. A simple and nonexpensive chemical treatment of mineral/protein removal from chitin is usually used with HCl/NaOH reagents, respectively, and chitosan is chemically or enzymatically produced. They vary only on the acetyl group container, which is designated by the degree of acetylating (DA) designating the percentage of acetylated units relative to the number of total units. The term chitosan applies to any copolymer whose DD is greater than 50%. Each chitosan is therefore characterized by the fraction of residual N-acetamide groups (DA) or by the relative amount of amino groups of the chitosan molecule (DD = 1-DA) [16]. It is important to distinguish between the degree of acetylation (DA) and the degree of deacetylation (DD). One being the opposite of the other, that is to say that chitosan having an 85% DD, it has 15% of acetyl groups and 85% of amine groups on its chains. The degree of deacetylation (DD) of chitosan is a dominant structural parameter that significantly influences the physicochemical properties of chitosan such as solubility, overall charge, reactivity, and mechanical properties such as elongation, breaking, and tensile strength. This parameter also influences biological properties [17] such as biocompatibility and biodegradability. For determination of the degree of deacetylation (DD), several analytical methods have been employed. Infrared spectroscopy [18, 19] and UV spectrophotometry [20] as analytical tools offer advantage over other traditional techniques which are expensive and destructive to the sample. FTIR spectroscopy is a quick technique for a quantitative evaluation of the DD through the determination of absorption ratios. FTIR analysis is attractive due to its nondestructive character, fastness, sensitivity, and suitability for both soluble and nonsoluble samples. Among the solution methods, first-derivative UV spectrophotometry draws attention owing to its simplicity and effectiveness in providing accurate results for highly deacetylated chitin. It was conceived by Muzzarelli and

Rocchetti [20] and relies on simple reagents and instrumentation. In addition, the results obtained from this method are reasonably independent of protein contamination. Alonso et al. [21] established the possibility to determine the acetylation degree with the use of empirical correlations based on the weight losses associated with the main decomposition peaks. A similar approach has been adopted to investigate if there was any relationship between the weight loss of the sample and its DD. According to Yu et al. [22], the conductometric assay is an adequate and accurate method for determining the degree of deacetylation of chitosan, except for some samples that have a high degree of crystallization. The conductometric method can be carried out in basic and acid medium. Other methods have also been used such as SEM and NMR for magnetic properties of certain atomic nuclei and the determination of DD.

The objective of this chapter is to present a bibliographic synthesis on the use of spectroscopy FTIR in order to study and optimize the reaction of deacetylation by calculating the chitosan DD. At the end of the chapter, a simple comparison between IR and other methods of DD determination is presented to find the most reliable formula of DD calculation.

2. How to extract chitin

Chitin is the structural polymer of exoskeletons of all arthropods (crustaceans and insects) and endoskeletons of cephalopods (cuttlefish, squid, etc.). The cuticles of various crustaceans, mainly crabs and shrimp, are the main sources of raw material for the production of chitin (**Table 1**). Chitin is found as part of a complex network of proteins on which calcium carbonate is deposited to form the rigid shell in crustaceans or more specifically in shellfish. The interaction between chitin and proteins is very intimate and there is also a small fraction of proteins involved in a polysaccharide-protein complex [24]. Thus, the preparation of chitin from shellfish requires the elimination of the two main constituents, namely proteins by deproteinization and calcium carbonate by demineralization, as well as small amounts of pigments and lipids generally removed during the two steps. An additional fading step is applied to remove residual pigments. Many methods have been proposed and used over the years to prepare pure chitin; however, no standard method has been

Lower plants	Annelid	Mushrooms	Molluscs
Algae Lichen yeasts	Ascomycetes (Class) Penicillium Blastocladiales (Family) Chytridiaceae (Family)	Earthworm Leech	Cuttlefish Octopus
Arthropods			
Crustaceans	**Arachnids**	**Insects**	
Lobsters Crabs Shrimps Scampi Krill	Octopus Scorpions	Spiders Ants Cockroaches Coleoptera (Order)	

Table 1.
Sources of chitin [25].

adopted. Deproteinization and demineralization can be carried out using chemical or enzymatic treatments. In the case of shrimp, the shell wall is thinner, which facilitates the isolation of chitin compared to other types of shells. The selected shells are then cleaned, dried, and ground into small shell pieces. Shrimp carapaces have the following average mass composition:

- 75% water

- 12% protein

- 9% mineral salts

- 4% chitin

- traces of lipids and organic pigments

We then develop the two essential steps for the preparation of chitin from the carapaces, namely deproteinization and demineralization.

2.1 Chemical deproteinization

The deproteinization of chitin consists in eliminating proteins; it is difficult because there is a breakdown of the chemical bonds between chitin and proteins. This is done using basic solutions in a heterogeneous way. Complete protein isolation is particularly important for biomedical applications. A wide range of chemicals have been tested as deproteinization reagents, including NaOH, Na_2CO_3, $NaHCO_3$, KOH, K_2CO_3, $Ca(OH)_2$, Na_2SO_3, $NaHSO_3$, $CaHSO_3$, Na_3PO_4, and Na_2S. The reaction conditions vary considerably in each study. NaOH is the preferred reagent and is applied at a concentration ranging from 0.125 to 12 M, at different temperatures (up to 20°C) and a duration of treatment (from a few minutes to a few days). In addition to deproteinization, the use of NaOH results in partial deacetylation of chitin and hydrolysis of the biopolymer, which decreases its molecular weight.

2.2 Chemical demineralization

Demineralization is a necessary step to produce chitosan. It consists of dissolving minerals, mainly calcium carbonate bound to chitin. Demineralization is generally carried out by acid treatment using HCl, HNO_3, H_2SO_4, CH_3COOH, and HCOOH [26, 27]. Of these acids, the preferred reagent is dilute hydrochloric acid. Demineralization is an acid–base reaction between carbonate ions and acids in water with the release of carbon dioxide, as indicated in the following equation:

$$2\,HCl + CaCO_3 \rightarrow CaCl_2 + H_2O + CO_2 \tag{1}$$

All the other minerals present in the crustacean cuticle react in the same way and give soluble salts in the presence of acid. Then, the salts can be easily separated by filtering the solid phase of chitin, followed by washing with distilled water.

Chemically, a kilogram of fresh shells provides about 40 g of dry chitin. After the chemical treatment, the grinding and sieving processes to obtain a homogeneous chitin cause losses of about 40%. The final yield is 2.5%, 25 g of chitin per kilogram of shell. **Table 2** summarizes the operating conditions of chitin extraction according to different sources.

| | Deproteinization | | | | Demineralization | | | |
Source	C_{NaOH}*	T°C	Number of baths	Duration (h)	C_{HCL}	T°C	DURAT-ION (h)	
Shrimp	0.125 M 0.75 M	100 100	1 1	0.5 0.5	1.25 M	Room	1	[28]
Shrimp	1.25 M	100	1	0.5	1.57 M	20–22	1–3	[29]
Shrimp	1%	65	1	1	0.5 M	Room	—	[30]
Shrimp	3%	100	1	1	1 M	Room	0.5	[31]
Shrimp	4%	100	1	1	5%	Room	—	[32]
Shrimp	7.5–12.5 M		1	30–180 mn	0.3–3.5 M	Room	24	[23]
Shrimp	1 M	Room	1	24 h	1 M	Room	24	[3]
Crab	0.5 M	65	1	2	1.57 M	Room	5	[33]
Crab	1 M	80	1	3	1 M	Room	12	[34]
Crab	1 M	100	1	36	2 M	Room	48	[28]
Crab	1 M	100	3	72	1 M	Room	—	[35]
Crab	1.25 M	85–90	3	24	1.37 M	Room	24	[36]
Crab	1 M	50	1	6	1 M	20	3	[37]
Crab/ lobster	2.5 M	Room	3	72	11 M	−20	4	[38]
Lobster	1 M	100	5	12	2 M	Room	5	[39]
Lobster	5%	80–85	2	0.5	5%	70	4	[40]
Lobster	10%	100	1	2.5	10% HCl 90% formic	Room	18	[41]
Krill	0.875	90–95	1	2	0.6 M	Room	2	[42]
Krill	3.5%	25	1	2	3.5%	20	1.5	[43]
Crawfish						Room		[44]

Table 2.
Chitin extraction conditions.

3. How to extract chitosan from chitin

Chitosan represents a family of polymers obtained at varying degrees after deacetylation of chitin. In fact, the degree of acetylation (DA), which reflects the balance between the two types of residues (**Figure 1**), differentiates chitin from chitosan. When the DA (expressed as molar percentage) is less than 50 mol%, the product is called chitosan, and it is characterized by its solubility in acidic solutions [45]. During deacetylation, the amides are protonated and the acetyl groups are removed, but a depolymerization reaction, indicated by the changes in the molecular weight of the chitosan, is also produced.

Chitin can be converted to chitosan by enzymatic preparations [46–49] or by a chemical process [50, 51]. Chemical methods are widely used for commercial purposes for the preparation of chitosan because of their low cost and their ability to mass produce [51].
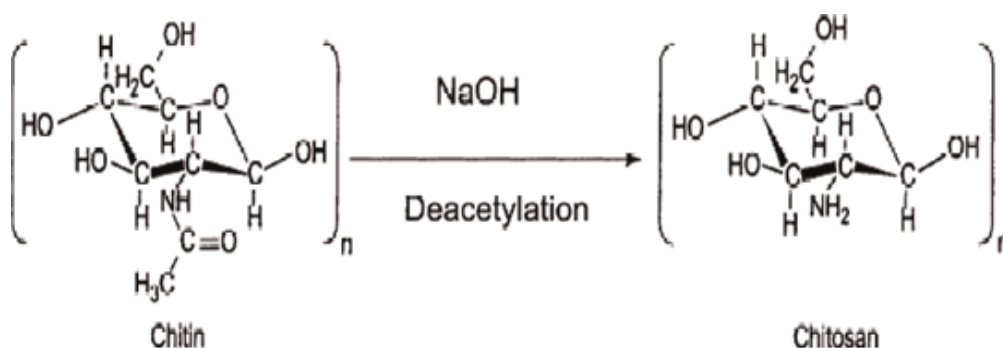
Figure 1.
Equation of chitin conversion to chitosan by deacetylation.

3.1 Chemical deacetylation

To deacetylate chitin chemically, acids or bases are used. However, glycosidic bonds are very sensitive to acid treatment [51, 52]; therefore, alkaline deacetylation is often used. The deacetylation reaction is heterogeneous [53] or homogeneous [54]. Generally, in the heterogeneous process, chitin is treated with hot concentrated NaOH solution for a few hours and chitosan is produced as an 85–99% deacetylated insoluble residue. According to the homogeneous method, the alkaline chitin is prepared after dispersion of the chitin in concentrated NaOH (30 g NaOH/45 g H_2O/3 g Chitin) at 25°C for 3 h or more and then dissolved in crushed ice around 0°C. This method gives a soluble chitosan with an average degree of acetylation of 48–55% [50]. This process produces a deacetylation with acetyl groups uniformly distributed in the chains, for example chitosan with DA = 10% after 580 h at 25°C [54].

The details of the process parameters employed by various researchers that include the number of demineralization, deproteinization, and deacetylation step have been reviewed and summarized in **Table 3**.

3.2 Enzymatic deacetylation

The main disadvantages of chemical deacetylation are energy consumption, waste concentrated alkaline solutions and thus increased environmental pollution. In order to avoid these disadvantages, an alternative enzymatic method exploiting chitin deacetylases has been explored. The use of chitin deacetylase offers the possibility of a nondegradable controlled process, leading to the production of well-defined chitosan [56]. This method is especially used to prepare chitosan oligomers.

Chitin deacetylase catalyzes the hydrolysis of N-acetamido linkages in chitin to produce chitosan. The presence of this enzymatic activity has been reported in several fungi [57, 58] and insect species [59]. The most studied enzymes are those extracted from the mushrooms Mucor rouxii [46, 56, 57], Absidia coerulea [60, 61], and Aspergillus nidulans [62, 63], and two strains of Colletotrichum lindemuthianum [64, 65]. All enzymes are glycoproteins and are secreted either in the periplasmic region or in the culture medium. In addition, all the enzymes exhibit remarkable thermal stability at their optimum temperature (50°C) and a very high specificity for the bound N-acetyl-D-glucosamine polymers.

4. How to follow the transformation reaction of chitin into chitosan (deacetylation) and calculate DD by IR spectroscopy

The determination of average DD for chitosan may be performed by different techniques: infrared spectroscopy, elementary analysis, potentiometric titration,

Source		Number of deproteinization baths	Number of demineralization baths	DA
		0.3 M; NaOH 80°C; 1 h	0.55 M HCl; 25°C; 2 h	
Cirripedia	Anatife	4	2	51
Reptantia	Red crab	3	5	48
Brachyura	Marbled crab	3	3	50
Reptantia	Spider crab	3	3	47
Macrura	Lobster	3	3	—
Natantia	Crayfish	7	3	51
	Slipper lobster	3	2	—
	Freshwater cayfish	3	2	—
	Pink shrimp	3	3	51
	Gray shrimp	2	2	51
Stomatopoda	Squilla	3	3	51
Cephalopoda	Squid	2	2	51

Table 3.
Comparison of chitosan production from different sources according to Tolaimate et al. [55].

and ^{1}H liquid state and solid state ^{13}C-NMR. But FTIR technique has specifically proved to be useful for the analysis of chitin due to its limited solubility in most of the solvents. Nevertheless, FTIR needs a calibration versus an absolute technique like nuclear magnetic resonance (NMR). Identifying the right combination of bands and baselines required a lot of effort, which led the authors to preface a large number of methods in the literature. In fact, several methods have been tried to determine the degree of deacetylation (DD) by FTIR [18, 66–69]. Below is a description of the major three types used to calculate DD by FTIR.

4.1 Formula 1

El ouahli [70] calculated from the absorption bands at 1320 and 1420 cm^{-1}. The first band is characteristic of the acetylated amine or amide function, while the second band is chosen as the reference band. The following equation is used to determine DD of chitosan:

$$DD\% = 100 - \left(\frac{A1320}{A1420} - 0.3822 \right) * 1/0.03133 \qquad (2)$$

4.2 Formula 2

This IR characterization formula for chitosan is based on the relationship between the absorbance (A) value of the primary amide at 1655 cm^{-1} and that of the hydroxyl at 3450 cm^{-1}. The degree of deacetylation (DD) was calculated by Eq. (3), [66, 69]:

$$DD\% = 100 - \left[\left(\frac{A_{1655}}{A_{3450}} \right) * 115 \right] \qquad (3)$$

4.3 Formula 3

In our study, a ratio had been already proposed [68, 71, 72]. However, choosing an appropriate calculation procedure was not an easy task since the choice of the baseline on FTIR spectra, reference, and the probe bands was difficult. In our work, the average DD was determined by the following formula:

$$DD\% = 100 - \left[\left(\frac{A_{1655}}{A_{3450}} \right) * \frac{100}{1.33} \right] \qquad (4)$$

where A_{1655} is the absorbance at 1655 cm^{-1} of the amide I band as a measure of the N-acetyl group content and A_{3430} is the absorbance at 3430 cm^{-1} due to hydroxyl group as an internal standard. The value 1.33 represents the ratio of this absorbance for a fully acetylated compound. An appropriate baseline in each spectrum was determined by using origin software.

The baseline problem for reference peaks has been studied and summarized in **Table 4** and **Figure 2**. **Table 4** shows that there are other formulas for calculating chitosan DD by FTIR and that these formulas have been approved by other techniques such as NMR.

A synthesis of the study on the effect of the operating conditions to know temperature concentration of the base and the time on the reaction of deacetylation will be presented using IR.

DA calibration curve	Method for DA standard	Ref.
1. DA = (A1655/A3450) × 155	Titration	[66]
2. (A1320/A1420) = 0.3822 + 0.03133 × DA	1H NMR, 13C NMR	[67]
3. (A1320/A3450) = 0.03146 + 0.00226 × DA	1H NMR, 13C NMR	[67]
4. (A1560/A2875) = 0.2 + 0.0125 × DA	Elemental analysis	[71]

Table 4.
Calibration curves from absorption ratios versus standard DA values [73].
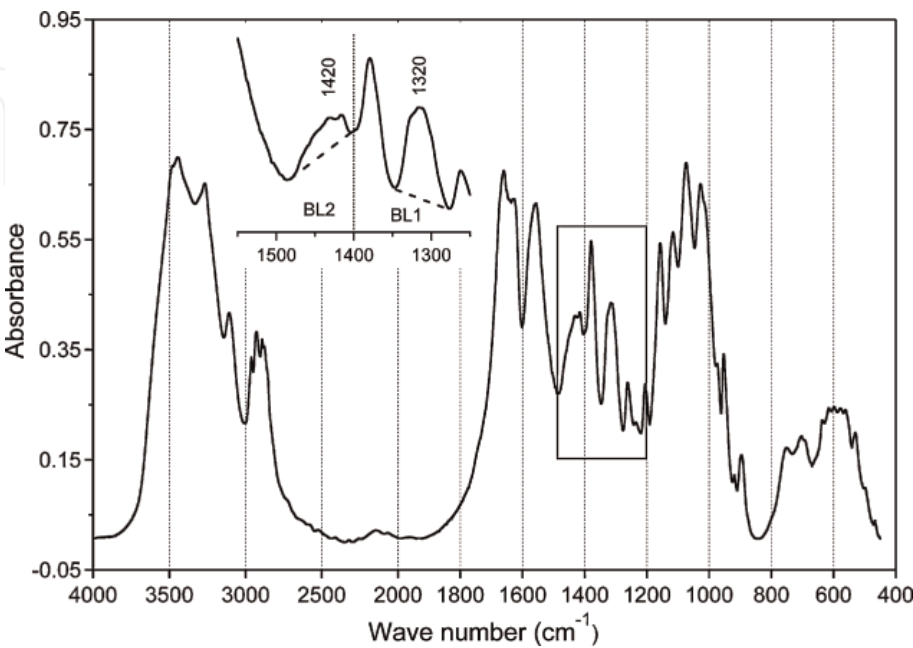

Figure 2.
FTIR spectrum of chitin and the baseline [73].

5. Optimization of chitosan extraction by IR

5.1 IR study of the effect of alkaline concentration on the chitin deacetylation

In this deacetylation reaction, it is an interrelation between the following variables: the concentration of the basic solution, the temperature, and the reaction time. The deacetylation reaction in basic medium is summarized in Eq. (5):

$$R\text{-}NHCOCH_3 + OH^- \rightarrow R\text{--}NH_2 + CH_3COO^- \qquad (5)$$
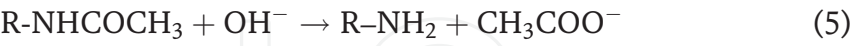
For C_{NaOH} = 12 N, the DD values calculated from the FTIR shown in **Table 5** do not exceed 55% and stabilize after a critical time t < 60 min. At a low concentration of NaOH, the equilibrium time became higher, which shows that the chitosan DD is low, which means that the deacetylation reaction is highly dependent on the concentration of NaOH, due to the inaccessibility of acetamide groups in the polymer chain. This type of behavior, observed by other authors, is explained firstly by the fact that the N-deacetylation occurs preferably at the level of the amorphous region of chitin and then passes from the edge to the interior of the crystalline region [26, 27]. The second reason concerns the equilibrium of the reaction and the degradation of chitosan. Other authors [10, 22, 28] have assumed that it can be controlled by both reaction and diffusion. The low deacetylation of chitin has also been attributed to the rearrangement of acetyl groups in the monomer unit with respect to the OH hydroxyl group [9]. Analysis of the results of the measurement of the DD (**Table 5**) shows that this increased by approximately 8% (66–74% DD), at a high concentration of NaOH (10 M) and with a thermal increase of 20°C (100–120°C). This variation of DD seems less important, only +2% (26–28% DD) at low concentration of NaOH (7.5 M). This increase in DD is particularly significant (13%) with a base concentration increase of 2.5 M (10 M to 12.5 M). Thus, the variation in NaOH concentration more significantly influences the reaction rate of DD than temperature.

5.2 IR study of the effect of temperature on the chitin deacetylation

Table 6 shows the effect of temperature on deacetylation; the more the temperature increases, the more the deacetylation increases, but the total conversion of

Temperature of DD	Concentration of the base (M)	Time (mn)	DD
25°C	8	180	38
	10	120	45
	12	60	55
80°C	4	38	35
	8	38	52
	10	38	55
	12	38	63
120°C	8	50 min	40
	12	50 min	50
	8	300max	65
	12	300max	70

Table 5.
Evolution of DD as a function of the concentration of the base at different temperatures [3].

Ref. [23]				Ref. [3]		
T°C/C_{NaOH} mol/l	7.5	10	12.5	C_{NaOH} mol/l	T°C	DD
40	—	1	2	8	180	38
60	10	14	20	10	120	45
80	24	60	67	12	60	55
100	26	68	78	8	180	38
120	28	74	90	10	120	45

Table 6.
Evolution of DD in relation to temperature at different concentrations of the base [3, 23].

chitin to chitosan is only carried out at the temperature of 80°C and for high concentrations of the base [23]. Analysis of the results of the measurement of DD shows that it has increased approximately 8% (66–74% DD), at high concentration of NaOH (10 M) and a thermal increase of 20°C (100–120°C). This variation of DD seems less important, only +2% (26–28% DD) at low concentration of NaOH (7.5 M). This discrepancy can be considered as an error of analysis. However, this increase in DD is particularly significant (13%) with a base concentration increase of 2.5 M (10–12.5 M). The temperature in this step is a factor that weakens the binding of the acetyl groups and accelerates the deacetylation reaction. Ahlafi and al [3] also found that the DDs increase as the NaOH concentration and temperature increase as the time of reaction increases. These reached a maximum of 63% and 71% for C_{NaOH} = 12 N at T = 80 and 120°C, respectively. On the other hand, it can be observed that the critical time decreases when the temperature and the concentration of NaOH increase. It can be seen in the case of C_{NaOH} = 12 N and T = 120°C. The increase of DD values with these parameters can be attributed to the change of the structure during deacetylation of chitin at height temperature, as confirmed by FTIR studies. These results confirm the hypothesis that the interaction of concentration and thermal energy are the main criteria to be taken into account for the deacetylation reaction.

5.3 FTIR study of the effect of time on the chitin deacetylation

The analysis of the results (**Table 7**) shows that the best deacetylation started after 60 min, for the three concentrations of NaOH studied. At this time, the activation energy for deacetylation can be reached and the chemical bond break occurs. However, this breakdown of the acetyl groups is significant only at the level of the high alkaline concentration (> 10 M). Diffusion of the alkaline solution on the shell substrate increases with time. These results confirm the hypothesis that there is an interaction between the concentration of NaOH and the reaction time on DD.

At the lowest NaOH concentration (7.5 M), the percentage of deacetylation increases from about 60°C but remains low even at 120°C (**Table 7**). The extracted product remains predominantly in the form of chitin. The deacetylation is terminated after a period ranging from 90 to 120min depending on the concentration of NaOH. Moreover, for a lower concentration of NaOH (8.75 M), this reaction is incomplete even after 3 h for different authors [74]. Therefore, in the case of a low concentration of NaOH (less than 10 M), the deacetylation does not occur completely. Thus, to obtain a good-quality chitosan, it is necessary to resume the treatment several times using NaOH solution [75, 76]. This reprocessing scheme is technically not easy and economically unviable on a large scale. So, for better deacetylation, 1 h is sufficient for concentrations greater than 10 M.

		[23]			120 °C [3]		
C mol/l/t mn	8.75	10	11.25	12.5	C mol/l/t mn	8	12
30	8	23	42	45	50	40	50
60	13	60	70	78	100	48	52.5
90	18	75	81	86	150	53	54
120	23	82	86	91	200	55	67
150	26.5	84	88	92	250	64	68
180	30	86	90	93	300	65	70

Table 7.
Evolution of DD as a function of time at different concentrations of the base [3, 23].

T(°C)	25	80	120
k_2 (min^{-1}) 2nd step	1.70×10^{-5}	2.23×10^{-4}	22×10^{-4}

Table 8.
Apparent rate constant [3].

The rate of deacetylation therefore depends not only on the concentration of NaOH used but also on the temperature for a breakdown of the acetyl bonds. However, a high temperature leads to a degradation of chitosan, which causes the viscosity η to drop and the molecular weight M (η = kM a) to drop, which affects the solubility of chitosan. The variation of the concentration of NaOH does not affect its molecular weight [76].

5.4 Determination of apparent rate constant k and energetic activation (Ea)

The chitin deacetylation process followed the pseudo–first-order kinetics for all the temperatures studied (25, 80, and 120°C) and at the same alkaline concentration (12 N) [3]. The **Table 8** showed that the values decreased as a function of temperature. This indicates that the speed of the deacetylation reaction is faster at the beginning of the reaction t < 60 min, but it was very slow at the end of reaction.

The apparent activation energy was estimated at about 48.76 kJ/mol from the straight line of the Arrhenius plot (in k vs. (1/T)) [3]. This value is in the same order of magnitude as that found by other authors for heterogeneous N-deacetylation performed between 80 and 120°C [10, 12, 22, 26]. The concentration of NaOH significantly influences the variation of the reaction temperature. Rinaud and al. [14] mention that at a NaOH concentration of 10–15 M, the energetic activation (Ea) of the deacetylation is, respectively, about 22–50 kJ/mol, which makes it possible to increase the degree of deacetylation. The results indicated that the reaction at higher concentration and temperature proceeded easier than that at their lower values.

5.5 Mechanism of the chitin deacetylation

The reagent diffusion mechanism represents the second step in deacetylation of chitin. Recently, Sarhan et al. [28] have proposed a mechanism in which heterogeneous N-deacetylation is controlled by both reaction and diffusion: the first step involves the reaction of the onium salt, designated (Q$^+$ X$^-$), with NaOH to give the

corresponding onium hydroxide ($Q^+ OH^-$) capable of diffusing from the aqueous phase to the organic phase to start the deacetylation process by attacking the C = O of the acetyl group and then at the end of the reaction of hydrolysis, the resulting onium acetate ($CH3COO^- Q^+$) will diffuse into the aqueous phase to be regenerated to a new onium hydroxide by the reaction with NaOH. From our results, the DD/C_{NaOH} ratios remain constant at each temperature at longer deacetylation times, which means that the deacetylation reaction is complete.

6. IR statistic study for optimization of deacetylation conditions

The results obtained under various conditions were analyzed statistically using multilinear regression analysis (uncertainty value =0.05) [23]. The NaOH concentration, temperature, and reaction time were chosen as independent parameters for the three-variable and three-level (maximum, mean, and minimum) factorial design. The best equation obtained for the chitosan extraction process in this study is Eq. (6). Shrimp at a higher temperature is not reliable. In addition, the production of chitosan at high temperature causes degradation of the container and therefore involves manipulations more difficult to adapt for industry. To ensure the quality of the chitosan product, the digestion temperature must be kept constant at 110°C.

$$\%DD = 72, 56 + 5, 6 * (NaOH) + 3, 78 * (temperature) + 24, 86 * (temps) - 11, 92 * (temps) \tag{6}$$

The values of this equation indicate that the effect of three factors studied influences DD in the following order: reaction time (24.86) > NaOH concentration (5.6) > reaction temperature (3.78). Further, a reaction time of 152 min ($\approx$2.5 h) is predicted from Eq. (5) to reach 90% DD using a NaOH concentration of 12.5 M and a temperature set at 110°C. This time predicted by the factorial plane seems consistent with the actual deacetylation reaction time (120 min) with an error of 27%.

The production of chitosan from dried shrimp exoskeletons can be done in 1 day instead of the 3 days required by the conventional method. The results obtained show that the extracted chitosan has a DD greater than 90% under optimal conditions by the hydrothermal-chemical technique in two stages at 110°C, that is, 11.25 M NaOH for 3 h or 12.5 M NaOH during 2 h. For the extraction of chitosan at an SD of 85% in the context of water treatment, the transformation procedure must be carried out under the following optimal conditions:

• Demineralization at 50°C for 2.5 h in 2 M HCl

• Deproteinization and deacetylation at 110°C with 11.25 M NaOH for 2 h

7. Deacetylation of chitin by compression method study by FTIR

A new method of producing very high DD content chitosan under low concentration alkaline conditions has been introduced by Xiaofei et al. [79]. The synthesis was produced at high temperatures and pressures. The results in **Table 9** were deduced from IR spectra. Compared to traditional methods, low concentration alkaline and short reaction time are excellent benefits. In addition, compared to the enzymatic and organic solvent treatment method, the pressure method was inexpensive and convenient without further purification. Excellent repeatability and simplified operation increased its availability in production and large application

C/%	CT:NaOH	RT/min	T/C	Productivity/%	DD/%
5	1:20	120	120	88.95	A
5	1:20	240	120	90.48	A
10	1:20	120	120	88.41	A
10	1:20	240	120	90.88	A
15	1:20	120	120	86.76	A
15	1:20	240	120	88.73	A
20	1:20	120	120	87.66	A
20	1:20	240	120	86.94	A
25	1:20	120	120	85.69	A
25	1:20	240	120	85.88	A
30	1:20	120	120	85.19	A
30	1:20	240	120	86.53	A
30	1:20	360	120	85.44	A
35	1:20	120	120	84.17	85.05
40	1:20	120	120	82.00	90.13
40	1:20	120	120	81.27	94.26
40	1:20	120	120	81.93	89.19

A: Undissolved in HCl.

Table 9.
The effect of different reaction conditions on DD value of chitosan [79].

chitosan scale with large quantity production. In order to produce chitosan with a very high content of DD by chitin in a more efficient and more environmentally friendly way, Xiaofei et al. [79] have changed the pressure and have used the multistep method for the deacetylation of chitin in alkaline at low concentration. They adopted the alkaline recycling model using alkaline waste in the next deacetylated step. In this model, they added an alkali according to the different demands of each phase and eliminated acetyl in a timely manner, in case the high concentration of the acetyl group would inhibit the deacetylation. In addition, the use of the base in several steps will be effective not only to control the degree of deacetylation but also to control the molecular weight of the resulting product.

Working under high pressure and with low concentrations of the base, they were able to extract chitosan with DD of 100%. In fact, only 15% of alkali solution and a ratio of 1:10 chitosan powder to NaOH solution and to a pressure of less than 0.11–0.12 MPa for 120 min lead to 100% DD. When the alkali concentration varies from 5–15%, the very high value DD chitosan (up to 95%) is produced. The method under pressure to prepare 100% deacetylated chitosan with less environmental pollution is very interesting.

8. Deacetylation of chitin under microwave irradiation effect

To extract a large quantity of chitosan, industrialists need high temperatures and chemicals in large quantities. In addition, the conventional process requires a lot of time and consumes a lot of energy, which would harm the environment. Recently, microwave irradiation has been used as an unconventional energy source in chemical reactions. The objective of this study is to synthesis chitosan under microwave

Chitin treatment	NaOH conc. (%)	DD%	Mw (k Daltons)	Solubility (%)
20 mesh	30	$67.58^F \pm 0.92$	2415.09	$66.31^H \pm 0.35$
	40	$75.77^E \pm 3.54$	1476.21	$74.94^G \pm 0.79$
	50	$78.83^D \pm 1.05$	1267.11	$96.77^{BC} \pm 0.17$
40 mesh	30	$76.89^{DE} \pm 0.89$	866.03	$99.05^A \pm 0.05$
	40	$78.64^D \pm 0.86$	1107.50	$92.79^D \pm 0.01$
	50	$83.05^C \pm 0.29$	2160.88	$95.60^C \pm 0.87$
60 mesh	30	$88.39^B \pm 0.49$	949.95	$83.28^F \pm 0.87$
	40	$89.17^B \pm 0.28$	1274.85	$85.57^E \pm 1.37$
	50	$95.19^A \pm 0.74$	4467.05	$97.73^{AB} \pm 0.95$
Commercial chitosan				
		$85.00^C \pm 0.66$	300	$99.00^A \pm 0.72$

Data are the mean.
Mean values in the same column bearing the same superscript do not differ significantly.

Table 10.
Degree of deacetylation (DD), molecular weight (mw), and solubility of chitosan samples extracted from shrimp wastes using microwave technique [81].

irradiation in order to reduce the impact of environmental pollution due to excessive use of chemical treatments [80–82]. The study will examine the effect of chemical addition, reaction time, operating temperature on manufacturing, and chitosan DD under microwave irradiation. These results will be compared to those from conventional heating methods to compare results. As part of this research, they developed the design and manufacture of a proton prototype for the production of chitosan from shrimp shell waste. Research has concluded that microwaves will accelerate reaction time.

The results showed that the demineralization condition of shrimp waste was achieved at the concentration of HCl 3,5 N solution with the weight ratio of shrimp shell waste and HCl solution of 1: 5 (w/v), at a temperature of 50°C during 1 h heating. In those conditions, the ash content was 8.06%. Ash content decreases to 5.4% if the demineralization reaction is carried out under microwave irradiation with 130 watts for 10 min. The optimum condition of the deproteinization process was achieved by heating at a temperature of 70°C for 2 h and at 4% NaOH concentration for shrimp waste ratio: a NaOH solution of 1:5 (w/v). In this condition, they obtained nitrogen levels of 1.882% (11.763% protein content). If the deproteinization reaction was performed under microwave irradiation with 130 W of power for 15 min, the nitrogen content obtained was 1.833% (11.461% protein content) (**Table 10**).

9. Comparison between IR and other techniques for calculating DD

To differentiate chitin from chitosan, it is necessary to define the degree of acetylation (DA), that is to say the ratio of the number of units comprising an acetyl group on the number of units in the molecule. We can also speak of degree of deacetylation (DD) such that: DD = 100 - DA in%.

The calculation of DD was made according to several methods described in the literature: acid and basic conductometric method, pH-metric method, UV method, and IR spectroscopy method. In a recent study [70], an attempt was made to

compare IR and other analytical methods to compute DD. The analysis was performed by acidic conductometric assay. Basic conductometric dosing and pH-metric dosing techniques will be compared to IR.

9.1 Determination of the degree of deacetylation by conductometric assay

According to Yu et al. [22], the conductometric assay is an adequate and accurate method to determine the degree of deacetylation of chitosan. It was carried out in basic and acid medium.

9.1.1 Basic conductivity measurement

A solution of chitosan was prepared by dissolving a mass of 150 mg of chitosan in 10 ml of hydrochloric acid (0.1 N) and then the volume was adjusted to 200 ml by addition of distilled water. The prepared solution is titrated with stirring with sodium hydroxide solution (0.1 N). **Figure 3A** shows the change in the volume of sodium hydroxide as a function of the conductivity of the chitosan solution. The curve has two points of inflection. The difference in the volume of NaOH between these two points corresponds to the amount of HCl required to dissolve the chitosan, which is to say to transform the $-NH_2$ groups into $-NH_3^+$.

The degree of deacetylation (DD) of chitosan is then determined from the following relationship [70]:

$$DD = 203 \times (V2 - V1) \times \frac{N}{m + 42 \times (V2 - V1) \times N} \times 100 \qquad (7)$$

where N is the normality of the NaOH solution (mol/l); V2 and V1 are the equivalent volumes of NaOH representing two inflection points, respectively; M is the mass of chitosan; 203 (g/mol) is the molar mass of the acetyl monomer; and 42 (g/mol) is the difference between the molecular weight of the acetyl monomer and the molecular weight of the deacetylated monomer.

$$DD = 203 \times (9.4 - 2.7).10 - 3 \times \frac{0.1}{0.15 + 42 \times (9.4 - 2.7).10 - 3 \times 0.1} \times 100$$

$$(8)$$

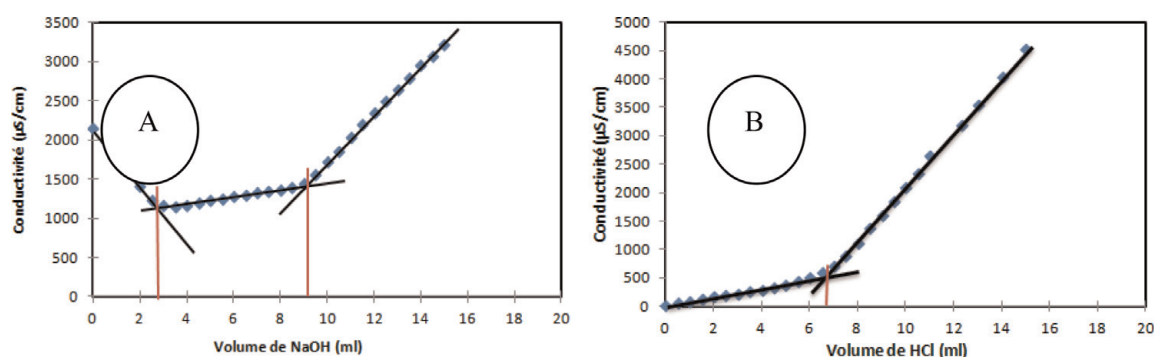The degree of deacetylation according to the conductometric method is: DD = 76.35%.

Figure 3.
(A) Variation of the conductivity of the chitosan solution as a function of the volume of the base (B) variation of the conductivity of the chitosan solution as a function of the volume of the acid solution.

9.1.2 Acidic conductivity dosing

The determination of the DD by acid conductometric assay is carried out as follows: a mass of 150 mg of chitosan is dispersed in 200 ml of distilled water; while stirring, the mixture is titrated with 0.1 N HCl solution. **Figure 3B** shows the evolution of the conductivity of the chitosan solution as a function of the volume of HCl poured. The point of inflection corresponds to the amount of HCl consumed by the amine groups of chitosan. The DD will be calculated from the following equation:

$$DD = (203 \times V \times N)/(m + 42 \times V \times N) \times 100 \qquad (9)$$

where N is the normality of the HCl solution (mol/l), V is the volume corresponding to the inflection point as shown in **Figure 3B**, m is the mass of chitosan (g), and 42 (g/mol) is the difference between the molecular weight of the acetylated monomer and the molecular weight of the deacetylated monomer.

DDA calculation gives:

$$DD = (203 \times 6.8 \times 10 - 3 \times 0.1)/(0.15 + 42 \times 6.8 \times 10 - 3 \times 0.1) \times 100$$
$$DD = 77.30\%. \qquad (10)$$

9.2 Determination of the degree of deacetylation by pH-metric determination

The determination of the DD by pH-metric assay was carried out according to the method described in the literature [77, 78].

A solution of chitosan was prepared by dissolving a mass of 125 mg of chitosan in a solution of excess HCl (0.1 N) and then neutralizing this solution with sodium hydroxide solution (0.05 N). **Figure 4A** shows the titration curve of chitosan, and **Figure 4B** shows the corresponding secondary derivative. From this last curve, the amount of hydrochloric acid necessary to protonate the amine groups is determined. The degree of deacetylation calculated from this method is 77.10%.

The results of the DDA obtained by the different assay methods are shown in **Table 11**.

The average value of DD calculated for the chitosan prepared during this work is 77.32%. So, IR spectroscopy remains the simplest and most economical technique for calculating DD.
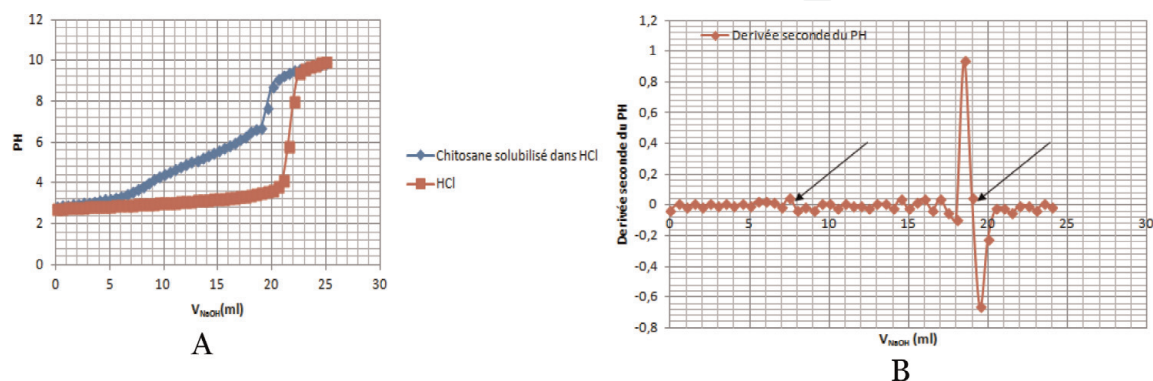

Figure 4.
(A) Variation of the pH according to the volume of soda poured for the solution of the chitosan and the solution of the hydrochloric acid, (B) secondary derivative curve corresponding to the assay.

Dosing method	DDA (%)
Basic conductivity meter	76.35
Conductometric acid	77.30
pH-metric	77.10
Infrared spectroscopy	78.50
Calculated average	77.32

Table 11.
DD values obtained with different assay methods [70].

Method	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
	Part-I				
DDTitration1	90.82	90.57	94.93	93.04	92.48
DDTitration2	92.85	94.62	93.97	95.40	94.99
DDTitration3	93.10	94.45	94.86	94.35	94.66
DD-UV1	95.97	95.96	99.42	98.18	99.34
DD-UV2	94.44	94.87	97.72	97.07	97.00
DD-UV3	95.02	95.36	98.07	97.42	97.99
	Part-II				
DD-UV1	82.00	95.96	97.68	100.00	100.00
DD-UV2	82.27	95.86	97.73	100.00	100.00
DD-IR1658/3450	80.43	94.50	94.60	95.37	96.33
DD-IR1320/1420	87.64	100.00	100.00	100.00	100.00

Table 12.
Comparison of DD value measured by different methods [79].

9.3 Comparison to UV: visible

Another study presented by Xiaofei and al [79] gave a comparison between IR spectroscopy and other techniques and showed the importance of FTIR for calculating DD (**Table 12**). They have been measured for 3 or 2 times.

10. Conclusion

The calculation of DD and the number of amine present in chitosan by FTIR allows first to follow the transformation reaction of chitin into chitosan and others by finding the optimal conditions for the synthesis of chitosan by studying the effect of different parameters, namely the concentration of the base, the temperature, and the duration of the reaction. The valorization of shrimp exoskeletons by extraction of chitosan according to the hydrothermal-chemical technique proposed in two stages makes it possible to reduce the production time by at least four times compared to the conventional technique in three stages (3–4 days). In addition, the consumption of digestion and energy chemicals is also significantly reduced. The chitosan obtained by the two-step technique is of good quality. Indeed, the degree

of deacetylation is greater than 90% under the optimal conditions for the simultaneous deproteinization and deacetylation.

The compression method for preparing 100% deacetylated chitosan with less environmental pollution was studied by FTIR. The 100% fully deacetylated chitosan was produced in low-concentration alkali and high-pressure conditions, which only requires 15% alkali solution and 1:10 chitosan powder to NaOH solution ratio under 0.11–0.12 MPa for 120 min. When the alkali concentration varied from 5–15%, the chitosan with ultra-high DD value (up to 95%) is produced.

In parallel, the FTIR calculation was also used to show that the microwave could be used in the extraction step of chitosan from chitin. From these results, it could be concluded that shrimp waste is an excellent source for chitin, and the yields of chitosan increased with decreasing the chitin particle size and increasing the concentration of NaOH solution used in deacetylation step. The highest degree of deacetylation was obtained from chitin samples at particle size of 60 mesh deacetylated by 50% NaOH solution, and it was 95.19% compared with 85% for commercial chitosan. Based on this synthesis, it is concluded that FTIR is an effective and reliable technique for the determination of DD and the study of the deacetylation reaction of chitin. All the formulas quoted are valid for calculating DD of chitosan, but the most reliable formula and the formula most approved by other techniques is formula 3 quoted in our works.

Author details

Boukhlifi Fatima
Equip, Materials and Applied Catalysis (MCA), Faculty of Science, Moulay Ismail University, Meknes, Morocco

*Address all correspondence to: boukhlifi1@yahoo.fr

IntechOpen

References

[1] Okazaki S, Tachibana T, Naganuma A, Mano N, Kuge S. Multistep disulfide bond formation in Yap1 is required for sensing and transduction of H_2O_2 stress signal. Molecular Cell. 2007;**27**(4):675-688

[2] Boukhlifi F, Mamouni FZ, Razouk R. Chitin/Chitosan's bio-fertilizer: Ch 16: Usage in vegetative growth of wheat a nd potato crops. In: Dongre RS, editor. Chitin-Chitosan – Myriad Functionalitie s in Science and Technology. Rijeka: IntechOpen; 2018. p. 75208

[3] Ahlafi H, Moussout H, Boukhlifi F, Echetna M, Naciri Bennani M, Slimane SM. Kinetics of N-deacetylation of chitin extracted from shrimp shells collected from coastal area of Morocco. Mediterranean Journal of Chemistry. 2013;**2**(3):503-513

[4] Ouattara B, Simard RE, Piette G, Begin A, Holley RA. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. International Journal of Food Microbiology. 2000;**62**:139-148

[5] Muzzarelli RAA, Muzzarelli C. Chitosan chemistry: Relevance to the biomedical sciences. Advances in Polymer Science Journal. 2005;**186**: 151-209

[6] Alves MN, Mano JF. Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. International Journal of Biological Macromolecules. 2008;**43**:401-414

[7] Dzung NA. Chitosan and chitosan derivatives as potential adjuvants for influenza vaccine. In: Kim SK, editor. Chitin and Chitosan Derivatives: Advances in Drug and Discovery and Developments. CRC Taylors & Francis; 2014

[8] Ngo DN, Kim SK. Antioxidant, antimicrobial properties of chitin, chitosan, and their derivatives. Advances in Food and Nutrition Research. 2014;**73**:15-31

[9] Boukhlifi F, Bencheikh A. Study of the competitive adsorption of heavy metals on crude chitin: Application to wastewater from a chemical industry. The Water Tribune. 2001;**55**(611/3): 37-43

[10] Boukhlifi F, Bencheikh A. Characterization of natural biosorbents used for the depollution of waste water. Annales de Chimie Science des Materiaux. 2000;**256**:153-160

[11] Boukhlifi F. Study of the retention of metallic micro-pollutants (Pb, Cd, Cu and Zn) on new biosorbent materials: liquid industrial effluent purification tests [doctoral thesis]. Morocco: Chouaib Doukkali El University jadida; 2000

[12] Boukhlifi F, El Akili C, Moussout H, Benzakour A, Ahlafi H. Treatment of global rejection of electroplating industry by raw chitin. International Journal of Applied Environmental Sciences. 2013;**8**:13-23

[13] Boukhlifi F, Bencheikh A, Ahlafi H. Characterization and adsorption of chitin toward copper Cu^{2+}. Physical and Chemical News. 2011;**58**:67-72

[14] Rinaudo M, Milas M, Ledung P. Characterization of chitosan—Influence of ionic-strength and degree of acetylation on chain expansion. International Journal of Biological Macromolecules. 1993;**15**(5):281-285

[15] Wei W, Bo SQ, Li SQ, Wen Q. Determination of the mark–Houwink equation for chitosans with different degrees of desacetylation. International

Journal of Biological Macromolecules. 1991;**13**(5):281-285

[16] Dash M, Chiellini F, Fernandez EG, Piras AM. Statistical approach to the spectroscopic determination of the desacetylation degree of chitins and chitosans. Carbohydrate Polymers. 2011;**86**:65-71

[17] Chatelet O, Damour A, Domard A. Influence of the degree of acetylation on some biological properties of chitosan films. Biomaterials. 2001;**22**:261-268

[18] Domszy JG, Roberts GAF. Evaluation of infrared spectroscopic techniques for analyzing chitosan. Makromolekulare Chemie-Macromolecular Chemistry and Physics. 1985;**186**(8):1671-1677

[19] Sannan T, Kurita K, Ogura K, Iwakura Y. Studies on chitin. 7. Infrared spectroscopic determination of degree of desacetylation. Polymer. 1978;**19**(4): 458-459

[20] Muzzarelli RAA, Rocchetti R. Determination of the degree of acetylation of chitosans by 1st derivative ultraviolet spectrophotometry. Carbohydrate Polymers. 1985;**5**(6): 461-472

[21] Alonso IG, Peniche-Covas C, Nieto JM. Determination of the degree of acetylation of chitin and chitosan by thermal-analysis. Journal of Thermal Analysis. 1983;**28**(1):189-193

[22] Yu JH, YM D, Zheng H. Blend films of chitosan/gelatin. Journal of Wuhan University (Natural Sciences Edition). 1999;**45**:440-444

[23] Truong TO, Hausler R, Monette F, Niquette P. Valorisation des résidus industriels de pêches pour la transformation de chitosane par technique hydrothermochimique. Revue des Sciences de l'Eau. 2007;**20**(3): 253-262

[24] Horst MN, Walker AN, Klar E. The pathway of crustacean chitin synthesis. In: Horst MN, Freeman JA, editors. The Crustacean Integument: Morphology and Biochemistry. Boca Raton, FL: CRC, USA; 1993. pp. 113-149

[25] Younes I, Rinaudo M. Review chitin and chitosan preparation from marine sources. Structure, properties and applications. Marine Drugs. 2015;**13**: 1133-1174

[26] No HK, Hur EY. Control of foam formation by antifoam during demineralization of crustacean shell in preparation of chitin. Journal of Agricultural and Food Chemistry. 1998; **46**:3844-3846

[27] Percot A, Viton C, Domard A. Characterization of shrimp shell deproteinization. Biomacromolecules. 2003;**4**:1380-1385

[28] Muzzarelli RAA, Tanfani F, Emanuelli M, Gentile S. The chelation of cupric ions by chitosan membranes [Callinectes sapidus, blue crab shell]. Journal of Applied Biochemistry. 1980; **2**:380-389

[29] Moorjani MN, Achutha V, Khasim DI. Parameters affecting the viscosity of chitosan from prawn waste. Journal of Food Science and Technology. 1975;**12**:187-189

[30] Wu ACM, Bough WA. A study of variables in the chitosan manufacturing process in relation to molecular-weight distribution, chemical characteristics and waste-treatment effectiveness. In: Proceedings of the 1st International Conference on Chitin/Chitosan, Boston, USA. 1977

[31] Bough WA, Salter WL, Wu ACM, Perkins BE. Influence of manufacturing variables on the characteristics and effectiveness of chitosan products 1. Chemical composition, viscosity, and molecular-weight distribution of

chitosan products. Biotechnology and Bioengineering. 1978;**20**:1931-1943

[32] Sluyanarayana Rao SV, Yashodha KP, Mahendrakar NS, Puttarajappa P. Deacetylation of chitin at low temperature by a novel alkali impregnation technique. Indian. Journal of Technology. 1987;**25**:194-196

[33] Mima S, Miya M, Iwamoto R, Yoshikawa S. Highly desacetylated chitosan and its properties. Journal of Applied Polymer Science. 1983;**28**: 1909-1917

[34] Shimahara K, Ohkouchi K, Ikeda M. In: Roberts GAF, editor. Chitin Chemistry. London, UK: Macmillan Press; 1992. p. 56

[35] Younes I, Rinaudo M. Review chitin and chitosan preparation from marine sources. Structure, properties and applications. Marine Drugs. 2015;**13**: 1133-1174

[36] Broussignac P. Un haut polymère naturel peu connu dans l'industrie, Le chitosane. Chimie and Industrie Genie Chimique. 1968;**99**:1241-1247

[37] Hakman RH, Goldberg M. Light-scattering and infrared-spectrophotometric studies of chitin and chitin derivatives. Carbohydrate Research. 1974;**38**:35-45

[38] BeMiller JN, Whistler RL. Alkaline degradation of amino sugars. The Journal of Organic Chemistry. 1963;**27**: 1161-1164

[39] Hakman RH, Goldberg M. Light-scattering and infrared-spectrophotometric studies of chitin and chitin derivatives. Carbohydrate Research. 1974;**38**:35-45

[40] Blumberg R, Southall CL, van Rensburg NJ, Volckman OB. South African fish products. XXXII—The rock lobster: A study of chitin production

from processing wastes. Journal of the Science of Food and Agriculture. 1951;**2**: 571-576

[41] Muzzarelli RAA, Priser ER, editors. Mit Sea Grant Program. MA, USA: Cambridge; 1978. pp. 54-63

[42] Anderson GG, de Pablo N, Romo C. Antartic krill (*Euphausia superba*) as a source of chitin and chitosan. In: Proceedings of First International Conference on Chitin and Chitosan; 2001

[43] Brzeski MM. Concept of chitin chitosan isolation from Antartic krill (*Euphausia superba*) shells on a technical scale. In: Hirano S, Tokura S, editors. Proceedings of the Second International Conference on Chitin and Chitosan. Sapporo, Japan: The Japan Society of Chitin and Chitosan; 1982. pp. 15-29

[44] Tolaimate A, Desbrieres J, Rhazi M, Alagui A. Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties. Polymer. 2003;**44**:7939-7952

[45] Roberts GAF. Structure of chitin and chitosan. In: Roberts GAE, editor. Chitin Chemistry. London, UK: Palgrave Macmillan; 1992. pp. 85-91

[46] Kurita K, Sannan T, Iwakura Y. Studies on chitin, 4: Evidence for formation of block and random copolymers of N-acetyl-D-glucosamine and D-glucosamine by hetero- and homogeneous hydrolyses. Makromolekulare Chemie. 1977;**178**: 3197-3202

[47] No HK, Meyers SP. Preparation and characterization of chitin and chitosan —A review. Journal of Aquatic Food Product Technology. 1995;**2**:27-52

[48] Kafetzopoulos D, Martinou A, Bouriotis V. Bioconversion of chitin to chitosan: Purification and characterization of chitin deacetylase

from Mucor rouxii. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**: 2564-2568

[49] Aiba SI. Preparation of N-acetylchitooligosaccharides by hydrolysis of chitosan with chitinase followed by N-acetylation. Carbohydrate Research. 1994;**265**: 323-328

[50] Hajji S, Younes I, Ghorbel-Bellaaj O, Hajji R, Rinaudo M, Nasri M, et al. Structural differences between chitin and chitosan extracted from three different marine sources. International Journal of Biological Macromolecules. 2014;**65**:298-306

[51] Chang KLB, Tsai G, Lee J, Fu WR. Heterogeneous N-desacetylation of chitin in alkaline solution. Carbohydrate Research. 1997;**303**:327-332

[52] Sannan T, Kurita K, Iwakura Y. Studies on chitin, 2. Effect of desacetylation on solubility. Makromol Chem. 1976;**177**:3589-3600

[53] Van de Velde K, Kiekens P. Struc ture analysis and degree of substitution of chitin, chitosan and dibutyrylchitin b y FT-IR spectroscopy and solid state 13C NMR. Carbohydrate Polymers. 2004;**58**: 409-416

[54] Chen R, Wang X, Yao X, Zheng X, Wang J, Jiang X. Near-IR-triggered h otothermal/photodynamic dual-moda lity therapy system via chitosan hybrid nanospheres. Biomaterials. 2013;**34**(33): 8314-8322

[55] Tolaimate, A. Exploration des gisements chitineux de la faune marine marocaine. Procédé d'extraction de chitines fortement acétylées. Préparation de chitosanes à caractéristiques contrôlées. [Ph.D. Dissertation] Marrakech, Maroc: Cadi Ayyad University; 2000

[56] Tsigos I, Martinou A, Kafetzopoulos D, Bouriotis V. Chitin deacetylases: New, versatile tools in biotechnology. Trends in Biotechnology. 2000;**18**:305-312

[57] Araki Y, Ito E. A pathway of chitosan formation in Mucor rouxii: Enzymatic desacetylation of chitin. European Journal of Biochemistry. 1975; **189**:249-253

[58] Martinou A, Kafetzopoulos D, Bouriotis V. Isolation of chitin deacetylase from Mucor rouxii by immunoaffinity chromatography. Journal of Chromatography. 1993;**644**: 35-41

[59] Sundara RG, Aruchami M, Gowri N. Natural desacetylation of chitin to chitosan in the abdominal cuticle of the physogastric queen of Macrotermes estherae. In: Tokura S, Hirano S, editors. Proceeding Second International Conference Chitin/Chitosan, Sapporo, Japan, 12–14 July 1982. Tottori, Japan: Japanese Soc. Chitin; 1982

[60] Gao XD, Katsumoto T, Onodera K. Purification and characterization of chitin deacetylase from *Absidia coerulea*. Journal of Biochemistry. 1995;**117**: 257-263

[61] Younes I, Nasri R, Bkahiria I, Jellouli K, Nasri M. New proteases extracted from red scorpionfish (*Scorpaena scrofa*) viscera: Characterization and application as a detergent additive and for shrimp waste deproteinization. Food and Bioproducts Processing. 2014;**94**:453-462. DOI: 10.1016/j.fbp.2014.06.003

[62] Kaur S, Dhillon GS. Recent trends in biological extraction of chitin from marine shell wastes: A review. Critical Reviews in Biotechnology. 2015;**35**: 44-61

[63] Boukhlifi F, El Akili C, Moussout H, Benzakour A, Ahlafi H. Treatment of

global rejection of electroplating industry by raw chitin. International Journal of Applied Environmental Sciences. 2013;**8**:13-23

[64] Tsigos I, Bouriotis V. Purification and characterization of chitin deacetylase from Colletotrichum lindemuthianum. The Journal of Biological Chemistry. 1995;**270**: 26286-26291

[65] Tokuyasu K, Kameyama MO, Hiyashi K. Purification and characterization of extracellular chitin deacetylase from Colletotrichum lindemuthianum. Bioscience, Biotechnology, and Biochemistry. 1996; **60**:1598-1603

[66] Baxter A, Dillon M, Taylor KDA, Roberts GAF. Improved method for IR determination of the degree of N-acetylation of chitosan. International Journal of Biological Macromolecules. 1992;**14**(3):166-169

[67] Brugnerotto J, Lizardi J, Goycoolea FM, Arguelles-Monal W, Desbrieres J, Rinaudo M. An infrared investigation in relation with chitin and chitosan characterization. Polymer. 2001;**42**(8):3569-3580

[68] Duarte ML, Ferreira MC, Marvao MR, Rocha J. An optimised method to determine the degree of acetylation of chitin and chitosan by FTIR spectroscopy. International Journal of Biological Macromolecules. 2002;**31**(1–3):1-8

[69] Khan TA, Peh KK, Ch'ng HS. Reporting degree of desacetylation values of chitosan: The influence of analytical methods. Journal of Pharmacy and Pharmaceutical Sciences. 2002;**5**(3): 205-212

[70] ELOUAHLI Abdelaziz Préparation et caractérisation de poudre pure de phosphate tricalcique. Application à la mise au point d'un nouveau nano-composite phosphate tricalcique apatitique/chitosane pour usage orthopédique et dentaire; 2017. THESE Présentée A Universite chouaib doukkali Faculte des sciences El jadida

[71] Kasaai MR. A review of several reported procedures to determine the degree of N-acetylation for chitin and chitosan using infrared spectroscopy. Carbohydrate Polymers. 2008;**71**: 497-508

[72] Ren D, Yi H, Wang W, Xiaojun M. The enzymatic degradation and swelling properties of chitosan matrices with different degrees of N-acetylation. Carbohydrate Research. 2005;**340**: 2403-2410

[73] Dash M, Chiellini F, Fernandez EG, Piras AM, Chiellini E. Statistical approach to the spectroscopic determination of the desacetylation degree of chitins and chitosans. Carbohydrate Polymers. 2011;**86**:65-71

[74] Miya M, Iwamoto R, Mima S. FTIR study of intermolecular interactions in polymer blends. Journal of Polymer Science. 1984;**22**:1149-1151

[75] Fenton DM, Eveleigh DE. Purification and mode of action of a chitosanase from *Penicillium islandicum*. Journal of General Microbiology. 1981; **126**:151-165

[76] Wu ACM. Determination of molecular-weight distribution of chitosan by high-performance liquid chromatography. Methods in Enzymology. 1988;**161**:447-452

[77] Tolaimate A, Desbrieres J, Rhazi M, Alagui A. Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties. Polymer. 2003;**44**:7939-7952

[78] El-Sherbiny IM. Synthesis, characte rization and metal uptake capacity of a

new carboxymethyl chitosan derivative. European Polymer Journal. 2009;**45**: 199-210

[79] He X, Li K, Xing R, Liu S, Hu L, Li P. The production of fully desacetylated chitosan by compression method. Egyptian Journal of Aquatic Research. 2016;**42**:75-81

[80] Titik D, Susanto H, Rokhati N. Influence of microwave irradiation on extraction of chitosan from shrimp. Shell Waste Reaktor. 2018;**18**(1):45-50

[81] Samar M, Khaloufi M. Physicochemical, functional antioxidant and ntibacterial properties of chitosan extracted from shrimp wastes by microwave technique. Annals of Agricultural Science. 2013;**500**(1):33-41

[82] Horowitz ST, Roseman S, Blumental HJ. Preparation of glucosamine oligosaccharides. 1. Separation. Journal of the American Chemical Society. 1957;**79**:5046-5049