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Chitin and chitosan: study of the possibilities of their production by valorization of the waste of crustaceans and cephalopods rejected in Essaouira

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Abstract

In this paper, we study the possibility of valorization of the waste of crustaceans and cephalopods, rejected by the seafood restaurants in Essaouira, by the production of chitin and chitosan. It is part of the continuity of previous works which have been interested in the exploration of the chitinous sources of the Moroccan coast and in the preparation of chitins and chitosan with controlled physico-chemical characteristics. We realized an investigation about fishing activity in Essaouira and the quantities of waste from marine sources that could be valorized, Sources chosen further to this investigation were the object of reactions of extraction and of N-deacetylation to prepare the chitin and the chitosan. The followed processes are chosen to obtain products of good quality; in particular chitosan of varied molar weight but highly deacetylated (low degree of acetylation DA) were targetted. An estimation of the annual quantities of chitin and chitosan can be produced only from the waste of restaurants in this small coastal city will be presented.

1. Introduction

Chitin is the second most abundant natural polymer after cellulose; it exists especially in Arthropods (crustaceans, mollusks, insects ...) in the animal kingdom and in the fungi in the plant's kingdom. In the industry, it is mainly produced from the shells of crabs and shrimps, in these cases it is α -chitin. When ß -chitin that is desired, the squid pens that are used. Chitin consists of a linear chain of (1 → 4) linked 2-acetamido-2-deoxy-β-D-glucopyranose units. Chitosan is its deacetylated derivative [1,2] (Figure 1).In most of the studied cases, the monomers of chitin will not be,in fact, all acetylated, and similarly for chitosan, the monomers will not all be deacetylated. In fact, chitin and chitosan are distinguished from one another by their degree of acetylation (DA), which reflects the relative proportion of N-acetyl-glucosamine monomers present in the polymer. When the DA is greater than 50%, the polymer is practically insoluble in dilute acid solutions and corresponds to chitin. When the DA is lower than 50 %, the polymer is soluble in these acid solutions and the name of chitosan is attributed to it [3].

The properties of the chitin and especially the chitosan (fixation of the negative electrolytes, biodegradability, oxygen permeability, stimulating of the immune system …) allow numerous applications in so varied and diversified domains that those of the water treatment (removing pesticides from water)[4,5], the biomedical, of cosmetic; The list of uses of these substances is long and could be endless [6].

2. Experimental and technical methods of analysis

2.1.*Investigation for the estimation of waste of crustaceans and cephalopods susceptible to be exploited to Essaouira*

First, we were interested to the quantities of marine chitinous sources (crustaceans and cephalopods) recorded at the port of Essaouira. The port services provided us with the productivity statistics for 2012 and 2013. According to these statistics we have concluded that the productivity of shrimp is the most important in Essaouira (as a chitinous source) followed by the varieties of squid and then the cuttlefish.

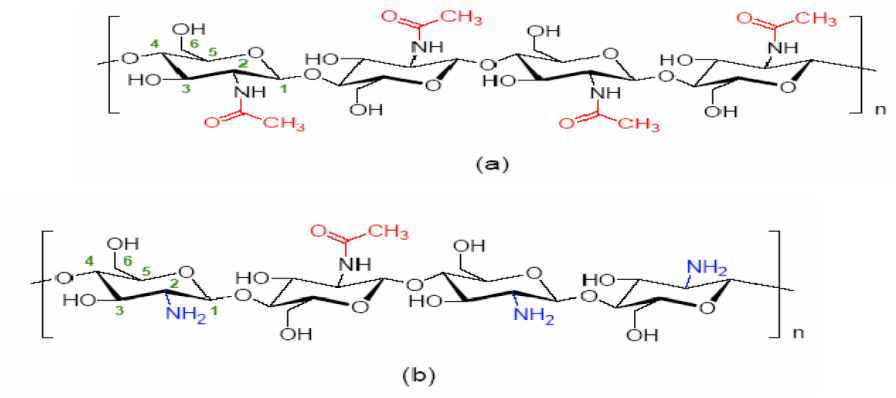

Figure 1: comparison of the structures of chitin (a) and the chitosan (b)

These data, although they give an idea of the productivity of chitinous sources in Essaouira, do not allow to estimate the quantities of waste in the city likely to be valorized by chitinous derivatives. This is due to the fact that a large part of these products is either exported or marketed in other cities of Morocco.
We extended our investigation to get an idea about the sources of the chitinous waste discharged in the city.
In the absence of industrial units (shrimp decortication, squid treatment), we turned to the chefs of the main restaurants of the city (5 restaurants), who agreed to receive us and answer our questions.

2.2. Chitin isolation

To extract the chitin from the chosen marine sources, following the results of the investigation, we adopted the process of isolation developed by Rhazi *et al* [7,8] and Tolaimate *et al.* [9,10,11,12]. This makes possible to obtain pure chitins that are highly acetylated and very similar to their native form.
The demineralization is carried out at room temperature using repeated baths of hydrochloric acid (0.55 M), 100 mL of acid are used per 10 g of raw material. The number of baths and their duration (between 15 and 60 min) depend upon the source.
The reaction is followed by measuring the pH of the reaction medium, the evolution toward neutrality reflects the consumption of the quantity of acid in the bath and indicates that there is a need for a new treatment. The end of the repeated series of baths was indicated by the persistence of acidity in the medium.
Once the raw material is demineralised, we proceed to deproteinization. Deproteinization is carried out using the principle of repeated alkaline baths. The NaOH concentration of each bath is 0.3M and the reaction is carried out at a temperature between 80-85 ° C. The number of alkaline baths and their duration is determined according to the evolution of the color of the reaction medium. At the end of the last bath, the recovered solution must be colorless. When the extracted chitin sometimes remains slightly colored, the bleaching step can be carried out using H_2O_2 in an acid medium.

2.3. Preparation of chitosans

According to all the results of the previous work [7-12], we adopted the following conditions for preparing the chitosan:

- For β-chitin from squid and cuttlefish we used NaOH 40%, at 80 ° C, under a nitrogen atmosphere, in the presence of $NaBH_4$. The reaction is carried out in three steps of three hours each [7].
- As for the α-chitin fromthe squilla, the spider crab and the pink shrimp, we applied the following processes:
    - ✓ The Broussignac process [13]; using a glycol-potassic mixture consisting of KOH (50%), ethanol (25%), monoethylene glycol (25%), at a temperature between 110 °C- 120 °C, in a single step. The duration varies depending on the source.
    - ✓ The Kurita process [14]; using NaOH (50%), at a temperature between 110 °C- 120 °C, in three steps of three hours each.

2.4 Characterization of prepared chitin and chitosan

Chitins and chitosans are prepared according to the processes described in a previous work by Tolaimate *and al* [9-12] and M. Rhazi *et al.* [7,8]. In these works, the polymers obtained were characterized using:

- Element analysis to determine the residual mineral content for the extracted chitins and prepared chitosan.

- The CP-MAS ^{13}C NMRto characterize chitin and determine, especially, their physical structure and their DA.
- The proton NMR spectroscopy to determine the DA of the prepared chitosan.

The chitin and chitosan samples prepared in this work were characterized by infrared spectroscopy.
The DA of the chitosan was determined by IR spectroscopy and potentiometric titration.

➢ Infrared Spectroscopy:

Infrared spectra of chitin were obtained by scanning from 400to4000 cm^{-1} in KBr pellets (0.0001 g chitin / 0.0999 g KBr).
The FTIR analysis allows to obtain the vibrational spectra of the groups constituting a molecule. The analysis is carried out on solid samples.
In IR, the spectrum of chitin showed two absorption bands at 1655 and 1625 cm^{-1}characteristic of hydrogen amide groups [15]. These two bands disappear gradually depending on the progress of deacetylation.
The DA value is calculated after determining the absorbance ratio of the two peaks at 1655 cm^{-1} and at 3450 cm^{-1}, according to the following relation:

$$DA\ (\%) = (A1655/A3450) * 115$$

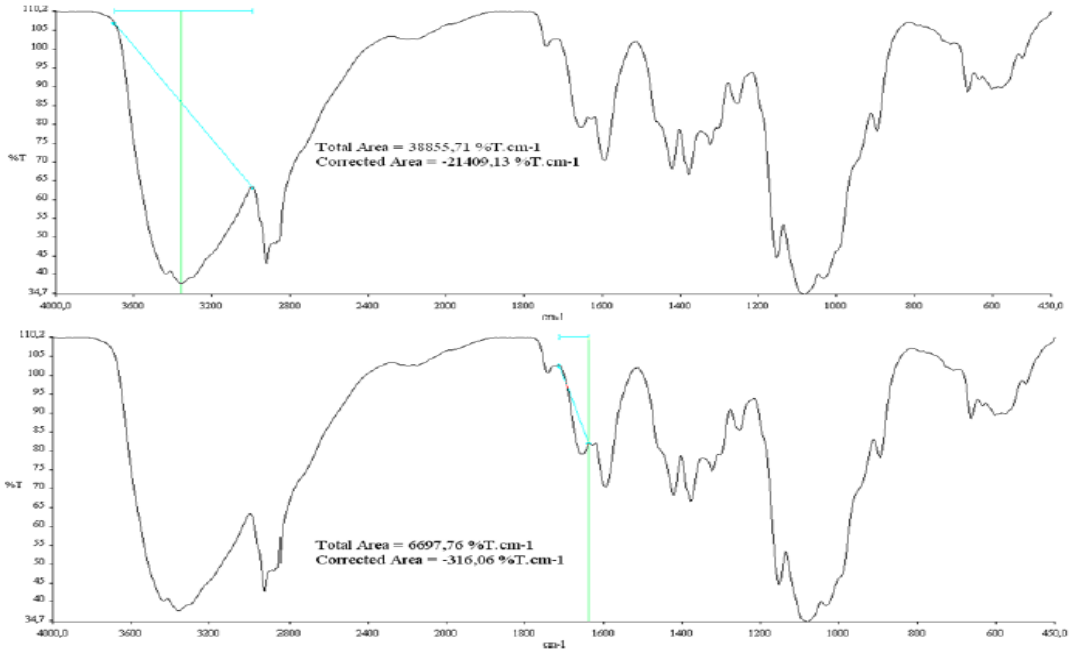

Figure 2: Presentation of the area corresponds to the peaks 1655 cm^{-1} and 3450 cm^{-1} of the IR spectrum of chitosan [16].

➢ Potentiometric titration :

The chitosan was dissolved in a known amount of acid (in excess). From the titration of this solution with a 0.1 M sodium hydroxide solution a curve with two inflexion points was obtained. The difference of NaOH solution volumes corresponding to these points corresponds to the acid consumed for the salification of amine groups and allows the determination of the DA of the chitosan. The titration was performed with a pH-meter Minisis 6000 from Radiometer (France) [11]. The DA was calculated from the relation:

$$\%\ NH_2 = \frac{16{,}1\ (x_2 - x_1)}{m - m'} \times N = \text{1-DA}$$

With :
$x_2 - x_1$: Value of the abscissas of the two inflection points.
m : weight of the chitosan sample
m' : weight of the water in the sample.
N : Normality of sodium hydroxide (0.1 N)
16,1 Corresponds to the weight of NH_2 group
DA : Degree of acetylation

3. Results and discussion

3.1 Investigation of the productivity of chitinous sources in Essaouira

To estimate the quantities of waste discharged and in the absence of industrial units for shrimp decortication or squid treatment, we turned to the five main seafood restaurants of of Essaouira. The results of the investigation are summarized in Table 1:

Source	Lobster	Spiny Lobster	Shrimp	Squilla	Spider crab	Squidpens	cuttlebone
Quantities of waste discharged (Kg/year)	5,310	4,500	17,370	22,680	43,740	378	378

Table 1: The quantity of waste discarded annually by major restaurants in Essaouira.

Following this investigation, we chose to extract the α-chitin from the exoskeletons of the spider crab, the squilla and the pink shrimp. The choice of these sources can be explained by the following facts:

- Their abundance regard to other crustaceans.
- The low commercial value of the squilla and spider crab.
- In addition, α-chitin has been extracted mainly from shrimp and it would be interesting to diversify the sources of this chitin.

As for β-chitin is extracted from the squid pens and the cuttlebone. This choice is due to the great reaction interest of β-chitin, which is practically only extracted from squid, the study of the extraction of this chitin from the cuttlebone would allow to explore a second source of β -chitin.

3.2 Extraction of chitin, preparation of chitosan and characterization of prepared products

The demineralization of the spider crab and the cuttlefish required seven acid baths, the squilla and the pink shrimp three baths while the squid required only one bath (this proves that the different sources of Chitin do not require the same treatment). The same observation is valid for the stage of the deproteinization.

So, whether for demineralization or deproteinization, the number and duration of baths depend on the source and its initial content of proteins and minerals. The efficiency of the demineralization and the deproteinization is more important when we proceed by repeated treatments that when we apply a continuous treatment in a single stage [9].

Table2: Reaction conditions of the extraction and chitin contents obtained.

Source ofchitin		Number of acid baths 0.55M HCl room temperature	Number of alkaline baths 0.3M NaOH; 80°C	% of part eliminated after demineralization	% of part eliminated after deprotéinization	Chitin content (% by weight)
ReptantiaBradyma	Spider crab	7	5	44%	43%	13%
Natantia	Pink shrimp	3	3	21%	57%	22%
Stomatopoda	Squilla	3	4	53.9%	20.3%	25.8%
Cephalopoda	Cuttlefish	7	3	80.5%	7.8%	11.7%
	Squid	1	2	12.4%	47.1%	40.5%

From extracted chitin, we proceeded to the preparation of chitosan according to the conditions indicated in Tables 3 and 4. Examination of the IR spectra and the curves of potentiometric titration show that the chitosan prepared have a very low degree of acetylation; in most of the cases it is lower than 10 %. The DA values obtained are generally in agreement with those expected determined by ^{1}HNMR in the previous work [7,9, 10,11,12] as observed with squid or squilla [7, 9].

Table 3: Preparation of chitosan according to the Kurita process [14].

Sources	type of chitin	Reaction Conditions					Titration (DA%)	IR (DA%)
		NaOH %	T ° C	Treatment Duration	$NaBH_4$	Under nitrogenatmosphere		
Squid	β	40	80°C	3h + 3h + 3h	+	Oui	0	___
cuttlefish	β	40	80°C	3h + 3h + 3h	+	Oui	6.2	6.2
Squilla	α	50	120°C	3h + 3h + 3h	+	Oui	13	10
Pink shrimp	α	50	120°C	3h + 3h + 3h	+	Oui	10	__

Table 4: Preparation of chitosan according to the Broussignac process [13], and comparison of the DA values of the chitosan samples determined respectively by potentiometric titration and IR spectroscopy.

Sources	type of chitin	Reaction Conditions					Titration (DA%)	IR (DA%)
		KOH %	Ethanol %	Monoethylene glycol %	T ° C	Treatment Duration		
Squilla	α	50	25	25	120°C	4h	12	10
Spider crab	α	50	25	25	120°C	16h	8	__

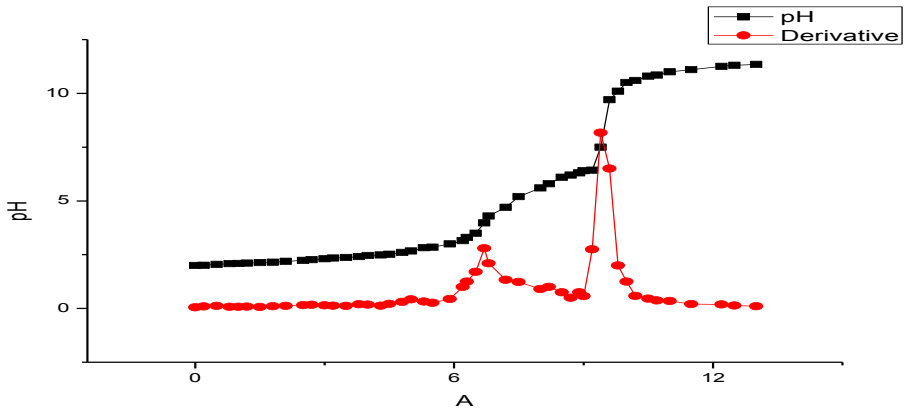

Figure 3: Potentiometric titration curve obtained for the chitosan prepared from the β-chitin of the cuttlefish bone after three three-hour treatment according to the Kurita process (x_1 = 6.64 and x_2 = 9.38, DA ≈ 6.2%).

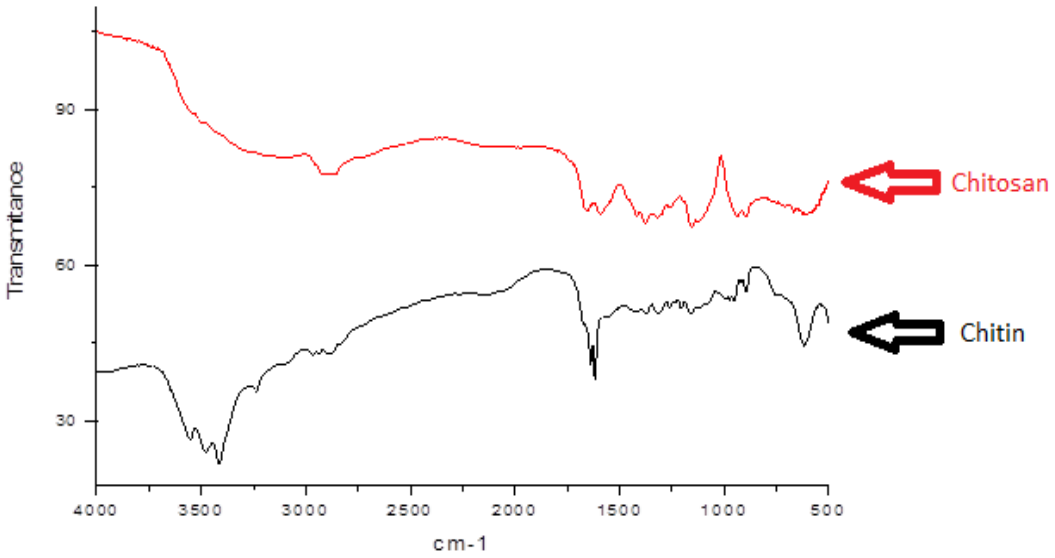

Figure 4: IR spectra obtained for the chitosan prepared from α -chitin of the squilla after three treatment of 3 h according to Kurita process (DA ≈ 10.4%)

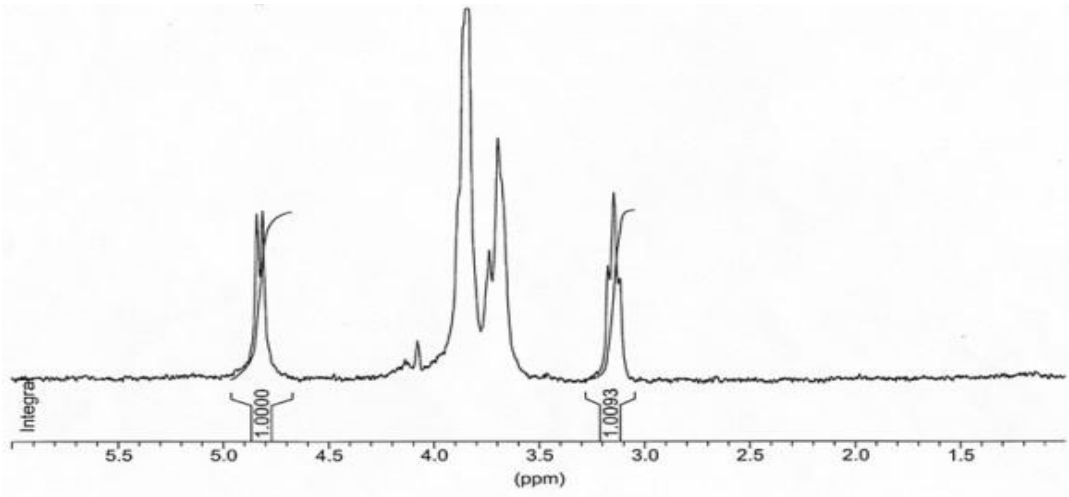

Figure 5: NMR spectra of the proton of the sample of totally desacetyled chitosan prepared from the ß-chitin in the following reaction conditions: NaOH 40% in the presence of $NaBH_4$ 80 ° C., 3 h x 3, under nitrogen (DA ≈ 0%) [11].

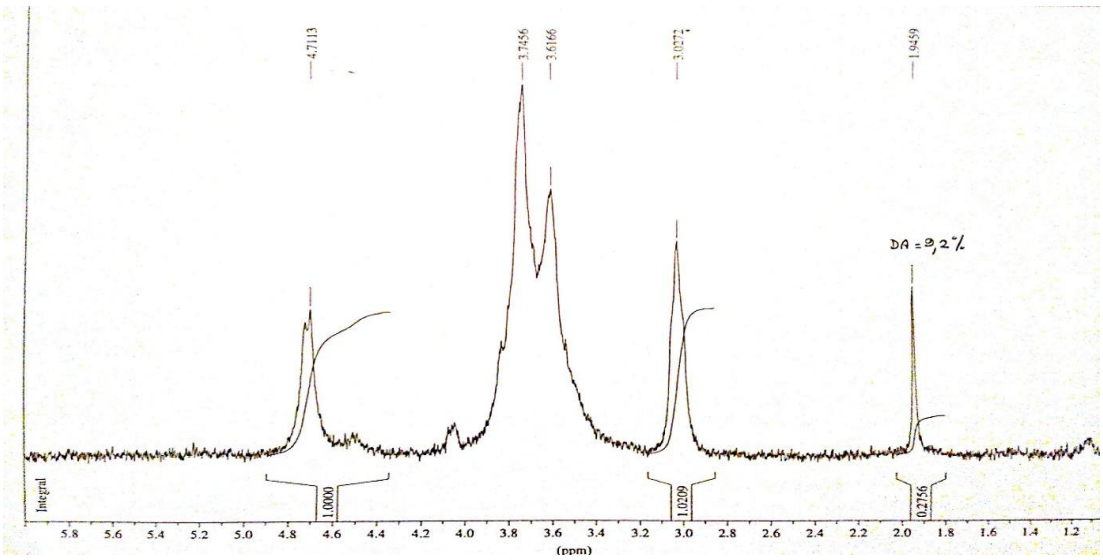

Figure 6: NMR spectra of the proton of the chitosan sample prepared from chitin α in the following reaction conditions: KOH %, 25% ethanol, 25% monoethylene glycol, 120 ° C, 4h, (DA ≈ 9.2%) [11].

3.3 Possibilities of production of chitin and chitosan by valorization of waste rejected in Essaouira

On the basis of the chitin content obtained for each of the sources studied (Table 2) and the quantities of waste discharged from the restaurants in the port of Essaouira (Table 1), we estimated the quantities of chitin and chitosan that could be produced, the results are presented in Table 5:

Table 5: Estimated quantities of chitin and chitosan that can be produced annually from waste rejected in Essaouira

Source of chitin		Total quantity of waste (kg/year)	Total quantity of the dry waste (kg/year)	Quantity of chitin (kg/year)	Quantity of chitosan (kg/year)
Reptantia Bradyma	Spider crab	43,740	34,992	4,549	3,639
Natantia	Pink shrimp	17,370	8,685	1,911	1,528
Stomatopoda	Squilla	22,680	11,340	2,923	2,339
Cephalopoda	cuttlefish	378	155	18	14
	Squid	378	302	122	98
Total		84,546	64,474	9,523	7,618

In order to obtain the quantities of dry waste, we multiplied the quantity rejected by a coefficient of 0.5 in the case of the squilla and the shrimp, a coefficient of 0.8 for the spider crab and the squid and a coefficient of 0.41 for the cuttlefish (the exploitable part constitutes 41% of the cuttlebone).

Thus, the valorization of this waste would allow to produce more than 9,500kg of chitin per year. These quantities could be transformed by reactions of N-deacetylations with an average yield of 80%, to about 7,600 kg of chitosan.

Chitin, medium quality, are sold by "Sigma - Aldrich" with prices varying from 170 to 400 euros/kg while the prices of chitosan, presenting a DA from 25 to 30 %, vary from 780 to 1,400 euros/kg.

On the basis of these prices, the annual sales of the quantities of chitin and chitosan that could be produced by valorization of waste would be between 1,615,000 EUR and 3, 800, 000 EUR for chitin and between 5,928, 000 EUR and 10,640,000 EUR for chitosan. It is obvious that more these products will be pure and present a good quality, more their sale prices will be raised.

Table 6: Prices of chitin

Selling prices (According to regions and purity)	170 EUR/kg	400 EUR /kg
Chitin 9,500 Kg/year	1, 615, 000 EUR	3,800,000 EUR

Table 7: Prices of chitosan

Selling prices (According to regions and purity)	780 EUR/kg	1400 EUR/kg
Chitosan 7,600 Kg/year	5 ,928,000 EUR	10,640,000 EUR

Conclusions

Thus, the quantities of waste rejected by restaurants of the coastal cities such Essaouira can be the object of valorization. Production could take place within a cooperative of workers in the field. In the context of a project related to the Social and Solidarity Economy, while supervision and scientific monitoring could be provided by the university structures of the region.

Morocco which has great fishing potential and several units of decortication of shrimps and treatment of squid and the cuttlefish should integrate the club of the producers of chitin and chitosan.

The valorization of the enormous quantities of the chitinous waste rejected in the sea or in the landfills has become both an ecological and an economic necessity. This is also in line with the objectives of the development program of fisheries sector *"halieutis"*, as it is consistent with the official commitment to strengthen the link between research and development.

References

1. Muzzarelli R.A.A., *"chitin",*Pergamon Press,New York, 1997 .
2. Roberts G. A. F, *"Chitin Chemistry»*, Macmillan Press, London, 1992.
3. Rinaudo M., Milas M., *le Dung P., Int. J. Biol. Macromol.*, 15 (1993) 281.
4. Rissouli L., Benicha M., Chabbi M., "*Contribution to the elimination of Linuron by the adsorption process using Chitin and Chitosan biopolymers* », *J. Mater. Environ. Sci.*, 7 (2016) 531-540.
5. Rhazi M., Tolaimate A. and Y. Habibi « *Interactions of Chitosan with Metals for Water Purification* » in « *Polysaccharide Building Blocks* » chapter 4,127-141, published by John Wiley and Sons 2012
6. Desbrières J., *"Dérivés amphiphile de la chitine et du chitosane, propriétés rheologiques, interfaciales et applications"*dans *"chitine et chitosane, du biopolymére à l'application "* chapitre 4,(2009), 89-104.
7. Rhazi M., Desbrières J, Tolaimate A, AlaguiA., Vincendon M., VottéroP., *"Investigation of different natural sources of chitin: Influence of the source and the deacetylation process on the physicochemical characteristics of chitosan". Polymer International*,49, (2000),337-344.
8. El Montassir D., Taourirte M., Eladlani N., Rhazi M., "*Extraction and Characterization of Chitin and Chitosan from Parapenaeuslongirostris from Moroccan Local sources"*. *International Journal of Polymer Analysis and Characterization*. 19, Issue 4 (2014) 342-351
9. Tolaimate A., Desbrières J., Alagui M., vincendon M., Vottérop., "On the influence of the deacetylation process on the physicochemical characteristics of chitosan from squid chitin". *Polymer*, 41 (2000) 2463-2469
10. Tolaimate A., Desbrieres J., Rhazi M., Alagui A. "Contribution to the preparation of chitins and chitosans with controlled physico- chemical properties" *polymer* 44 (2003) 7939–7952
11. Tolaimate A., *« Exploration des gisements chitineux de la faune marine marocaine. Procédé d'extraction de chitines fortement acétylées. Préparation du chitosane à caractéristiques contrôlées. »*, Thèse de doctorat, option: chimie Bio-Organique, (2000), 256, C200 Université Cadi Ayyad. Maroc
12. Tolaimate A., Rhazi M., A. Alagui, J. Desbrières, Rinaudo M., «Valorization of waste products from fishing industry by production of the chitin and chitosan» *Phys. Chem. News*, 42 (2008) 120-127.

(2017) ; http://www.jmaterenvironsci.com