BEETOSAN/CHITOSAN FROM BEES - PREPARATION AND PROPERTIES

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Abstract- Nowadays polysaccharides such as chitosan are becoming more and more popular. The main raw material for the preparation of chitosan is chitin. This organic compound is a fundamental building block of external skeletons of arthropods. However, due to the growing demand for chitosan (and hence chitin) new sources are sought and this source turn out to be honeybees. In order to receive chitin from these insects multistage chemical tretment is necessary. An important issue is the removalfrom the bodies of bees substances such as waxes, mineral substances, proteins and natural pigments (such as melanin).The selecting of conditions for each of stages has a significant impact on the quantity and physicochemical properties of the resulting chitin.

Keywords- Beetosan, chitosan, chitin, deacetylation

I. INTRODUCTION

Chitosan is a polysaccharide consisting of two kinds of polymers; ie. glucosamine and N-acetylglucosamine. This naturally occurring biopolymer is obtained by deacetylation of chitin. Process of deaetylation comprises hydrolysis of N-acetylglucosamine groups and may take place on an enzymatic method or a chemical one. In the first of the mentioned chitin deacetylase is applied. This enzyme is a part of the cells of filamentous fungi of the genus Mucoraceae and hydrolyses the bond between the amino group and the acetyl group. In the chemical deacetylation material is treated with sodium hydroxide and the reaction proceeds at an elevated temperature. The amount of N-acetylglucosamine groups that are hydrolyzed to those not hydrolyzed is defined by the degree of deacetylation. The value of this degree, viscosity and molecular weight determine the properties of chitosan This chitin derivate is insoluble in solutions at neutral and alkaline. What is important, chitosan is capable of forming salts with inorganic and organic acid (ie. acetic, lactic, hydrochloric) [1,2,6]
Chitosan is non-toxic and is characterized by a biocompatibility and biodegradability. Thanks to its unique properties is becoming more and more popular and is applied in many fields. It is worth noting in particular the use of this polysaccharide in medicine, pharmacy, cosmetics and even in biomedical engineering.Chitosan can be used as a drug carrier but also as a substance used in the treatment of patients suffering from hypercholesterolemia. The derivate of chitin proves to be useful in the purification of bloodbut also in the preparation of artificial organs [3,6].
Chitin, which is a raw material for obtaining of chitosan, is a major component of external skeletons of marine organisms such as shrimp, crab, squid, oysters or krill. Nowadays new sources of this polysaccharide are sought. Insects become an alternative option.Alternative materials such as naturally died bees are sourced in spring.
What is important, body of bees include among others proteins, mineral substances, chitin and natural pigment. Several steps for removing unwanted substances are necessary in order to isolate the chitin. The important components of the bodies of bees, that are removed, are salts, notably calcium salts. They constitute approx. 3-5% of the total weight of the insect. Waxes should be removedbecause due to their hydrophobic properties they can hinder the reactions taking place in an aqueous medium [4,5,6].
Based on the origin (ie. the bees) obtained chitosan is defined as “Beetosan".

II. EXPERIMENTAL PART

In order to obtain beetosan from naturally died honeybees is necessary to carry out some steps aimed at removing waxes, mineral salts, proteins and melanin (natural pigment that occurs in the body of bees). Before the chemical treatment bees were dried at 37°C. After every step a loss of weight was verified. The parameters of each step were chosen to obtain as small as possible loss of material while maintaining the effectiveness of the process.
What is more, a structure of attained material was defined by means of Fourier Transform Infrared Spectroscopy (FT-IR) and X-ray diffraction (XRD).

1.1. Removing of waxes

Waxes from the bodies of naturally died bees were removed by extraction in the Soxhlet apparatus. The process was considered to be completed after five cycles.Ethyl alcohol was used as a solvent.The obtained fractions were dried at room temperature.

1.2. Removing of mineral substances

In order to remove mineral substances material deprived of waxeswas treated with the solution of

hydrochloric acid (with ratio 1g/10ml) with constant stirring. After a treatment with acid the solid material was filtered and washed with distilled water to neutral filtrate. The last step was drying of attained fraction in room temperature. Different concentrations of HCl solution and a different reaction time were applied.

1.3. Deproteinization

To remove proteins from material obtained after the first two stages it was treated with sodium hydroxide. The reaction was carried out at 80°C for a specified time (10, 20, 30 and 40h). Further proceedings was the same as in the case of the previous stage (filtering, washing and drying).

1.4. Depigmentation

Substance responsible for the color of insects, including honeybees, is melanin. In order to remove this natural pigment material deprived of waxes, mineral substances and proteins was treated with solution of hydrogen peroxide at different concentrations. Process was conducted at 80-85°C at various time.

III. RESULTS AND DISCUSSION

1.5. Removing of waxes

Table 1. Results of removal of waxes from bodies of naturally died honeybees

Time [h]	Number of cycles	Initial mass [g]	Final mass [g]	Weight loss [%]
1.,5	5	7.003	5.180	26.0
1.5	5	7.007	5.257	25.0
1.5	5	7.010	4.971	29.1
1.5	5	6.993	5.089	27.2
1.5	5	7.032	5.195	26.1
1.5	5	7.020	5.301	24.5

It was established that carrying out of the extraction process at the time of 1.5 hours (5 cycles) results in minimal loss of raw material. Therefore these conditions were considered to be the most favorable.

1.6. Removing of mineral substances

Table 2. Results of removal of mineral substances from bees deprived of waxes

Weight after extraction [g]	C_{HCl} [mol/l]	Time [h]	Weight after HCl[g]	Weight loss [%]
5.807	1	1	4.505	22.4
5.070	1	1	4.066	19.8
5.209	1	0.5	4.324	17.0
4.508	1	0.5	3.808	15.5
5.180	1	0.5	4.213	18.7
4.850	1	0.5	4.040	16.7
5.008	1	0.5	4.112	17.9

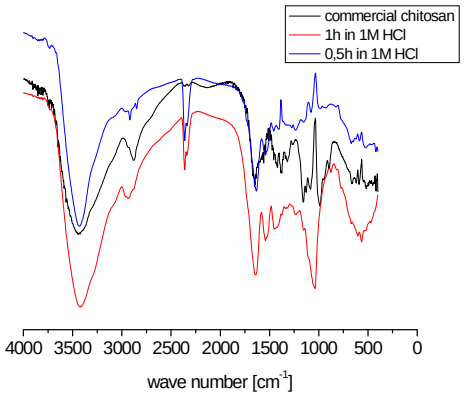


Fig.1. FT-IR spectra of the samples after removal of mineral substances.

The lowest loss of weight was obtained when the process was carried out using 1M HCl for 0.5h. HCl. What is more, material obtained by a process that was carried out in the above-mentioned conditions is characterized by a structure similar to that of commercial chitosan, as shown in Fig. 1.

1.7. Removal of proteins

Table 3. Results of deproteinization.

m_{before} [g]	C_{NaOH}[mol/l]	t [h]	$m_{afterdeproteinization}$ [g]	loss of weight [%]
8.132	1	40	1.260	84.1
8.213	0.5	40	2.845	65.4
8.813	1	20	4.173	52.6
7.882	0.5	20	2.793	64.5
8.454	1	30	2.368	72.0
8.587	0.5	30	1.741	79.7

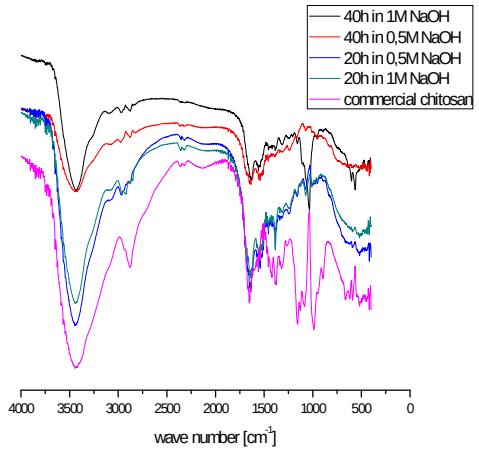


Fig.2. FT-IR spectra of the samples after process of deproteinization

On the basis of studies it was found that the material of the preferred structure is obtained using 1 M NaOH at the time of 20 hours.In addition, under these conditions the lowest loss of material was determined.

1.8. Removal of natural pigment – melanin

Table 4. Results of removal of natural pigment – melanin

m_{before} [g]	C_{H2O2} [%]	t [h]	m_{after} [g]	Weight loss [%]
1.048	9	2.5	0.673	35.78
1.146	18	2.5	0.833	27.31
1.738	18	1.5	1.269	26.99
3.589	18	1	2.812	21.65

The most favorable concentration of hydrogen peroxide that results in the lowest loss of weight is 18%. What is important, the resulting material has a different color as compared to the starting material.

CONCLUSIONS

Naturally died honeybees after suitable chemical treatment constitute an alternative source of chitosan. The process of obtaining of this polysaccharide is a multi-step, and each step requires the selection of conditions to ensure the desired physicochemical properties of the final product. Beetosanobtained in this way is characterized by a chemical structure that is very similar to that of commercial chitosan.

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