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STUDIES OF PHYSICO-CHEMICAL PROPERTIES CHITOS AN *APIS MELLIFERA*

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Abstract: *The article presents methods for studying the physicochemical properties of chitosan synthesized from the dead bee Apis Mellifera. The molecular weight, viscosity, moisture content, degree of deacetylation, and amount of nitrogen in the polymer were determined.*

Key words: *molecular weight, viscosity, humidity, degree of deacetylation, amount of nitrogen.*

Determining the moisture content of*Apis Mellifera* chitosan. The humidity of the samples was determined according to the operating instructions of the instrument METTLERTOLEDOLP16. The method is based on drying the sample by heating it to a constant weight. In this experiment, we found that the moisture content of *Apis Mellifer*a chitosan was 10.3%.

The mass fraction of the sol was determined by burning the sample and weighing the residue. The calculation of the experimental results was performed according to the following form:

$$X_z = \frac{(m_2 - m_1)}{m} \text{x} 100\%$$

Where:
X_z – is the mass fraction of sol, %;
m_2 – mass of ash with crucible, g;
m_1– mass of dry crucible, g;
m – is the mass of the test sample, g.

Determining the degree of deacetylation of *Apis Mellifera* chitosan. We determined the degree of deacetylation of chitosan using a conductometric titration method. To do this, a sample of chitosan (50–100 mg) was suspended in 5 ml of 0.1 n HCl and 25 ml of distilled water. The resulting solution is titrated with 0.1 M NaOH, adding 0.1 ml every 30 seconds, stirring constantly. We calculated from the graph that the amount of alkali required for titration of free amino acids per ml depends on the volume of alkali of the electrical conductivity of the solution. The calculation of DAD was performed according to the following formula:

$$\%DAD = \frac{M_x \text{x V x N}}{p + (M_x - M_{xt})} \text{x} 100\% = \frac{203 \text{ x V x N}}{\text{p} + 42 \text{ x V x N}} \text{x } 100\%$$

Where:
M_x - is the molecular weight of the chitin
M_{xt} – is the molecular weight of the chitin
V - is the difference in NaOH volumes determined from the graph, ml;
N - is the normality of NaOH;
P - is the mass of chitosan, mg;

The experiment was performed three times and found that the deacetylation rate of *ApisMellifera* chitosan was in the range of% DAD = 70-80%.

Determination of viscosity of *ApisMellifera* chitosan polymer. To determine the viscosity of the polymer, we determined it by dissolving it in a 2% solution of acetic acid and measuring it with aUbbelohde viscometer with a capillary diameter of 0.5 mm. In this case, we diluted the polymer solution in series and determined the average of the experiment three times (average transition time of the solution at 30 ^{0}C for 22 sec). The relative (η_n) and measured ($\eta_{o'}$)viscosity values of chitosan are calculated according to the following formulas:

$$\eta_n = \frac{\tau_1 - \tau_0}{\tau_0}$$

$$\eta_{o'} = \frac{\eta_n}{c}$$

Where:
τ_1 – flow time of the polymer, sec;
τ_0 – solvent flow time, sec;
c – is the concentration of the solution, %.

According to the calculations, the viscosity of the polymer ApisMellifera chitosan is $3.28 \cdot 10^{-3}$ Pa · s.

Determination of molecular mass of ApisMellifera chitosan polymer.

ApisMellifera was calculated using the Mark-Howing equation for chitazan with different levels of deacetylation obtained from inanimate bees:

$[\eta] = k \times M^{\alpha}$

Where:

$[\eta]$ – is the viscosity of the chitosan solution

M – is the molecular mass of chitosan.

$k$vaα– are constants determined by the formulas:

$k = 1{,}64 \times 10^{-30} \times DAD^{14{,}0}$

$\alpha = -1{,}02 \times 10^{-2} \times DAD + 1{,}82$,

Where:

DAD – is the degree of deacetylation of the sample,%.

The method for the determination of $[\eta]$ of chitosan samples is based on the graphical determination of the kinematic viscosity of an infinitely dilute polymer solution. For this purpose, the viscosity of solutions of different polymer concentrations obtained by successive dilution of the initial solution with 2% acetic acid was measured, after which $[\eta]$ was determined. The viscosity of the solutions was measured on aUbbelode viscometer at 25 °C (capillary diameter 0.56 mm). 0,2 M sodium acetate solution was introduced as the electrolyte.

Detection of protein residues in chitosan polymer derived from apis Mellifera inanimate bees.

The determination of the total amount of protein was performed according to the Bradford method. A 750 mkl solution containing the protein to be detected in the solution is mixed with an equal volume of dye solution (Coomassie G - 250 3% HClO4). 0,1 M Na-acetate buffer pH 6,5 was used instead of protein solution to prepare the test solution. The solutions are stored for 15-20 minutes until the development of purple color stops. Subsequently, the absorption of the protein solution was measured at 600 nm relative to the control. According to the calibration curve, the protein was determined with albumin in µg / ml.

Protein composition was determined by analysis of amino acids using the standard method on a BiotronikLC-3000 analyzer (Eppendorf-Biotronik, Germany) in a Biotronic buffer system using VT-2410 ion exchange resin, the elution rate was 0.2 ml / min. The samples were hydrolyzed by the Pierce method (6 N HCl; 2,5 h; 140 °C).

Analysis of chitosan polymer element content obtained from apis Mellifera inanimate bees.

Elemental composition is determined by the standard method of thermal decomposition of substances. Nitrogen content was measured at 380 °C after decomposition in the presence of mercury sulfate. Determination of C and H content was performed by burning the samples in an oxygen stream and then freezing the carbon dioxide and water formed. The detection was performed on an EA 1108 CHNS analyzer from Karl-Erba. Measurement error 0.3%.

Qualitative reactions to determine the nature of the dark melanin pigment were performed as follows:

· A 10% solution of H_2O_2 was added to a 0,1% solution of melanin in 0,1 N NaOH solution, and oxidation and discoloration of melanins were observed during the day;

· When $KMnO_4$ reagent is added to 0,1% solution of melanin in 0,1 N NaOH solution, the color changes from brown to green, then the color of the solution changes and a precipitate is formed;

When 5 M $FeCl_3$ is added, a flocculant precipitate is formed from 0,01% melanin solutions containing 0,01 N NaOH, which dissolves when excess $FeCl_3$ is added.

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