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The Study of the Microstructure of Dairy Products Stabilizers.

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ABSTRACT

The following properties of carboxymethylcellulose have been studied: bulk density, viscosity, ratio of insoluble residues, content of micro voids, specific volume, specific surface area, and characteristical diameter. Using photomicrography, dynamics of structural changes in course of interaction with the solvent has been assessed. According to the results of spectrophotometric profile analysis, mass fraction of chemical elements (oxygen, nitrogen, carbon, sodium, chlorine) was estimated.

Keywords: carboxymethylcellulose, stabilizers, konjac gum, dairy products, microstructure.

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INTRODUCTION

Many stabilizing agents are presented in biological objects in the form of a hydrated mesh of chains, for example, bacterial capsules, cell walls of different ages of plant tissues, connective tissues of animals, etc [1].

Stabilization systems are widely used in the confectionery industry in the manufacture of marmalade-pastila group [2, 3]. They provide one of the most important functions of the technological process – the transformation of complex visco-current system in the gel [4, 5]. The main task in the production of this product is a stable gel producing, the formation of the desired rheological properties and providing the necessary organoleptic characteristics for the final product [6, 7]. The organization of technological processes, allowing solving the above-mentioned tasks is possible only on the basis of knowledge about the microstructure of structure stabilizers and its component composition [8, 9].

OBJECTS AND METHODS

The objects of a research were: cow's raw milk of the second grade and higher according to the State standard - GOST 13264; cream, obtained by separation of cow's milk (GOST R 52054); skim cow's milk, without foreign tastes and odors, acidity - not more than 20°T, obtained by separation of cow's raw milk of the exact grade; skimmed milk powder (GOST 10970); lyophilized culture of a direct application FD-DVS CH-N-19 (consisting of *Lactococcus lactis spb. cremoris, Lactococcus lactis spb. lactis, Leuconostoc mesenteroidis spb. cremoris, Lactococcus lactis spb. lactis biovar diacetylactis*) *и* EZAL U-D MYE 96 (consisting of *Streptococcus termophilus, Lactobacterium delbrueckii spb. bulgaricus*); starter cultures for immediate application, designed specifically for the Russian market of "DELVO-YOG" series (name: CY-346/347; FVV-21; CY DSL; FVV-31); «AiBi» (name: LbS 22.11(R4); LbS 22.11(R2); LbS 22.11(Y3); LbS 22.11(Y2)) and «Lactoferm» (names: KEFIR-30; YO-441; MSO-11; RENNET; PROTEK); the stabilizers of the structure of the following types: CMC Akucell 3265; CMC 4500-6000; konjac gum [10]; CMC 6000-9000; pectin ARA 105; carob pulp gum; sodium alginate HO4-600; sodium pyrophosphate SAPP 28; the CMC Akucell 2785 [11]; sodium pyrophosphate SAPP 40; xanthan gum; drinking water GOST 2874; natural flavourings or identical to natural aromatic food essences, essential oil, domestic or imported, approved for use by the CPS (Rospotrebnadzor) of the Russian Federation; sorbic acid according to TU 6-14-358 and TU 6-14-22-206.

In carrying out the work were used conventional, standard and original methods.

Were performed the selection of milk and dairy products, preparation for analysis according to GOST 26809-86 and sampling for microbiological studies according to GOST 9225-84.

Titratable acidity was performed according to GOST 3524-92. The method is based on neutralization of acids and their salts contained in the product by a solution of caustic alkali in the presence of phenolphthalein indicator. An active acidity was measured on the potentiometric analyzer according to GOST 26781-85.

Evaluation of taste and smell was performed according to GOST 28283-92.

Determination of moisture content and dry matter was performed according to GOST 3626.

Total protein content was performed according to GOST 23327-78.

Mass fraction of sucrose was determined by iodometric titration according to GOST 3628 (arbitration method). The method is based on oxidation of reducing sugars containing aldehyde group with iodine in an alkaline environment. Mass fraction of sucrose was calculated by the difference between the amount of taken and unspent iodine determined by titration.

Were used modern instrumental, physical, physico-chemical and rheological methods. Below are the basics of the research methods used in the work, which helped to receive the most essential characteristics of a structured cheese product.

The method of determining galactosidase activity of lactic acid bacteria is as follows: was prepared a 5% solution of lactose in buffer solution with a pH of 4.2 or 7.0; was prepared a 1 % solution of the enzyme; was determined the activity of the enzyme cryoscopic method.

For this, into 1 cm^3 of the prepared solution was added 4 cm^3 of substrate, then it was mixed. After that was taken away 1 cm^3 of a solution for a control sample. The remaining solution was incubated at 30 °C for 30 minutes. Was taken away 1 cm^3 of the experimental sample and then was measured a temperature of its freezing. Then a galactosidase activity was calculated.

Method for the determination of proteolytic activity is based on the hydrolysis of sodium caseinate to peptides by the studied enzyme preparation, with its following definition. As the unit of proteolytic activity is adopted the ability of the enzyme to convert in one minute at 30° the sodium caseinate into the non-sediment condition by the trichloroacetic acid in the amount corresponding to 1 mkmoll of tyrosine (GOST 20264.2-88).

A determination of nonprotein nitrogen was performed in the filtrate after precipitation of proteins by the photometric method. Fractionation of nitrogenous substances and the study of their composition and properties were carried out according to well-known methods. Sample preparation includes the following operations: hydrolysis with hydrochloric acid at a temperature of 110±10°C, a pressure of 0.8 MPa during 12-15 h, centrifugation through a membrane with a defined pore size for the removal from solution of various impurities.

Mass fraction of fat was determined by the Gerber`s acid method according to GOST 5867. The method is based on the isolation of fat from milk and dairy products under the action of concentrated sulfuric acid and isoamyl alcohol followed by centrifugation and measurement of the volume of the released fat into the graduated part of a fat-metric device.

Determination of the mass fraction of lactose, glucose, galactose, and oligosaccharides was carried out by the high performance liquid chromatography on the device "Color 500 M.

Mass fraction of macroelements and microelements was determined by atomic absorption spectrophotometry. The principle of the method is based on the ability of dissociated atoms of elements absorb light in a narrow spectral region. The study was carried out on the device Hitachi (Japan) according to the enclosed instructions.

The activity of proteolytic enzymes in the environment and the cells of the producer was assessed by the change in the content of the reaction mixtures of ninhydrin-positive substances. The principle of the method is in the observation and a subsequent calculation of changes in the content of ninhydrin-positive products in the reaction mixture and either of the extracellular enzyme of a studied microorganism that accumulates in the environment by the method of Chebotarev.

The degree of microbiological purity was assessed by counting of colonies grown on Petri dishes with nutrient media. As a nutrient media was used a mastopathy agar, potato and malt agars. To determine the total contamination of the enzyme preparation was prepared its water solution in the ratio 1:100, 1:1000 and 1:10000. From each of the prepared solution was made the inoculation per six Petri dishes. For this with a sterile pipette near the flame was taken 0.3 ml of enzyme solution and transferred to the surface of the agar. After that, the inoculated Petri dishes were incubated for 48 h at a temperature of 37±2°C.

The total amount of bacteria in the enzyme preparation was calculated as the arithmetic mean of the number of colonies of microorganisms per 1g of the preparation for all dilutions.

Microbiological parameters were determined taking into account the requirements specified in SanPiN 2.3.2.1078-01, according to the standard and accepted methods, taking into account the requirements specified in MU 4.2.727-99 "Hygienic assessment of shelf life of food products": bacteria of intestinal sticks (E.Coli) group, the number of mesophilic aerobic and facultative anaerobic microorganisms according to GOST 9225; pathogens, including bacteria of the genus Salmonella - GOST 30519, GOST R 50480; Staphylococcus aureus - GOST 30347; mold and yeast according to GOST10444.12.

Total protein analysis was conducted using Duma`s method, that is based on measurement of thermal conductivity of molecular nitrogen formed upon combustion of the test sample at a temperature of about 1000° C in an oxygen atmosphere and subsequent recovery of all the resulting nitrogen oxides with a reducing agent (copper), using the protein nitrogen analyzer RAPID N Cube (Elementar, Germany). The determination process is fully automated. At the stage of sample preparation the sample is tableted using a foil that was not containing nitrogen. The weighed sample is 250 mg. To obtain reliable results were carried out from three to five parallel analyses of each sample.

Determination of the mass content of water-soluble vitamins, inorganic anions was carried out by capillary zonal electrophoresis using the system of capillary electrophoresis "Capel 105". The method is based on the migration and separation of the ionic forms of analyzed components under the influence of an electric field due to a different electrophoretic mobility with the subsequent registration.

A determination of a content of toxic elements, pesticides, antibioticks and radionuclides: lead – according to GOST R 51301, GOST 26932 and GOST 30178, GOST 30538 and MUK 4.1.986; arsenic – GOST R 51766, GOST 26930 and GOST 30538; cadmium – according to GOST R 51301, GOST 26933, GOST 30178, GOST 30538 and MUK 4.1.986; mercury – GOST 26927 and MU 5178; residual quantities of organochlorine pesticides – according to GOST 23452 and MU 6129; aldrin – on methods for determination of trace amounts of pesticides in food, feed and the environment; antibiotic - MU 3049, MR 4.18/1890, MUK 4.2.026; radionuclides of strontium-90 and cesium-137 – MUK 2.6.1.1194.

The experimental results were processed by methods of mathematical statistics. The adequacy of the experimental data was tested with a Fisher`s test. The confidence error of the regression equation coefficients was calculated using the Student`s criterion.

To study the composition of the structure stabilizers was used the analyzing station JEOL JED-2300. With a help of this station by means of the method of x-ray microanalysis were obtained the spectrometric profiles, allowing to determine the chemical composition of the structure stabilizers.

The ability to the formation of the coarse system (CS) (foaming capacity) was determined by the method of P. A. Rebinder (by a multiplicity of pins) and was expressed in percent [11].

The stability of the (CS) during a certain length of time was calculated as the ratio of initial height to the final (CS) and was expressed in percents [11].

Dispersion of CS, the size and the number of air bubbles were determined by the method of microphotography, with pre-freezing the samples in an atmosphere of liquid nitrogen. The results were processed according to the techniques presented in [11].

Water binding, fat emulsion, ability to retain fat and the ability of milk protein concentrate -UV were studied by traditional methods that were given in the workshop on colloid chemistry.

Fig. 1 shows the microstructure of konjak gum at a magnification of 100, 200 and 500 times. The bulk density of the stabilizer structure is 540 g/dm3. Fig. 1(a) shows that the microstructure of konjak gum consists of large and small granules of irregular shape, the size of which varies from 10 to 250-300 µm. The microstructure is presented with granules with a crystalline rough surface, and with a smooth surface (Fig. 1c).

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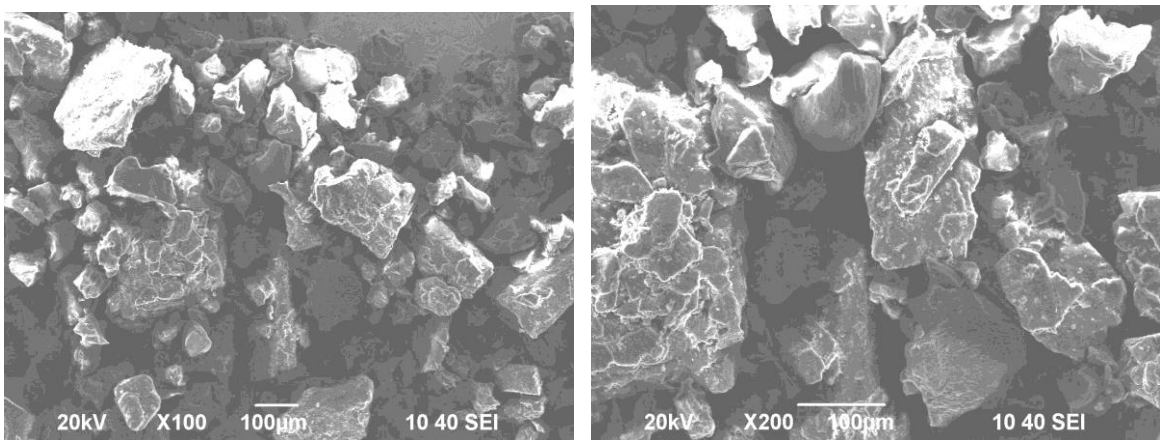
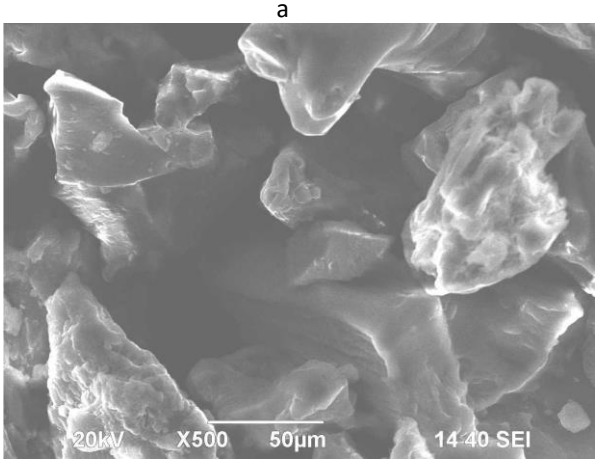


a b



c

Fig. 1. The microstructure of a konjac gum with a magnification of: a - 100 times; b - 200 times c - 500 times

When a konjak gum is dispersing in the hot and cold water are forming the viscous solutions with pH from 4 to 7 (tab. 1).

Table 1 The pH of the aqueous solution of the konjak gum

The mass fraction of konjak gum in the solution	pH
0,5	6,7
1,0	5,9
1,5	5,4
2,0	4,7
2,5	4,1

With the increasing of concentration of konjac gum decreases the pH of the aqueous solution. While stirring and heating, the solubility increases. This stabilizer is composed of monomers of D-mannose and D-glucose that are linked by β-glycoside bond.

In a hot water konjac gum has a better solubility than in the cold water that can be demonstrated by the given dependence of the fraction of dry residue of konjak gum in an aqueous solution after 60 min of diffusion at different temperatures.

In the organic solvents that structure stabilizer is insoluble. According to the properties of the thickener the konjac gum is between the xanthan and guar gum. A distinctive feature of this structure stabilizer is its high level of viscosity even at low doses of concentration. The main component of the composition of matter is chitosamine. It is responsible for the dispersion of the particles of the structure stabilizer in the water. In addition, the composition of konjac gum contains alcohol, which is involved in deposition and drying of glucomannan.

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In the food industry the konjac gum is often used as a fat substitute in the manufacture of nonfat and low-fat meat products. It also helps to form a dense consistency in the production of jellies, puddings, drinks, yoghurts etc.

Fig. 2 shows the spectrometric profile of determining the composition of konjac gum. The component composition is shown in table. 2.

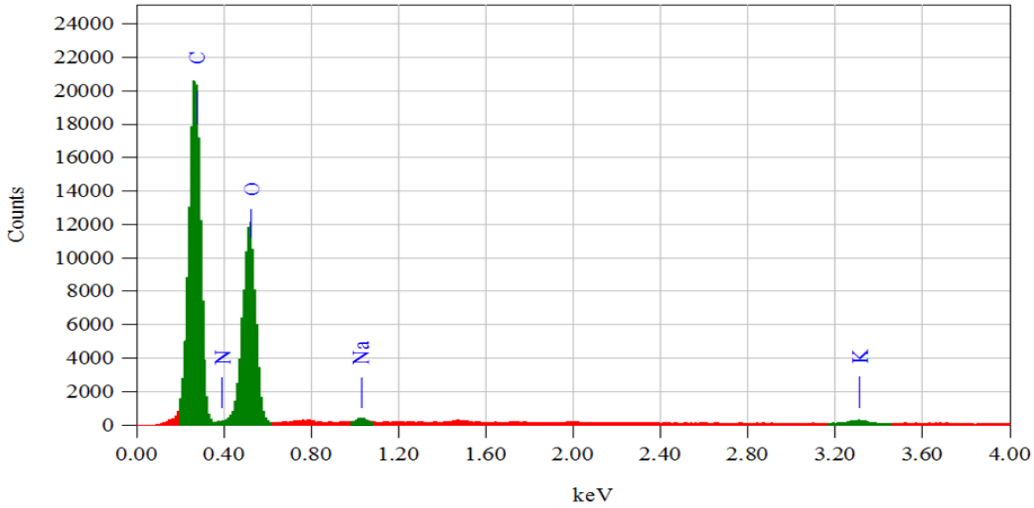


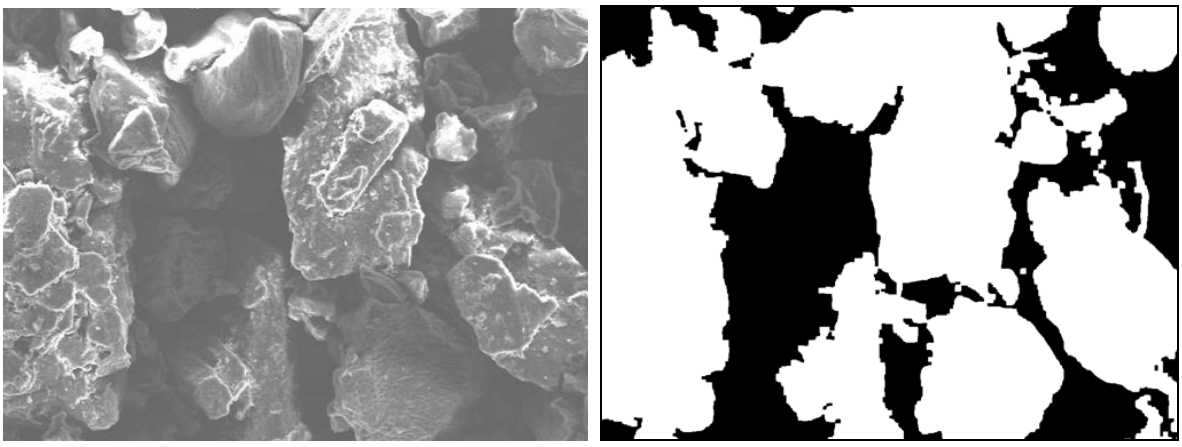
Fig. 2. Spectrometric profile of the component composition of konjac gum

Table 2 The component composition of konjac gum

Element	The relative weight, %
Carbon	29,79±0,89
Nitrogen	22,29±0,67
Oxygen	47,73±1,43
Potassium	0,17±0,005
Sodium	0,02±0,001

In konjac gum there are such elements as carbon, nitrogen, oxygen, potassium and sodium. The content of carbon and oxygen is similar to that observed in CMC 4500-6000. Konjac gum differs from the previously considered structure stabilizers by the presence of potassium and a lack of sodium.

To determine the micro hollows in konjac gum was used a microphotograph with a magnification of 200 times (Fig. 3 b). The required content of micro hollows was of 33,87±1,1%. When there was a need to increase the contrast of the image to filter particles presented in the background.



a b

Fig. 3. Results of the fraction determination of micro hollows in konjac gum: a micrograph with a magnification of 200 times; b - mask of the micrograph shown in Fig. 3 (a).

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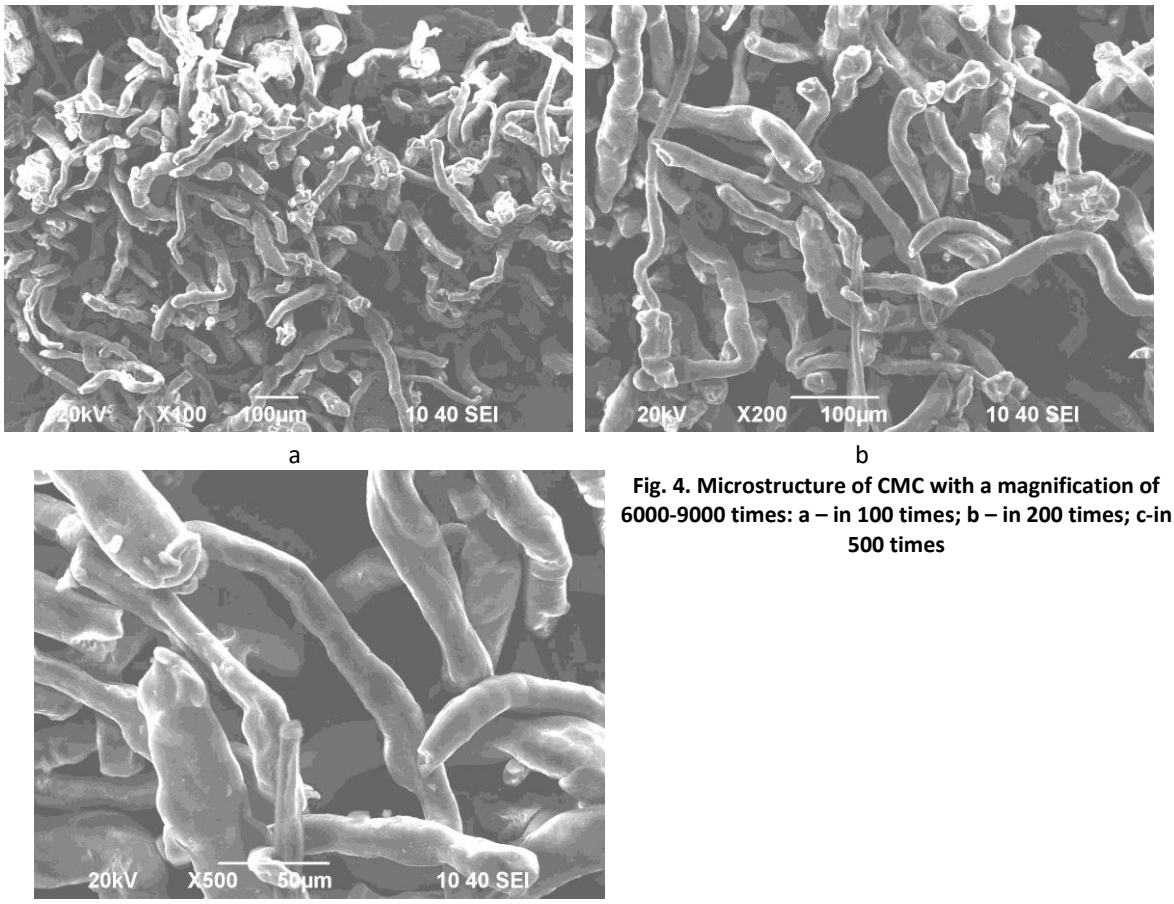
Thus, the elements of the konjac gum are presented in the form of granules of the irregular shape; its size varies from 10 to 300 microns. The component composition of a konjac gum contains carbon, nitrogen, oxygen, potassium and sodium. According to the results of mathematical processing the content of micro hollows in konjac gum is 33,87±1,1%.

In Fig. 4 is given a microphotograph of the structure of CMC 6000-9000 at a magnification of 100, 200 and 500 times.

From the obtained microphotographs it follows that the structure of CMC 6000-9000 is similar to the structure of CMC 4500-6000. The bulk density of CMC 6000-9000 is 550 g/dm3.

Unlike of CMC 4500-9000 in the structure of CMC 6000-9000 are missing plexus fibers, their shape is more rounded and has a diameter of 15-35 μm. On the surface of the fibers of CMC 6000-9000 are observed white crystal-like structures, ranging from 3 to 15 μm, in CMC 4500-6000 they are practically absent.



a b c

Fig. 4. Microstructure of CMC with a magnification of 6000-9000 times: a – in 100 times; b – in 200 times; c-in 500 times

Spectrometric profile is similar to the profile that is observed in CMC 4500-6000 (Fig. 4). Component composition of CMC 4500-6000 is given in table. 3.

Table 3 Component composition of CMC 6000-9000

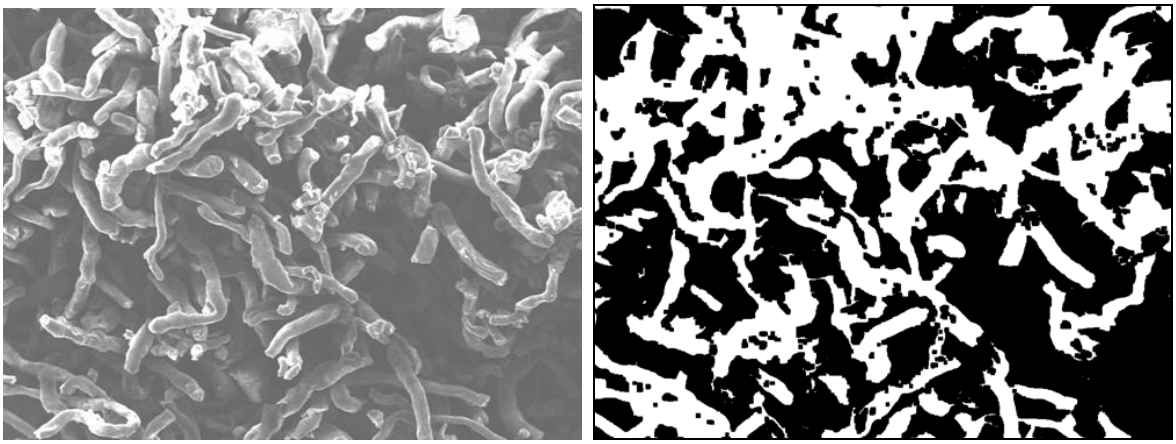
Element	The relative weight, %
Carbon	29,60±0,89
Nitrogen	16,23±0,49
Oxygen	48,00±1,44
Potassium	5,95±0,17
Sodium	0,23±0,007

A comparative analysis of the obtained data (table 2 and 3) allows concluding that the structure stabilizers - CMC 6000-9000 and CMC 4500-6000 are characterized by almost identical structure of all the

elements with a difference in the percentage from 0.01 to 1.06%.

To determine micro hollows in CMC 6000-9000 was used a microphotograph with a magnification of 100 times. The results of determination of CMC 6000-9000 micro hollows are shown in Fig. 5.

To create a mask was used a color selection, and then was applied the reduction of the border selection on three pixels, a manual correction and the subsequent extension of the boundaries of the mask. According to the results of the histogram of the obtained mask (Fig. 5 b) was determined the content of CMC 6000-9000 micro hollows, which was 55,56±2,3%.



a b

Fig. 5. The results of determining of the fraction of micro hollows in CMC 6000-9000: a microphotograph with a magnification of 100 times; b - mask of the micrograph shown in Fig. (a)

INSIGHTS

From the obtained results it follows that the structure of CMC 6000-9000 contains elements in the form of fibers of irregular shape, with a diameter of 15-30 microns. On its surface there are crystal-like structures with a size of 3-15 microns. The composition of the stabilizer structure is presented with carbon, nitrogen, oxygen, sodium and chlorine. The share of micro hollows in CMC 6000-9000 55,56 is±2.3 percent.

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