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Biopolimeric Nano Structural Compositions Based on Caramelized Honey

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The article deals with chemical properties of honey as well as wax and beeglue before and after the process of caramelization. The research has been done to study chemical reactions of caramelization of honey and other bee products; the biochemical properties of the obtained substances have been investigated. It has been revealed that biological activity of the compound after thermochemical heating of honey in the presence of special catalysts was discovered. Infrared spectra, moisture, viscosity, size and distribution of nanoparticles, elemental composition, oxymethylfurfural content, diastase number of caramelized honey have been investigated. The difference between the sizes of nanoparticles of raw and caramelized honey has been revealed, namely caramelized honey (1.5-2.0 times) of smaller size encourages their biochemical activity. On the basis of the results obtained from caramelized honey, wax and beeglue as well as their properties, the chewing gum with biologically active components for the prevention and treatment of periodontal tissue diseases (gingivitis, periodontitis and periodontal disease) was invented. Clinical studies on chewing gum with honey, wax, and beeglue have confirmed that it provides health benefits among 72 % of patients.

Keywords: honey, wax, beeglue, caramelization, biochemical properties, chewing gum, prophylaxis, gingivitis, periodontitis, periodontal disease, treatment.

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Introduction

It is a well-known fact that bee products serve as additional food, medicines and beauty aids. It has mild antiseptic properties and has been used in the treatment of ulcers and wounds, diseases of the internal organs, eyes, skin, it helps to reduce blood pressure, it also has the capacity to stop the growth of pathogens as well as to treat neuroses and insomnia.

Honey is a part of traditional medicine in many cultures, although it is most widely used as a sweetener. It contains at least 180 components and is water soluble with fructose (≥ 38 %) and glucose (31%); the moisture content is about 17.7-21%, the total acidity is 0.08% (pH=3,4-4,3) and the ash is 0.18%. The wax contains 200-300 components, namely esters, free fatty acids,

saturated hydrocarbons, mineral dyes and aromatic substances, namely: esters of ceric acid - 76.0% of cholesterol esters 1.0%, coloring 0.3%, lactones 0.6%, free alcohol of 1.25%, free cerin to-you 13.5%, carbohydrates 10,5-13,5%. Pure wax is lighter than water, melting point 62-69°C density 0.96g / cm³. it is insoluble in water but well soluble in gasoline, chloroform, ether and boiling alcohol. [1]

Beeswax is a product of the bees' wax glands, formed by the use of pollen, nectar and honey. Bees build honeycombs in a beehive. The beeswax is obtained, after removal of the honey, by melting the honeycomb, straining the wax to remove impurities, and pressing the residue to extract any remaining wax.

Beeglue is a unique natural substance that has the biological properties of antibiotics that cannot be

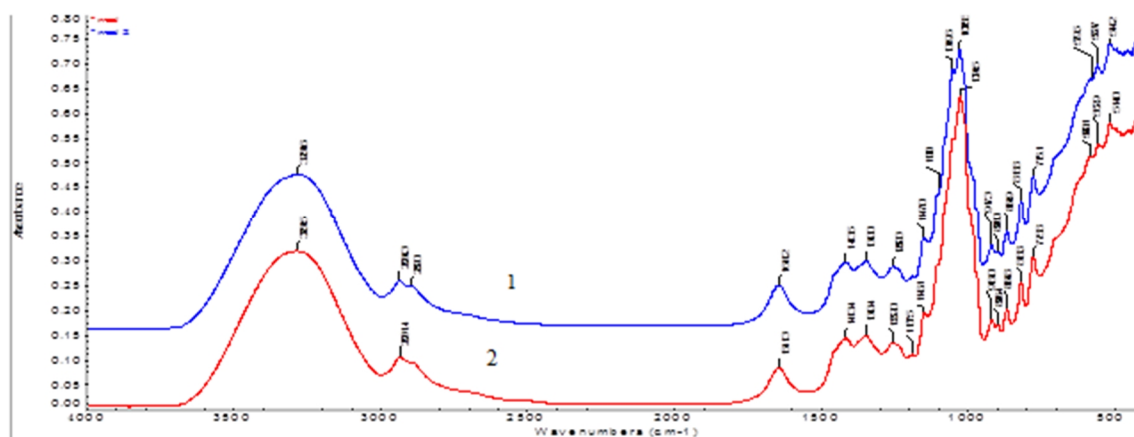


Fig.1. IR spectra of honey 1-red line – honeycomd, 2-blue line - caramelized honey.

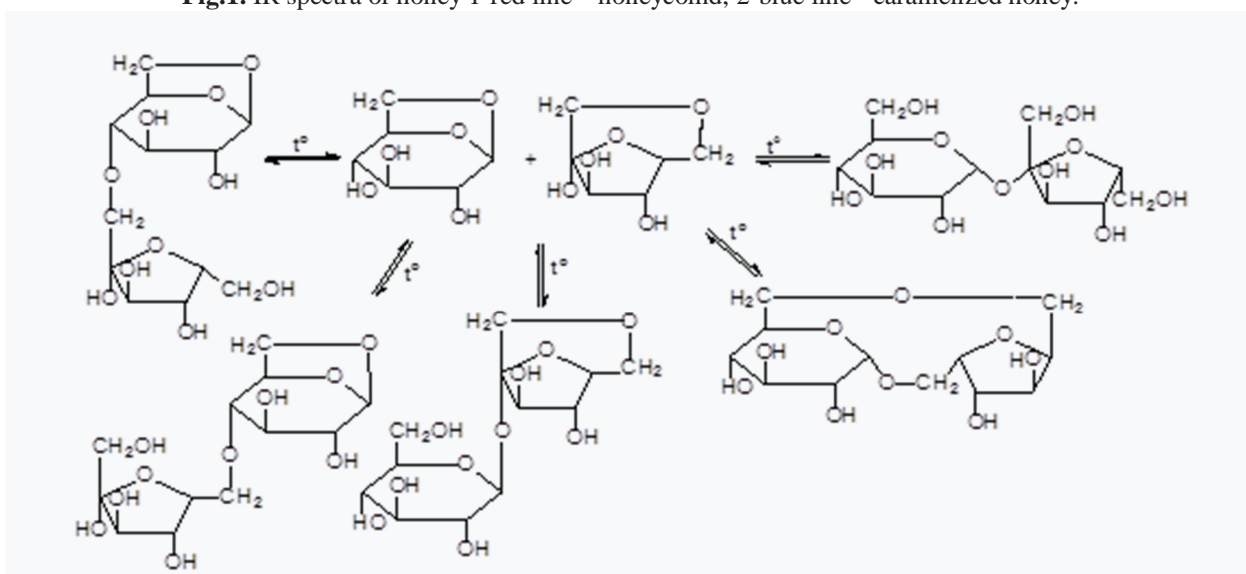


Fig. 2. Reactions and possible products of cycloformation and condensation- caramelization of sugars.

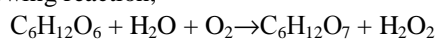
synthesized artificially, its taste is bitter, slightly burning. At a temperature of 20-40°C dense less than 20°C - solid. The content of wax in propolis is less than 28%, the oxidation rate is 22%, the iodine number is less than -35. Beeglu has bactericidal and antiviral activity against new viruses and bacteria that have “adapted”. Beeglu includes: natural resins (40-55%), beeswax and fatty acids (20-35%), aromatic oils (about 10%), pollen (about 5%) and other components. Today it is experimentally proven that beeglu has a wide range of pharmacological activity. It has bactericidal, local anesthetic, bacteriostatic, antifungal, antiviral, antitoxic, immunostimulating, anti-inflammatory effect on the human body. Therefore, beeglu-based drugs are successfully used in surgery, dentistry, pediatrics, gerontology. Nowadays beeglu is the most widely applied in dentistry: 2-4% of beeglu alcohol extracts are effective in the treatment of fungal diseases of the oral cavity, abscesses on the gums and in the complex therapy of periodontitis and gingivitis. Due to local anesthetic

effect, beeglu can be used as an analgesic in case of hypersensitivity of the tooth.

Bee pollen contains the following minerals in mg / 100g: ascorbic acid -1.4-205.2 mg, phosphorus 50-610 mg, vitamin B (thiamine) 0.4-1.5 mg, potassium 130-1140 mg, riboflavin 0.54-1.9 mg, calcium 30-1180 mg, B5- nicotinic acid 4.8-21.0mg, magnesium 60-380 mg, B6 (pyridoxine) 0.5-0.9mg, sodium 28 -44mg, folic acid 0.1-0.68 mg, copper 0.6-1.57, H (-biotin) 0-0.25mg, iron 0.2-4.2 mg, pantothenic acid 0.32-5, 0 mg /, E (á-tocopherol) 0.3-170, water-3-4%, recovered sugars 20-40%, non-recovered sugars 0-20%, fats 1-20%, proteins 11-35%, amino acids 10-45%, antibiotics + vitamins 5.75-10.8 mcg / g, riboflavin 16.3-19.2 mcg / g, pi ridoxin 0-9 µg / g [1].

The antibacterial property of honey was first recognized in 1892 and it was discovered that the honey concentration must range from 1.8% to 10.8% (v/v). Thus, honeycomd had sufficient antibacterial potency to stop bacterial growth if diluted at least nine times [2].

Important factors which influence the antibacterial effectiveness of honey are as follows: Its hygroscopic properties: This effect is based on high osmotic properties so it can extract water from bacterial cells and cause them to die. Honey, like other saturated sugar syrups and sugar pastes, has an osmolarity sufficient to inhibit microbial growth. Its acidic pH: Honey is characteristically quite acidic, its pH being between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens. The optimum pH for growth of these species normally falls between 7.2 and 7.4. Hydrogen peroxide: The major antibacterial activity in honey has been found to be due to hydrogen peroxide produced enzymatically in the honey. The glucose oxidase enzyme is secreted from the hypopharyngeal gland of the bee into the nectar to assist in the formation of honey from the nectar. The hydrogen peroxide and acidity produced by the following reaction,



These substances serve to preserve the honey. The hydrogen peroxide produced would be of effect as a sterilizing agent only during the ripening of honey. Full-strength honey has a negligible level of hydrogen peroxide because this substance is short-lived in the presence of the transition metal ions and ascorbic acid in honey which catalyze its decomposition to oxygen and water. The enzyme has been found to be practically inactive in full-strength honey, it giving rise to hydrogen peroxide only when the honey is diluted. This is because the acidity produced in the action of the enzyme drops the pH to a point which is too low for the enzyme to work anymore. On dilution of honey the activity increases by a factor of 2.500 – 50.000, thus giving a "slow-release" antiseptic at a level which is antibacterial but not tissue-damaging [3]. Therefore, the research work on properties of bee products and particle changes that occur during caramelization of honey is very urgent both for Chemistry and Medicine.

I. Experimental

The authors of the given article suggested and carried out the experiment of low-temperature honey caramelization [4]. The properties of caramelized honey and its composition and structure were studied by using IR spectra measurements with honeycomd on a FTIR spectrometer Nicolet S50; ThermoScien'fic. The results obtained are summarized below (see Figure 1). The comparisons of the IR spectra of raw (red line) and caramelized honey (blue line) after low-temperature isothermal treatment make it possible to state that there is almost no difference in the chemical composition of the products, with the exception of some areas of the IR spectrum ($1600\text{-}500\text{ cm}^{-1}$).

This can be explained by the appearance in the structure of caramelized honey of supramolecular changes, namely condensed, caramelized mono-, di- and polysaccharides into cyclic monosaccharides, in which the chemical structure is almost unchanged, and the molecular weight and degree of polymerization,

polycondensation of sugars.

In this case, intramolecular and intermolecular cycles of sugars can occur. Subsequent heating causes the sugars and their formed anhydrides to condense. As a result of longer heating and caramelization, a system of condensed (colored) compounds is formed, among them are caramelan ($\text{C}_{12}\text{H}_{18}\text{O}_9$), caramelen ($\text{C}_{36}\text{H}_{50}\text{O}_{15}$) [5].

For a detailed study of the supramolecular structure of caramelized honey we have applied the automatic analyzer to compare it with the structure of honeycomd, we have also used the method of determining the size and size distribution of nanoparticles of macromolecules of oligomeric compounds of honey, including polysaccharides. The size and distribution of nanoparticles in static and dynamic states were studied by laser scattering (400 nm - 700 nm) of particles in aqueous solutions, dispersions, emulsions and solids, sizes from 0.3 nm to 100000 nm (10 μm) (Size analyzer of particles CILAS Nano DS is an innovative optical device that allows you to measure the size of nanoparticles without having to replace the optics or calibrate the device). As a result we have a series of particle distribution represented in lines shown in Fig. 3.

The data obtained (see Fig. 3) let us assume that honeycomd has 4 types of macromolecules, which differ in (1.5-2.0 times) size of nanoparticles if compared to caramelized honey (Fig. 3). The highest content is 51.5% of particles with an average diameter of 13594 nm (1.359 μm) (blue line in Fig. 3). Then there are smaller nanoparticles (blue line in Fig. 3) - 21.4% - 12392 nm (1.239 μm) and even smaller particles of honey sugars (red line in Fig. 3) 18.9% - 6721 nm (0.672 μm), and the smallest honey nanoparticles (green line Fig. 3) - 8.9% - 258 nm.

According to the obtained results we can conclude that caramelized honey (see Fig. 4) also has 4 types of macromolecules that differ in much smaller nanoparticle sizes compared to honeycomd (Fig. 3). The highest content, 50.6%, was found in particles with an average diameter of 7469.5 nm (0.746 μm) (red line in Fig. 4), 34.7% - in the larger nanoparticles of 8655.1 nm (0.865 μm) (blue line in Fig. 4) and smaller (216,2 nm) - 9,7% (green line in Fig. 4).

As we can see from the nanoparticle distribution (see Figs. 3, 4) described above, a new peak appears in caramelized honey in the area of smaller nanoparticles - 713.7 nm, the amount of which is 5.3% and even less caramelized sugar content of 216.2 nm - 9.7%. The total content of nanoparticles of caramelized honey (Fig. 4) which have a size greater than 1000 nm is 85% that is significantly less than in honeycomd - 92% (Fig. 3).

According to the obtained data, a diagram of the distribution of nanoparticles of raw and caramelized honey (Fig. 5) is constructed, which leads to the conclusion that two types of reactions occur during caramelization of honey. Depolymerization of the polysaccharides with decreasing particle size, on the one hand, is confirmed by the blue diagrams in Figs. 5, they are smaller in three diagram ranges. Only in the range of 6721-7469.2 nm we can observe a small (748 nm) increase in the size of macromolecules of caramelized

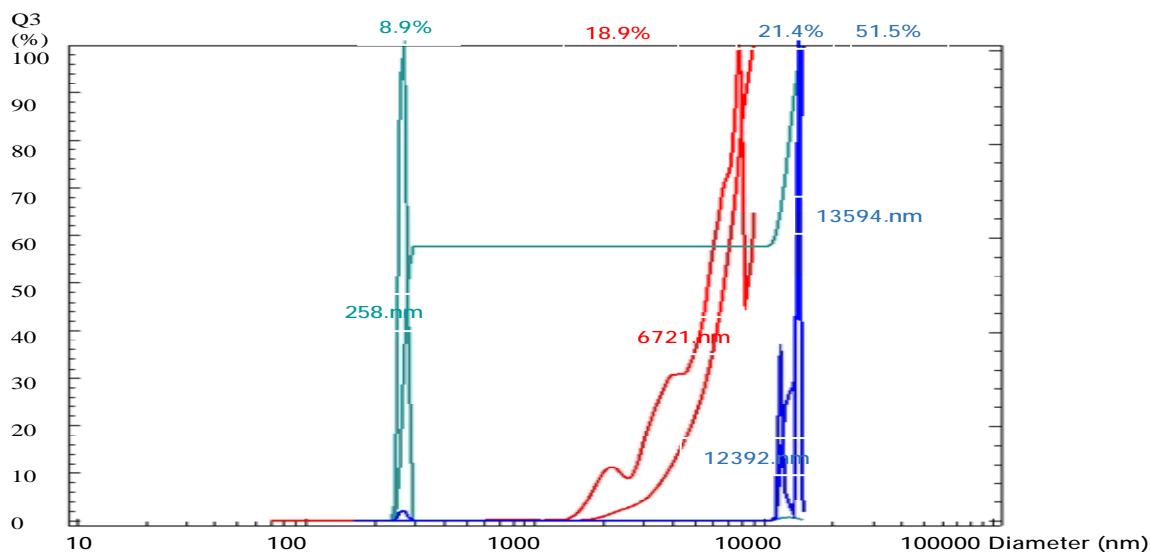


Fig. 3. Distribution of raw honey macromolecules by size, in dynamic and static conditions of diameter measurement (nm) on a CILAS Nano DS device.

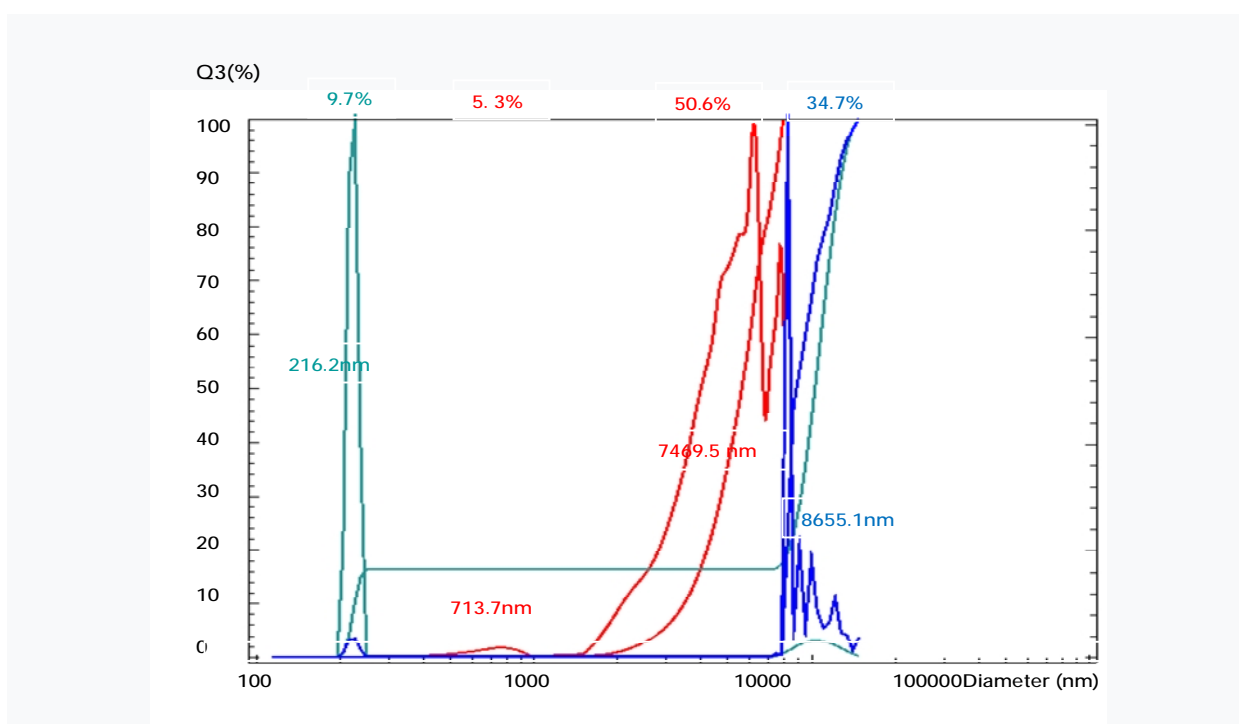


Fig. 4. Size distribution of nanoparticles by the diameter of macromolecules of caramelized honey under dynamic and static conditions.

honey, it can be explained by the polymerization and polycondensation reaction of mono- and disaccharides (reaction scheme, Fig. 2).

The obtained results show that the content of particles with a diameter bigger than 13594 nm in honeycomd responds to 54.5 %, (Fig.5 red line), and at the same time the diameter of the particles for caramelized honey is approximately 2 times smaller (7469 nm) and their content does not exceed 50.6 % (Fig.5 blue line). As we can see, the honey caramelization decreases the size of honey nanoparticles

by 1.5-2.0 times to 258-8655 nm, in our opinion, this is the key to their being biological active in dentistry. According to the results of X-ray fluorescence analysis, we display the histograms of the composition of the elements (see Fig. 6 and their oxides (see Fig. 7) in raw (N) and caramelized honey (N1, N2, N3).

Thanks to obtained results we confirm our hypothesis that at low-temperature catalytic caramelization of honey, its elemental composition hardly changes, but at the same time the supramolecular structure and structure of macromolecules (di- and

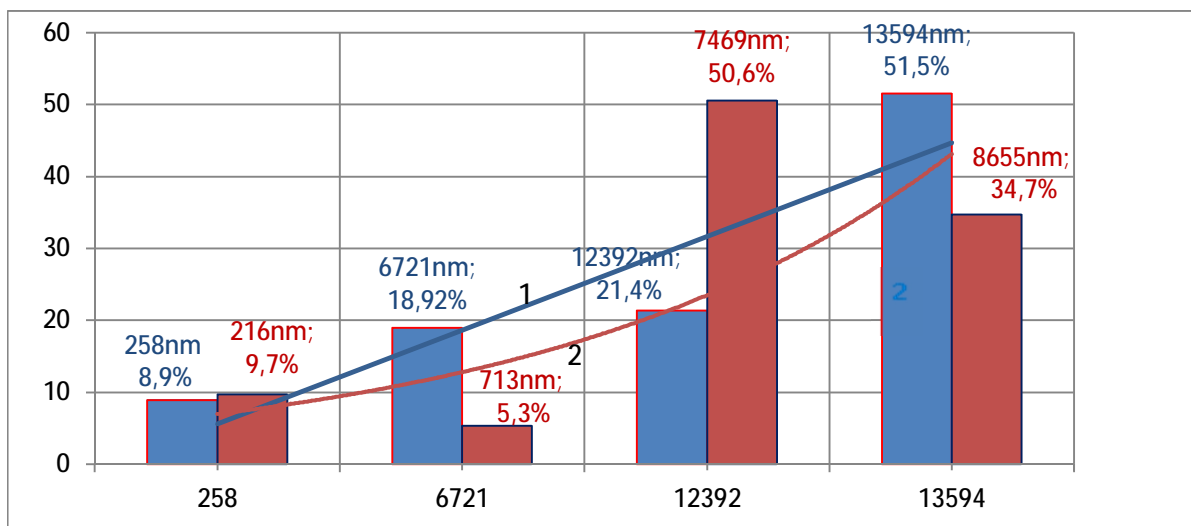


Fig. 5. Histograms of the comparative size distribution of nanoparticles of raw honey (1 - blue line) and caramelized honey (2 - red line).

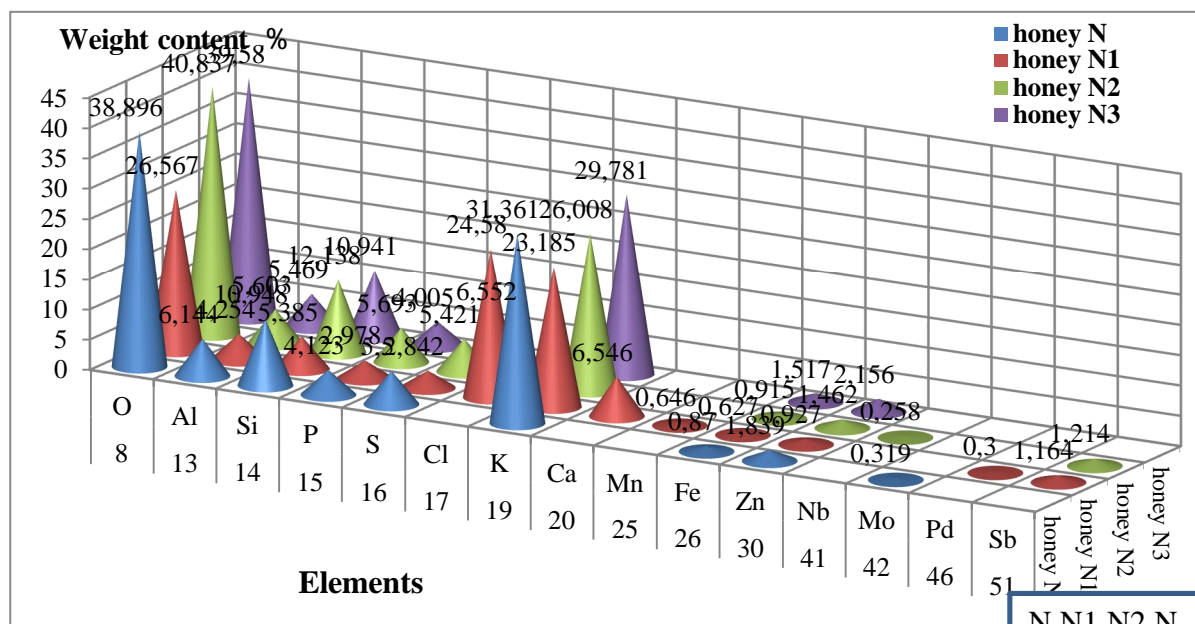


Fig. 6. Results of X-ray fluorescence analysis of elemental composition of raw N and caramelized honey: N1, N2, N3. (EDX-7000 energydispersive x-rayfluorescencespectrometer, Shimadzu Na-U , vacuum).

polysaccharides) of honey change.

The histograms in Fig. 7 show that in all samples of raw and caramelized honey there is the highest content of such elements as: Oxygen (26-40%), Silicon (5-12%), Potassium (23-31%), Aluminum (4-6%) and Phosphorus (3-6%). While caramelization process the content of these elements (except for Fe) changes slightly.

Histograms of oxide content in raw and caramelized honey are shown in Fig. 7. We can conclude that in all samples of honey, the following oxides as SiO₂-11-26 %, K₂O-27-37 % , Al₂O₃- 4-6 % and P₂O₅- 6-13 % are present in considerable quantity: It should be noted that the content of oxides of most of the elements changes while caramelization process slightly, except for the oxides: SO₃, Fe₂O₃ and ZnO, their content increases

compared to their content in honeycomd (N). Applying chemical and physical and chemical methods we analyzed the content of pure Fe and the total content of oxides (FeO, Fe₂O₃, Fe₃O₄) in the composition of raw and caramelized honey, the results are presented in the histogram, Figure 8.

As we can see in Fig. 8, the content of Fe rum compounds after caramelization first decreases from 1.244 % and then increases approximately 2 times up to 2.168 %. This may occur due to the effect of the Fe-containing catalyst on the honey caramelization process [4].

We have carried out the analysis of honey, mainly the content of undesirable hydroxymethylfurfural and sufficient value of diastasis, as the main indicators of

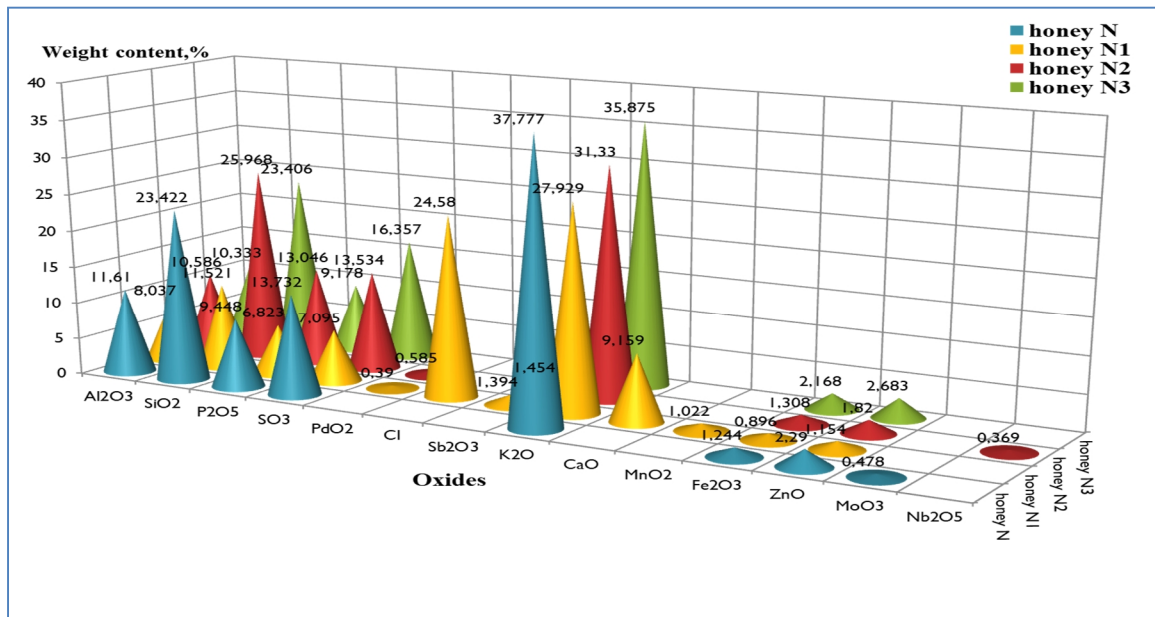


Fig. 7. Results of X-ray fluorescence analysis of oxide content in samples of raw N, and caramelized honey: N1, N2, N3. (EDX-7000 energydispersive x-rayfluorescencespectrometer, Shimadzu Na-U, vacuum).

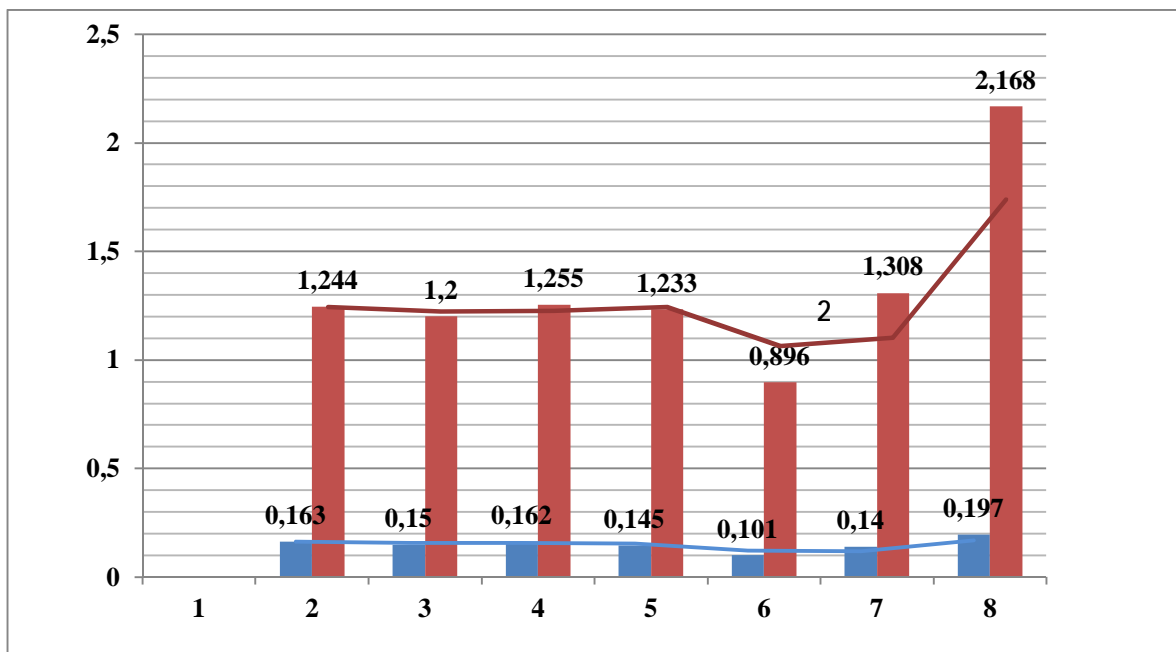


Fig. 8. Histograms comparing Fe content (1-blue histogram), Fe₂O₃ (2- red histogram) of raw and caramelized honey (byluminescent X-ray and chemical analysis).

quality and biological activity of honey before and aftercaramelization process (Fig. 9). As we can seein the histograms of these indicators (Fig. 9), at low-temperature isothermal caramelization of honey the hydroxymethylfurfural content increases from 5 to 21.5, but it does not exceed its allowable values up to 25 mg / kg according to State standard of Ukraine 4497: 2005 for honeycomd (Fig. 9, blue line).

But when re-treated (caramelized), its content increases to 26 and 43 mg / kg, which exceeds the

permissible State standard of Ukraine (10-25 mg / kg). In this case, the diastasis number in units of Gote partially decreases from 10-16 units. According to State standard of Ukraine, Gota is up to 8,7-13 after thermochemical treatment - caramelization (samples 1.2.2. in Fig. 9 red histograms). This confirms the fact that honey can be subjected to low-temperature thermochemical treatment, but the overheating of the honey over 75°C during 15-20 minutes cannot be allowed.

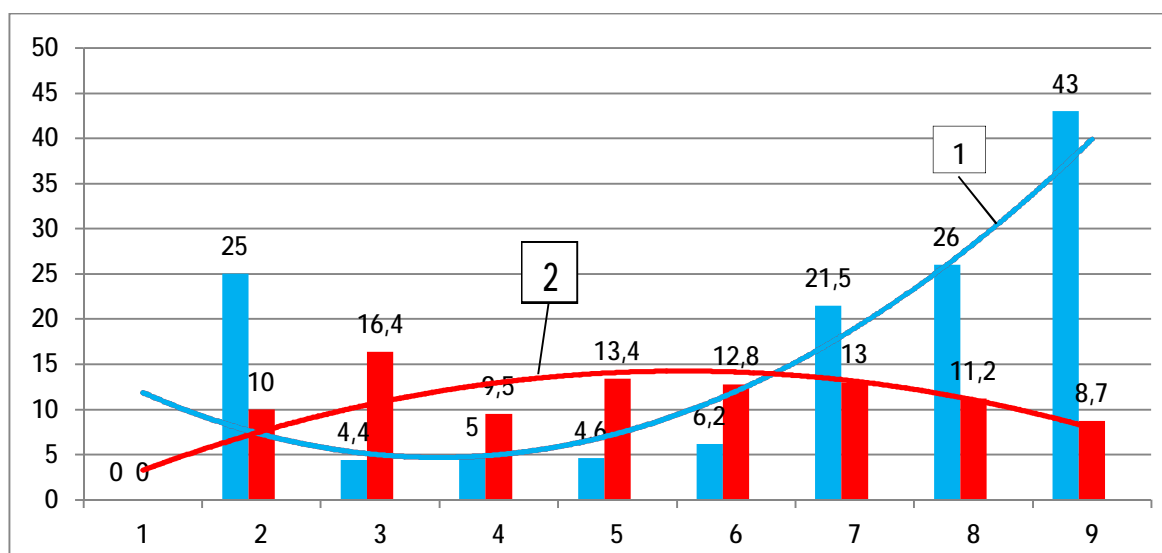


Fig. 9. Histograms of oxymethylfurfural content (1 – blue histogram) and diastase number (2 – red histogram) of raw and caramelized honey.



1

2

Рис. 10. (1,2). Пилкок у натуральному меді - 1, (D -частинки пилку = 0,05 - 0,1 мм) і карамелізованому меді - 2, (D -частинки пилку=0,01-0,01 мм), (термообробка при $T = 60-75^{\circ}\text{C}$., $\tau = 45-90$ сек. + каталізатор), мікроскоп МБС-20 (x56 раз) [6].

Issues on crystallization of honey and its viscosity are of great interest to both producers and consumers. Liquid, non-crystallized honey is in the greatest demand. The microscopic crystals of glucose, pollen and barley are the primary centers of crystallization in honey. Reducing the amount of pollen leads to a decrease in crystallization, this can be achieved by special heat treatment –caramelization of honey. The particle size of the pollen thus varies from 0.1 mm to 0.01 mm. (see Fig. 10 (1,2)). The samples of 10% solutions of different varieties of honey were analyzed for oxygen content. The obtained data show that the dissolved oxygen content in 10 % aqueous solutions of different honey varieties decreases after low temperature treatment, but in caramelized honey the oxygen content (0.37 mg / l) is higher than in the raw one (0.37 mg / l), and less than in a honeycomb (1.22 mg / l). The results of studies of the particle size of the pollen contained in all honey combs have shown that after low-temperature chemical treatment (caramelization), the pollen particle size

decreases by 5-10 times compared to the pollen diameter of honeycomb (see Fig. 10).

Conclusion

The technology of honey caramelization employing the method of low-temperature thermochemical heating has been proposed. The reactions of honey caramelization and other bee products have been studied for the first time. The biochemical properties of the obtained products have also been investigated, the capacity to transform into biologically active compounds during thermochemical process of honey in the presence of catalysts has been investigated. The IR spectra and elemental composition (by using X-ray fluorescence analysis) of raw and caramelized honey, which are almost the same, have been studied. The diameter of macromolecules and the size distribution of nanoparticles for caramelized honey are determined; the nanoparticles

of caramelized honey are 1.5-2.0 times smaller than those of honeycomd, for this very reasonit determines their biochemical activity. The content of hydroxymethylfurfural and the diastase number of caramelized honey have been analyzed, they satisfy State standard of Ukraine. Clinical studies carried out center University Dental Clinic of Uzhhorod National University, (Ukraine), confirmed the high efficiency (72%) of "Medivnyk" chewing gum based on caramelized honey, wax and beegleue for the prevention and symptomatic treatment of dental diseases.

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Біополімерні наноструктурні композиції на основі карамелізованого меду

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Вивчено хімічні властивості меду, воску, прополісу до та після процесу карамелізації меду. Вперше досліджено реакції, що відбуваються при карамелізації меду та інших продуктів бджільництва, вивчено біохімічні властивості отриманих речовин. Виявлено посилення біологічної активності композиції при термохімічній обробці меду в присутності спеціально підібраних каталізаторів. Досліджені ІЧ спектри, вологість, в'язкість, розміри та розподіл за розмірами наночастинок, елементний склад, вміст оксиметилфурфурулу, діастазне число карамелізованого меду. Виявлена різниця між розмірами наночастинок натурального та карамелізованого меду, а саме: значно менші (1,5 - 2,0 рази) розміри частинок карамелізованого меду зумовлюють його біохімічну активність. На основі вивчених властивостей та отриманих результатів з карамелізованого меду, воску і прополісу створена жувальна гумка з біологічно-активними компонентами для профілактики і лікування захворювань тканин пародонту (гінгівіту, пародонтиту і пародонтозу). Проведені клінічні дослідження із застосуванням жувальної гумки на основі меду, воску і прополісу для лікування ротової порожнини підтвердили позитивний ефект у 72 % пацієнтів.

Keywords: мед, віск, прополіс, карамелізація, біохімічні властивості, жувальна гумка, профілактика, гінгівіт, пародонтит, пародонтоз, лікування.