UDC 57.085.2+353

PHYSICOCHEMICAL PARAMETERS AND PROTEIN CONTENT IN THE MUCUS OF *HELIX ASPERSA*

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Since 2015 snail culture of species Helix aspersa Müller have been successfully introduced into Ukrainian farming. Snail mucus contains biologically active components that determine its widespread using in pharmaceutical and cosmetic products. In this work, the moisture content (98,43±0,2%) and pH = 8.2±0,1 in the snail mucus obtained from a farm company, were determined. The water-soluble fraction presence of complex proteins was determined by the biuret test, the obtained index was found to be 23.2 g/L. Experiment results are important for improving the lyophilic drying technology of mucus and further targeted applying of product.

***Keywords**: mucus, Helix aspersa, moisture content, pH, biuret test*

Introduction. Snail mucus has been used in medicine since ancient times for burn injuries and different diseases treatment. Research on land helixes secretions has confirmed that the mucus contains a unique ingredients combination with beneficial and therapeutic qualities for human skin. Some investigators then also reviewed the pharmacological activities of the land snail *Helix pomatia* mucus, such as mucolytic activity, its inhibiting effect on pathogenic bacteria and spasmolytic activity in the respiratory tract. The authors concluded that the effect of helix extract on the trachea is partially related to the release of a relaxant prostaglandin, but its cellular origin within the pulmonary tract remains to be determined [1]. The various chemical components of *Helix* can also be analyzed using enzyme procedures thus revealing:

more than 30 enzymes within the digestive mucus and several enzymes within the pancreostomach, the muscle, and lymphatic fluid [2].

Using an anion-exchange column chromatograpgy the different glycosaminoglycans were identified containing uronic acid, hexosamine and sulfate was shown to be present in helix mucus [3]. Such components are important ones for using in burn injury therapy and cosmetology for moisturizing and soothing effect. Other useful components of snail mucus, that are used in cosmetic products, are allantoin and glycolic acid [4]. Due to research *in vitro* impact of mucus extract on fibroblast cultures, has been found that compounds of Helix complex are lacked of cytotoxicity, protect cells from apoptosis and, that is more important, was are able to significantly induce cell proliferation and migration through direct and indirect mechanisms. Such effects were associated to morphological changes, cytoskeleton reorganization and release of cytokines in mammalian fibroblasts [5]. Snail mucus in quantities from 5% to 50 have also used in special device "socks" for preventing, treating and curing ulcers on a diabetic foot. A gauze soaked by the mixture, that can be formed as a cream or a gel and shaped as a bandage or sock to be applied over ulcerous wounds or other skin-like conditions. Mentioned composition also contains natural extracts such as marigold (calendula) extract, propolis and vegetable oils. Based on such content it was preparing a medicine or device for prevention or treatment illnesses of ulcers or poor prognosis wounds caused by metabolic diseases such as diabetes, gout, Lesch Nyhan Syndrome or other conditions to the skin, including keratosis and onychomycosis [6].

Helix aspersa Müller is one of edible land snail species, which is wide breeding in agriculture. It is also known as «small grey snail» or French «petit gris escargot». *H. aspersa* is native habitant of to the shores of the Mediterranean and up the coast of Spain and France. In the early 19th century the French brought this snail into California, where it has become a serious pest. These snails are now common throughout the United States. It was introduced into several Eastern and Gulf states even before 1850 and, later introduced into other countries such as Australia, South Africa, New Zealand, Mexico, and Argentina. *H. aspersa* has a life span of 2 to 5

years. This species is more adaptable to different climates and conditions than many snails, and is found in woods, fields, sand dunes, and gardens. This adaptability not only increases species range, but it also makes their farming easier and less risky. In Europe, recent annual consumption of snails is about 100 000 tons. These mollusks are consumed in many countries but mainly in France (about 40000 tonns/year) and Italy (about 6000 tonns/year). Snail meat is very low in fat (0.5 to 0.8%) and relatively low in calories (60-80 cal/100 g), but has a biological high value of protein (12-16%) minerals (1.5%) and nitrogen (2.5%) [7].

In 2015, the first snail farm opened in Ukraine. Production was, and remains, almost entirely for export, there being no consumer market for snails in the country. In 2020 Ukraine had more the 400 farms. Export was decimated in 2020, however, by quarantine lockdowns related to the COVID-19 pandemic. Thus, a most of farm companies is redirected their production on obtaining snail mucus as demanded ingredient for modern cosmetic industry. Snail mucus are collected, settled, filtrated, concentrated and lyophilized at low temperature under vacuum evaporation.

The aim of current research is analysis of native mucus samples collected in one Ukrainian breeding farm on physical chemical properties (pH, water content) and content of water soluble proteins. Mentioned parameters are valuable for estimation of native mucus quality and optimization of its conservation technology and storage.

Material and methods. Native mucus collected on a snail farm was freezing (-19 ^{0}C for 48 h) for preventing product deterioration. Before test conduction the mucus samples were defrosted and immediately used. For estimation of protein concentration the thawed mucus and mucus conserved in ethanol solution (1:1) have been used.

Estimation of water content and pH of mucus

The water content was determined by drying the samples in glass boxes placed on laboratory hot air oven (+105 ^{0}C) to constant weight.

pH measurement were carried out on the pH meter 150-MI with glass electrode. Device was calibrated by line of standart buffer solutions with known hydrogen ion activity before experiment.

All testing were conducted in five replication for obtaining representative results.

Analysis of water soluble protein fraction

The biuret reagents diagnostic set for protein content in blood serum estimation produced by Genesis LLC (Ukraine) has been used. According to instructions, the ampoule with sterile albumin solution was opened and gradual line of solution in concentration, such as 40 g/L, 60 g/L, 80 g/L, 100 g/L was prepared. All these solutions were mixed with biuret reagent; optical density (D) was measured using photoelectric concentration colorimeter (CPC-2), quartz cuvettes with 1 cm optical path length at light wavelength of 540 nm. Calibration graph was created on the obtaining results (fig 1).

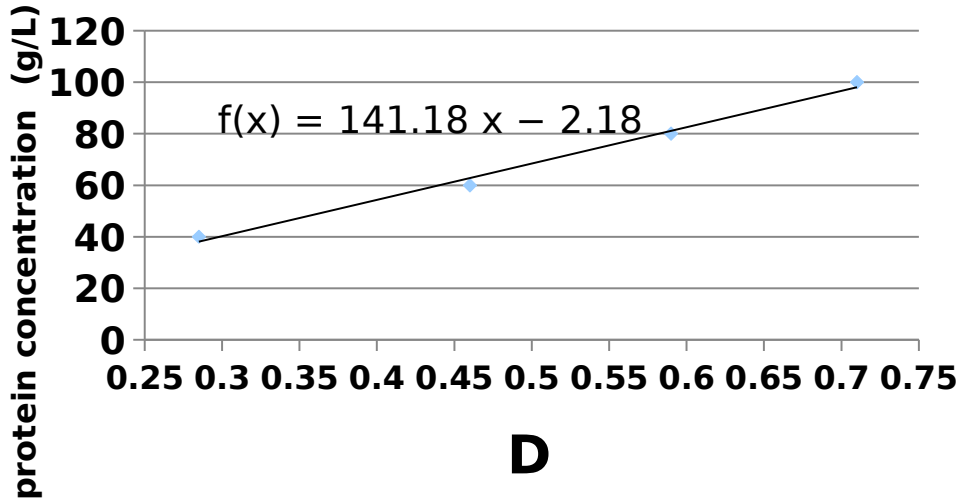

Fig. 1. Optical density of albumin solution for calibration graph

Linear calibration graph is described by a equation:

$$y = 141{,}18\,x - 2{,}178$$

This equation we used for calculation of water-soluble protein concentration after measures optical density of samples with biuret reagent + mucus prepared according the instructions.

Next step was preparing mixed solutions of mucus and biuret reagent. According to instruction of Genesis LLC diagnostic set it was necessary to add 2 ml of snail mucus to 0.04 ml (40 μl) of biuret reagent. Unfortunately, snail mucus had a

thick viscous consistency and it was difficult to quantitatively transfer the amount of mucus with a capillary micropipette. As a result, several samples of thawed mucus and one sample mucus in ethanol were prepared:

- three samples (№ 1, № 2, № 3) according to the standard scheme: 2 ml of reagent + ≈ 0.04 ml of mucus;
- one sample (№ 4), in a large test tube, where the reagents were increased × 12.5, times, as to 25 ml of biuret reagent was added 0.5 ml of mucus;
- mucus in ethanol (sample № 5) as biuret reagent (2 ml) and 0.04 ml of mucus in ethanol.

Color variety of solutions from blue to violet is depending on different protein content inside (fig 2).

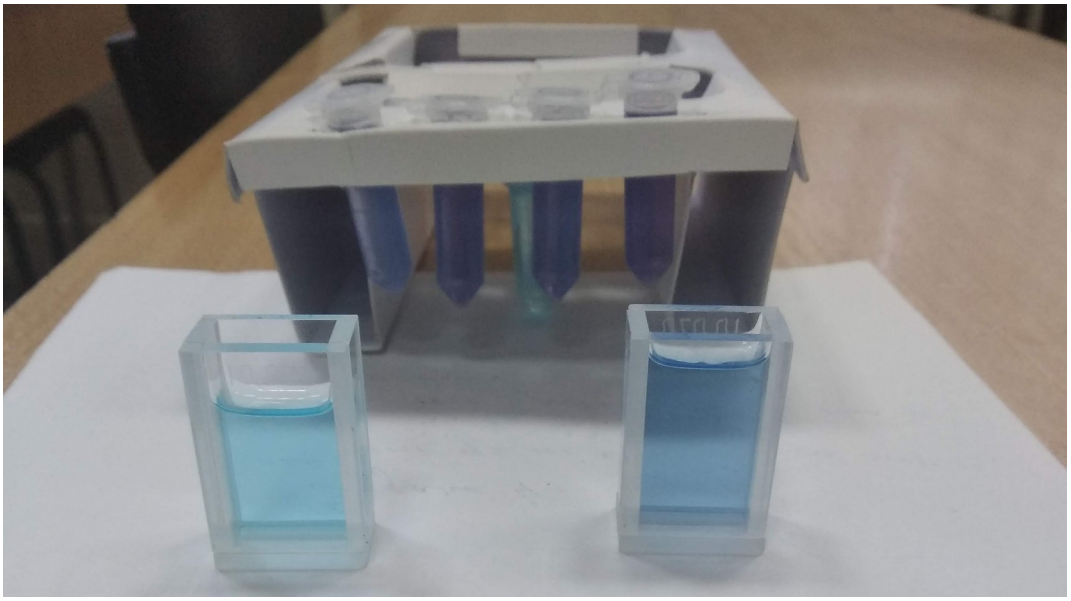

Fig. 2. Cuvette on the left with pure biuret reagent; cuvette on the right - biuret reagent + mucus from a large test tube (sample № 4); photo background is calibration solutions (albumin in different concentrations + biuret reagent)

Results and discussion. Experimental results of water content in mucus are presented in table 1.

Table 1

Water content in mucus samples

№ sample	mucus mass before drying (g)	mucus mass after 11 hour drying at 105 ^{0}C (g)	water content, %
1	30.8269	0.489	98.41
2	30.7678	0.4933	98.40
3	31.235	0.4899	98.43
4	30.9522	0.4789	98.45
5	30.2413	0.4721	98.44

So, the middle value of water content mucus is 98,43±0,2 %.

Series pH measurements of mucus and their correction according to the calibration graph showed that the average value is pH = 8,2±0,1.

Results of optical density and calculation of protein concentration according equation presented in table 2.

Table 2

Protein content in mucus measured by biuret test

№ sample	Optical density value	Protein concentration (g / L)
1	0,115	14.1
2	0,12	14.8
3	0,1	11.9
4	0,18	23.2
5	0,1	11.9

Snail mucus has a complex fractional composition and low solubility of some glycoprotein fraction, which can be explained primarily by their high molecular weight. Features of micro quantitative analysis significantly affect the accuracy of measurement. Thus, as final results we taking into account the sample № 4, where 0.5 ml of mucus was analyzed: the content of water-soluble fraction of protein and peptide components in the native mucus frozen at -18^{0}C and thawed up to +20 ^{0}C is 23.2 g/L. Ethyl alcohol is a more polar solvent than water, but we obtained close results for sample № 5 to samples № 1–3. So, we assume the feasibility of using an

alcoholic solution of mucus in the composition of future cosmetics or pharmacy combining with native mucus.

All obtained result of physicochemical parameters and protein content will be used for optimization of technology for mucus storage and preservation for further applying in cosmetic and medicine products.

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ВИЗНАЧЕННЯ ФІЗИКО-ХІМІЧНИХ ПОКАЗНИКІВ ТА ВМІСТУ БІЛКІВ В СЛИЗІ РАВЛИКА *HELIX ASPERSA*

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Культуру равлика Helix aspersa Müller з 2015 року активно впроваджують в українське фермерське господарство. Слиз равлика містить біологічно активні компоненти, які обумовлюють його широке застосування в фармацевтичній і косметичній продукції. В даній роботі визначено вміст вологи (98,43±0,2%) та pH = 8,2±0,1 в нативному слизі равлика, який було одержано від вітчизняного виробника. Біуретовим методом визначено вміст водорозчинної фракції складних білків, одержаний показник склав 23,2 г/л. Визначені параметри є важливими для удосконалення технології ліофільного висушування слизу та подальшого цільового використання продукту.

Ключові слова: слиз, *Helix aspersa, вміст вологи, pH, біуретовим тест*

ОПРЕДЕЛЕНИЕ ФИЗИКО-ХИМИЧЕСКИХ ПОКАЗАТЕЛЕЙ И СОДЕРЖАНИЯ БЕЛКОВ В СЛИЗИ УЛИТКИ *HELIX ASPERSA*

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Культуру улитки Helix aspersa Müller с 2015 года активно выращивают фермерские хозяйства Украины. Слизь улитки содержит биологически активные компоненты, которые обусловливают его широкое применение в фармацевтической и косметической продукции. В представленной работе определено содержание влаги (98,43±0,2%) и pH = 8,2±0,1 в нативной слизи, полученной от отечественного производителя. Биуретовым методом определено содержание водорастворимой фракции сложных белков, полученный показатель составил 23,2 г/л. Определенные параметры важны для усовершенствования технологии лиофильной сушки слизи и дальнейшего целевого использования продукта.

Ключевые слова: слизь, *Helix aspersa, содержание влаги, pH, биуретовый тест*