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# ИЗВЕСТИЯ

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АО «Институт топлива, катализа и
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Институт органического катализа и электрохимии им. Д. В. Сокольского,
каб. 310, тел. 291-62-80, факс 291-57-22, e-mail:orgcat@nursat.kz
Адрес типографии: ИП «Аруна», г. Алматы, ул. Муратбаева, 75

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**[1]B.Sh. Ismailkhodjaev, [2]B.A. Khalmurzayeva, [3]M.I. Satayev, [3]R.S. Alibekov**

[1]Tashkent Institute of Irrigation and Agricultural Mechanization, Qori Niyozov street, 39, Tashkent, 100000, Uzbekistan
[2]University of Friendship of Peoples named after academician A. Kuatbekov, Tolebi street, 32, Shymkent, 160000, Kazakhstan
[3]M.Auezov' South-Kazakhstan State University, Tauke Khan avenue, 5, Shymkent, 160012, Kazakhstan

# STUDY OF VITAMINS CONTENT OF MICROALGAE

**Abstract.** The efficiency of using microalgae biomass for practical purposes is determined by their physiological and biochemical features. The growth and development of algae are closely interrelated with external factors, under the influence of which the structural, functional and biochemical characteristics of the cell change. Selection of new highly active microalgae-producers of essential compounds in the natural conditions would expand the area of their practical application. The present work shows a comparative study of the amount of vitamins — provitamin A, vitamin C, vitamin E in the ten types and strains of green and *Euglena* microalgae, isolated from the local flora that grown in semi-industrial terms and under the open atmospheric conditions: *Chlorella pyrenoidosa ChickYA-1-1; Scenedesmus obliquus YA-2-6; Ankistrodesmus angustus YA-3-1; Ankistrodesmus braunii; Chlamydomonas reinhardii YA-5-16; Chlamydomonas reinhardii 449; Euglena gracillis YA-4-17; Euglena YA-4-19; Dunaliella salina; Dunaliella minuta UA-5-10*. It is shown that the vitamins content depends on the type of algae strain and the seasons, the duration of cultivation and the type of mixing. The developed biomass with a high content of vitamins allow to increase the biotechnological potential of microalgae that will provide an opportunity for the problems solving related to the provision of various sectors of the national economics by algae-rich raw materials: animal husbandry, poultry farming, fish farming, as well as food and pharmaceutical industries.

**Keywords:** biosynthesis, cultivation technology, microalgae, strain, vitamins.

## INTRODUCTION

Currently, in the field of algal biotechnology, the studies are based on the isolation of new species and strains of microalgae [4, 16]. Mostly, the studies of their physiological, biochemical and environmental features that enhance productivity and the synthesis of biologically active substances are applied [6]. The demand for food having beneficial effects on human health is also increasing in Kazakhstan [10].

As known, vitamins play an important function in normal metabolism and vital activity of organisms. They are essential for human nutrition, animals and other organisms, but unlike proteins, fats and carbohydrates, the quantitative needs of the body for vitamins are negligible. Vitamins often ensure the functioning of biological catalysts. For the catalytic activity of many enzymes, certain non-protein cofactors are required, the function of which can be performed by vitamins [5].

Microbiological synthesis is most suitable for the production of vitamins for livestock and crop production, and it became as basis of their industrial production for these purposes [2].

Algal biomass is rich in various vitamins, including those typical for plants. However, their content varies in different algae. For example, the content of provitamin A or Carotene is the most promising for *Dunaliella* (1100 mg %), the content of which exceeds all known vegetable foods. The amount of Carotene in *Chlorella* reaches 140-200 mg % that is 3 times more than in grass meal [15].

Algae are rich in vitamin C or Ascorbic acid, the content of which is as much as in lemon and 100 times more than milk. Algae contain in significant amounts of Tocopherol, Riboflavin, Nicotinic acid and other biologically active compounds. Algae contain Thiamine and Pyridoxine as much as corn, barley and oats 5.6 & 5.3 (μg/g), respectively. Vitamin B12 and D are found in significant amounts of green algae biomass. For instance, 100 g of dry *Dunaliella* biomass consists in 7.5 μg/g of vitamin B12, and 100 mg of vitamin D in *Chlorella* 100 mg of green algae are currently found [16].

Along with chemotrophic microorganisms, phototrophic microorganisms, in particular microalgae can also be a source of a complex of various groups of vitamins [11].

One of the essential compounds is vitamin C (Ascorbic acid) that is involved in a number of redox reactions and provides vital processes in the human body. Presently, in assessing the mechanism of action of Ascorbic acid, great importance is attached for its possible participation in the prevention of oxidation of active sulfonate hydroxyl groups, including proteins with biocatalytic activity. This function is performed by the reduced form of Ascorbic acid [13].

Recently, more attention of researchers acquires vitamin E (Tocopherol) due to its influence on the function of the sex glands in reproduction. It is also known that it takes part in the regulation of a number of vital processes in living organisms. Therefore, it is often used as a vitamin supplement to feed [8].

Tocopherol can input to the body only by food. Its absence in the organism leads to the disruption of the activity of not only the reproductive system, but also of many other organs and tissues. By preventing the oxidation of saturated fatty acids, the most important component of cell membranes and organelles, Tocopherol thereby maintains a structural integrity of the cells and consequently of the whole living organism [7].

Therefore, in the production of food based on microalgae, the main requirement is a high content of vitamins in them.

**Purpose of the study:** the purpose of this work was a comparative study of the content of three important vitamins from biomass of 10 species and strains of microalgae isolated from the local nature and producers of the above mentioned compounds with the determination of the change in the amount of these vitamins depending on the season of the year and the duration of cultivation.

## OBJECTS AND METHODS

In the flora of Kazakhstan, there are more than 70 species of plants, many species have plant medicinal plants and may be required for the pharmaceutical industry [9].

The objects of the study were 10 types and strains of microalgae: *Chlorella pyrenoidosa ChickYA-1-1, Scenedesmus obliquus YA-2-6, Ankistrodesmus angustus YA-3-1, Ankistrodesmus braunii; Chlamydomonas reinhardii YA-5-16; Ch.reinhardii 449; Euglena gracillis YA-4-17; Euglena proximaYA-4-19; Dunaliella salina, Dunaliella minuta UA-5-10.*

For the cultivation, phytoplanktons of the Shardara reservoir (Turkestan region, Kazakhstan) were used as a source material in the laboratory studies [12]. Under the open atmospheric conditions, algae were cultivated in a horizontal installation of a tray type with a volume of 1000 liters. For the growing microalgae, the following standard mineral nutrient media were used - 04, (for the cultivation of *Chlorella*) Tamiya, (*Scenedesmus*) Yaguzhensky, (*Ankistrodesmus*) Gromov, (*Chlamydomonas*) Artari (*Dunaliella*), UzA (*Euglena*) that are optimal for the cultivation of these algae and determined in laboratory studies. The growth and morphological state of the cells was checked under observation and counting in the Goryaev chamber by using the MBI-3 microscope. The productivity of algae was determined on a dry mass by the gravimetric method [11].

Carotene content in fresh pasta of microalgae was determined by the method of Murri. Tocopherol was determined by the iron-dipyridyl method in the modifications of Devyatina and Solunina [3]. Ascorbic acid was determined by titration of the extract with 2,6-dichlorophenolindophenol [8].

## RESULTS AND DISCUSSION

Studies shown that the content of vitamins, depending on the type and strain of microalgae, varies widely: Carotene - 90.6÷1100 mg%, Ascorbic acid - 62.2÷182.7 mg%, Tocopherol - 25.2 ÷123.4 mg% of dry weight. The high contents of Carotene, Ascorbic acid, Tocopherol are related with *Dunaliella* species, where its amount reached 1100 mg%. In the intensive culture and in conditions without stirring the suspension under the open atmospheric conditions, in the accumulations of carotene *D.salina* exceeds *D.minuta* (Table 1).

As seen Table 1, the highest amounts of Ascorbic acid and Tocopherol were for *E.proxima* and *D.salina* that respectively: 108.7 & 123.4 (mg%). The minimum amount of these vitamins was observed in *Chlorella* and *Scenedesmus*: 68.5 &77.3 (mg%) for Ascorbic acid and 25.2&48.0 (mg%) for Tocopherol. Comparison of microalgae of the Tocopherol content with various known objects showed following: soybean oil - 114.0 mg%, cotton oil – 99 mg%, sunflower oil - 67.0 mg%, olive oil - 13.0 mg%, hazelnut - 25.5 mg%, walnut-23.0 mg%, corn - 31.9 mg%, green dried lucerne I$^{st}$ crop - 12.5 mg%,

reeds flour - 12.5 mg%, barley - 2.0 mg%. Consequently, microalgae are rich in vitamin E than the above mentioned objects.

Table 1 - Efficiency and vitamin content in microalgae that grown under the conditions of open mass cultivation (of absolute dried mass)

| Culture | Productivity g/m$^2$ per day | Carotene, mg% | Ascorbic acid, mg% | Tocopherol, mg% |
|---|---|---|---|---|
| *Chlarella pyrenoidosa YA-1-1* | 22,0 | 220,3 | 68,5 | 25,2 |
| *Scenedesmus obliquus YA-2-6* | 17,2 | 90,6 | 77,3 | 48,0 |
| *Ankistrodesmus angustus YA-3-1* | 15,0 | 160,4 | 80,2 | 58,5 |
| *Ankistrodesmus braunii* | 14,9 | 140,7 | 74,6 | 55,0 |
| *Chlamydomonus reihardii 449* | 23,6 | 125,0 | 66,4 | 52,0 |
| *Chlamydomonus reinhardii YA-5-16* | 18,5 | 152,0 | 62,2 | 46,5 |
| *Euglena graulis YA-4-17* | 18,0 | 137,0 | 182,7 | 67,8 |
| *Euglena Proxima YA-4-19* | 16,0 | 120,3 | 108,7 | 60,0 |
| *Dunaliella minuta* | 20,0 | 1100,0 | 100,5 | 103,8 |
| *Dunaliella salina* | 21,0 | 815,7 | 120,0 | 123,4 |

In addition, *Dunaliella* species outnumber the three vitamins from other tested forms of algae. Therefore, the changes in the amount of these vitamins in *Dunaliella* were investigated further depending on the seasons, the cultivation and the type of mixing.

In order to introduce them into industrial cultivation in the Turkestan region of Kazakhstan, the development of the open-air cultivation of the halophilic alga *Dunaliella minuta* has begun, in a wooden tray installation of 50 l and 500 l with 1-5(m$^2$) illuminated surface without mixing and with suspension mixing (circulating, bubbling) on *Arthari* culture medium containing different concentrations of NaCl.

Studies shown that the amount of vitamins in *D.minuta* depends both on the season of the year and on the temperature and light intensity (Table 2).

Carotene content in *D.minuta* biomass grown in open-air conditions from February till September varied in the range of 171-672.9 (mg%). The most favorable for the development of this seaweed were the April-May and August-September months. The accumulation of Carotene in these months in biomass reached 405-599.0 (mg %), and in *D.minuta* cells grown in June-July, it increased by 15% or 654-672 (mg %). Perhaps this is due to the fact that, with an increased intensity of light, a grow in the biosynthesis of Carotene is due to its participation in the protection of chlorophyll against oxidation at high light intensities. Therefore, to obtain a *D.minuta* biomass with a high content of Carotene, the culture should be grown in summer in open-type plants at high light intensity. Similar data were obtained for *D.salina* [6].

In the biomass of *D.minuta* grown in April, May and August, the increasing in the amount of Ascorbic acid and Tocopherol occurs at the same time. Synthesis of Ascorbic acid correlated with changes in temperature and light intensity. In May, it was two times more in *Dunaliella* biomass than in cells that grew in March (48 mg % versus 92.4 mg %). Moreover, at high temperature (T=34$^0$C) and light intensity, suppression of the synthesis of Ascorbic acid was observed.

Thus, the maximum accumulation of valuable substances over the seasons of the year (vitamins et al) in the holofilous algae *D.minuta* when it is cultivated in the open plants conditions of the Turkestan region has scientific and practical importance.

Table 2 - Influence of the seasons on the vitamins content in *Dunaliella minuta* biomass (of absolute dried mass)

| Months | Temperature, $^0$C | Illumination, kilolux | Carotene, mg% | Ascorbic acid, mg% | Tocopherol, mg% |
|---|---|---|---|---|---|
| February | 8-14 | 40-60 | 171±8,1 | 35,6±0,95 | 30,5±1,00 |
| March | 10-16 | 60-80 | 202,9±10,2 | 48,0±1,12 | 60,4±1,27 |
| April | 14-24 | 70-90 | 405,1±16,4 | 75,3±1,42 | 80,8±1,40 |
| May | 16-26 | 80-100 | 444,6±15,0 | 92,4±2,0 | 103,8±1,90 |
| June | 24-34 | 100-140 | 672,9±20,3 | 63,8±1,10 | 50,4±1,31 |
| July | 22-30 | 100-130 | 654,0±22,4 | 80,2±1,67 | 62,3±1,05 |
| August | 19-28 | 90-120 | 599,0±16,1 | 71,5±1,00 | 41,5±1,10 |
| September | 15-24 | 70-100 | 450,5±17,3 | 85,2±1,72 | 95,0±1,42 |

The study of the dynamics of the accumulation of vitamins in *Dunaliella* with open-air mass cultivation showed (Table 3) that at the cultivating under conditions without moving in the first 10 days of cultivation, the amount of vitamins gradually increases, and starting from 12 days the content of Ascorbic acid increases by 25% or102 mg% compare with 9 days or 75 mg%. During the same periods (on the 12th day) of cultivation, the dry matter (1.7 g/l) and Tocopherol (60.2 mg %) accumulate to the maximum. This is apparently due to the fact that at the beginning of the life cycle of algae, the directionality of their synthetic processes changes from structural to the middle and end of the life cycle towards the synthesis of reserve substances. The greatest amount of Carotene was noted on the 15th day and can reach 410 mg%.

Table 3 - Effect of cultivation duration on dry matter and vitamins in *Dunaliella minuta* at the open air conditions (of absolute dried mass)

| Duration of cultivation | Dry matter, g/l | Carotene, mg% | Ascorbic acid, mg% | Tocopherol, mg% |
|---|---|---|---|---|
| without mixing | | | | |
| The beginning of the experiment | - | - | - | - |
| 3$^{d}$ day | 0,45±0,01 | 213,5±8,1 | 43,7±0,55 | 26,4±0,15 |
| 6$^{th}$ day | 0,80±0,02 | 247,0±6,2 | 63,7±0,72 | 37,3±0,25 |
| 9$^{th}$ day | 1,08±0,05 | 273,5±5,0 | 75,0±0,94 | 50,0±0,85 |
| 12$^{th}$ day | 1,70±0,12 | 325,0±9,0 | 102,0±0,85 | 60,2±0,93 |
| 15$^{th}$ day | 1,52±0,10 | 410,0±9,5 | 72,5±0,78 | 55,0±0,72 |
| 18$^{th}$ day | 1,00±0,09 | 370,5±9,3 | 48,2±0,42 | 43,1±0,48 |
| with mixing (bubbling) | | | | |
| The beginning of the experiment | 0,88±0,02 | 250,7±5,3 | 72,3±0,85 | - |
| 2$^{d}$ day | 1,20±0,05 | 258,1±8,4 | 100,4±0,92 | 35,7±0,32 |
| 4$^{th}$ day | 1,42±0,09 | 375,5±9,2 | 118,8±0,97 | 42,4±0,82 |
| 6$^{th}$ day | 1,73±0,12 | 580,0±10,0 | 135,4±0,80 | 60,5±0,65 |
| 8$^{th}$ day | 2,65±0,15 | 935,2±12,1 | 142,0±0,94 | 75,0±0,96 |
| 10$^{th}$ day | 3,00±0,18 | 1100,0±22,6 | 129,2±0,72 | 78,5±0,90 |
| 12$^{th}$ day | 2,75±0,12 | 1015,4±19,2 | - | 65,6±0,77 |

Therefore, under conditions without mixing of the suspension in the middle of 12-15 days of cultivation, *Dunaliella* cells are in the most active state. At the bubbling method of cultivation of *Dunaliella*, the periods of culture transition to the active state reduced, and the maximum content of vitamins is observed in the cells on the 10$^{th}$ day of cultivation (3.0 g/l of dry matter, 1100 mg% Carotene and 78.5 mg% Tocopherol). The maximum accumulation of Ascorbic acid was noted for two days earlier. After 10 days of growth, the amount of dry matter of Carotene and Tocopherol in *Dunaliella* decreases and amounts to 2.75 g/l, 1015 mg% and 65.6 mg%, respectively.

Thus, during the cultivation of *Dunaliella* by bubbling mixing in biomass, the vitamins content is optimized after 8-10 days of cultivation. Similar data for the optimal cultivation were obtained by other authors regarding the biosynthesis of Eicosapentaenoic acid from other algae [1].

## CONCLUSION

Thus, the study results showed that the microalgae consist in irregular amount of vitamins and depending on the type and strains. The high content of provitamins A (Carotene) is characteristic for species of the genus *Dunaliella* 935.2÷1100.0 (mg%), of absolute dry weight. The greatest amount of vitamin C (Ascorbic acid) and vitamin E (Tocopherol) was in the species *Euglena* 182.7 & 67.8 (mg%) and *Dunaliella* 120.0 & 123.4 (mg%), respectively. In terms of productivity, it is also not inferior to *Chlamydomonas*. The minimum amount of these vitamins was observed in the *Chlarella* species or 68.5 & 25.2 (mg%) and *Chlamydomonas* or 62.2 and 46.5 (mg%), respectively. As can be seen, by the number of studied vitamins, *Dunaliella* and *Euglena* species are more promising producers of these compounds and can serve as sources of vitamin A, vitamin C and vitamin E. Following contents are discovered: provitamin A - 90.6÷1100 (mg%); vitamin C - 62.2÷120 (mg%); vitamin E - 25.2÷123 (mg%) of dry mass. The productivity and content of the three vitamins in *Dunaliella* depends on the season of the year, the duration of cultivation and the type of mixing. The highest productivity and the maximum amount of vitamins were observed during cultivation in May-June, at 8-10 days of cultivation by the bubbling cultivation method. The developed biomass with a high content of vitamins allow to increase the

biotechnological potential of microalgae that will provide an opportunity for the problems solving related to the provision of various sectors of the national economics by algae-rich raw materials: animal husbandry, poultry farming, fish farming, as well as food and pharmaceutical industries. In this regard, the need for the expanding of number and range of cultivated algae by searching for and isolating their local forms, especially promising species, as well as identifying sensitiveness and resistance to wide ecological ranges have a significant value.

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**[1]В.Sh. Исмаилходжаев, [2]Б.А. Халмурзаева, [3]М.И.Сатаев, [3]Р.С.Алибеков**

[1]Ташкент ауыл шаруашылығын ирригациялау және механикаландыру инженерлері институты, Кори-Ниязов көшесі 39, 100000, Ташкент қ., Өзбекстан;
[2] Академик А. Қуатбеков атындағы Халықтар Достығы университеті, Төле би көшесі 32, Шымкент қ., 160000, Қазақстан;
[3]М.Әуезов атындағы Оңтүстік Қазақстан мемлекеттік университеті, Тәуке хан даңғылы, 5, Шымкент қ., 160012, Қазақстан

## МИКРОБАЛДЫРЛАРДЫҢ ДӘРУМЕНДІК ҚҰРАМЫНЫ ЗЕРТТЕУ

**Аннотация.** Микробалдырлардың биомассасын тәжірибелік мақсатта пайдалану тиімділігі олардың физиологиялық және биохимиялық ерекшеліктерімен анықталады. Балдырлардың өсуі мен дамуы сыртқы факторлармен тығыз байланысты, олардың әсерінен жасушаның құрылымдық, функционалдық және биохимиялық сипаттамалары өзгереді. Табиғи жағдайларда жаңа жоғары белсенді микробалдырлар-алмастырылмайтын қосылыстардың продуценттерін іріктеу олардың тәжірибелік қолдану саласын кеңейтуге мүмкіндік береді. Осы жұмыста жергілікті флорадан бөлінген жасыл және Euglena микробалдырлардың он түрі мен штаммдарында С дәруменінің, Е дәруменінің А продәруменінің санын салыстырмалы зерттеу көрсетілген, олар жартылай өнеркәсіптік режимдерде және ашық атмосферада өсірілген: *Chlorella pyrenoidosa ChickYA-1-1*; *Scenedesmus obliquus YA-2-6*; *Ankistrodesmus braunii; Chlamydomonas reinhardii YA-5-16; Chlamydomonas reinhardii 449; Euglena gracillis YA-4-17; Euglena YA-4-19; Dunaliella salina; Dunaliella minuta UA-5-10*. Дәрумендердің құрамы балдырлар штаммының түріне және жыл уақытына, өсіру ұзақтығына және араластыру түріне байланысты екендігі көрсетілді. Әзірленген дәрумендік құрамы жоғары биомасса микробалдырлардың биотехнологиялық әлеуетін арттыруға мүмкіндік береді, бұл мал шаруашылығы, құс шаруашылығы, балық шаруашылығы, сондай-ақ тамақ және фармацевтика өнеркәсібін халық шаруашылығының әртүрлі салаларын шикізатпен қамтамасыз етуге байланысты міндеттерді шешуге мүмкіндік береді.

**Түйін сөздер:** биосинтез, өсіру технологиясы, микробалдырлар, штамм, дәрумендер

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**[1]Б.Ш. Исмаилходжаев, [2]Б.А. Халмурзаева, [3]М.И.Сатаев, [3]Р.С.Алибеков**

[1]Ташкентский институт инженеров ирригации и механизации сельского хозяйства, ул.Кори-Ниязова, 39, 100000, г.Ташкент, Узбекистан;
[2]Университет Дружба Народов имени академика А.Куатбекова, г.Шымкент, ул.Толеби, 32, 160000, Казахстан;
[3]Южно-Казахстанский Государственный Университет имени М.Ауэзова, проспект Тауке Хана, 5, г.Шымкент, 160012, Казахстан

## ИССЛЕДОВАНИЕ ВИТАМИННОГО СОСТАВА МИКРОВОДОРОСЛЕЙ

**Аннотация.** Эффективность использования биомассы микроводорослей в практических целях определяется их физиологическими и биохимическими особенностями. Рост и развитие водорослей тесно связаны с внешними факторами, под влиянием которых меняются структурные, функциональные и биохимические характеристики клетки. Отбор новых высокоактивных микроводорослей-продуцентов незаменимых соединений в естественных условиях позволит расширить область их практического применения. В настоящей работе показано сравнительное исследование количества витаминов - провитамина А, витамина С, витамина Е в десяти типах и штаммах зеленых и *Euglena* микроводорослей, выделенных из местной флоры, выращенных в полупромышленных режимах и в условиях открытой атмосферы: *Chlorella pyrenoidosa ChickYA-1-1*; *Scenedesmus obliquus YA-2-6*; *Ankistrodesmus braunii; Chlamydomonas reinhardii YA-5-16; Chlamydomonas reinhardii 449; Euglena gracillis YA-4-17; Euglena YA-4-19; Dunaliella salina; Dunaliella minuta UA-5-10*. Показано, что содержание витаминов зависит от типа штамма водорослей и времени года,

продолжительности выращивания и типа смешивания. Разработанная биомасса с высоким содержанием витаминов позволит повысить биотехнологический потенциал микроводорослей, что даст возможность для решения задач, связанных с обеспечением различных отраслей народного хозяйства богатым водорослями сырьем: животноводство, птицеводство, рыбоводство, а также пищевая и фармацевтическая промышленности.

**Ключевые слова:** биосинтез, технология культивирования, микроводоросли, штамм, витамины

**Information about the authors:**

Bakhodir Ismailkhodjayev – Doctor of Biological Sciences, Professor, Ecology Department, [1]Tashkent Institute of Irrigation and Agricultural Mechanization, Tashkent, Uzbekistan, e-mail: ismailkhodjaev@mail.ru, ORCID: https://orcid.org/0000-0002-6670-0259;

Bibiosiya Khalmurzaeva – PhD in Biology, As.Professor, Chemistry and Biology Department, University of Friendship of Peoples named after academician A.Kuatbekov, Shymkent, Kazakhstan, ba.khalmurzayeva@mail.ru, ORCID: https://orcid.org/0000-0002-5254-5331;

Marat Satayev – Doctor of Technical Sciences, Corresponding member of the National Academy of Republic of Kazakhstan, Professor, Vice-Rector in Science and Innovations, M.Auezov' South-Kazakhstan State University, Shymkent, Kazakhstan, e-mail: maratsatayev@mail.ru, ORCID: https://orcid.org/0000-0002-5819-4480;

Ravshanbek Alibekov - PhD in Chemistry, As. Professor, Food Engineering Department, M.Auezov' South-Kazakhstan State University, Kazakhstan, e-mail: ralibekov@hotmail.com, ORCID: https://orcid.org/0000-0002-0723-3101

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