ՀԱՅԱՍՏԱՆԻ ՀԱՆՐԱՊԵՏՈՒԹՅԱՆ ԳԻՏՈՒԹՅՈՒՆՆԵՐԻ
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НАЦИОНАЛЬНАЯ АКАДЕМИЯ НАУК РЕСПУБЛИКИ АРМЕНИЯ
NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF ARMENIA

Հայաստանի քիմիական հանդես
Химический журнал Армении **72, №3, 2019** Chemical Journal of Armenia

УДК 547.96

COMPARATIVE CHARACTERISTICS OF GREEN MICROALGAE PARACHLORELLA KESSLERI AND CHLORELLA VULGARIS AS A PROTEIN ADDITIVE

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The comparative evaluation of green unicellular microalgae *Parachlorella kessleri* and *Chlorella vulgaris* biomass as an additional protein and essential amino acids source has been performed.

The total protein amount determined by the modified Kjeldahl method showed up to 43.3% and 55% protein content for the *P. kessleri* and *C. vulgaris* biomass, respectively.

Qualitative and quantitative analyses of the *P. kessleri* and *C. vulgaris* biomass amino acids were carried out by the amino acid analyzer. 7 and 15 different amino acids were detected, respectively.

Figs. 4, references 13.

Introduction

Some microalgae such as *Nostoc, Arthrospira* (*Spirulina*) and *Aphanizomenon* have been used in the human diet as a protein-rich supplement since thousands of years [1]. The Spanish described the consumption of blue-green cake made from *Arthrospira* by the Aztecs [2]. Although in the early 1940s there was some progress in microalgae research, only in 1952 at the Algae Mass-Culture Symposium the microalgae were suggested as a food and source for technological production. The first facilities for commercial production of *Chlorella* were established in Japan. Mexico was the first country of *Arthrospira* cultivation in the 1970s [1]. The number of microalgae species in nature is estimated between 200,000 and

800,000, but only a small percent of this amount can be used for human nutrition [3].

Currently, the use of microalgae as food supplements becomes more and more popular because of their high nutritional value. The human protein demand is increasing [4] and nowadays, more than one billion people suffer protein deficiency. Furthermore, scientists consider that conventional sources of protein may be insufficient in the nearest future [5]. The existence of a significantly increasing protein demand was reported years ago [4]. The amount of currently produced protein must at least be doubled to reach the needs of the expected population of around 9.8 billion people in 2050 [6]. Plant-based proteins account for the majority of protein intake worldwide used for food and feed. In the EU, animal-based proteins are consumed in a greater quantity than plant-based proteins, however, concerns about health and environmental issues as well as animal welfare could give a boost to plant-based sources.

Microalgae can be a promising sustainable alternative protein source. By the middle of 21 century, algae protein may constitute around 18% of the global protein market. However, aspects related to food safety of algae are still not well investigated, especially the presence of contaminants, allergens, or hazardous substances [7].

It is well-known that some species of *Chlorella* and *Arthrospira* accumulate high-quality proteins. They have well-balanced amino acid profiles according to the WHO/FAO/UNU recommendations regarding human requirements for essential amino acids. The amino acid profiles of both species are similar to other conventional protein sources such as eggs and soybean [4].

Materials and Methods

For this study the green microalgae strain *P. kessleri* (isolated from the Armenian river waters) and *C. vulgaris* (maintained in the Algae Culture Collection of the Laboratory of Energy Alternative Sources) have been investigated.

Both strains were cultivated in modificated Tamiya's nutrient medium [9, 10]. Cultivation was carried out in the photobioreactor under the following conditions: 24-26^{o}C, 2000 lux constant illumination. Illumination was provided by Phillips LED 19W/865 lightbulbs, the Netherlands). Culture mixing of was performed by the pressed air (108 $L \cdot h^{-1} \cdot L^{-1}$).

The presence of the Kjeldahl nitrogen in the microalgae biomass was determined by a modified Kjeldahl method [11], using 400 *mg* of freeze-dried microalgae biomass. Protein content was calculated by multiplying Kjeldahl nitrogen content by the conversion factor of 5.95 [12]. 400 *mg* of sample and 50 *mL* of digestion reagent (per liter: 134 *g* K_2SO_4 +

650 *mL* H_2O + 200 *mL* H_2SO_4 + 2 *g* HgO/25 *mL* H_2SO_4 6N) were added in a Kjeldahl tube. The mixture was digested in a Buchi Digestor Unit K-424 for 4-4.5 *h*. After cooling, it was diluted with 100 *mL* of distilled water and distilled in the distillation unit for 6 *min* with 50 *mL* of sodium hydroxide-sodium thiosulfate (per liter: 500 *g* NaOH + 25 *g* $Na_2S_2O_3 \cdot 5H_2O$). The distillate was collected in an Erlenmeyer flask containing 50 *mL* of boric acid indicator solution and was titrated with a stock solution of 0.02N H_2SO_4.

The hydrolysis of proteins to amino acids was performed by hydrochloric acid: 5 *ml* 6.6 M hydrochloric acid was added to 0.5 *g* dry biomass. The mixture was put to the digestion for 20 *hours* with reverse refrigerator at the temperature of 100°C. The solution was filtered, the ash was washed with distilled water.

The extraction of free amino acids was performed by ethyl alcohol. 50 ml of 30% ethyl alcohol was added to 0.5 *g* of a dry biomass sample. The mixture was put on a water bath for 30 *min*, then again 50 *ml* of 30% ethyl alcohol was added and left for 30 *min* in the bath.

In both solutions obtained the presence of amino acids was first determined by thin layer chromatography. The chromatographic plate with the samples was put in acetone : ethyl alcohol : butyl alcohol : ammonia : water system (40:20:30:30:10 v/v). Visualization was performed by the 0.5% ninhydrin solution.

The amino acid composition of hydrolyzed and extracted samples was analyzed by amino acid analyzer "Shimadzu Nexera X2" (Japan). For separation of amino acids Novo-Pak C 18, 4 *μm*, 3.9–150 mm chromatographic column was used. Amino acid separations were carried out in a gradient elution mode. The following reagents were used as the mobile phase: A) acetonitrile:methanol:water (45:40:15 -*v*/*v*); B) phosphate buffer pH = 7.0; flow rate was 0.5 *ml*/*min*, detection was carried out at a wavelength of ex350-em450 *nm*, column temperature – 30°C, injection volume 10 *μl*; chemicals and eluents: MeCN, MeOH, Na_2HPO_4, NaH_2PO_4, HCI, ortho-phthaldialdehyde reagent CAS: 643-79-8 (Sigma-Aldrich with purity> 99.9%).

The Results and Discussion

The Kjeldahl nitrogen analysis of *P. keslerii* and *C. vulgaris* microalgae biomass showed up to 43.3% and 55% protein content, respectively.

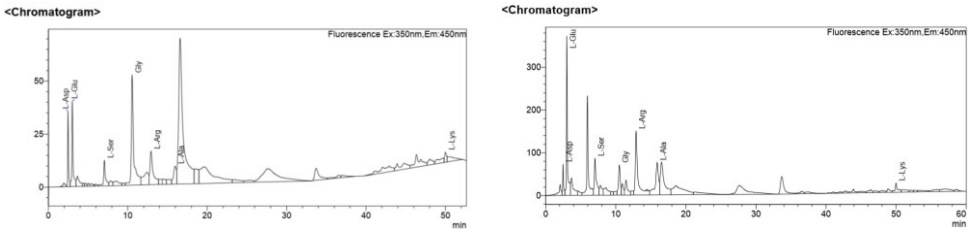
The Kjeldahl method is a method for the quantitative determination of nitrogen contained in organic substances plus the nitrogen contained in the inorganic compounds ammonia and ammonium (NH_3/NH_4^+). Without modification, other forms of inorganic nitrogen, for instance, nitrate are not included in this measurement. The conversion factor 5.95, the rate obtained for the microalgae biomass, is considered to be the total ammonium/amino

acids. This method gives an idea on all protein/amino acids content, but it does not give any information about the amino acid composition of these proteins, specifically, what amounts of these amino acids are included in the protein composition and what amounts are free amino acids.

It is well-known that microalgae contain all protein amino acids [8]. From this point of view nowadays the inclusion of microalgae in the vegetarian diet has become very important. A large amount of protein content allows to use micoalgae biomass as a food suplement for the additional source of proteins. Thus, it is very important to know the range of amino acids, which are present in these microalgae biomass.

Visualization of the thin layer chromatogram with ninhydrin solution has shown the presence of amino acids for both microalgae. The thin layer chromatography is just quality analysis and does not give any specific information about the amino acid profile and quantity.

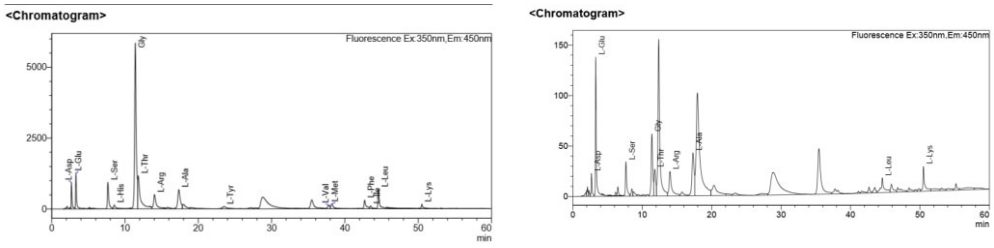
To obtain more accurate results, the amino acid composition was analyzed by amino acid analyzer. For the biomass of *P. kessleri* microalgae hydrolysate (a) and alcohol extract (b) the following results were obtained (Fig. 1):



a) HCl hydrolysate **b) alcohol extract**

Fig. 1. Chromatograms: *P. kessleri.*

For the biomass of *C. vulgaris* microalgae hydrolysate (a) and alcohol extract (b) the following results were obtained (Fig. 2):



a) HCl hydrolysate **b) alcohol extract**

Fig. 2. Chromatograms: *C. vulgaris.*

7 amino acids: L-Aspartic acid, L-Glutamic acid, L-Serine, Glycine, L-Arginine, L-Alanine, L-Lysine were detected in *P. kessleri* biomass by amino acid analyzer. The analyses of chromatograms of the HCl hydrolysate

and alcohol extract of the first sample have shown that 57.59% of total amino acids of *P. kessleri* biomass are bound amino acids, which means that they are included in different proteins composition. Summarizing the results of these experiments and those of Kjeldahl nitrogen determination (protein content is 43.3%), the following picture of amino acid composition of *P. kessleri* biomass was observed. The amount of each amino acid (*g* in 100 *g* dry biomass) of *P. kesleri* biomass is presented in Fig.3.

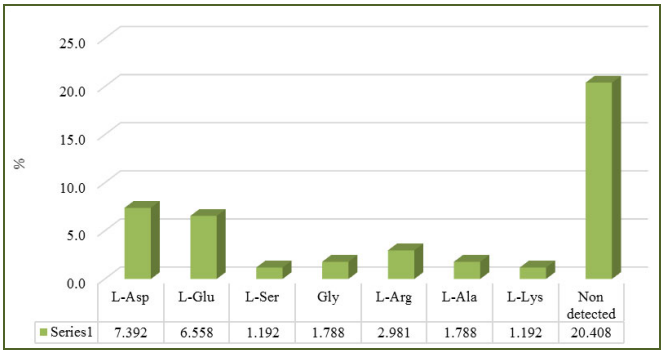


Fig. 3. Amino acid composition of *P. kessleri* biomass, %.

The analyses of the chromatograms of the HCl hydrolysate and alcohol extract of the *C. vulgaris* biomass were carried out similarly. 15 Amino acids: L-Aspartic acid, L-Glutamic acid, L-Serine, L-Histidine, Glycine, L-Threonine, L-Arginine, L-Alanine, L-Tyrosine, L-Valine, L-Methionine, L-Phenylalanine, L-Isoleucine, L-Leucine, L-Lysine were detected. The results have shown that 88.18% of amino acids of the *C. vulgaris* are protein bound. Therefore, they are included in different protein compositions. The amino acid amounts (*g* in 100 *g* dry biomass) of *C. vulgaris* biomass are presented in Fig.4:

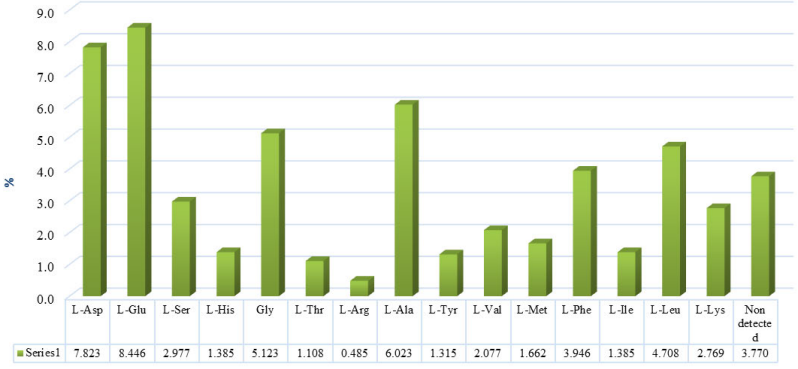


Fig. 4. Amino acid composition of *C. vulgaris* biomass, %.

Conclusion

These amino acids have a wide application in medicine, pharmacy, agriculture, food production, etc. Besides, they play a vital role in the human body where they synthesize some essential biopolymers, like enzymes, vitamins, hormones, and immunoglobulin. Thus, it is very important to include them in a diet [13], especially of the vegans who exclude any animal origin food and have a big deficiency in proteins/amino acids. From this point of view, the microalgae are excellent food additives due to high protein content of non-animal origin. The studied microalgae contain significant amounts of protein/amino acids, which means that they can be excellent food additives for obtaining daily requirement of amino acids.

Acknowledgement

The authors express the acknowledgement to the Head of the Laboratory of Galenic and New Galenic Preparations Dr. S.A. Dadayan and the Head of the Laboratory of Bioactive Substance Purification and Certification Dr. A.H. Tsaturyan for the valuable support.

PARACHLORELLA KESSLERI ԵՎ CHLORELLA VULGARIS ԿԱՆԱՉ ՄԻԿՐՈՋՐԻՄՈՒՌՆԵՐԻ ՀԱՄԵՄԱՏԱԿԱՆ ԲՆՈՒԹԱԳԻՐԸ՝ ՈՐՊԵՍ ՍՊԻՏԱԿՈՒՑԱՅԻՆ ՀԱՎԵԼՈՒՄ

Ն. Ք. ՔԱԼԱՆԹԱՐՅԱՆ, Լ. Ա. ՍՏԵՓԱՆՅԱՆ, Ա. Մ. ԴԱԴԱՅԱՆ, Է. Վ. ՄԻՆԱՍՅԱՆ և Վ. Բ. ԳՈԳԻՆՅԱՆ

Իրականացվել է Parachlorella kessleri և Chlorella vulgaris կանաչ միկրոջրիմուռների կենսազանգվածների համեմատական գնահատում՝ որպես լրացուցիչ սպիտակուցների և անփոխարինելի ամինաթթուների աղբյուր:

Կեղտալի եղանակով P. kessleri և C. vulgaris միկրոջրիմուռների կենսազանգվածներում որոշվել է ընդհանուր սպիտակուցների քանակությունը, այն կազմել է չուրջ 43.3 և 55%, համապատասխանաբար:

P. kessleri և C. vulgaris միկրոջրիմուռների կենսազանգվածների ամինաթթուների քանակական և որակական անալիզներն իրականացվել են ամինաթթվային անալիզատորով: Համապատասխանաբար հայտնաբերվել են 7 և 15 տարբեր ամինաթթուներ:

СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА ЗЕЛЕНЫХ МИКРОВОДОРОСЛЕЙ PARACHLORELLA KESSLERI И CHLORELLA VULGARIS В КАЧЕСТВЕ БЕЛКОВЫХ ДОБАВОК

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Проведена сравнительная оценка зеленых одноклеточных микроводорослей *Parachlorella kessleri* и *Chlorella vulgaris* в качестве дополнительных источников белка и аминокислот, в том числе незаменимых.

Общее содержание белка определено модифицированным методом Къельдаля, которое составило около 43.3 и 55% для биомассы *P. kessleri* и *C. vulgaris*, соответственно.

Качественный и количественный анализы аминокислот в биомассе *P. kessleri* и *C. vulgaris* выполнены с помощью аминокислотного анализатора. Идентифицировано соответственно 7 и 15 аминокислот у исследованных культур.

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