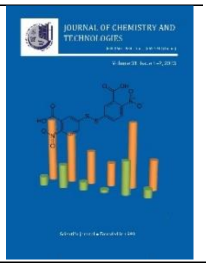
Journal of Chemistry and Technologies

pISSN 2663-2934 (Print), ISSN 2663-2942 (Online).

journal homepage: http://chemistry.dnu.dp.ua



UDC 664.022:582.263

CHLORELLA AS A BIOLOGICALLY ACTIVE COMPONENT IN THE TECHNOLOGY OF HEALTH AND WELLNESS PRODUCTS

Olha V. Kozhemiaka*, Lyudmyla V. Peshuk
Oles Honchar Dnipro National University, 72 Haharina Ave., Dnipro, 49010, Ukraine
Received 7 March 2023; accepted 20 June 2023; available online 25 July 2023

Abstract

Aim. Develop technology for meat pâtés using the microalgae *Chlorella*. Investigate the effect of *Chlorella* on the qualitative and quantitative composition of essential amino acids, macro- and microelements in the finished product. Methods. The finished food products were studied for organoleptic, physicochemical, and toxicological parameters using standard methods of analysis. The macro- and microelement composition was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The results of experimental studies were subjected to statistical processing using standard Microsoft Office software packages. Results. Developed pâtés technology using microalgae *Chlorella*, which is cultivated for industrial production in Ukraine and used as a food additive in dry form (powder) and as live microalgae in the form of a suspension – the *Chlorella* wellness drink. The effect of *Chlorella* on the sensory characteristics of the product, protein content, macro- and microelement composition, including toxic elements, was investigated. To characterize the biological value of the product, the amino acid score, the amount of essential amino acids, the coefficient of difference of amino acid scores (CDAS), the coefficient of utilization (U), and the coefficient of comparative redundancy of essential amino acids (σ) were calculated. Conclusions. The enrichment of meat pâtés with K, Mg, Ca, Cu, Mn, Zn; an increase in the amount of lysine and tryptophan; and a significant decrease in the content of Cd were found. The coefficient of utilization and the coefficient of comparative redundancy of essential amino acids (EAA) showed a better balance in terms of essential amino acids. Thus, the use of *Chlorella* to fortify the mineral and amino acid composition has been scientifically substantiated and experimentally confirmed, which will lead to an increase in the functional properties of meat pâtés and an expansion of the range of health-promoting pâtés products.

Keywords: microalgae; *Chlorella* wellness drink; meat pâtés; protein; essential amino acids; biological value.

CHLORELLA – БІОЛОГІЧНО АКТИВНИЙ КОМПОНЕНТ В ТЕХНОЛОГІЇ ПРОДУКТІВ ОЗДОРОВЧОГО ТА ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО ПРИЗНАЧЕННЯ

Ольга В. Кожемяка, Людмила В. Пешук
Дніпровський національний університет імені Олеся Гончара, пр. Гагаріна, 72, Дніпро, 49010, Україна

Анотація

Мета. Розробити технологію м'ясних паштетів з використанням мікроводорості *Chlorella*. Дослідити вплив *Chlorella* на якісний та кількісний склад незамінних амінокислот, макро- та мікроелементів у готовому продукті. Методи. Дослідження готової харчової продукції проводили за органолептичними, фізико-хімічними та токсикологічними показниками з використанням стандартних методів аналізу. Визначення макро- та мікроелементного складу проводили методом оптичної емісійної спектрометрії з індуктивно зв'язаною плазмою (ICP-OES). Результати експериментальних досліджень піддавали статистичній обробці за допомогою стандартних програмних пакетів Microsoft Office. Результати. Розроблено технологію паштетів з використання мікроводорості *Chlorella,* яка культивується для промислового виробництва в Україні та використовується як харчова добавка в сухому вигляді (порошок), так і жива мікроводорость у вигляді суспензії – wellness-напій *Chlorella*. Досліджено вплив *Chlorella* на сенсорні характеристики продукту, вміст білків, макро- та мікроелементний склад, в тому числі токсичних елементів. Для характеристики біологічної цінності продукту розраховано амінокислотний СКОР, кількість незамінних амінокислот, коефіцієнт різниці амінокислотного СКОР (КРАС), коефіцієнт утилітарності (U), коефіцієнт порівняльної надлишковості незамінних амінокислот (σ). Висновок. Виявлено збагачення м'ясних паштетів K, Mg, Ca, Cu, Mn, Zn; збільшення кількості лізину та триптофану; суттєве зменшення вмісту Cd. Коефіцієнт утилітарності та коефіцієнт порівняльної надлишковості НАК показали кращу збалансованість за незамінними амінокислотами. Отже, науково обґрунтовано та експериментально підтверджено використання *Chlorella* для фортифікації мінерального та амінокислотного складу, що в результаті приведе до підвищення функціональних властивостей м'ясних паштетів та розширення асортименту паштетної продукції оздоровчого призначення.

Ключові слова: мікроводорость; wellness-напій *Chlorella*; м'ясні паштети; білок; незамінні амінокислоти; біологічна цінність.

**Corresponding author: e-mail address: olgakogem@ukr.net*
 doi: 10.15421/jchemtech.v31i2.275148

Introduction

Food shortages and limited global resources are the most significant challenges facing humanity in the 21st century. According to FAO estimates, by 2050, the demand for animal products will grow rapidly, especially due to protein consumption [1]. Increased consumption of animal proteins could have a negative impact on the livestock industry and natural resources. Soil degradation, water shortages, abrupt climate change, natural disasters, and military operations in Ukraine are also problems for the livestock sector.

A sharp increase in hunger and food insecurity has led to the fact that every 10th person in the world does not have enough food, and the World Bank predicts that every percentage point increase in global food prices will lead to an increase in the number of people in the world who will be in extreme poverty (UN, 2022) [2]. To meet the shortage of traditional animal protein resources, scientists around the world are searching for alternative sources. Recently, microalgae have been considered as a new source of protein, including the enrichment of food products with biologically valuable substances. Microalgae contain functional elements such as peptides, carbohydrates, lipids, pigments, vitamins and minerals that can provide consumers with a wide range of health benefits.

The environmental aspects of microalgae, such as CO_2 consumption, no need for arable land or drinking water, have made them a promising alternative source of food. In addition, microalgae have a significantly high growth rate and can adapt to a wide range of growing conditions compared to land-based crops [3].

Microalgae have been part of the human diet for many centuries. For example, Nostoc was used in Asia more than 2000 years ago; Spirulina was used by some tribes in Africa, the indigenous population of Mexico and other indigenous peoples [4]. Later, commercial forms of microalgae (*Chlorella* sp. and Spirulina sp.) were consumed as a healthy food in Japan, Taiwan and Mexico [5].

The cultivation and production of microalgae is mainly concentrated in East and Southeast Asia. More than 70 companies in the world are engaged in the cultivation of *Chlorella*, and the largest producer is Taiwan *Chlorella* Manufacturing and Co. (Taipei, Taiwan), which produces 400 tonnes of dried biomass/year [5].

There are also enterprises producing *Chlorella* microalgae in Ukraine – *Chlorella* Ukraine LLC (Bila Tserkva) and U Samvela Farm (Odesa region).

Problem statement. Today, there is a deformation of human diets, which leads to low consumption of complete proteins, essential fatty acids, complex carbohydrates, vitamins and minerals.

People are beginning to realise that it is necessary to pay attention to foods that benefit health, ensure and maintain the body's vital processes at a high level under various living conditions, and enable people to remain active for a long time. Foods that have health-promoting properties should be included in the regular diet on a regular basis.

In East Asia, seaweed and microalgae are widely consumed as an additional source of protein and mineral components. In Japan, China, France, the USA, Indonesia and other countries, the microalgae *Chlorella* is consumed as a "green food". In other regions of the world, microalgae are included in the diet of health-conscious people as a food supplement [6]. In particular, in Ukraine, there is no culture of consuming microalgae as food and they are usually presented on the market in the form of tablets, capsules, powders and a "live *Chlorella*" drink (wellness drink). We see the prospect of enriching traditional food products with this microalgae.

Analysis of recent research and publications. The number of products containing microalgae is constantly growing. Between 2015 and 2019, about 13,090 new food products containing algae or algae-derived components were registered worldwide. These new products accounted for 79 % of food and 21 % of beverages [6].

Microalgae are added to cookies, biscuits, ice cream, processed cheeses, yoghurts [7], candies, chewing gum, snacks, pasta, noodles, breakfast cereals and drinks, etc. [8]. Microalgae have also been used in meat products, such as sausages [9], hamburgers [10], pork liver pâté, turkey cutlets, boiled turkey breast, fresh pork sausages and marinated turkey breast [11], beef cutlets [12].

In Ukraine, there is some information on the use of *Chlorella* as an enrichment component. For example, in the Odesa region, U Samvela farm cultivates the microalgae strain Chlorella vulgaris IFR No C-11 and *Chlorella* vulgaris BIN. For the *Chlorella* suspension, the regulatory documentation is TU U 03.0-37613791-001:2017 "*Chlorella* suspension. Technical specifications". Food additives with *Chlorella* are used by the farm in baking bread, cooking meat, poultry, fish, soups and other dishes in its own restaurants in Odesa [13].

Journal of Chemistry and Technologies, 2023, *31*(2), 230-239

The companies "Proper honey" and "Living *Chlorella*" have produced an original product – real green honey, based on cream honey from flower herbs and a suspension of *Chlorella* microalgae [14].

A technology for the production of mayonnaise and sauces using the algae *Chlorella* vulgaris has been developed [15], as well as a technology for meat breads involving nutria, rabbit, poultry meat and the addition of *Chlorella* microalgae [16].

Microalgae contain a unique complex of nutrients necessary for the human body. The biochemical components of the microalgae *Chlorella* vulgaris mainly consist of proteins, lipids, carbohydrates, pigments, minerals and vitamins [17; 18].

Proteins and peptides are one of the main groups of food components that significantly affect the functional and biological activity of the product [19].

Peptides are specific protein fragments that are attributed with antioxidant, antihypertensive, immunomodulatory, anticarcinogenic, hepato-protective and anticoagulant properties [20].

A unique nucleotide-peptide complex is found in the cell nucleus and is called C.G.F (*Chlorella* Growth Factor). It is a water-soluble extract consisting of substances such as essential amino acids, peptides, proteins, vitamins, sugars and nucleic acids [21].

Protein is one of the key components that determines the biological value of meat. The quality and quantity of proteins in raw materials affect the emulsification, gelation, viscosity and texture properties of promising meat products.

The protein content in microalgae is higher than in most foods. For example, beef contains 17.4 % protein; fish – 19.2–20.6 %; chicken – 19-24 %; peanuts – 26 %; wheat germ – 27 %; Parmesan cheese – 36 %; skimmed milk powder – 36 %; soy flour – 36 %; brewer's yeast – 45 %; chicken egg – 47 %; *Chlorella* sp. – 50-60 %; Spirulina sp. – 60–70 % [22].

Microalgae are a promising protein resource and have been studied as food components for many years. To compare the main types of microalgae, Table 1 shows their macronutrient composition [23].

Table 1

Composition of different types of microalgae in terms of dry matter of biomass [23]

Type of microalgae	Composition (% dry matter)		
	Protein	Lipids	Carbohydrates
Anabena cylindrica	43–56	4–7	25–30
Aphanizomenon flos-aquae	62	3	23
Chaetoceros calcitrans	36	15	27
Chlamydomonas rheinhardii	48	21	17
Chlorella vulgaris	51–58	14–22	12–17
Chlorella pyrenoidosa	57	2	26
Diacronema vlkianum	57	6	32
Dunaliela salina	57	6	32
Dunaliela bioculata	49	8	4
Euglena gracilis	39–61	22–38	14–18
Haematococcus pluvialis	48	15	27
Isochrysis galbana	50–56	12–14	10–17
Porphyridium cruentum	28–39	9–14	40–57
Prymnesium parvum	28–45	22–38	25–33
Scenedesmus obliquus	50–56	12–14	10–17
Scenedesmus dimorphus	8–18	16–40	21–52
Scenedesmus quadricauda	47	1.9	21–52
Spirogyra sp.	6–20	11–21	33–64
Spirulina maxima	60–71	6–7	13–16
Spirulina platensis	46–63	4–9	8–14
Synechococcus sp.	63	11	15
Tetraselmis maculata	52	3	15

The table shows that microalgae are a rich source of proteins, fats, and carbohydrates, which is why they are attracting the attention of scientists around the world who are working on developing new types of products.

Microalgae protein contains all the amino acids necessary for normal human metabolism, including essential amino acids that cannot be synthesised in the human or animal body. Becker [24] states that the content of amino acids (lysine, methionine, tryptophan, threonine, valine, histidine and isoleucine) in some microalgae is comparable to that of an egg or soybean (Table 2).

Table 2

Amino acid profile of standard protein sources and microalgae (g/100 g of dry matter) [23; 25]

Source	Egg	Chicken breast	Soybean	*Chlorella* sp.	*Chlorella* vulgaris
Essential amino acids					
Histidine	2.4	4.5	2.6	2.4	2.0
Isoleucine	6.6	3.24	5.3	4.4	3.8
Leucine	8.8	6.4	7.7	9.2	8.8
Lysine	5.3	7.9	6.4	8.9	8.4
Methionine	3.2	2.5	1.3	2.2	2.2
Phenyl alanine	5.8	3.2	5.0	5.5	5.0
Threonine	5.0	3.7	4.0	4.7	4.8
Tryptophan	1.7	-	1.4	-	2.1
Valine	7.2	3.46	5.3	6.1	5.5
Total	**46.0**	**34.9**	**39.0**	**43.4**	**42.6**
Non-essential amino acids					
Tyrosine	4.2	3.65	3.7	4.2	3.4
Alanine	-	4.7	5.0	8.3	7.9
Arginine	6.2	5.8	7.4	7.1	6.4
Asparagine	11.0	7.8	1.3	9.4	9.0
Glutamic	12.6	11.2	19.0	12.9	11.6
Glycine	4.2	3.4	4.5	5.4	5.8
Proline	4.2	3.2	5.3	4.8	4.8
Serine	6.9	3.4	5.8	4.0	4.1
Cystine	2.3	1.1	1.9	0.4	1.4
Total	**51.6**	**44.25**	**53.9**	**56.5**	**54.4**

The table data shows that the total content of essential and non-essential amino acids of *Chlorella* is higher than that of chicken breast and soya beans, and the total content of essential acids of chicken egg is lower compared to *Chlorella*.

Minerals are inorganic substances that the human body needs for proper functioning. *Chlorella* is rich in potassium, sodium, calcium, magnesium, manganese, zinc, and selenium [26–29]. Selenium is an essential trace element and is a component of selenoproteins, which have a variety of pharmaceutical activities, including anti-tumour and anti-aging effects. The main mineral composition is shown in Table 3.

Table 3

Mineral content in the microalgae *Chlorella* [26; 28]

Chemical element	*mg/kg* dry biomass	Chemical element	*µg/kg* dry biomass
Na	10.4	Fe	1.185
K	11.0	Zn	24.7
Mg	3.53	Cu	6.21
Ca	2.3	Mn	77.8
P	19.2	Cr	1.38
--	--	B	27.5

The mineral content generally depends on the growing conditions such as season, temperature, physiological state, geographical variations, growing process, nutrient composition, etc.

Chlorella is a source of polysaccharides and oligosaccharides, which is why it is recommended as a prebiotic [21]. One of the most important polysaccharides is β-1.3 glucan, an active

Journal of Chemistry and Technologies, 2023, *31*(2), 230-239

immunostimulant that reduces free radicals and cholesterol in blood [5].

Polyunsaturated fatty acids (ω-3, ω-6), which are not synthesised in the human body, are of great nutritional and medical importance and are used in the treatment of heart disease, asthma, and arthritis. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from microalgae are a promising source and alternative to fish oil [30].

The vitamins and vitamin-like substances contained in Chlorella are: alpha-carotene, beta-carotene, vitamins of group B, C, E, D, K, folic acid, pantothenic acid, biotin, inositol, choline. *Chlorella* products contain a significant amount of vitamins D_2 and B_{12}, which are not found in plants. This vitamin complex is a key element for cell growth and differentiation in the human body, a major participant in the activity of enzymes involved in metabolism; it has antioxidant, anticarcinogenic properties, supports healthy skin, hair and muscles, etc.

Chlorella is considered one of the main sources of chlorophyll a and b. It can synthesise chlorophyll in large quantities, reaching 1–2 % of its dry biomass [31]. Some of the health benefits of chlorophyll include healing wounds, ulcers, haemorrhoids, helping with haemophilia, improving diabetes and asthma, etc. Because chlorophyll is very similar in structure to heme, which combines with proteins to form haemoglobin, it has been shown to be beneficial in increasing the number of red blood cells and plays an important role in cancer prevention [33].

Chlorella is a rich source of carotenoids (astaxanthin, lutein, β-carotene, lycopene and canthaxanthin), accounting for about 1.3 % of dry biomass. *Chlorella* vulgaris is reported to produce lutein as the main carotenoid [32].

It has been shown that the biologically active ingredients of *Chlorella* have a positive effect as antihypertensive, antiallergic, antiasthmatic, antidiabetic, anticancer and preventive cardiovascular agents. Moreover, *Chlorella* sp. has GRAS (Generally Recognised as Safe) status in accordance with the requirements of the US Food and Drug Administration (FDA) [34].

Thus, *Chlorella* can be confidently used as an enrichment component of food products for therapeutic and prevention purposes.

As for meat products, they play an important role in human nutrition. This is a group of food products consumed by a wide range of people around the world. The development of meat pâtés using microalgae is promising.

Pâté is a product that has been known since ancient times. France is considered to be the birthplace of this product, where pâtés have three varieties: pâté, pâté aux terrines, and pâté aux croutons. French chefs skilfully combined various ingredients, mixing minced meat or fish, offal with spices, wrapped in dough and baked in an oven. Pâté made without dough in special moulds is called terrine, rillon, or riette. In the Middle Ages, pâtés baked in dough were made from veal, poultry, fish, venison, capon, etc. Nowadays, meat pâtés are in demand due to their high nutritional value, pleasant specific taste and delicate texture. In addition, they can combine raw materials of animal and vegetable origin, which have health benefits.

The results of the literature analysis indicate that the use of microalgae in meat food products, taking into account the amino acid balance and their mineral enrichment, remains poorly understood.

Purpose and objectives of the research. The aim of the work was to develop and improve the technology of meat pâtés using the microalgae *Chlorella* as a natural macro- and micronutrient enrichment to expand the range of meat-based health food products; conduct organoleptic and physicochemical tests, study the effect of the microalgae *Chlorella* on the qualitative and quantitative composition of proteins, mineral composition and content of toxic elements.

Materials and methods of the research

The technology of the cold method of preparing pâtés using the microalgae *Chlorella* vulgaris was chosen as the *object of research*. The technology of pâtés included veining, grinding of meat raw materials, hydration of *Chlorella* powder, cutting, moulding, heat treatment (t = 72 °C), cooling, and storage.

Subject of research. We developed five recipes for meat pâtés using Chlorella vulgaris from 0.5 % to 3.0 %. The microalgae was added in the form of a powder (hydro-module 1 : 3) from the Ukrainian manufacturer *Chlorella* Ukraine LLC; and in one recipe, "live *Chlorella*" was added – replacing the broth according to the recipe with a *Chlorella* suspension (wellness drink) produced by U Samvela Farm. The pâté recipe from DSTU 4432:2005 "Meat pâtés. Technical specifications". In the recipe, the liver and pork meat were replaced with chicken liver and meat, and sunflower oil was added.

Research methods. Organoleptic evaluation of the quality of pâtés was carried out in accordance with DSTU 4823.2-2007 "Meat products. Organoleptic evaluation of quality indicators.

Part 2. General requirements" on a five-point scale.

Protein was determined in the laboratory by the Kjeldahl control method – DSTU ISO 937:2005 "Meat and meat products. Determination of nitrogen content (control method)". The determination of total ash was carried out according to DSTU ISO 936:2008 "Meat and meat products. Method for determination of the mass fraction of total ash".

The macro- and microelement composition was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using a PerkinElmer Avio 200 atomic emission spectrometer (USA). The signals from the detectors were processed by a computer system using standard Microsoft Office software packages.

The amino acid composition of the experimental samples was calculated using reference materials. The biological value of protein components in the experimental samples was assessed according to the indicators and criteria proposed by N. N. Lipatov [36].

Results and discussion

At the first stage of the study, after manufacturing, heat treatment and cooling, a sensory analysis was carried out according to the following indicators: appearance, consistency, appearance of minced meat in a cut, taste, smell (Table 4). The control sample was a pâté recipe without *Chlorella*.

Table 4

Organoleptic characteristics of meat pates using *Chlorella*

Indicator name	Recipe 1 without *Chlorella* Control	Samples with *Chlorella* content				
		Recipe 2 0.5 % *Chlorella* powder	Recipe 3 1.0 % *Chlorella* powder	Recipe 4 2.0 % *Chlorella* powder	Recipe 5 3.0 % *Chlorella* powder	Recipe 6 suspension of live *Chlorella*
Appearance	Homogeneous mass of light brown colour	Homogeneous mass of light green colour	Homogeneous mass of light green colour	Homogeneous mass of green colour	Homogeneous mass of dark green colour	Homogeneous mass of light brown colour, darker than the control
Consistency	Delicate, smeary	Delicate, smeary	Delicate, smeary	Denser than the control sample	Denser than the control sample	Delicate, smeary
View of minced meat in a cut	Homogeneous structure	Homogeneous structure	Homogeneous structure	Homogeneous structure	Homogeneous structure	Homogeneous structure
Taste	The taste is pleasant, typical of pâtés	The taste is pleasant, typical of pâtés	The taste is pleasant, typical of pâtés	Barely perceptible taste of algae	A tangible taste of algae	The taste is pleasant, typical of pâtés
Smell	No foreign smell	No foreign smell	No foreign smell	No foreign smell	No foreign smell	No foreign smell

Samples 2, 3 and 6 are close in terms of organoleptic characteristics to the sample of pate without *Chlorella* (control). Based on the results of the sensory analysis, a profilogram of pâtés with *Chlorella* was constructed (Fig. 1).

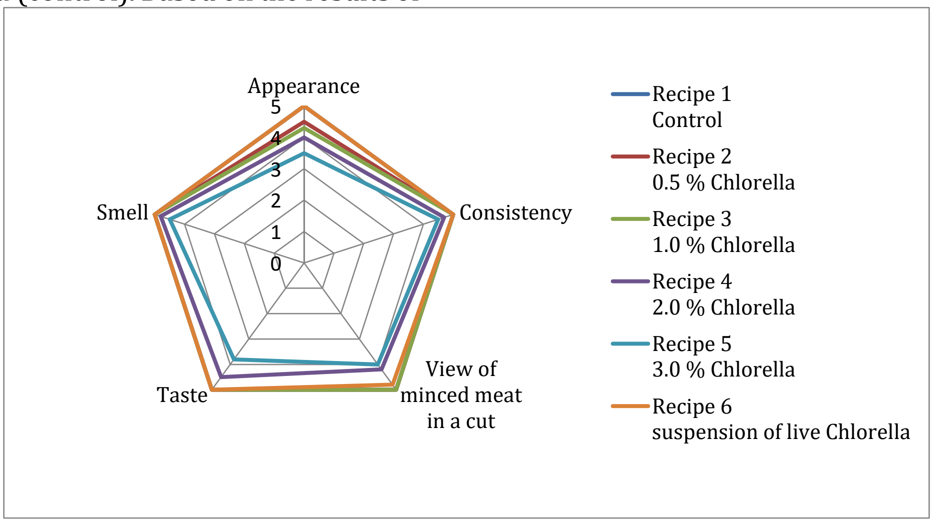


Fig. 1. Organoleptic profile of pâtés with Chlorella compared to the control sample without Chlorella

The samples of pâtés met the sensory characteristics, colour and taste characteristics were decisive in this study, and the samples of pâté with 0.5 %, 1.0 % *Chlorella* and with a suspension of live *Chlorella* were the closest to the control sample according to the above criteria.

When microalgae are added in powder form up to 1.0 %, the colour of the paste turns light green and does not affect the taste and smell.

For further laboratory tests, we chose pâtés with a *Chlorella* content of 0.5 % and a suspension of live *Chlorella*.

The results of the tests for protein and total ash content are presented in Table 5.

There is a positive trend in the increase in the amount of proteins, and an increase in the ash content indicates enrichment with macro- and microelements (Fig. 2).

Table 5

Content of mass fraction of protein and total ash in pâtés

Indicator	Recipe 1 without *Chlorella* Control	Recipe 2 0.5 % *Chlorella* powder	Recipe 6 suspension of live *Chlorella*
Mass fraction of protein, %	15.39±0.1	15.69±0.1	15.61±0.1
Mass fraction of ash, %	1.29±0.076	1.44±0.076	1.53±0.076

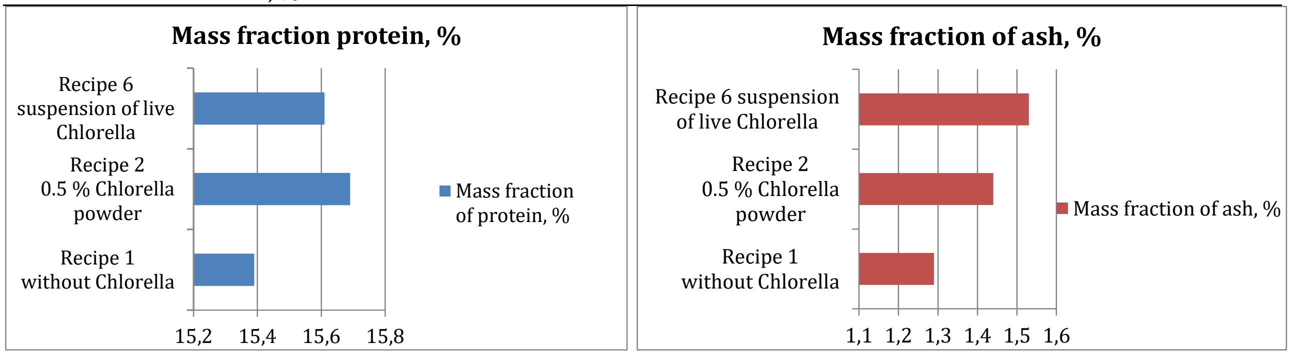


Fig. 2. Content of mass fraction of protein and total ash in pates

As can be seen from the table and figures, the amount of proteins increased in the paste with 0.5 % *Chlorella* and with a suspension of live *Chlorella* by 1.95 % and 1.43 %, respectively; the content of total ash increased by 11.6 % and 18.6 %, respectively.

Not only is the supply of a certain amount of essential components important, but also their quality. Proteins play a vital role as they comprise the major structural elements of all cells and catalyze virtually all chemical reactions in the body.

Biological value is a complex characteristic that shows the quality of the protein components of a product. The lower the coefficient of difference of amino acid score (CDAS), the higher the biological value of the protein in the product.

We calculated the biological value – the amino acid score, and compared the degree of balance of essential amino acids against the standard proposed by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO).

For the experimental pâtés with a *Chlorella* content of 0.5 % (recipe 2), 1.0 % (recipe 3) and a suspension of "live *Chlorella*" (recipe 6), the CDAS coefficient, the utilization coefficient (U) and the comparative redundancy of EAA (σ) were calculated (Table 6).

Table 6

Parameters of amino acid balance of pates

Amino acid	FAO / WHO g/100 g protein (2011)	Control pâté "For breakfast" according to DSTU 4432		Recipe 2 0.5 % *Chlorella* powder		Recipe 3 1.0 % *Chlorella* powder		Recipe 6 suspension of live *Chlorella*	
		g /100 g	Amino acid score, %	g /100 g	Amino acid score, %	g /100 g	Amino acid score, %	g /100 g	Amino acid score, %
Protein content,%		12.73		15.43		15.61		15.30	
Valine	4.0	6.9	174.6	5.8	144.7	5.9	146.4	5.7	143.3
Isoleucine	3.0	4.6	152.1	4.8	159.1	4.8	160.1	4.7	158.2
Leucine	6.1	8.9	145.6	8.9	145.5	9.0	147.4	8.8	143.9
Lysine	4.8	7.9	164.0	9.3	193.5	9.4	195.1	9.2	192.2
Threonine	2.5	4.7	189.9	4.4	174.3	4.4	177.0	4.3	172.0
Tryptophan	1.0	1.3	140.9	1.8	176.8	1.8	179.8	1.7	173.4

Continue the Table 6

Phenylalanine + tyrosine	4.1	8.1	198.7	8.3	203.5	8.4	206.1	8.2	201.2
Methionine + cystine	2.3	4.0	174.3	3.6	155.8	3.6	157.8	3.5	154.1
Index of essential amino acids		1.66		1.68		1.70		1.66	
Amount of essential amino acids, mg/g		466.1		467.8		473.2		463.1	
Biological value, %		73.37		75.64		75.20		76.01	
Coefficient of difference of amino acid score (CDAS), %		26.63		24.36		24.80		23.99	
Coefficient of utilization (U)		0.84		0.86		0.86		0.86	
Coefficient of comparative redundancy of essential amino acids (σ), g/100 g of reference protein		5.29		4.52		4.52		4.52	
Limiting amino acid score, %		not available		not available		not available		not available	

Replacing the ingredients in the recipe according to DSTU 4432:2005 (control) and adding *Chlorella* microalgae to the pâtés had a positive effect on increasing lysine by up to 19 % and tryptophan by up to 38 %. Lysine is involved in the synthesis of proteins, hormones and enzymes, promotes tissue regeneration, affects growth, blood circulation, etc. Tryptophan regulates protein synthesis in the liver and is a precursor of biologically active compounds such as serotonin, melatonin, tryptamine, and the coenzymes NAD and NADP.

Liver and chicken meat are easily digestible products that contain a significant amount of proteins, iron, nitrogenous extractives, minerals (potassium, calcium, zinc, copper, selenium, etc.), vitamins A, C and B.

The CDAS coefficient shows the excess of essential amino acids used for catabolic purposes in the human body. In the developed pâtés, this indicator is decreased by an average of 10 % compared to the control sample. This demonstrates a positive trend in the use of essential amino acids for the body's nutritional needs. The coefficient of utility and the coefficient of comparative redundancy of EAA showed a better balance of essential amino acids in the developed pâtés [37].

The table shows that the amount of protein in the developed pâtés increased by 20.2–22.6 % compared to the control sample. Obviously, this result is also due to the use of the microalgae *Chlorella*, which contains up to 60 % protein in terms of dry biomass.

Microalgae are known to be high in minerals. The samples of pâtés were tested for macro- and microelements, including the determination of toxic elements Pb, Cd, Hg, As. The results are presented in Table 7.

Table 7

Content of macro- and microelements in ready-made pates

Chemical element	Recipe 1, without *Chlorella*, Control	Recipe 2, 0.5 % *Chlorella* powder	Recipe 6, suspension of live *Chlorella*
		content, mg/kg	
Na	2531.98	3117.88	3286.42
K	2125.154	2235.77	2244.20
Mg	213.33	236.704	183.77
Ca	108.948	127.029	90.481
Fe	48.462	41.854	27.715
Zn	11.668	11.468	14.669
Cu	1.076	1.124	0.874
Mn	1.076	1.202	2.185
Cd	0.153	0.031	0.031
Pb	0.092	0.092	0.092
Hg	less than 0.001	less than 0.001	less than 0.001
As	less than 0.001	less than 0.001	less than 0.001

The sample of pâté containing 0.5 % *Chlorella* powder (recipe 2) increased Ca by 16.6 %, Mg by 11.0 %, and Cu by 4.5 %.

In the sample of paste with a suspension of live *Chlorella* (formulation 6), Zn was increased by 25.7 %, and Mn – by 203.0 % (a 2-fold increase).

Toxic elements (Pb, Cd, Zn, Hg, As) did not exceed the maximum permissible levels in accordance with the requirements of regulatory documents. The study revealed a significant decrease in the amount of Cd in the experimental pâtés with the microalgae Chlorella. Fig. 3 shows the results of comparative studies of pates for Cd (II) content.

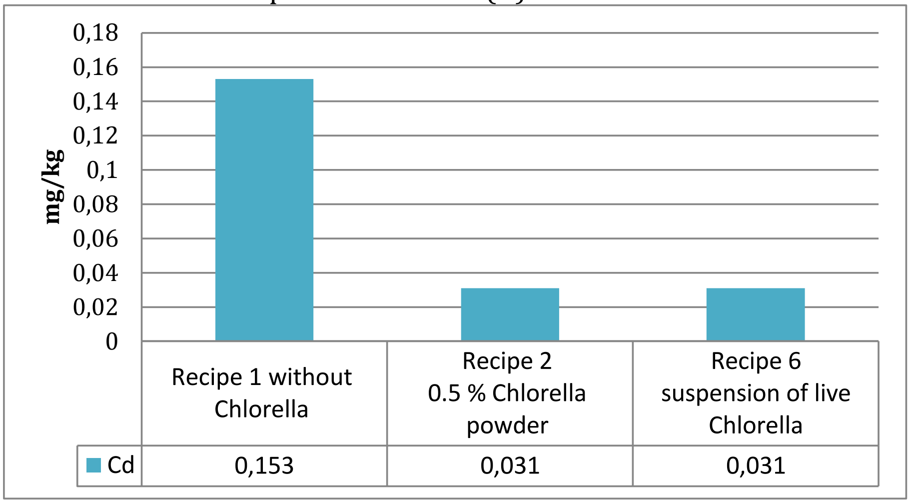


Fig. 3. Cd content in pâtés, mg/kg

In the samples of pâtés using microalgae, a 5-fold reduction in Cd (II) compared to the control sample (recipe 1) demonstrates the ability of *Chlorella* to cleanse natural environments and remove toxic elements from the cycle of substances, which has been repeatedly reported in the literature [38; 39].

Conclusions

According to the results of the research, it can be concluded that meat pâtés with *Chlorella* microalgae have a great advantage over traditional food due to their enrichment with essential amino acids (lysine, tryptophan), macro- and microelements (K, Mg, Ca, Cu, Mn, Zn).

Chlorella patés are in line with an important food design principle that the set of protein-containing ingredients should maximize the use of essential amino acids for anabolic purposes while minimizing their energy consumption. This indicates the high nutritional and biological value of *Chlorella* pâtés.

Thus, by using *Chlorella* in meat paste technology, we are expanding the range of health products with therapeutic and preventive effects aimed at improving public health.

References

[1] OECD/FAO (2022). *OECD-FAO Agricultural Outlook 2022-2031.* OECD Publishing. Paris. https://doi.org/10.1787/19991142.

[2] UN Press. (2022). Conflict, Humanitarian Crisis in Ukraine Threatening Future Global Food Security as Prices Rise, Production Capacity Shrinks, Speakers Warn Security. https://press.un.org/en/2022/sc14846.doc.htm.

[3] Fu, Y., Chen, T., Chen, S. H. Y., Liu, B., Sun, P., Sun, H., Chen, F. (2021). The potentials and challenges of using microalgae as an ingredient to produce meat analogues. *Trends in Food Science & Technology*, 112, 188–200. doi:10.1016/j.tifs.2021.03.050.

[4] Chacón-Lee T. L., González-Mariño, G. E. (2010). Microalgae for "Healthy" Foods—Possibilities and Challenges. *Comp Rev Food Sci Food Safety*, *9*(6), 655–675. doi:10.1111/j.1541-4337.2010.00132.x.

[5] Sathasivam, R., Radhakrishnan, R., Hashem, A., Abd_Allah, E. F. (2017). Microalgae metabolites: A rich source for food and medicine. *Saudi Journal of Biological Sciences*. *26*(4), 709–722 doi:10.1016/j.sjbs.2017.11.003.

[6] Boukid, F., Castellari, M., 2021. Food and beverages containing algae and derived ingredients launched in the market from 2015 to 2019: a front-of-pack labeling perspective with a special focus on Spain. *Foods, 10*, 1. doi:10.3390/foods10010173.

[7] Koyande, A.K., Chew, K.W., Rambabu, K., Tao, Y., Chu, D.T., Show, P.L. (2019). Microalgae: A potential alternative to health supplementation for humans. *Food Sci Hum Wellness*, 8, 16–24. https://doi.org/10.1016/j.fshw.2019.03.001.

[8] S. Liang, X. Liu, F. Chen, Z. Chen, Current microalgal health food R & Dactivities in China, in: P.O. Ang (Ed.), Asian Pacific Phycol. 21st Century: Prospects and Challenges, Springer, Netherlands, Dordrecht, 45–48. https://doi.org/10.1023/b:hydr.0000020366.65760.98

[9] Marti-Quijal, F.J., Zamuz, S., Tomašević, I., Gómez, B., Rocchetti, G., Lucini, L., Remize, F., Barba, F.J., Lorenzo, J.M. (2019). Influence of different sources of vegetable, whey and microalgae proteins on the physicochemical properties and amino acid profile of fresh pork sausages. *LWT - Food Sci Technol*, 110, 316–323. doi:10.1016/j.lwt.2019.04.097.

[10] Marti-Quijal, F.J., Zamuz, S., Tomašević, I., Rocchetti, G., Lucini, L., Marszałek, K., Barba, F.J., Lorenzo, J.M. (2019). A chemometric approach to evaluate the impact of pulses, Chlorella and Spirulina on proximate composition, amino acid, and physicochemical properties of turkey burgers. *J Sci Food Agric*, 99, 3672–3680. doi:10.1002/jsfa.9595.

[11] Zamuz, S., Purriños, L., Galvez, F., Zdolec, N., Muchenje, V., Barba, F.J., Lorenzo, J.M. (2019). Influence

of the addition of different origin sources of protein on meat products sensory acceptance. *J Food Process Preserv*, 43, 1–12. https://doi.org/10.1111/jfpp.13940.
[12] Zugcic, T., Abdelkebir, R., Barba, F. J., Rezek-Jambrak, A., Gálvez, F., Zamuz, S., Granato, D., Lorenzo, J.M. (2018). Effects of pulses and microalgal proteins on quality traits of beef patties. *J Food Sci Technol*, 55, 4544–4553. doi: 10.1007/s13197-018-3390-9.
[13] Sharylo, Yu. Ye., Derenko, O. O., Diudiaieva, O. A. (2020). [Using algae of the Chlorophyta species as a biological method of water purification]. *Vodni bioresursy ta akvakultura – Aquatic bioresources and aquaculture*, 1, 88–102. https://doi.org/10.32851/wba.2020.1.8 (in Ukrainian)
[14] Green honey with algae made in Ukraine. https://crispy.news/2020/10/29/busines/zeljonyj-mjod-s-vodorosljami-sdelali-v-ukraine/
[15] Bakhmach, V. A., Peshuk, L V., Chernushenko, E. A., Savchenko, A. M., Petrenko, S. A. (2022). [Use of innovative technologies and components in the manufacture of emulsion products]. *Visnyk Natsionalnoho Tekhnichnoho Universytetu «ХПІ»,* 1, 18–22. https://doi.org/10.20998/2220-4784.2022.01.03 (in Ukrainian).
[16] Peshuk, L.V., Simonova, I. (2022). [The trend of modernity is health-improving products with microalgae]. *Scientific bulletin LNUVMB im. S.Z. Gzhitsky*, *24*(97), 33–38 (in Ukrainian).
[17] Ru, I. T. K., Sung, Y. Y., Jusoh, M., Wahid, M. E. A., Nagappan, T. (2020). Chlorella vulgaris: a perspective on its potential for combining high biomass with high value bioproducts. *Appl. Phycol. 1*(1), 2–11. https://doi.org/10.1080/26388081.2020.1715256.
[18] Safi, C., Zebib, B., Merah, O., Pontalier, P. (2014). Morphology, composition, production, processing and applications of Chlorella vulgaris: A review. *Renewable and Sustainable Energy Reviews*, 35, 265–278. doi:10.1016/j.rser.2014.04.007.
[19] Carrasco-Castilla, J., Hernández-Alvarez, A.J., Jiménez-Martínez, C., Gutiérrez-López, G.F., Dávila-Ortiz, G. (2012). Use of proteomics and peptidomics methods in food bioactive peptide science and engineering. *Food Eng Rev*. 4, 224–243. doi: 10.1007/s12393-012-9058-8
[20] Caporgno, Martín P.; Mathys, Alexander (2018). Trends in microalgae incorporation into innovative food products with potential health benefits. *Frontiers in Nutrition*, 5, 58. doi:10.3389/fnut.2018.00058/.
[21] Ferreira, A., Guerra, I., Costa, M., Silva, J., Gouveia, L. (2021). Future perspectives of microalgae in the food industry. *Cultured Microalgae for the Food Industry*, 387–433. doi:10.1016/b978-0-12-821080-2.00008-3.
[22] Christaki, E., Florou-Paneri, P., Bonos, E. (2011). Microalgae: a novel ingredient in nutrition, *Int. J. Food Sci. Nutr. 62*, 794–799, http://dx.doi.org/10.3109/09637486.2011.582460.
[23] Krishna Koyande, A., Chew, K. W., Rambabu, K., Tao, Y., Chu, D.-T., Show, P.-L. (2019). Microalgae: A potential alternative to health supplementation for humans. *Food Science and Human Wellness*, *8*(1), 16–24. doi:10.1016/j.fshw.2019.03.001.
[24] Becker, E. W. (2007). Microalgae as a source of protein. *Biotechnology Advances*, 25, 107–210. doi:10.1016/j.biotechadv.2006.11.002.
[25] FAO – News Article: World's future food security "in jeopardy" due to multiple challenges, report warns (n.d.). http://www.fao.org/news/story/en/item/471169/icode.
[26] Mobin, Saleh M.A.; Chowdhury, Harun; Alam, Firoz (2019). Commercially important bioproducts from microalgae and their current applications – A review. *Energy Procedia*, 160, 752–760. doi:10.1016/j.egypro.2019.02.183.