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## DEVELOPMENT OF TECHNOLOGY FOR EXTRACTION OF PROTEIN ISOLATE FROM PUMPKIN MEAL WITH ITS FURTHER USE AS EMULSIFIER IN MAYONNAISE RECIPE

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### Abstract

The technology of protein isolate extraction from raw material waste of plant origin is being considered in this work. In order to optimize the performance of all organs and systems in the human body, nutrition must be complete and balanced. This task is achieved by improving the formulation and production techniques of existing foods in order to preserve the nutritional properties of the product components or by adding new characteristics to the product. Plant proteins are of particular importance for human health. Protein deficiency in the human body leads to the breakdown of body's own proteins. Protein products have high biological value and emulsifying capacity, their use in food products allows for stable emulsions. For research pumpkin meal produced after extracting oil from pumpkin high-protein naked-seeds by cold-pressing was studied. The obtained protein isolate was used in the recipe of mayonnaise as a substitute for egg powder in the amount of 8.5 % and 10 %. Mayonnaises with stable emulsion were obtained according to both recipes. Mayonnaise with a lower emulsifier content was selected for further study. The developed mayonnaise has good organoleptic and physical and chemical indicators and is a promising product for the food market in Ukraine. When analysing the growth of microorganisms on the surface of the Sabouroud's medium, yeast colonies were discovered. Mold fungi or their spores, as well as colonies typical of coliform bacteria, were not found in the mayonnaise samples examined. Based on the determination of the change in acid number and the persistence of the emulsion in storage, the recommended storage period for mayonnaise is 35 days at a temperature of  $1 \pm 6$ . The development of industrial technology for the production of protein isolate from pumpkin meal is a promising development that will allow the complete replacement of mayonnaise emulsifier of animal origin to emulsifier of plant origin.

**Keywords:** pumpkin meal; protein isolate; alkaline dissolution; acid precipitation; emulsifier; mayonnaise.

## РОЗРОБКА ТЕХНОЛОГІЇ ВИЛУЧЕННЯ БІЛКОВОГО ІЗОЛЯТУ ІЗ ГАРБУЗОВОГО ШРОТУ З ПОДАЛЬШИМ ВИКОРИСТАННЯМ ЙОГО В ЯКОСТІ ЕМУЛЬГАТОРУ В РЕЦЕПТУРІ МАЙОНЕЗУ

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### Анотація

У роботі розроблено технологію вилучення білкового ізоляту з відходів сировини рослинного походження. Для оптимальної роботи всіх органів та систем в людському організмі харчування має бути повноцінним та збалансованим, це досягається вдосконалення рецептур та технологій виробництва існуючих харчових продуктів з метою збереження поживних властивостей компонентів продукту або надання продукції нових властивостей. Особливе значення для здоров'я людини мають білки рослинного походження. Дефіцит білків в організмі людини призводить до розпаду власних білків. Білкові продукти мають високу біологічну цінність та емульгуючу здатність, їх застосування у складі харчових продуктів дозволяє отримати стійкі емульсії. Для проведення дослідження використовували шрот голонасінного гарбуза з високим вмістом сирого протеїну, отриманий після вилучення олії з насіння методом холодного пресування. Одержаний ізолят білку використовували в рецептурі майонезу в якості заміни яєчного порошку в кількості 8.5% і 10%. За обома рецептурами одержали майонези зі стійкою емульсією. Для подальшого дослідження було обрано майонез з меншим вмістом емульгатору. Розроблений майонез має гарні органолептичні і фізико-хімічні показники і є перспективним продуктом для ринка харчових продуктів в Україні. При аналізі росту мікроорганізмів на поверхні середовища Сабуро були виявлені колонії дріжджів. Наявність пліснявих грибів або їх спор, а також колоній, типових для колиформних бактерій, у досліджених зразках майонезу виявлено не було.

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За результатами визначення зміни кислотного числа і стійкості емульсії під час зберігання встановлено рекомендований строк зберігання майонезу 35 днів при температурі 1÷6 °С. Розробка промислової технології виробництва білкового ізоляту з гарбузового шроту є перспективним напрямком, який дозволить провести повну заміну в рецептурі майонезу емульгатору тваринного походження на емульгатор рослинного походження.

*Ключові слова:* гарбузовий шрот; білковий ізолят; лужне розчинення; кислотне осадження; емульгатор; майонез.

## Introduction

Nutrition is one of the most important factors in human life, it directly affects the state of health, efficiency, physical and mental development. The body receives proteins, fats, carbohydrates, micro- and macro-elements necessary for normal functioning. In order to optimize the performance of all organs and systems in the diet, it must be complete and balanced. It can be achieved in several ways, one of them is the improvement of formulations and production technologies of existing foods in order to preserve the nutritional qualities of the product components, the other one is to give the product new properties.

Among the food components that are of particular importance for human health, plant-based proteins play a crucial role, as the Ukrainian population has recently undergone negative changes related to the reduction of most products. This has led to a growing shortage of essential nutrients: proteins, vitamins, minerals, polyunsaturated fatty acids. According to data correlated with the spread of the relevant diseases, and according to expert estimates, the deficit of food protein in Ukraine is about 30–40 %, vitamins is 40–60 % [1]. It is known that the lack of proteins in the diet results in the body's own proteins breakdown. In food products, plant proteins have a positive influence on the organoleptic parameters of food: type, color, taste and texture [2–7].

One of the most widely used sauces in the human diet is mayonnaise. It is used as a seasoning to improve the taste and digestibility of products, and as an additive for making first, second courses, and snacks. Traditionally mayonnaise is a product based on vegetable oil, egg powder, and lemon juice, as well as mustard, sugar, salt and other food and flavour additives that make up a multi-component emulsion system like «oil in water» [8–14]. The main trends in the product-line expansion of mayonnaise are a reduction in calorie intake in fat and sugar formulations, increasing the biological value by full or partial replacement of traditional components with natural biological and physiologically active substances [15–21].

One of such promising components is oilseed protein products. It is known that protein products have high biological value and emulsifying capacity that makes it possible to obtain stable emulsions of the consumer's usual consistency. It gives the possibility to replace the egg powder as the traditional emulsifier in the mayonnaise recipe. Egg powder is known to contain 9 % cholesterol, it is not recommended for the elderly and patients with hypercholesterolemia. Therefore, today it is important to develop recipes for low-calorie mayonnaise with complete replacement of the emulsifier of animal origin with an emulsifier based on protein products derived from vegetable waste.

There is currently a great deal of work on the production of protein products from sunflower and soybean meal [22–30]. Ukraine traditionally grows a large number of pumpkin plantations every year. The pumpkin fruit pulp is processed in a cannery, leaving a large amount of seeds as waste. Pumpkin seeds are used to make useful pumpkin oil, and low-fat pumpkin meal has not been widely used in the food industry. But compared to other oilseeds meal, it's the meal from the pumpkin seeds that contains a lot of protein [31–34].

In view of the above, the aim of this work was to develop a technology for producing a protein isolate from pumpkin meal and to use the resulting product as an emulsifier in mayonnaise.

In the mayonnaise recipe, a blend of oils from wheat germ and sunflower was used as a fatty base. To prevent diabetes diseases, sugar was replaced by fructose in mayonnaise recipe. In order to give mayonnaise functional properties golden flax seeds were used in the recipe.

## Experimental part

Naked-seeds pumpkin meal was selected for the study. Compared with other oilseeds meal, pumpkin seed meal contains a large amount of protein (Table 1). Pressing the pure kernel of pumpkin seeds allows to get a white meal with a high protein content, not subject to denaturation.

Table 1

Comparison of protein content in oilseed meal		
Nº	Oil plant	Crude protein content, %
1	Flax seed meal	29.96
2	Sesame seed meal	39.71
3	Hemp seed meal	26.35
4	Pumpkin seed meal	50.68
5	Coconut shavings meal	15.60

Naked-seed pumpkin meal was used in the work after extraction of oil from the seeds by cold pressing. Characteristics of pumpkin seeds meal is shown in table 2.

Table 2

Characteristics of naked pumpkin seed meal			
Nº	Indicator name	Actual value	Test method
1	Crude protein, %	50.68	DSTU 7169:2010
2	Crude fat, %	23.31	DSTU ISO 6492:2003
3	Moisture content, %	8.60	DSTU 7621:2014
4	Crude fiber, %	5.17	DSTU ISO 6865:2004
5	Acid number of fat, mgCON / g	1.50	DSTU 7618:2014
6	Raw ash, %	6.50	DSTU ISO 5984:2004

Mayonnaise was produced and tested by standard methods according to DSTU4487: 2015 "Mayonnaise and mayonnaise sauces. General technical conditions".

During a microbiological study of the types of mayonnaise the following characteristic was determined: the total microbial number in 1 g of the product, the nature of the microflora, the coliform bacteria, the quantity of yeast and mold according to known methods [35; 36]. The Sabouraud's medium was used to determine the amount of yeast and mold in mayonnaise. The Endo medium was used to determine coliform bacteria. The 10 g mayonnaise was prepared with the initial and a series of ten times dilution. For each dilution, three parallel sowings were made at the Petri dish. After incubation, microscopic studies of microorganism colonies were carried out.

In the course of the research, a five-fold repetition of experiments was used. Statistical processing of the results was carried out using EOM and MS Excel software. The confidence interval was 95–96 %.

## Results and discussion

In the first stage, the protein isolate of the pumpkin meal was extracted using hexane as a solvent. Next, the proteins were extracted from the meal: extractant ratio of 1:10 with constant stirring, maintaining the process temperature in

the range of 38–40 °C for 1 hour. A 12.5 % solution of NaCl in hydroxide buffer (pH 10.0) was used as an extractant. Proteins from the obtained extract were precipitated by isoelectric precipitation, adjusting the pH to 3.9–4.2 1N with HCl solution. The protein residue was separated from the serum water by centrifuging at 1500 g/min. for 15 minutes.

The precipitate obtained was washed with distilled water to remove excess sodium chloride and acid. The precipitate was separated from the wash water by centrifuging at 1500 rpm. for 15 min and dried at a temperature of 40–42 °C to constant weight. The scheme of obtaining protein isolate from pumpkin meal is shown in the figure below.

The obtained protein isolate was used in the formulation of mayonnaise as an egg powder replacement in the amount of 8.5 % and 10 %. Recipes for mayonnaise using protein isolate are given in Table 3.

The taste of the obtained product was determined during the mayonnaise production. The obtained mayonnaise had a sufficient taste of salt (this can be explained by not fully removal of salt during protein paste washing), so salt was excluded from the recipe. Mayonnaises with a stable emulsion were obtained according to both recipes.

Table 3

Prescription ratios of mayonnaise test samples			
Nº	Raw stuff	Sample №1	Sample №2
1	Sunflower oil, %	45.50	45.50
2	Wheat germ oil, %	19.50	19.50
3	Protein isolate, %	8.50	10.00
4	Kitchen salt, %	-	-

5	Fructose, %	0.62	0.62
6	Mustard powder, %	2.00	2.00
7	Lemon juice, %	2.10	2.10
8	Flax seeds, %	0.50	0.50
9	Purified water, %	remaining portion	remaining portion

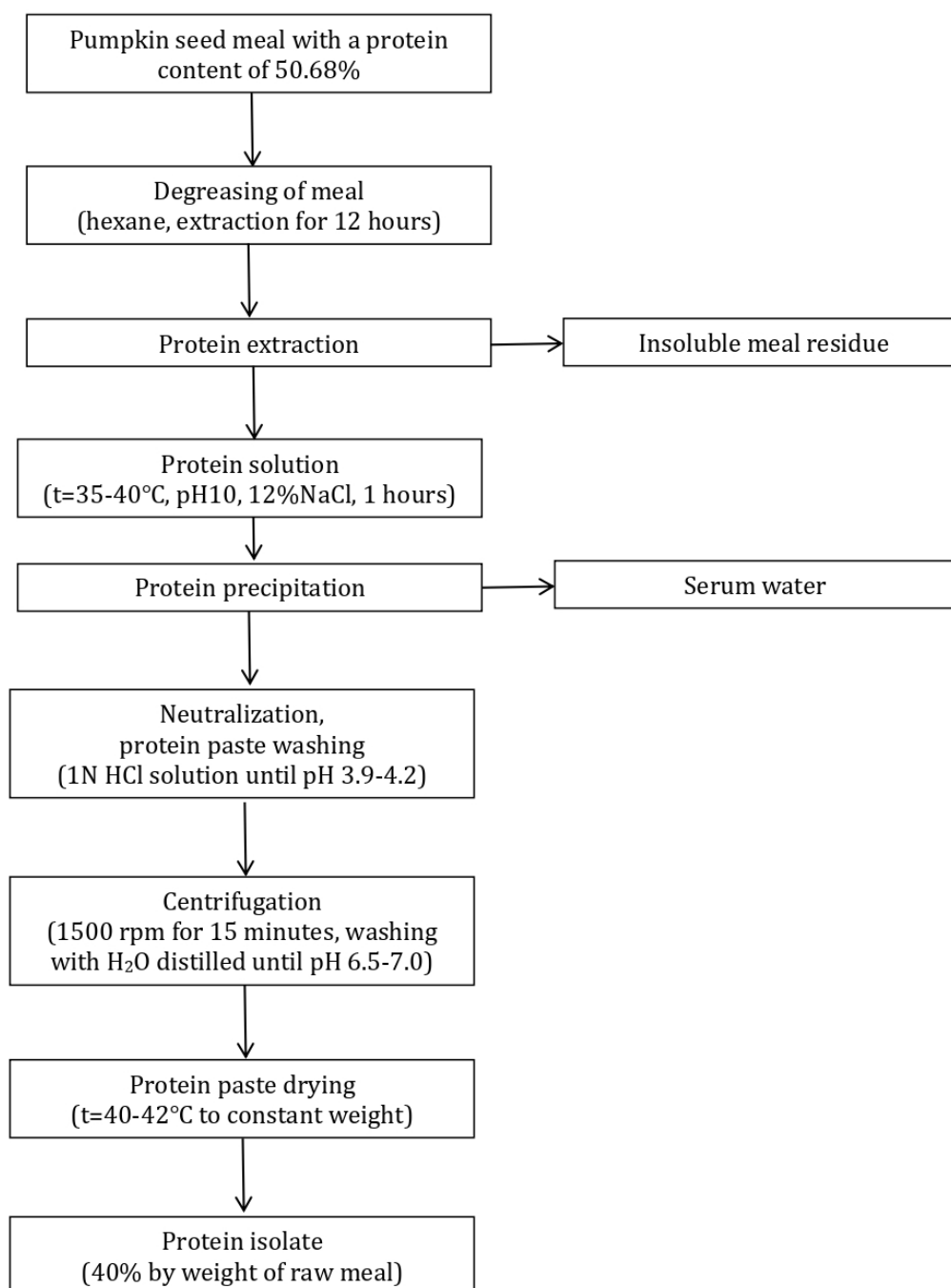


Figure. Scheme of obtaining protein isolate from naked-seed pumpkin meal

Table 4

## Organoleptic and physical and chemical parameters of experimental mayonnaises

Indicators	Sample №1	Sample №2
Appearance, consistency	Homogeneous creamy product with single air bubbles	
Color	Homogeneous, creamy white	
Taste	Inherent in traditional mayonnaise	
Scent	Inherent in traditional mayonnaise	
Mass fraction of moisture, %	16.86	17.31

Mass fraction of fat, %	65.00	65.00
Acid number, %	0.14	0.15
Emulsion stability, %	99	99
pH medium	4.2	4.2

A quality study was carried out immediately after the mayonnaise was manufactured. The results of mayonnaise experimental samples quality are given in Table. 4. Mayonnaise №1

sample with a lower emulsifier content was selected for further study.

The results of mayonnaise microbiological analysis are shown in Table 5.

Table 5

#### Microbiological evaluation of mayonnaise using a plant emulsifier

Indicator	Value
Bacteria of coli group (coliforms) in 0.01 g	not revealed
Pathogenic microorganisms, including <i>Salmonella bacteria</i> , in 25 g	not revealed
Yeast, CFU in 1 cm <sup>3</sup>	1·10
Molds, CFU in 1 cm <sup>3</sup>	not revealed
Opportunistic micro-organisms (staphylococcus)	not revealed

In the analysis of the growth of microorganisms on the surface of the Sabouraud's medium for mayonnaise samples using plant-based emulsifiers of 1:10 dilution, colonies of white yellow color and slippery consistency were found, morphologically similar to yeast colonies.

A microscopic study of the colonies by cell size and morphology confirmed their belonging to yeast. Not fluffy cobwebs on the surface of Sabouraud's medium were found in any of the Petri dishes, which indicates the absence of

molds or their spores in the studied samples of mayonnaise. No colonies typical of coliform bacteria were found on the surface of the Endo medium. Therefore, it can be concluded that the microbiological indicators of all types of mayonnaise are within the norm.

The acidity and stability of the emulsion of the developed mayonnaise was determined within 35 days in order to establish the optimal shelf life of the finished product. The results of the study are shown in Table 6.

Table 6

#### Determination of acidity and stability of the emulsion of the test mayonnaise

Shelf life	Mayonnaise acidity of, %	Emulsion stability, %
1 day	0.14	99
7 days	0.16	98
14 days	0.17	97
21 days	0.18	96
28 days	0.19	94
35 days	0.21	94

When analyzing the obtained data, we can see an increase in the acidity of mayonnaise and a decrease in emulsion stability, but the obtained data do not exceed the standard values. Based on the above data, the recommended shelf life for mayonnaise is 35 days at a temperature of 1 ÷ 6 °C.

### Conclusions

The technology for producing a protein isolate from naked-seed pumpkin meal has been developed. The work shows the possibility of replacing the animal emulsifier with a plant-based emulsifier. The required stability of the

emulsion is achieved with a lower content of emulsifier based on waste vegetable raw materials compared to traditional emulsifier. The mayonnaise produced has good organoleptic and physical and chemical indicators and is the latest product of the food market in Ukraine. The development of industrial technology for the production of protein isolate from pumpkin meal is a promising development that will allow the complete replacement of animal mayonnaise emulsifier with plant-based emulsifier formulations.

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