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VITAMIN C ACCUMULATION IN FLAX SEEDS DURING GERMINATION

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Abstract. Research of biologically active plant raw materials for the creation of healthy food products is in recent years one of the trends in the correction of nutritional status of the population. The article describes the qualitative composition and quantitative content of valuable food products in flax seeds, due to the biochemical composition and biological value of protein. Thus, the bio activation of seeds by germination allows to activate the enzyme system. The course of biochemical processes leads to an increase in water-soluble protein fractions, the accumulation of free amino acids and fatty acids, as well as easily soluble reducing sugars. According to the increase in the value of flax and improving the functional properties of the protein present in it. The influence of parameters and duration on the process of germination of flax seeds in aqueous solution is determined. The step-by-step process of conversion of carbohydrates into vitamin C, as well as the accumulation of vitamin C in seeds during germination described. A comparative table by ascorbic acid content in some cultivars of flax, namely "Vruchiy", "Blue-orange", "Eureka", "Original" is given. The proposed method allows to increase the content of vitamin C by more than 90%, as well as to use all the anatomical parts of the seed in the technology of health products. It was found that the amount of synthesized vitamin C depends on the cultivar of germinated seeds, so the highest rate of ascorbic acid in flax seeds of the cultivar "Original", 16.56 mg/100g.

Keywords: vitamin C, flax, sprouts, germinated flax seeds, bio activation.

Introduction. Formulation of the problem

Common flax (Linum usitatissimum L.), which belongs to the Lineaceae family, has regained its popularity as a natural functional food product due to its biochemical composition and biological value. With its unique natural qualitative properties, flax is recommended for use in the prevention and treatment of a significant number of diseases. Especially for supporting the body's immune system, treating cardiovascular diseases, diabetes, diseases of the gastrointestinal tract and thyroid gland, for removing toxins from the body, as an anti-inflammatory agent, and many others. This fact is confirmed by the famous Indian figure Mahatma Gandhi in his statements about flax seeds. He believed that flax seeds should become a regular component of people's nutrition and, accordingly, their health should improve thanks to flax. Today, there are quite a few conclusions from scientists from different countries about the importance of daily consumption of flax seeds, which, in their opinion, should become part of the food culture. Adding flax seeds increases immunity and reduces the risk of various types of chronic diseases, which is quite relevant during the COVID-19 pandemic and constant stressful loads in the life of a modern person.

Today, flax seeds are recognized as a potential source of functional ingredients due to the following bioactive components: ω-3 fatty acids; lignans; dietary fiber and complete protein. The seeds have a special texture and a pleasant nutty taste. Flax is one of the best sources of ω-3 fatty acids for a vegetarian diet [1, 2]. Due to the content of several groups of physiologically functional substances in flax seeds, it is a valuable raw material for the production of functional food products, in particular bakery products, since they are products of daily consumption [3]. To increase the content or activity of a certain physiologically functional ingredient in flax seeds, it is subjected to pre-treatment. When using seeds without additional cleaning from the shells, various methods and methods of flattening, extrusion, germination are most often used. Germination is a technological technique that is widely used due to its ability to reduce the level of anti-nutritional factors in seeds, improve the taste appeal and availability of biologically active substances, and activate its enzyme system. Studies on the bioactivation of flax seeds have shown that during germination the content of vitamin C increases several times.

Sprouted flax seeds are widely used in food technologies to increase the nutritional value of products. Currently, cereals, breakfast cereals, various types of bread and flour confectionery are produced in the industry based on sprouted flax. These products are characterized by high increased nutritional value and a positive effect on human health.

Analysis of recent research and publications

Vitamin C (L-ascorbic acid) is an essential nutrient that cannot be synthesized in the human body. However, vitamin C is not only an antioxidant, it is also a cofactor for numerous enzymes involved in plant and human metabolism. In the human body, vitamin C is involved in various physiological processes such as iron absorption, collagen synthesis, immune stimulation and epigenetic regulation. Due to the functional loss of the gene encoding L-gulonolactone oxidase, humans cannot synthesize vitamin C. Therefore, humanity mainly uses plant-based foods to meet its vitamin C needs.

Vitamin C (L-ascorbic acid) was isolated from the adrenal cortex by Albert Szent-Gyorgyi in 1928. Szent-Gyorgyi A. demonstrated that this compound, which can act as a powerful reducing agent, has the empirical formula $C_6H_8O_6$ with a molecular weight of 178 ± 3 and is a lactone with an acidic hydrogen atom. Due to its similarity to simple sugars and acidic properties, Szent-Gyorgyi A. named this compound "hexuronic acid". In 1932, Charles Glen King isolated an anti-scurvy (anti-scurvy) compound from lemon juice, which was recognized as "hexuronic acid" discovered by Szent-Gyorgyi. At the same time, Szent-Gyorgyi A. showed that 1 mg of "hexuronic acid" per day provides sufficient protection against scurvy. The final structure of vitamin C, which is an aldono-1,4-lactone of hexonic acid with an enediol group at C2 and C3, was determined by Norman Haworth in 1933. Evidence that this compound could prevent scurvy led to its renaming from "hexuronic acid" to "ascorbic acid" [5]. Without exaggeration, vitamin C is one of the most important nutrients and a key element in the metabolism of almost all living organisms. In humans, vitamin C performs numerous functions, mainly acting as an antioxidant and a cofactor for monooxygenases and dioxygenases. The role of vitamin C as an antioxidant in humans has been established on the basis of a large body of scientific evidence. Vitamin C protects DNA, proteins, and lipids from oxidative damage by blocking free radicals [6]. Vitamin C is used as an antioxidant throughout the body, but may have specific functions in certain organs. For example, vitamin C is required by the eyes in millimolar concentrations to ensure protection against oxidative damage from solar radiation. Vitamin C is also important for the bioavailability of iron, reducing non-heme trivalent iron (Fe^{3+}) to the divalent (Fe^{2+}) form, which is more easily absorbed in the intestine. For this reason, this vitamin is indirectly required for protection against anemia. Vitamin C also affects iron metabolism by stimulating ferritin synthesis and inhibiting ferritin degradation [7]. The authors of [8] revealed that vitamin C, as a cofactor of peptidyl-glycine alpha-amidating monooxygenase, is involved in the biosynthesis of many signal peptides, such as oxytocin, vasopressin, cholecystokinin and calcitonin. Vitamin C functions as a cofactor of many dioxygenases, reducing the iron in the active site of these enzymes to Fe^{2+}. A group of scientists [8] noted the contribution of vitamin C to the proper formation of collagen through post-translational modifications of procollagen. Therefore, vitamin C, as a cofactor of hydroxylase, is used for the synthesis of noradrenaline and carnitine.

Vitamin C enters the human body as a result of food consumption through a proper diet, and plant products are the main source of this vitamin. Although synthetic vitamin C is chemically no different from the plant vitamin, fruits and vegetables have various trace elements and phytochemicals that can affect its bioavailability [9].

The authors [2, 4, 5, 6, 7] have disclosed the results of biochemical processes in seeds during germination. They found that during biochemical processes, there is an increase in water-soluble protein fractions, accumulation of free amino acids and fatty acids, as well as easily soluble reducing sugars. This leads to an increase in the value of the raw material and improvement of the functional properties of the protein present in it.

Flax seed sprouts are used to increase the content of ω-3 polyunsaturated fatty acids and reduce cholesterol in chicken eggs. Sprouted flax seeds synthesize tocopherols, vitamin C, and lignans are also released from it to combat a number of diseases, including cancer [4]. Sprouting also leads to the degradation of anti-nutritional factors - α-galactoside oligosaccharides, inhibitors of pancreatic proteinases and lipase, phytohemagglutenins, tannins, phytates, cyanogenic glycosides [1, 7, 8, 9, 10, 11].

Increasing the nutritional and biological value through germination allows you to expand the range of products. At the same time, determining the duration and other parameters of the germination process, establishing increased biological activity of flax seeds, which changes due to the accumulation of hydrolytic cleavage products of biopolymers, is of practical value. Despite research and significant results in the field of seed germination biochemistry, scientific and practical interest in these processes does not weaken. In this regard, there are relevant studies of the dynamics of macronutrients in the process of flax seed germination.

The purpose and objectives of the research. The purpose of the work is to study the synthesis of vitamin C and its accumulation in flax seeds during germination. In accordance with the goal, the main research objectives were formulated:

• to determine the optimal parameters of flax germination;

• to establish the accumulation of vitamin C under the optimal parameters of flax germination.

Materials and methods.

The research was conducted on several varieties of flax seeds, which were included in the Register of Plant Varieties of Ukraine in the period from 2003 to 2007: "Eureka", "Blue-orange", "Original" and "Vruchy". Flax seeds were germinated in laboratory conditions in a specially designed installation at a temperature of 18-20ºC and at a water:seed ratio of 2: 1. The seeds were germinated for 2 days with periodic moistening. Germinated flax seeds had sprouts from 2 to 5 mm long. The germination process was stopped by drying to a seed moisture content of no more than 5%. Sampling of germinated seeds was carried out daily. Vitamin C was determined by high-performance liquid chromatography. Chromatographic analysis of vitamin C was determined on a high-performance liquid chromatograph UltiMate 3000 (Germany), Dionex, with a photodiode matrix detector, on a chromatographic column Acclaim 120, C18 (Dionex). The analyses were performed in 3-fold replication.

The composition of flax seeds significantly depends on the variety, growing conditions and processing methods of plant raw materials [2]. A study of several varieties of flax seeds included in the Register of Plant Cultivars of Ukraine in the period from 2003 to 2007: "Eureka", "Blue-Orange", "Original" and "Vruchiy".

The following were used: standard, generally accepted, special and modified physicochemical, microbiological and organoleptic methods that allow determining the chemical composition, biological value. Flax seeds were cleaned of damaged specimens, dust, dirt and other foreign impurities. A comprehensive study of the production of sprouted flax seeds, which includes the analysis of its physical properties (size, shape, weight, color, density), consideration of extraction parameters and subsequent process properties, is presented in (Fig.1).

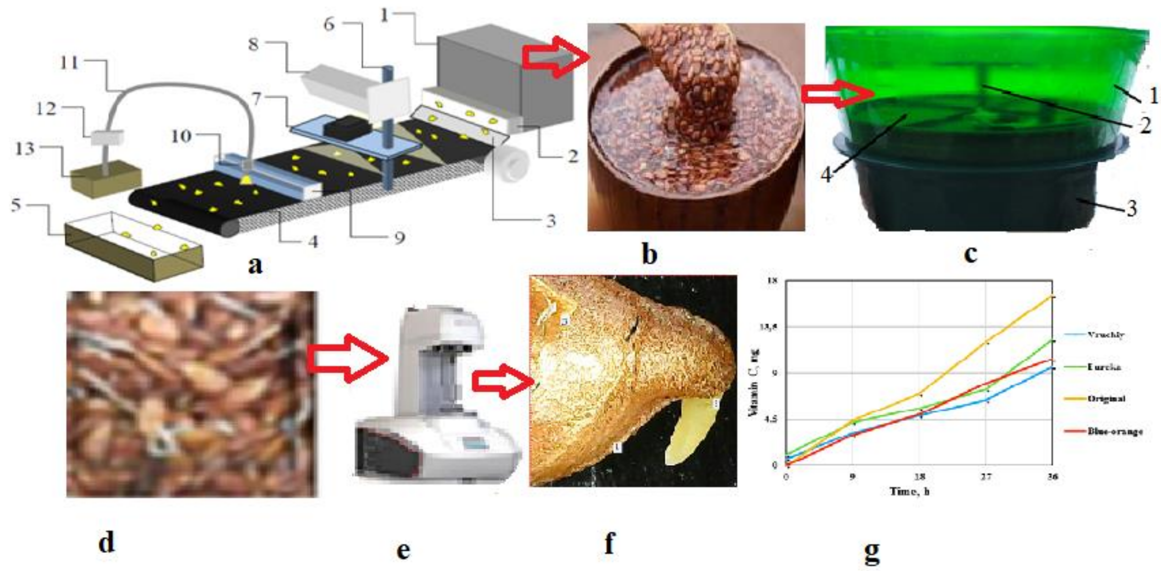

Fig. 1 - Scheme of the research methodology:
a – device for sorting flax seeds: 1 – loading hopper; 2 – dosing device; 3 – inclined tray; 4 – black conveyor belt; 5 – discharge tray; 6 – tripod; 7–8 – digital cameras; 9 – carriage; 10 – suction head; 11 – flexible hose; 12 – vacuum solenoid valve; 13 – accumulator; b – soaking flax seeds; c – device for germinating flax seeds:1 – flax vessel; 2 – working body; 3 – base with electric stirrer drive and warm air supply pump; 4 – sieve surface for germination; d – germination process on the sieve surface; e – digital microscope; f – flax seed sprout; g – accumulation of vitamin C in flax seeds during germination

Modern technical means for sorting raw materials were used Fig. 1. The device for sorting flax seeds provided effective and high-quality sorting of seeds before soaking with the ability to recognize their varieties. 400 seeds were counted, 100 or 50 (for large-seeded crops) pieces in each repetition. The seeds were evenly placed on moistened filter paper. To moisten, the paper was immersed in water, taken out and allowed to drain excess water and spread it on two layers of moistened paper placed in Petri dishes.

The germination process was studied and analyzed by changing the input factors and observing the outputs (Fig.2.(2,3,4)). Flax seed germination was carried out in the laboratory in a specially designed installation at a temperature of 18-20ºC and at a water: seed ratio of 2:1. The seeds were germinated for 2 days with periodic moistening. Germinated flax seeds had sprouts from 2 to 5 mm long. The germination process was stopped by drying the seeds to a moisture content of no more than 5%. Germinated seeds were sampled daily. Vitamin C was determined by high-performance liquid chromatography. Chromatographic analysis of vitamin C was determined on a high-performance liquid chromatograph UltiMate 3000 (Germany), Dionex, with a photodiode detector, on an Acclaim 120, C18 chromatographic column (Dionex). The analysis was performed in 3-fold replication.

It should be noted that the created conditions for flax seed germination affect the formation and development of seed fibres and their connection into a three-dimensional network.

The study used digital microscopes 500x SDM, CL PC camera 4.5, BW-400X with a resolution of 1280x 960 (1.3MP) with a magnification of 126 times with a field size of 4.2x3.15 mm. and a pixel count of 320+-0.5 (Fig. 1.(5)). Connection to a personal computer (PC) was made via a USB interface.

Results of the research and their discussion

It can be noted that for the germination of flax seeds, three simple conditions are necessary: moisture, access of oxygen, a certain minimum of heat. In our previous studies [11, 12] it was established that at a humidity of 95%, the temperature range that provides germination energy of not less than 99% is 25…34°C. When the temperature decreases to 24°C, the germination energy reaches 98%, at 22°C - 95%, and at 20°C - 91%. The permissible range of germination energy values is from 90 to 100%. Therefore, in production conditions, the process of germination of flax seeds can be carried out at temperatures from 20 to 30°C, if the ambient humidity is 95%. When the humidity decreases to 70%, the temperature range that provides germination energy of flax seeds within 90-100% is 22-32°C.

During germination, flax seeds undergo a number of transformations that activate the work of enzymes, as well as changes in the chemical composition of the raw material and structural and mechanical characteristics. With a sharp activation of enzymes, the breakdown of proteins and carbohydrates occurs [13].

The dry mass of the grain during germination decreases somewhat, since when soaking the seeds swell, and some organic substances react with water and pass into simpler forms and are lost. These losses are a consequence of the respiratory processes that occur during germination. The decomposition of basic nutrients is only part of the complex processes.

During germination, the absorption from water and the assimilation of the necessary macro- and microelements that form polyribosomes responsible for protein synthesis and active phytohormones work, accelerating growth and synthesizing vitamins.

The amount of antinutrients is significantly reduced, and the anti-inflammatory activity increases. Thus, when the grain is in a state of biological activity, high-molecular biopolymers are cleaved to low-molecular soluble substances. At the same time, the rate of their assimilation by living organisms increases sharply.

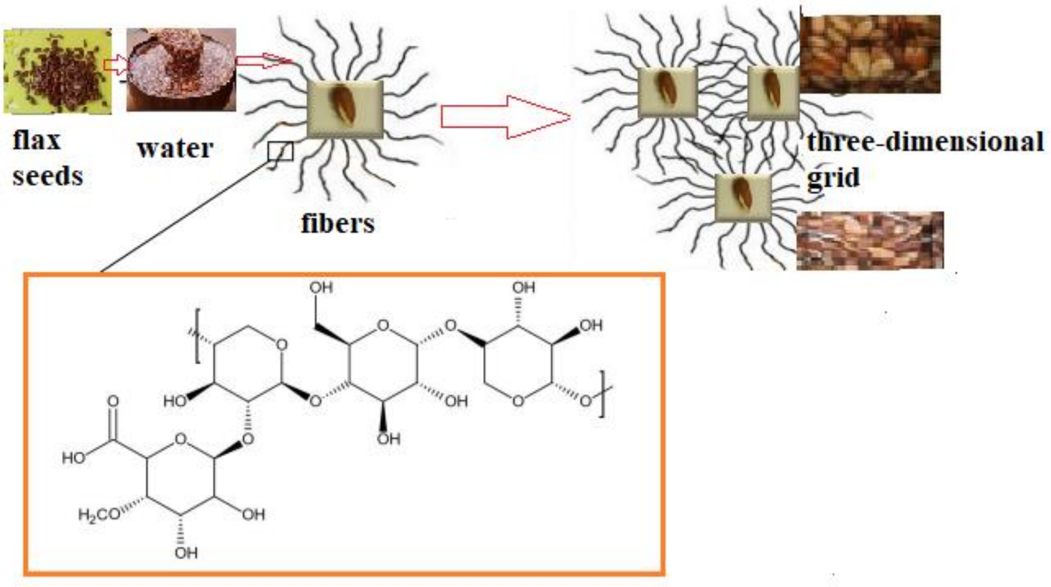

Fig. 2 - Modern ideas about the mechanism of gelation of mucous substances of flax seeds during germination

Therefore, when using sprouts in food, a person can obtain a complex of necessary nutrients in a simple accessible form. One of the main properties of sprouts is the ability to synthesize vitamin C, while in dry seeds its content is insignificant or completely absent. Considering the Reichstein-Grosser method formulated by Swiss chemists for the conversion of glucose into vitamin C, it was assumed that the same transformations occur in the process of germinating flax seeds. The entire process of obtaining ascorbic acid is mixed, that is, chemical-enzymatic and consists of six stages, including one stage of microbial synthesis (Fig. 3).

The presented scheme in Figure 3 reflects the transformation of glucose into vitamin C formulated by Swiss chemists. Flax seed carbohydrates are represented by: cellulose, lignans, hemicellulose, which in turn are partially or completely hydrolyzed to glucose during germination (in the presence of water), and glucose, having undergone a number of transformations, is transformed into ascorbic acid [11,17].

So, the accumulation of vitamin C in sprouts, depending on the germination time. Therefore, we stopped the germination process after 36 hours, since we were limited to a sprout length of 3 mm. In addition, the limitation of the germination duration is associated with the bioactivation of the grain and the formation of biologically active compounds. This is characteristic only for the beginning of germination. Further growth processes reduce the overall value of the product and the mass fraction of vitamins, minerals, plant enzymes and phytohormones in them [18,19,20].

Table 1 - Vitamin C content in the studied flax seed varieties before and after germination

No.	Variety	Vitamin C, mg/100g	
		Whole grain	Sprouted grain
1	"Vruchy"	0.72	9.61
2	"Original"	1.24	16.56
3	"Blue-orange"	0.88	10.40
4	"Eureka"	1.11	12.23

Measurements were carried out with an interval of 9 hours. The increase in vitamin C throughout the germination period is not uniform. The amount of synthesized ascorbic acid depends on the seed variety. Therefore, the obtained data of the study of the content of vitamin C in flax seeds before and after germination

In addition, we have constructed the dependence of the accumulation of vitamin C in flax seeds during germination. Fig. 4 shows quite clearly the accumulation of ascorbic acid in seedlings, which occurs quite intensively.

During 36 hours of germination, the content of vitamin C in flax seeds, depending on the variety, increased by 91-94.5%. The results of the study show that the greatest increase in vitamin C was obtained in flax seeds of the "Original" variety, and in the varieties Eureka", "Blue-orange", "Vruchy" it is correspondingly smaller and is almost within the same limits.

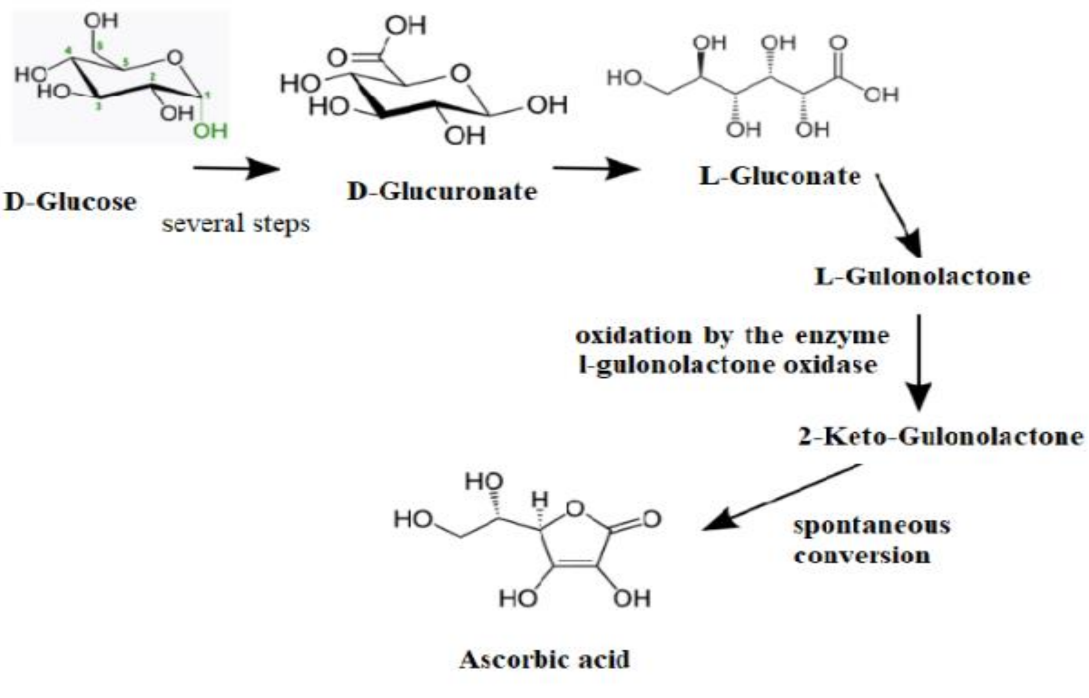

Fig.3. - Scheme of the conversion of glucose into vitamin C

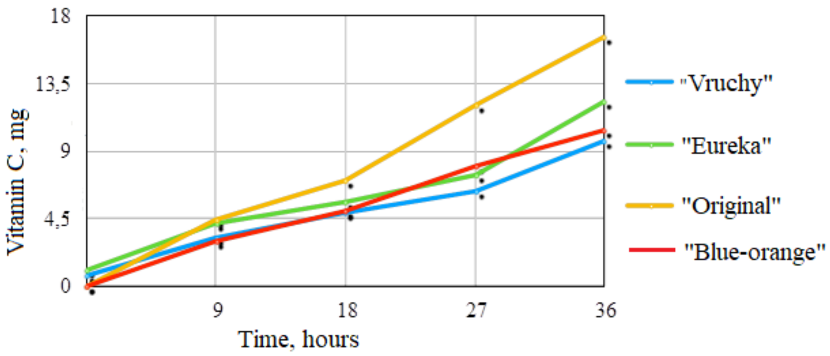

Fig. 4. Vitamin C accumulation in flax seeds during germination for 36 hours

Conclusion

The value of sprouts has been established in activating enzymes that control hydrolytic and oxidative processes. The germination period determines a number of chemical transformations that allow increasing the content of vitamin C by more than 90%. The amount of synthesized vitamin C depends on the variety of germinated seeds, and during the research it was determined that the highest indicator of ascorbic acid is in flax seeds of the "Original" variety, 16.56 mg/100g.

The rich physicochemical composition of sprouted flax seeds allows it to be used to enrich various categories of food products, namely: fermented milk products, bakery products, pasta and others.

The use of flax seeds in the food products of the population of Ukraine will allow improving the balance of diets in terms of vitamin and fatty acid composition, as well as expanding the range of food products for special dietary nutrition and using all anatomical parts of the seed in the technologies of health-improving products.

The obtained data provide the basis for the development of new food products with health-improving and preventive effects based on germinated flax seeds for different age groups.

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НАКОПИЧЕННЯ ВІТАМІНУ С У НАСІННІ ЛЬОНУ ПІД ЧАС ПРОРОЩУВАННЯ

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Анотація. Анотація. Дослідження біологічно активної рослинної сировини для створення продуктів здорового харчування є за останні роки одним із трендів корекції харчового статусу населення. У статті наведено характеристику якісного складу та кількісного вмісту цінного харчового продукту в насінні льону, завдяки біохімічного складу та біологічної цінності білка. Так біоактивація насіння пророщуванням дозволяє активізувати ферментну систему. Протікання біохімічних процесів призводить до збільшення водорозчинних білкових фракцій, накопичення вільних амінокислот і жирних кислот, а також легкорозчинних редукційних цукрів. Відповідно до підвищення цінності льону і поліпшення функціональних властивостей укладеного в ньому білка. Визначено вплив параметрів та тривалості на процес пророщування насіння льону у водному розчині. Описано поетапний процес перетворення вуглеводів на вітамін С, а також накопичення вітаміну С у насінні під час пророщування. Складено порівняльну таблицю за вмістом аскорбінової кислоти в деяких сортах льону, а саме «Вручий», «Блакитно-помаранчевий», «Евріка», «Оригінал». Запропоновано спосіб дозволяє підвищити вміст вітаміну С більш ніж на 90%, а також використовувати всі анатомічні частини насінини у технологіях продуктів оздоровчого призначення. Встановлено, що кількість синтезованого вітаміну С залежить від сорту пророщеного насіння, тому найбільший показник аскорбінової кислоти у насінні льону сорту «Оригінал», 16,56 мг/100г.

Ключові слова: Вітамін С, льон, паростки, пророщене насіння льону, біоактивація.