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STUDYING THE ACCUMULATION OF NITROGENOUS SUBSTANCES IN BIOFORTIFIED PUMPKIN VEGETABLES

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Доводимо доцільність використання біофортифікованих гарбузових овочів (гарбузів, кавунів, динь), що відрізняються природно підвищеним вмістом азотистих речовин (зокрема білка) у збалансованих за вмістом тваринного і рослинного білків харчових раціонах, безглютенових дієтах, а також для харчування вегетаріанців. Біофортифікацію овочів здійснювали шляхом застосування органічного, екологічно чистого добрива «Ріверм» під час їх вирощування

Ключові слова: біофортифікація, добрива, «Ріверм», білок, азотисті речовини, амінокислоти, гарбузові овочі, мікронутрієнти

Доказываем целесообразность использования биофортифицированных тыквенных овощей (тыкв, арбузов, дынь), отличающихся естественно повышенным содержанием азотистых веществ (в частности белка), в сбалансированных по содержанию животного и растительного белков пищевых рационах, безглютеновых диетах, а также в питании вегетарианцев. Биофортификацию овощей осуществляли путем применения органического, экологически чистого удобрения «Риверм» во время их выращивания

Ключевые слова: биофортификация, удобрения, «Риверм», белок, азотистые вещества, аминокислоты, тыквенные овощи, микронутриенты

1. Introduction

Physiological value of vegetables is manifested in their pronounced influence on the digestive organs – they both stimulate appetite and secretory activity of digestive glands and improve digestion and assimilation of meat, fish, bakery products, and cereals. Low-energy value of vegetables combined with their high biological value makes them indispensable in the treatment of people with different diseases.

Nitrogen compounds include, in particular, vegetable proteins, free amino acids, nucleic acids, enzymes, nitrogenous glycosides, and nitrates. The main share of these substances is comprised of proteins and free amino acids. Biological value of vegetable protein is lower than that of animal protein: they have a scarce content of some essential amino acids, and their absorption is on average 30 % [1]. However, simultaneous use of animal protein and vegetable protein increases the value of protein nutrition since vegetable proteins (the main source of nitrogen) alongside animal ones create quite active (in biological terms) amino acid complexes that provide interstitial synthesis. Meat proteins are most beneficial if they are combined with vegetable proteins: their combination ensures the necessary “dilution” and intercomplementary amino acid composition, which results in obtaining full-value proteins of potatoes and vegetables. The total protein amount should comprise 15 % of daily calories; vegetable proteins should make up almost half of the total protein amount, and the ratio of tryptophan, methionine and lysine should be 1:3:3.

The value of vegetable protein increases when it comes to vegetarian food, especially hard one (eating only plant products). In this case, plant products remain the sole supplier of protein to the human body. One more fact should be considered: at present more people are suffering from a hereditary celiac disease that is characterized by intolerance to gluten protein that is contained in wheat, rye, barley, and oats. Celiac disease is manifested in gastrointestinal disorders and requires rejecting food derived from the above crops, i. e. animal protein in the diet for this category of people can be balanced with proteins of other plants, particularly the one that is found in vegetables.

Bioenriched with nitrogenous substances (e. g., proteins and amino acids) pumpkin vegetables cannot fully satisfy the human need of proteins and essential amino acids, but they can significantly complement the diet. Moreover, the study is topical due to the fact that watermelons, melons and pumpkins are characterised by high nutritional value and have excellent organoleptic properties due to the harmonious combination of taste and flavour. These vegetables are also popular as a delicious dessert.

2. Analysis of the previous studies and formulation of the problem

Vegetable products that are grown in the world do not always contain enough nutrients. The main purpose of biofortification is to increase the content of minerals, vitamins, amino acids, improve the fatty acid composition, and increase antioxidant properties [2].

Biofortfication primarily involves creation of new food plants that would be able to accumulate higher levels of vitamins, minerals and other compounds via changes in the genetic apparatus (by genetic engineering or by using classic breeding techniques). The strategy of biofortification can also be applied in growing plants that would have signs of an improved mobilization of soil micronutrients due to their conversion in a bioavailable form or due to the use of fertilizers that contain target elements [3]. The strategy is considered to be promising and helpful in overcoming nutrient starvation of the poor as bioenriched vegetable crops can be grown during a long time.

Since agricultural produce is a source of many micronutrients for people, it is very important to introduce such changes in agricultural systems that would provide a consistent and adequate supply of these nutrients to the human [4]. Improving bioaccumulation of essential macro- and micronutrients in food crops is an important strategy both in overcoming their deficiency in food plants and strengthening the human health [5]. We need growing more food plants with the highest nutritional value. If this is neglected, the deficiency of vital compounds will lead to permanent physical and mental fatigue, increase in the number of deaths from infectious and chronic diseases, as well as neonatal malformations [6]. The lack of essential nutrients has become global because it directly affects 2 billion people [7, 8]. Biofortification must resolve the problems of a particular geographic area and become more profitable so that it might make alternative to commercial fortification and be a successful component of national nutrition strategies [9].

The advantages of the biofortification strategy (that involves fertilization) include easy implementation, low cost of each interference, and quick effect. The factors that complicate this process include methods of production, the effect of the soil composition and properties on the absorption of target elements by the plant, as well as the mobility and location of minerals' accumulation in the plant [7]. Despite the shortcomings, this strategy is often recommended by researchers from many countries for obtaining biofortified crops with high concentrations of micronutrients.

It is proved that the use of special fertilizers affects metabolic processes in cassava, which results in obtaining biofortified high-iron crops [10]. Biofortification with the iron of lentil (Lens culinaris L.) [11] and cowpea [12] also gives positive effects. A new method of wheat biofortification has been recently suggested – biofortification with zinc via direct soil fertilization or spraying of plant leaves (during the plant growth) with special zink-containing fertilizers [13–18]. Nowadays, many countries apply biofortification to obtain crops enriched with selenium – an important antioxidant and anticancerogen. Spraying pea leaves during the plant growth and development in the Mediterranean region resulted in biofortified crops that are recommended to overcome micronutrient fasting [19]. The effectiveness of the use of special fertilizers is proved by growing selenium-enriched lentils in Bangladesh [20] and by growing bioenriched turnips [21]. Biofortification with iodine-containing fertilizers is recommended for growing tomatoes [22] and salad [23, 24].

There is evidence that the application of specially selected fertilizers for biofortification may increase the content of mineral and nitrogenous substances, including amino acids, in plant products. Spraying the leaves of rice with special fertilizers during its growth leads to the accumulation of iron and amino acids – lysine, arginine, threonine, leucine, phenylalanine and glutamic acid – in grain [25]. The use of selenium-containing fertilizers in potato growing increases the content of amino acids – such as phenylalanine, glutamic acid, threonine and tyrosine – in potato tubers [26].

In Ukraine, vegetables are biofortified with the liquid, organic, environment-friendly fertilizer *Riverm* [27] that is distinguished by a peculiar composition (microorganisms, enzymes and growth substances) and specific effect that leads to the natural accumulation of biologically active compounds in plants. The use of the above mentioned fertilizer contributes to the accumulation of a greater amount of zinc, carotenoids and iron in tomato and pumpkin vegetables [28–30]. Pumpkin vegetables (watermelon, melon and pumpkin) biofortified with special fertilizers are new raw food materials in Ukraine; therefore, any previous studies do not provide information on the accumulation of major nutrients, particularly nitrogenous compounds, in them. Moreover, there is no information how the organic fertilizer *Riverm* that is used in growing pumpkin vegetables affects their protein and amino acid composition.

The value of research in this area is increasing also because such nitrogenous substance as vegetable protein remains basic for people whose nutrition concepts differ from the common ones because of religious and psychological views, or health problems. Thus, biofortified vegetables can contribute important nutrients to the diet of these categories of people.

3. The purpose and objectives of the study

The purpose of the research is studying the accumulation of nitrogenous compounds in biofortified pumpkin vegetables, which can be achieved via solution of the following tasks:

(1) examining the peculiarities of the accumulation of the total, protein and non-protein nitrogen as well as protein

in biofortified vegetables and comparing it with the concentrations of the above substances in reference samples;

(2) analysing the amino acid composition of protein in biofortified pumpkin vegetables;

(3) making conclusions about the possibilities of using biofortified pumpkin vegetables.

4. Materials and methods of studing the content of nitrogenous substances in biofortified pumpkin, watermelon and melon varieties

The objects of research are biofortified pumpkin vegetables: pumpkin varieties *Oleshkivskyi* and *Sviten*, melon varieties *Fortuna* and *Olvia*, watermelon varieties *Orphei* and *Atlant*. All samples were grown with the use of the liquid, organic, environment-friendly fertilizer *Riverm*. The reference samples are fresh vegetables that are grown by the standard technology, without the use of the above mentioned fertilizer. The content of the total nitrogen was studied with the Kjeldahl method in accordance with the GOST 26889-86 (ST CMEA) [4], whereas the content of protein nitrogen – with the Barnstein method [5]. The amino acid composition of protein was determined in accordance with the instruction to the *Alpha Plus* analyser after sample 6 n HCl's hydrolysis, 24-hour aging in an incubator at a temperature of 110 °C, dilution with a sodium citrate buffer with pH 2.2, and subsequent passing through an automatic analyzer according to the universal methods. The content of tryptophan was determined spectrophotometrically in an alkaline hydrolyzate.

5. Research findings on the content of nitrogenous substances

The study has revealed that biofortified pumpkins contain 1.31 % (*Oleshkivskyi*) and 0.91 % (*Sviten*) of protein. In addition, the vegetables contain 0.192 % (*Sviten*) to 0.341 % (*Oleshkivskyi*) of total nitrogen. The share of protein nitrogen in the total quantity is 76 % (*Sviten* pumpkins) and 62.5 % (*Oleshkivskyi* pumpkins). In the reference samples, protein content varies from 1.08 % (*Oleshkivskyi*) to 0.77 % (*Sviten*), which is 0.95 % and 0.14 % lower than in biofortified vegetables. The shares of protein nitrogen in the reference samples make up 73.6 % (*Oleshkivskyi*) and 74.5 % (*Sviten*) of its total amounts (Fig. 1).

Study of the contents of nitrogenous substances in biofortified *Fortuna* and *Olvia* melon varieties results in the conclusion that the samples contain 0.158–0.167 % of total nitrogen (including 0.117–0.123 % of protein nitrogen). The share of protein nitrogen in the total amount is 74.1–73.7 %. The reference samples have accumulated 0.126–0.158 % of total nitrogen, 0.098–0.115 % of which is protein nitrogen. Fortuna and Olvia melons grown with the use of the Riverm fertilizer contain 0.73 % and 0,77 % of protein (the reference samples – 0.61 % and 0.72 %), respectively. Thus, the protein content makes biofortified melons more valuable in comparison with the reference samples (Fig. 2).

The experimental samples of biofortified watermelons contain 0.157–0.165 % of total nitrogen, 77.0–80.3 % of which is protein nitrogen (0.126–0.127 %). The reference samples contain 0.143–0.148 % of total nitrogen, 0.114–0.115 % of which is protein nitrogen (77.7–79.7 %). It is found that the protein content in biofortified samples of watermelons reaches 0.79 %, which is higher than in the reference samples (Fig. 3).

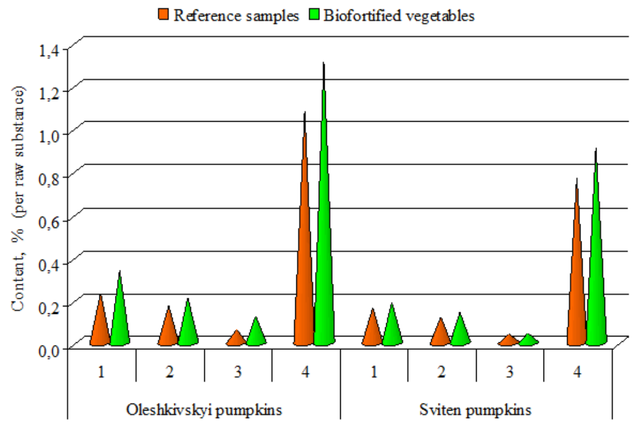

Fig. 1. The contents of total nitrogen, protein nitrogen, non-protein nitrogen and protein in the experimental samples of pumpkins, % (per raw substance): 1 – total nitrogen, 2 – protein nitrogen, 3 – non-protein nitrogen, 4 – protein

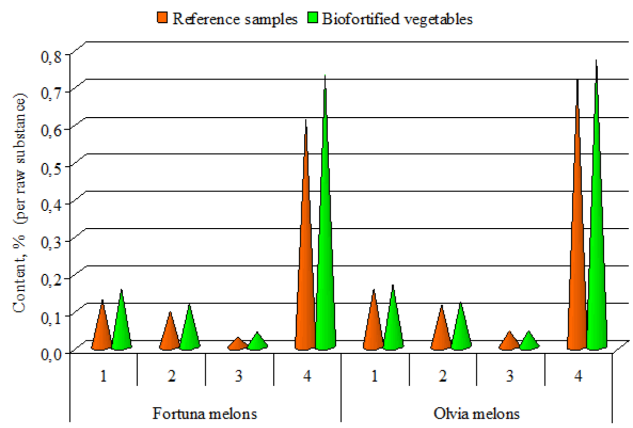

Fig. 2. The contents of total nitrogen, protein nitrogen, non-protein nitrogen and protein in the experimental samples of melons, % (per raw substance): 1 – total nitrogen, 2 – protein nitrogen, 3 – non-protein nitrogen, 4 – protein

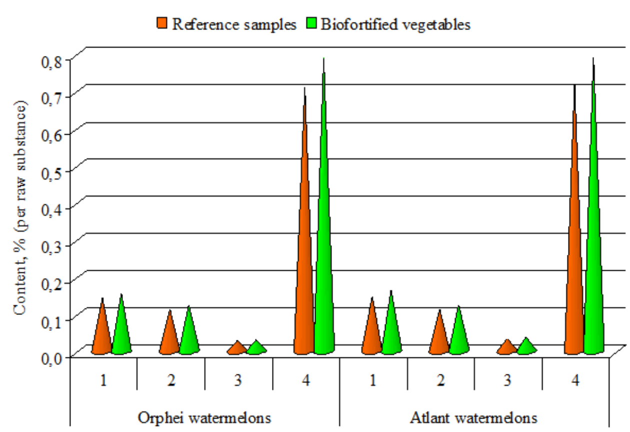

Fig. 3. The contents of total nitrogen, protein nitrogen, non-protein nitrogen and protein in the experimental samples of watermelons, % (per raw substance): 1 – total nitrogen, 2 – protein nitrogen, 3 – non-protein nitrogen, 4 – protein

Therefore, biofortified pumpkin vegetables – melons, watermelons and pumpkins – grown with the use of the *Riverm* fertilizer have high contents of total nitrogen, protein nitrogen, and protein.

We have studied the amino acid compositions of protein in biofortified melons, watermelons, and pumpkins. *Oleshkivskyi* pumpkins, *Olvia* melons and *Orphei* watermelons were taken as experimental ones since they contain the highest amounts of protein. The research findings show that the highest amount of essential amino acids is contained in the biofortified pumpkin variety *Oleshkivskyi* – 27.3 g / 100 g of protein (27.6 % of the total content). Somewhat lower amounts of essential amino acids are contained in the proteins of the biofortified melon variety *Olvia* – 18.4 g / 100 g of protein (18.6 % of the total amount of amino acids) and the watermelon variety *Orphei* – 18.6 g / 100 g of protein (23.1 % of the total content).

The *Oleshkivskyi* pumpkin protein is most valuable due to its composition, g / 100 g of protein: leucine – 5.4, valine – 4.6, and lysine – 5.4. It also includes g / 100 g: tryptophan – 0.9, methionine – 1.2, and phenylalanine – 3.2. *Olvia* melons contain dominant shares of lysine (4.5 g / 100 g of protein), phenylalanine (3.4 g / 100 g of protein), methionine (2.6 g / 100 g of protein), and isoleucine (2.4 g / 100 g of protein). Simultenuously, these melons contain lower amounts of leucine (1.8 g / 100 g of protein), threonine and valine (1.5 g / 100 g of protein), as well as tryptophan (0.7 g / 100 g of protein). The *Orphei* watermelon protein features the following content of essential amino acids, g / 100 g of protein: lysine – 5.5, phenylalanine – 3.4, isoleucine and methionine – 2.3, leucine – 1.8, valine – 1.4, threonine – 1.1, and tryptophan – 0.8.

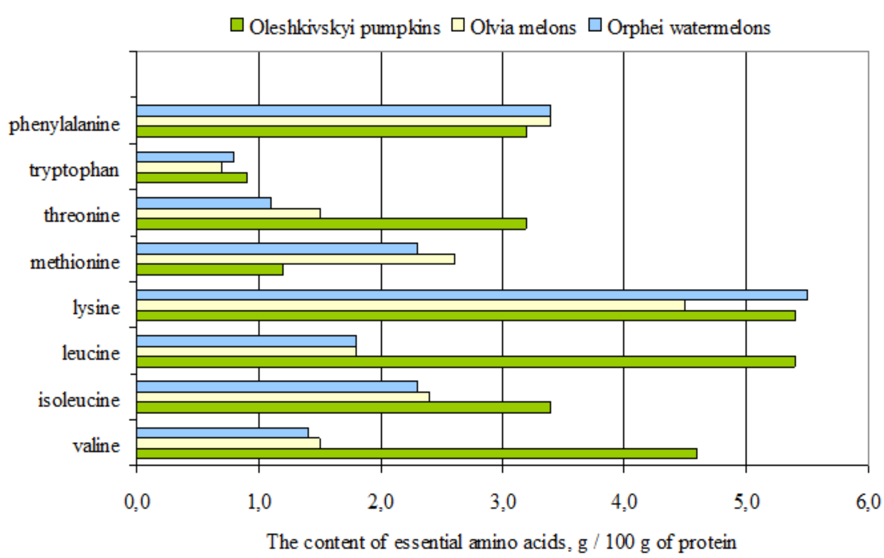

Fig. 4. The contents of essential amino acids in biofortified pumpkin vegetables, g / 100 g of protein

Thus, the compositions of *Olvia* melons and *Orphei* watermelons are dominated by lysine (4.5-5.5 g / 100 g of protein) and phenylalanine (3.4 g / 100 g of protein). All other essential amino acids are contained in relatively small amounts, the smallest of which are those of: tryptophan – 0.7–0.8 g / 100 g of protein, threonine – 1.1–1.5 g / 100 g of protein, and valine – 1.4–1.5 g / 100 g of protein. The most valuable due to its amino acid composition is the protein of biofortified pumpkins.

All samples of biofortified vegetables are leaders due to the contents of replaceable amino acids, such as aspartic and glutamic acids. Biofortified *Oleshkivskyi* pumpkins contain 16.6 g of such acids / 100 g of protein. *Olvia* melons and the *Orphei* watermelons are characterized by high contents of aspartic acid (44.1 g / 100 g of protein and 44.2 g / 100 g of protein, respectively) (Fig. 5).

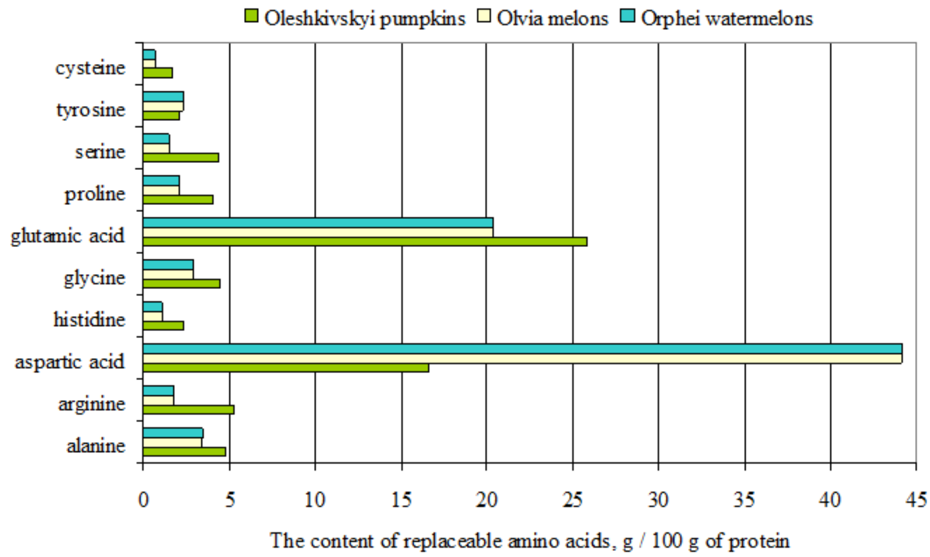

Fig. 5. The contents of replaceable amino acids in biofortified pumpkin vegetables, g / 100 g of protein

The glutamic acid content ranges from 20.4 g / 100 g of protein (*Olvia* melons and *Orphei* watermelons) to 25.8 g / 100 g of protein (*Oleshkivskyi* pumpkins). The experimental vegetable samples are characterized by the lowest levels of histidine (1.1–2.4 g / 100 g of protein) and cysteine (0.7–1.7g / 100 g of protein).

The PDCAAS evaluation of protein quality in the experimental biofortified samples has shown that the highest valine score is found in the protein of *Oleshkivskyi* pumpkins (92 %), whereas the lowest – in *Olvia* melons (30 %) and *Orphei* watermelons (28 %). The isoleucine scores in biofortified pumpkin vegetables are at the following levels: watermelons – 57.5 %, melons – 60 %, and pumpkins – 85 % (Fig. 6).

The leucine amino acid score is the highest in biofortified pumpkins – 77.1 %, it is a slightly lower in melons – 45.0 %, and the lowest in biofortified watermelons – 25.7 %.

The lysine amino acid score distinguishes biofortified watermelons – 100 % and pumpkins – 98.2 % among the experimental samples. The highest content of methionine is found in the protein of melons (amino acid score is 74.3 %) and watermelons (amino acid score is 65.7 %). The methionine amino acid score in pumpkins is 34.2 %; the threonine amino acid score in pumpkins is 80.0 %, in melons – 37.5 %, and in watermelons – 27.5 %, i.e. threonine is noticeably scarce in the proteins of *Olvia* melons and *Orphei* watermelons.

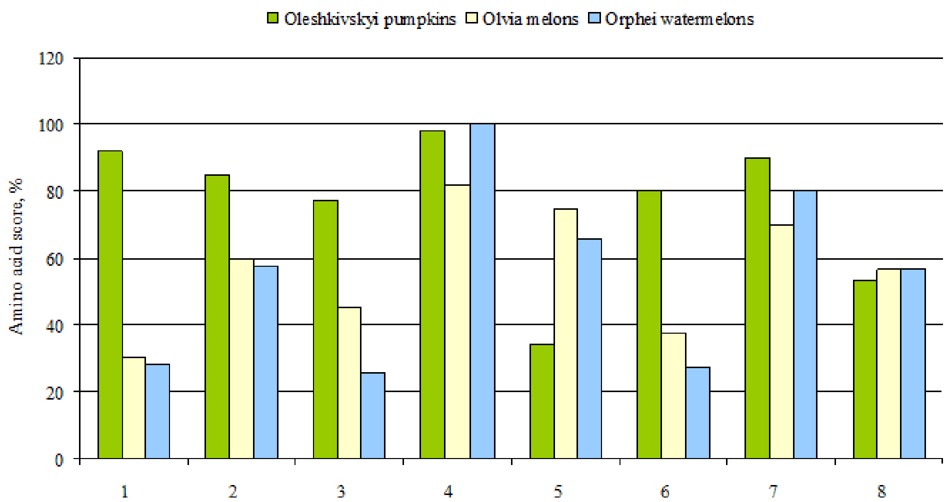

Fig. 6. Amino acid scores in biofortified pumpkin vegetables, %: 1 — valine, 2 — isoleucine, 3 — leucine, 4 — lysine, 5 — metionyn+cystine, 6 — threonine, 7 — tryptophan, 8 — phenylalanine+tyrosine

The tryptophan amino acid score in biofortified pumpkin vegetables is as follows: in pumpkins – 90.0 %, in watermelons – 80.0 %, and in melons – 70.0 %. The highest phenylalanine+tyrosine score is found in biofortified *Orphei* watermelons and *Olvia* melons (56.7 %). The protein of biofortified *Oleshkivskyi* pumpkins is less valuable in terms of the content of phenylalanine+tyrosine (amino acid score is 53.3 %).

Thertefore, the highest valine, isoleucine, leucine, threonine and tryptophan amino acid scores are found in biofortified pumpkins; the highest lysine and phenylalanine+tyrosine amino acid scores – in biofortified watermelons, and the highest methionine amino acid scores – in biofortified melons.

6. Dicussing the research findings on the contents of nitrogenous substances in biofortified varieties of pumpkins, watermelons and melons

The advantage of the carried out research is its relevance to a new raw substance in Ukraine – biofortified pumpkin vegetables grown with the use of the organic, environment-friendly *Riverm* fertilizer. Thus, the obtained findings on the content of nitrogenous substances and, in particular, proteins and amino acids in melons, watermelons and pumpkins provide additional characteristics of the biofortified crop and complement the previous findings on the above mentioned fertilizer that increases the contents of iron, zink, copper and caratinoids in vegetables [28–30]. However, the peculiarities of nitrogenous substances accumulation have been analysed not in all pumpkin vegetables but only in melons, watermelons and pumpkins (2 varieties of each species).

The research findings provide a more comprehensive picture of the peculiarities of the chemical compositions of biofortified pumpkin vegetables (melons, watermelons and pumpkins) and prove the existing statement that specially designed fertilizers can be used to biofortify plant products, i. e. bioenrich them with micro- and macronutrients. Biofortified vegetables are recommended to be used separately or with food of animal origin to overcome the nutrient deficiency in the diets of various categories of people and improve assimilation of the food components.

The research continues a series of studies of the peculiarities of the chemical compositions of pumpkin and tomato vegetables biofortified with the *Riverm* fertilizer. Perspective studies involve experimental research on more varieties of vegetables and investigation of the possibilities of pickling and freezing the biofortified raw substance as more valuable due to its micronutrient composition.

7. Conclusion

1. Biofortified pumpkin vegetables (*Oleshkivskyi* and *Sviten* pumpkins, *Olvia* and *Fortuna* melons as well as *Orphei* and *Atlant* watermelons) accumulate greater amounts of total nitrogen and protein nitrogen in their compositions and are characterized by high contents of protein (in comparison with the reference samples).

2. The comparison of the contents of essential amino acids in pumpkin vegetables grown with the *Riverm* fertilizer has revealed that their highest amount is found in biofortified *Oleshkivskyi* pumpkins, whereas the lowest – in *Orphei* watermelons. The protein of biofortified pumpkins is the most valuable due to the content of leucine, valine, and lysine. The chemical compositions of melons and watermelons are dominated by lysine and phenylalanine. All the vegetables are valuable due to the contents of aspartic acid and glutamic acid.

3. Since the experimental samples of biofortified pumpkins, melons and watermelons contain more proteins than the reference samples, they can be recommended for vegetarians, people suffering from celiac disease, as well as for consumption alongside animal protein to improve assimilation of the latter.

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RESEARCH INTO THE IMPACT OF ENZYME PREPARATIONS ON THE PROCESSES OF GRAIN DOUGH FERMENTATION AND BREAD QUALITY

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Досліджено вплив ферментних препаратів целюлази, ксиланази та глюкозооксидази на процеси дозрівання зернового тіста та якість зернового хліба. Встановлено, що внесення дослідних ферментних препаратів у зернове тісто під час його приготування сприяє інтенсифікації біохімічних і мікробіологічних процесів дозрівання. У результаті покращуються реологічні властивості тіста та підвищуються показники якості готових виробів

Ключові слова: ферментні препарати, зерновий хліб, приготування тіста, целюлаза, ксиланаза, глюкозооксидаза

Исследовано влияние ферментных препаратов целлюлазы, ксиланазы и глюкозооксидазы на процессы созревания зернового теста и качество зернового хлеба. Установлено, что внесение исследуемых ферментных препаратов в зерновое тесто при его приготовлении способствует интенсификации биохимических и микробиологических процессов созревания, улучшению его реологических свойств и повышению показателей качества готовых изделий

Ключевые слова: ферментные препараты, зерновой хлеб, приготовление теста, целлюлаза, ксиланаза, глюкозооксидаза

1. Introduction

One of the most acute problems of society today is a significant spread of diseases of alimentary origin, associated with deficiencies of essential nutrients in food rations, in particular dietary fibers. It is known that an efficient way of solving this problem is using the products of everyday consumption, rich in essential substances, including bakery products. From this perspective, very promising is expanding the range of bread made of whole grains, which, along with high content of dietary fibers, contains a significant amount of vitamins, minerals and other physiologically-functional ingredients [1]. However, due to the features of the technology and the high content of non-starch polysaccharides,