FORMATION OF INDIVIDUAL HUMAN HEALTH: MODERN BIOTECHNOLOGICAL TRENDS IN THE USE OF PROBIOTIC MICROORGANISMS IN FUNCTIONAL SOURDOUGH BAKERY PRODUCTS

Korniienko I. M., Kuznietsova O. O., Garkava K. H.

INTRODUCTION

In various regions of modern Ukraine, a study of the nutrition structure of the country's population was conducted, which revealed such violations of the nutritional status as a discrepancy between a low level of energy expenditure and a high level of consumption of high-calorie food products against the background of a significant decrease in the provision of the body of adults and children with essential nutrients (primarily micronutrients and minor biologically active food components)¹.

An increase in the number of deaths from neoplasms can be considered among the frequent causes of mortality among the population of our country² (51,817 people in 2020), and a significant part of the deaths are caused by diseases of the digestive organs (15,259 dead people in 2020). High level of morbidity of the population is due to the deterioration of people's health, caused by the influence of numerous adverse factors, among which an unhealthy lifestyle, including non-compliance with the principles of healthy nutrition. It is worth noting that a healthy lifestyle and, first of all, a healthy diet is a more important factor influencing human health than other ones (ecological, socio-economic). The possibility and necessity of preventing food-dependent diseases by correcting nutritional status is generally recognized and documented position stated in the Political Declaration of the High-Level Meeting of the United Nations General Assembly on the Prevention and Control of Non-communicable Diseases (A/RES/66/2, 2011).

Considering the fact that bread is the most consumed product for Ukrainians (takes 2nd place), especially in wartime, the introduction of biotechnological approaches to the development of recipes for functional health bread is an urgent and unresolved issue today.

¹ Neposhyvaylenko N., Kornienko I. Current problems of individual health of adolescents and the use of modern food biotechnology to solve them. Collective Monograph: Actual problems of natural sciences: modern scientific disscusions. Lublin : Universiti of life sciences in Lublin, 2020. P. 391–409.

² Смертність в Україні у 2020 році. *Офіційна статистика* : вебсайт. URL: https:// vyshneve-rada.gov.ua (Accessed: 17.12.22).

1. The concept of functional nutrition, the role of probiotics in the technology of obtaining specialized bread products

For the first time, the concept of "functional nutrition" and the corresponding term were proposed in 1984 in Japan as a result of research on the relationship between human health and nutrition, which was aimed at identifying those food products enriched with special ingredients that have useful physiological properties³. In Europe and the USA, the concept of functional nutrition in the food industry became widespread in the 1990s and is actively developing until now. Scientific research on functional nutrition is aimed at maintaining health, improving well-being and creating conditions for reducing the risk of diseases, especially cardiovascular, some types of cancer, allergies, and intestinal problems. However, there is increasing evidence that some food components that are not considered nutrients in the traditional sense can provide positive health effects and can be added to food, this led to the concept of working definitions that specify which products can be called functional ones. Table 1 presents the most common definitions of the term "functional foods" (FF) according to various organizations.

Table 1

working definitions of the term "functional foods							
Organization	Definition of the term						
1	2						
American Academy of	Foods defined as whole foods along with fortified,						
Nutrition and Dietetics	enriched, or enhanced foods that have a potentially						
	beneficial effect on health when consumed as part of a						
	varied diet on a regular basis at effective levels.						
International Food	Foods or dietary components that may provide a health						
Information Council	benefit beyond basic nutrition and may play a role in						
	reducing or minimizing the risk of certain diseases and						
	other health conditions.						
European Commission	A food that beneficially affects one or more target						
	functions in the body, beyond adequate nutritional						
	effects, in a way that is relevant to either an improved						
	state of health and well-being and/or reduction of risk of						
	disease. It is part of a normal food pattern. It is not a pill,						
	a capsule or any form of dietary supplement.						
Institute of Food	Foods and food components that provide a health						
Technologists	benefit beyond basic nutrition (for the intended						
	population).						

Working definitions of the term "functional foods"³

³ Martirosyan D., Brugger J., Bialow S. Functional food science: Differences and similarities with food science. *Functional Foods in Health and Disease*. 2021. Vol. 11, № 9. P. 408–430. URL: https://pdfs.semanticscholar.org.

Table 1 (ending)

1	2							
International Life Sciences	Foods that by virtue of the presence of physiologically							
Institute	active food components provide health benefits beyond							
	basic nutrition							
Health Canada	A functional food is similar in appearance to, or may be,							
	a conventional food, is consumed as part of a usual diet,							
	and is demonstrated to have physiological benefit							
	and/or reduce the risk of chronic disease beyond basic							
	nutritional functions.							
Japanese Ministry of Health,	f Health, FOSHU (food for specified health uses) refers to food							
Labor and Welfare	containing ingredient with functions for health and							
	officially approved to claim its physiological effects on							
	the human body. FOSHU is intended to be consumed							
	for the maintenance / promotion of health or special							
	health uses by people who wish to control health							
	conditions, including blood pressure or blood							
	cholesterol.							
Food and Agriculture	FF is a food product that is beneficial for health and							
Organization of the United	provides special benefits, including the prevention and							
Nations	treatment of diseases.							

All functional foods according to the American Academy of Nutrition and Dietetics can be divided into three main categories⁴:

1. Conventional food products that contain natural bioactive food compounds. Most vegetables, fruits, cereals, dairy products, fish and meat contain bioactive food compounds that ensure their predominant use in the human diet.

2. Modified food products that have been improved as a result of their enrichment with bioactive food compounds.

3. Synthesized food ingredients such as indigestible carbohydrates that provide the product with prebiotic properties, such as oligosaccharides or resistant starch, bran or meal.

In Japan, a special regulatory system has been established for "functional foods" that aims to inform the public about medical research on specific foods. FF have received an official legislative product category called FOSHU. FF in Japan must meet three requirements:

- proven effectiveness in clinical trials;
- safety in clinical and non-clinical studies;
- the results of determination of active components should be presented.

⁴ European Commission. Functional foods: website. URL: http://publications.europa.eu/ resource/cellar (Accessed: 18.12.22).

In accordance with the same, functional food ingredients include physiologically active, valuable and safe for health ingredients with known physicochemical characteristics, for which properties useful for preserving and improving health have been identified and scientifically substantiated, the daily physiological need has been established, for example, insoluble dietary fibers (pectin, etc.), vitamins, minerals, fats, polysaccharides, secondary plant compounds, probiotics, prebiotics and synbiotics⁵.

The range of tasks that functional food ingredients in diets are designed to solve is quite wide. Among the most important are:

1) filling the deficit of protein in diets and certain essential amino acids, lipids, fatty acids, carbohydrates and vitamins, macro- and microelements and other bioactive compounds;

2) regulation of the caloric content of the diet, which affects appetite and body weight;

3) increasing the body's immunity to various infections, reducing the risk of developing diseases and metabolic disorders;

4) maintenance of physiological homeostasis and normal body functions;

5) binding and removal of foreign substances, toxins and allergens;

6) maintaining the natural composition and functional activity of intestinal microflora.

A special place among functional foods is occupied by probiotic food products, which as a physiologically functional food ingredient contain specially selected strains of useful for humans (non-pathogenic and nontoxicogenic) live microorganisms that have a beneficial effect on the human body⁶. Today, the probiotic concept is the leading one in prescribing bacterial preparations for the prevention, diagnosis and treatment of infectious diseases and disorders of the body's natural ecology. Functional foods containing probiotics occupy a special position between ordinary food products and medicinal ones.

In 2001, during the joint FAO/WHO Expert Consultation, probiotics were defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". According to the opinion of physicians, when probiotics are introduced into the human body, there is a change in the endogenous microflora, which leads to its development in a

⁵ Platkin, C., Cather, A., Butz, L., Garcia, I., Gallanter, M., Leung, MM. Food As Medicine: Overview and Report: How Food and Diet Impact the Treatment of Disease and Disease Management. *Center for Food As Medicine and Hunter College NYC Food Policy Center:* website. URL: https://www.nycfoodpolicy.org/wpcontent/uploads/2022/04/foodasmedicine (Accessed: 19.12.22).

⁶ Hill, C., Guarner, F., Reid, G. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014. № 11. P. 506–514. URL: https://www.nature.com/articles/nrgastro.

natural direction and thus beneficial effects on the physiological functions and biochemical reactions of the host organism are revealed⁷.

As probiotics, strains of various types of microorganisms, such as bacteria, yeast and fungi, which differ in many physiological, morphological and other characteristics, are used. The most common types of probiotics are:

1) bacteria, genus Lactobacillus: acidophilus, sporogenes, plantarum, rhamnosus, delbruecki, reuteri, fermentum, lactus, cellobiosus, brevis, casei, farciminis, paracasei, gasseri, crispatus; genus Bifidobacterium: bifidum, infantis, adolescentis, longum, thermophilum, breve, lactis, animalis; genus Streptococcus: lactis, cremoris, alivarius, intermedius, thermophilis, diacetylactis; Leuconostoc mesenteroides; genus Pediococcus; genus Propionibacterium; genus. Bacillus; genus Enterococcus: Enterococcus faecium;

2) yeast and fungi: Saccharomyces cerevisiae, Saccharomyces bourlardii, Candida pintolopesii.

In order for the microorganisms included in the composition of probiotics intended for human administration to have the maximum therapeutic effect, the following requirements are imposed on them:

1) strains of bacteria used as probiotics must be isolated from humans based on ecological reasons that take into account the original environment. It is believed that such bacteria have a chance to compete with resident bacteria to establish a constant number in the host organism;

2) biological survival when passing through the digestive tract while maintaining the ability to bring a certain effect to the human body through growth and/or activity in the intestines, that is, microorganisms must be resistant to the action of gastric juice and bile;

3) probiotic strains of bacteria should have a high ability to adhere to the intestinal epithelium.

Mechanisms of action of probiotics on the host organism can be classified in three main ways⁸:

1) modulation of the innate and acquired immune system, which is important for the prevention and treatment of infectious diseases, as well as the treatment of chronic inflammation of the digestive tract and its individual parts; it has been proven that probiotics play a role in the proliferation and differentiation of epithelial cells, as well as in the development and homeostasis of the immune system;

⁷ Report of a Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food: website. URL: https://www.iqb.es/digestivo/pdfs/probioticos (Accessed: 19.20.22).

⁸ Benchimol E. I., Mack D. R. Probiotics in relapsing and chronic diarrhea. *Journal of pediatric hematology/oncology*. 2004. Vol. 26, № 8. P. 515–517.

2) influence of probiotic microorganisms on other microorganisms, namely, commensal and/or pathogenic, which is important for the prevention and treatment of infections, as well as restoring the microbial balance in the intestines;

3) impact on other microbial products, including toxins, and on human metabolites, such as bile acid salts, which leads to inactivation of toxins and detoxification of body components and food in the intestine.

The role of probiotics in human health, as well as their effectiveness, has been shown in many studies, especially concerning the treatment of intestinal disorders⁹. Some studies have shown their ability to inhibit gastric colonization and activity of *Helicobacter pylori* bacteria, which cause gastritis, peptic ulcer disease, and cancer. Probiotics have been proven to reduce the risk of certain types of cancer and hypertension, and regulate the state of the genitourinary tract. Sometimes probiotics participate in processes that the human body is unable to regulate, for example, supplementing lactase deficiency in digestion.

Most often, the clearest evidence of the positive effect of probiotic microorganisms on the human body was obtained in relation to bifido- and lactobacteria.

The probiotic effect of bifidobacteria is based on many of their properties. The main positive effect is the synthesis of various biologically active compounds. For example, the ability of bifidobacteria to produce lactic and acetic acid, as well as bacteriocins, contributes to antagonistic activity against pathogenic microflora. Also, bifidobacteria perform a vitamin-forming function, synthesize a number of essential amino acids, improve indicators of protein, lipid and mineral metabolism. It has been proven that bifidobacteria enhance phagocytosis, have a protective effect on the synthesis of immunoglobulin A, and increase the production of interleukins and g-interferon. Active components present in their cells, namely, glycoconjugates, which include various glycoproteins, polysaccharides, glycolipids, compounds with lipoteichoic acids and proteins, have immunostimulating and antitumor activity¹⁰.

The use of sourdough based on lactic acid bacteria (LAB) in baking is a modern approach in the field of healthy nutrition. LAB, which are included in the sourdough during the fermentation of the flour component, lead not only to an increase in the quality of bread, but also contribute to the extension of

⁹ Aiba Y. et al. Lactic acid-mediated suppression of Helicobacter pylori by the oral administration of Lactobacillus salivarius as a probiotic in a gnotobiotic murine model. *The American journal of gastroenterology*.1998. Vol. 93, № 11. P. 2097–2101.

¹⁰ Snigdha Misra, Debapriya Mohanty and Swati Mohapatra. Applications of Probiotics as a Functional Ingredient in Food and Gut Health. *Journal of Food and Nutrition Research*. 2019. Vol. 7, № 3. P. 213–223. URL: http://www.sciepub.com/JFNR/abstract/10244

the shelf life of the finished product due to the accumulated bacteriocins. It has been experimentally proven that sourdough bread has improved quality (porosity, taste), structure, increased stability due to the fermentation processes of cereals with a consortium of lactic acid bacteria. The improvement of the generally accepted health-giving properties of whole grain, fiber-enriched bread products, made on sourdough with the introduction of pure probiotic cultures of the LAB, is due to the slowing down of the assimilation of starch by the human body, which in turn leads to a decrease in the glycemic index due to a change in the level of bioavailability of biologically active compounds. Thanks to the fermentation of cereals by the LAB consortium under the influence of its own enzyme systems, polysaccharides unassimilable by the human body are formed, or the bioavailability of grain fibers for human intestinal microflora changes, which in turn acts as a prebiotic, which helps to increase the titer of LAB in the human intestine. Therefore, sourdough bread is recommended for celiac disease patients, as gluten biodegradation occurs under the influence of the powerful enzyme systems of the LAB.

A change in the cereals matrix potentially leads to an improvement in the quality of nutrition due to the introduction of such a functional bread product into the diet of patients, which is also recommended for use by patients with type 2 diabetes¹¹. LAB synthesize lactic acid, which also slows down the digestibility of starch, as the pH level of the dough changes, at which some endogenous enzymes change the bioavailability of minerals and phytochemical elements. This quality is very important for bread products enriched with cereal bran. The complex action of cereals enzymes and LAB leads to hydrolysis and solubilization of grain cell wall macromolecules such as proteins and polysaccharides. The combination of these factors leads to a change in the structure of the product, which affects the digestibility of nutrients and non-nutritive substances. And new bioactive compounds, namely, prebiotic oligosaccharides and other metabolites can be formed in the process of fermentation of cereals in sufficient quantities. During grain fermentation at a moderate temperature, the metabolic activity of the LAB consortium enters into a relationship with the components of the grain, and the LAB themselves produce lactic and acetic acids, so the pH value of the dough is not higher than 5.0. Then, enzymes are activated, the biosynthesis of amylases, proteases, hemicellulases, and phatases increases. Enzyme-induced changes together with LAB metabolites lead to the appearance of technological nutritional effects of fermented grain products.

¹¹ Korniienko I., Lutsenko O., Isaienko V., Baranovskyi M., Anatskyi A., Laricheva L. Optimization of technological parameters of nutrition mixture fermentation process with the use of spline interpolation. *Chemistry and Technologies*. 2021. Vol. 29, № 1. P. 118–136.

Sourdough bread is recommended for use as a specialized and functional product for diabetes (type 2), allergies of various etiologies, and as obesity dietary nutrition, since the fermentation of grain products by LAB enzymes slows down the digestibility of starch, which makes this product enriched with phytochemicals elements and endogenous enzymes, namely, xylanases, aspartic proteases, which hydrolyze such proteins as secalins, from which amino acids, small peptides (considered precursors of aromas) are generated, very useful. In addition, the use of bran in bread baking at the stage of preparation of sourdough with preliminary fermentation by LAB leads to an increase in the volume of loaves. Using self-cultivated bread sourdough with the addition of the probiotic preparation "Vivo", we obtained sourdough for the needs of the bakery industry, which contains a highly active consortium of strains of the LAB, namely streptococci, lactobacilli and bifidobacteria, which are presented to your attention in Fig. 1–3 in the form of photographs, performed during microscopic studies.

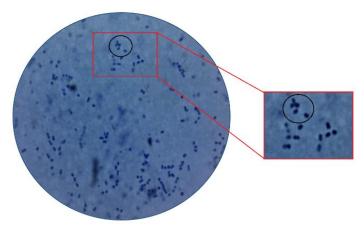


Fig. 1. Streptococcus thermophilus cells under a microscope, ×1000

Taxonomy of the strain *S. thermophilus* according to "Bergey's manual of systematic bacteriology" belongs to the domain *Bacteria*, phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Streptococcaceae*, genus *Streptococcus*. Morphological and cultural characteristics of *S. Thermophilus*: gram-positive, non-sporulating, facultatively anaerobic and immobile cocci. Microscopically, it looks like spherical or egg-shaped cells (with a diameter of 0.7–0.9 µm) in pairs or chains (Fig. 1).

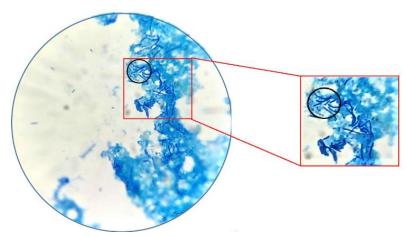


Fig. 2. Lactobacillus acidophilus cells under a microscope, ×1000

The taxonomy of the *L. acidophilus* strain according to "Bergey's manual of systematic bacteriology" is assigned to the domain *Bacteria*, phylum *Bacillota*, class *Bacilli*, order *Lactobacillales*, family *Lactobacillacea*, genus *Lactobacillus*. Morphological and cultural features: gram-positive, non-motile, non-sporulating bacillus with rounded ends, occurring as single cells, pairs or short chains. The typical size is $0.6-0.9 \times 1.5-6 \mu m$ (Fig. 2).

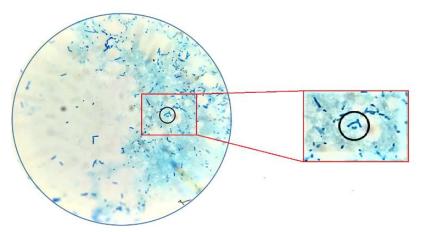


Fig. 3. Bifidobacterium lactis cells under a microscope, ×1000

Bifidobacterium animalis subsp. lactis is well established as a probiotic strain that has a beneficial effect on the health of the gastrointestinal tract and immune function of humans. At the time of isolation, this strain was thought to belong to the species *Bifidobacterium bifidum*. Modern methods of molecular classification reclassified it as *Bifidobacterium animalis* and then as a new species of *B. lactis*. The species *B. lactis* was later shown not to meet species criteria and was instead included in *B. animalis* as a subspecies. Therefore, according to "Bergey's Manual of Systematic Bacteriology", *B. lactis* belongs to the domain *Bacteria*, class *Actinobacterium*, species *B. animalis*. Morphological and cultural characteristics of *B. Lactis*: non-sporulating, heterofermentative gram-positive anaerobes. It is a catalase-negative rod-shaped bacterium. It is a slightly curved stick measuring $0.5-1.3 \times 1.5-8$ microns, has branches in the form of a Y- or V-shape, with club-shaped swellings at the ends (Fig. 3).

One of the ways to improve people's health with the help of food products is the industrial production of products belonging to the so-called "health" group, which currently includes functional and specialized products. Richer consumers living in big cities are creating a demand for non-traditional types of bread in accordance with the recommendations of nutritionists, namely, protein bread, yeast-free bread and bread with various additives. For new flavors of baked goods, Ukrainians and guests of the country go to cafes, pastry shops, and mini bakeries, which have a flexible assortment according to customer preferences.

However, as of today, bread products take second place after dairy products in the total volume of consumption, therefore the introduction of functional bread recipes is relevant today. Considering the importance of bread products, in particular flour products (a modern Ukrainian consumes an average of about 50-70 kg of bread per year), as well as the need for increase of their nutritional value Article 29 of the Law of Ukraine was adopted, which concerns the requirements for the production of food products for special dietary consumption and dietary supplements. Considering the fact that Ukraine, together with representatives of 159 countries of the world, adopted the "World Declaration and Plan of Action for Nutrition", taking upon themselves the responsibility to eliminate the chronic lack of essential vitamins, trace elements and other necessary compounds in the diet, as of today the law "On the safety and quality of food products" (Vidomosti of the Verkhovna Rada of Ukraine, 2005, N 50, Article 533; 2007, N 44, Article 1780; 2010, N 9, Article 83, N 46, Article 540; 2011, N 33, Article 326) is in force in Ukraine, which provides for the control of the circulation of food products for special dietary consumption, functional products and

dietary supplements that have not passed the state sanitary-epidemiological examination and state registration¹². The control of which is carried out in accordance with the Procedure for conducting a state sanitary and epidemiological examination, approved by the Order of the Ministry of Health of Ukraine dated 09.10.2000 No. 247. The application for the performance of work on the classification of the food product to the above categories, as well as the documents attached to it, are drawn up in accordance with paragraphs 4 and 5 of the Procedure approved by the Resolution of the CMU dated 26.07.2006 No. 1023. In order to assign a food product to the special category, the expert institution examines the documents, conducts relevant research, evaluates the special properties of the product, analyzes the manufacturer's recommendations for its consumption and prepares a report on the possibilities of classifying the food product to the special category, which is signed by the head of the expert institution.

2. Biotechnological aspects of fermentation of cereal products by lactic acid bacteria in the production technology of functional sourdough bread with the addition of oilseed meal

In 2007, the World Health Organization (WHO) presented the specially developed "The Second WHO European Action Plan for Food and Nutrition Policy 2007–2012", which defines the basics of healthy nutrition for Europeans. This plan concerns food technology and food products. These principles were also confirmed by the new third European Food and Nutrition Action Plan 2015–2020, which WHO presented in Denmark in September 2014.

Considering the trends of intensive development and spread of functional nutrition in highly developed countries, we determined the share of functional foods in the assortment of supermarkets of well-known retail chains in Ukraine. It was established that their share during 2015-2020 was only 1.5-2.0%. But in the last 2 years, this share has increased by about 0.6%. Taking into account the presence of a trend towards increasing demand for functional foods, it is possible to predict the growth of the corresponding segment at the level of 1-3% annually.

Taking into account the implemented programs and the significant update of the regulatory framework (Order of the Ministry of Agrarian Policy of Ukraine dated 20.03.2008 No164 on the approval of the sectoral program for the development of the bakery industry for the period until 2015; Concepts of the state scientific and technical program Biofortification and functional

¹² Іваніщева О., Пахомська О. Тенденції формування якості хлібобулочних виробів функціонального призначення. *Молодий вчений*. 2021. № 5 (93). Р. 159–163. DOI: org/10.32839/2304-5809/2021-5-93-30.

products based on plant raw materials 2012–2016¹³, as well as school nutrition reforms – Decree of the Cabinet of Ministers of Ukraine dated of 05.08.2020 No 1008-r), it can be noted that Ukraine needs new state programs for the development and support of the concept of popularizing functional bakery products.

Our own research results^{1,16,18} confirm that at the current stage of development of the bakery industry, one of the conceptual tasks of development is the qualitative improvement of the range of products produced, in the direction of increasing the share of therapeutic, preventive (i.e., specialized) and functional bakery products.

Analysis (in recent years) of foreign trends and practices regarding the presence of functional bread products in supermarkets in highly developed countries, which use wheat, rye, or oat bran, whole wheat bran (grain), oat and barley flour as part of recipes, as well as vegetable and fruit supplements and other components indicates that the share of "healthy" bread in the total volume of production increased from 18 to 34 % (in the United States), in Great Britain – by 68 %, and in Germany the production of such products doubled.

One of the promising trends in food biotechnology is the use of cereals as a substrate for probiotic bacteria to obtain new functional foods – healthy bakery products. It is known that cereals have a rich chemical composition that can satisfy not only the nutritional needs of probiotic microorganisms but can also have positive effects on human health due to the presence of certain substances in their composition, such as arabinoxylans. Products based on grain and cereal components make it possible to include in the human diet not only fermented prebiotics and fiber, but also LAB, that is, probiotics that remain active after baking bread products with a titer of 1×10^4 cells/g of the product, under the condition of using sourdough on the basis of LAB and protective media in the form of thistle, flax and other oil crops meal. One of the reasons for the increased interest in grain-based probiotic products is evidence of a correlation between whole grain consumption and health benefits, which have been shown to reduce the risk of type 2 diabetes, cardiovascular disease, obesity, and some cancers.

In order to obtain a biologically active sourdough for bread baking with a stable consortium of lactic acid bacteria, we suggest using a commercial probiotic preparation of pure cultures of the trademark VIVO (Ukraine), the quality of which is confirmed by the certificates of the International Organization for Standardization IS O 9001:2008, as well as IS O 22000:2005, which corresponds to European quality standards. VIVO biological

¹³ Біофортифікація: вебсайт. URL: https://www.nas.gov.ua/legaltexts/DocPublic/P-110608-189-1 (дата звернення: 19.12.22).

preparation is a symbiotic complex of pure cultures such as *Streptococcus* thermophilus, Lactobacillus delbrueckii ssp. Bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus paracasei, Bifidobacterium infantis. The sourdough technology is based on the fermentation of flour by a stable consortium of lactic acid bacteria of the VIVO probiotic preparation in the amount of 1 g of the preparation per 1 kg of flour at a temperature of 20-22 °C with the addition of 5 % oilseed meal. The sordough was divided into two parts, namely, for the preparation of the dough and for recovery.

In order to provide functional characteristics to sourdough and bread, and improve the rheological properties of the dough in the technology of obtaining functional sourdough bread with the introduction of pure cultures of the LAB, we recommend the use of meal from oil crops such as sesame and flax, as well as the introduction of a part of functional flour obtained from grain legumes such as soy and chickpeas, which contain valuable proteins in the amount of 10%. Meal is a protein defatted flour from which soluble non-protein components, which have highly active functional characteristics, have been isolated. It affects the structure, technological and nutritional properties of the finished product. It has a high moisture-binding effect, plasticity, adhesion, and sorption. Meal or crushed seeds of oil crops are characterized by a high content of nitrogenous components, sugars, dextrins, cinnaropicrin, cynarin, tannins, fiber, carotene, vitamins C, B1, B2, B3, B5, B6, vegetable fats, mineral salts (about 19) such as potassium, iron, manganese, phosphorus, as well as flavonoids and organic acids. The seeds of oil crops contain a large amount of vitamins of group B such as thiamin and pyridoxine in the amount ranging between 11 and 46 mg/kg of flour. It is known that these vitamins are key enzymes of carbohydrate metabolism in the cells of living organisms, which in this case is relevant for accelerating the fermentation process of the flour component. Meal contains nicotinic acid in the amount of 45-51 mg/kg of flour, which takes part in redox processes. To stimulate the growth of lactic acid bacteria, as well as to improve their immobilization in the fermentation process, it is recommended to use meal of oil crops, which contain a lot of fatty oils (omega-3 and omega-6). Meals of oil crops contain 30% of nitrogen-containing substances of organic and inorganic nature, which are represented by proteins and biogenic elements. Almost 90 % of nitrogenous components are represented by enzymes of biochemical processes, which perform the following roles:

- structural - biological membranes of organoids;

- energy - reserve proteins, which are used by microorganisms for metabolic processes.

These proteins are built from 20 amino acid residues located in different genetic sequences of the protein. These protein substances are represented by hydrophilic colloids, their density is 1.25 g/cm³. They do not distil, do not melt, do not dissolve in organic solvents. However, they swell in water, form gels and denature when the temperature rises or when frozen.

Some proteins are represented by glutelins (gluten is a group of storage proteins found in plant seeds, they are represented by proteins of the fraction of prolamins and glutelins), they are subject to hydrolysis in dilute alkalis and acids. Therefore, the use of methods of fermentation of the flour component by symbiosis of pure cultures of lactic acid bacteria is a reasonable technological solution from the point of view of the biosynthesis of organic acids by representatives of sourdough (mainly lactic, acetic), which promotes the hydrolysis of gluten, the use of which is contraindicated for patients with a special type of allergy (celiac disease), i.e. a hereditary defect in humans that causes intolerance to gluten. People with this type of allergy must follow a lifelong diet with the complete exclusion of all products containing gluten. It is believed that 6% of the world's population suffers from gluten allergy (this form of allergy is still poorly understood; symptoms include nausea, headache, weakness). But at the same time gluten is of great importance in the baking industry, its content in flour is a factor that determines such characteristics of the dough as elasticity when mixed with water and serves as a criterion for determining the quality of flour. It helps to obtain loose and porous flour products¹⁴.

Also, albumins that are soluble in water, prolamins that are soluble in alcohol as well as gobulins that are soluble in alkalis and acids can be attributed to an important group of proteins of such agricultural crops (Table 2), which can be used in the bakery industry.

Table 2

which are used in baking as functional components							
Oilseed crops and grain legumes	Albumins	Globulins	Prolamins	Glutelins			
Sesame	3	85	Trace	-			
Flax seeds	4	80	Trace	_			
Soybeans	2,5	95	Trace	_			
Chickpeas	2,0	85	Trace	_			

Fractional composition of oligomeric proteins of oil crops (sesame and flax) and grain legumes (soybeans and chickpea), which are used in baking as functional components

¹⁴ Пещук Л. В., Новеснко Т. Т. Біохімія та технологія оліє-жирової сировини : навчальний посібник. Київ : НУХТ, 2008. 296 с.

Proteins are evaluated by the indicator of biological value (the efficiency of the use of protein by a living organism) and the coefficient of protein efficiency (increase in the biomass of microorganisms). As a result of hydrolysis, proteins are broken down into amino acids. The amino acid composition of the proteins of sesame and flax seeds, grain legumes such as soybeans and chickpeas is shown in Table 3.

Table 3

Amino acid composition of the proteins of sesame and flax seeds and grain legumes such as soybeans and chickpeas

	Ma	Mass fraction of amino acids in proteins per dry substance, %										
Culture	Lysine	Threonine	Valin	Phenylalanine	Methionine	Isoleucine	Leucine	Arginine	Tryptophan	Histitdin	Proline	Tyrosine
Sesame	1,1	1,7	1,9	1,7	1,1	1,7	2,5	2,8	0,9	0,6	1,0	1,7
Flax seeds	1,1	1,7	1,7	1,9	0,5	1,6	2,0	2,1	1,3	0,5	0,85	1,7
Soybeans	8,2	5,5	7,5	4	1,9	6,6	9,0	-	-	-	_	-
Chickpeas	7,0	4,5	6,5	6,5	1,8	5,9	8,4	-	-	-	-	-

The value of the proteins of the cultures listed in Table 3 lies in the catalytic activity, namely:

- oxidoreductases - enzymes that catalyze redox reactions;

- transferases - catalyze reactions of intermolecular transfer of various chemical groups and residues;

- hydrolases hydrolytic cleavage of intramolecular bonds;
- lyases cleavage of groups with the formation of double bonds;
- lipases hydrolysis of acylglycerols.

Taking into account the fact that the specified (Tabl e 2) grain legumes do not contain gluten, their addition to the composition of bread products will lead to an increase in their functional properties and enrichment with essential amino acids during hydrolysis, but at the same time it leads to a deterioration of some important indicators of bread products, namely, elasticity, porosity as well as specific volume. Therefore, we have proposed the optimal use of these types of flour (chickpea or soybean) in the amount of 10 % of the total mass of flour. This is explained by the fact that an increase in the fermentation time (hydrolysis by organic acids) of these types of flour will lead to an improvement in the rheological properties of the dough. In order to activate

the fermentation processes of the dough, we suggested introducing additional substrates (separate use of each) such as lactulose, fructose and sorbitol at the stage of preparation of the dough. Considering the fact that it is not possible to use the indicated gluten-free types of flour in a sufficiently large quantity during the preparation of the dough, we conducted a study to establish the role of additional substrates such as lactulose, fructose and sorbitol in the fermentation processes of the flour component (wheat flour of the highest grade, wheat whole grain flour and spelled flour) regarding changes in the dynamics of the gluten content (methodology for determining gluten - DSTU, IS O 21415-1:2006, IDT, State Statistics Service of Ukraine, 2011, 15 p.), which can be hydrolyzed under the influence of the enzymes of the LAB sourdough. This study is very important from the point of view of understanding the role of LAB in the processes of gluten hydrolysis, because it causes allergies in a certain category of people, leading to an increase in histamine in the blood and immunoglobulin E. Factors such as intestinal dysbacteriosis, environmental pollution, weakening of the immune system and diseases of the digestive organs increase the risk of an active reaction of the body to gluten. Therefore, these studies are relevant (Fig. 4).

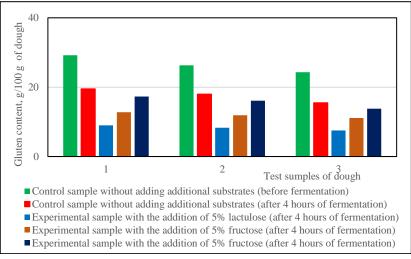


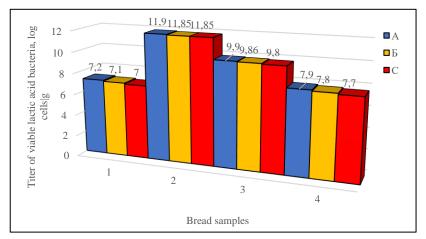
Fig. 4. Results of determination of gluten in test samples of dough prepared from different types of flour:

1 – sample of dough with the addition of only high-grade wheat flour; 2 – sample of dough with the addition of whole wheat and high-grade wheat flour in a ratio of 1:1; 3 – a sample of dough with the addition of spelled flour and wheat flour of the highest grade in a ratio of 1:1

Analyzing the presented research results (Fig. 4), it was established that the addition of lactulose (5% of the mass of flour used) as a lacto- and bifidogenic prebiotic (an additional growth-stimulating LAB substrate) accelerates fermentation processes, which are accompanied by a decrease in gluten content, which is very positive biotechnological approach that is accompanied by a significant increase in the titer of viable LAB, which is confirmed by our own research results (Fig. 4). The gluten content was reduced by 50% in the dough (with the addition of lactulose) at the end of the dough fermentation, which lasted for 4 hours. In a sample of dough with the addition of lactulose in the amount of 5 % relative to the mass of flour used. which was made with the addition of wheat flour of the highest grade (the type of flour with the maximum gluten content), the process of almost complete hydrolysis of gluten occurred during fermentation, the content of which was reduced in 4 hours fermentation from 29.2 g/100g to 9 g/100g of dough. The research results indicate the efficiency and completeness of the fermentation processes of the flour component, by using the symbiosis of pure cultures of the LAB in the technology of preparing functional bread with the addition of lactulose, flax meal, and a portion of chickpea or soybean types of flour. When adding fructose and sorbitol, which are recommended for use by diabetics as part of sweets and bakery products, partial hydrolysis of gluten occurs in the dough at the end of the fermentation time, which decreases compared to the control to 30 and 15 %. Considering the fact that human enzymes cannot break down lactulose, it does not lead to an increase in sugar in the blood of a person, since it contains 0 kcal, in addition, it is destroyed at a temperature above 260 ⁰C. Based on this, as part of functional bread, it enters the human intestines in an unchanged form, therefore it contributes to the increase of useful intestinal microflora and prevents the development of dysbacteriosis. The addition of sorbitol in order to replace sugar in the recipe of functional bread in the amount of 5% led to the deterioration of such indicators as the specific volume (decrease by 12–15 %), porosity (decrease by 12 %), which cannot be said about lactulose and fructose. Therefore, as a sugar substitute with a prebiotic effect, it is recommended to introduce no more than 5 % of lactulose into the recipe of functional bread, since its use is more effective than fructose and sorbitol. Thanks to the fermentation of the flour component by the LAB consortium, not only the reduction of gluten occurs, but also the release of fiber, which allows to restore the human intestines. Also, thanks to the fermentation by LAB, phytin is split due to lactic acid. Fermented bread contains amino acids with branched side chains.

The obtained research results (Fig. 5) indicate that lactulose leads to a significant increase in the titer of viable cultures of LAB, which is the main factor that affects the completeness of fermentation processes, namely, a

decrease in the content of gluten. Therefore, this type of functional bread is not only useful for humans, but also ensures compliance with all the standards of DSTU for bakery products according to indicators such as acidity, porosity and specific volume. Unfortunately, in the production of modern bread products with the use of accelerated fermentation technologies and improvers, gluten is stored in large quantities, that is, its hydrolysis does not occur, therefore, the use of sourdough bread with the addition of pure cultures of the LAB will allow its use by patients with celiac disease.





1 - control sample without the addition of additional substrates; 2 - experimental sample with the addition of lactulose (5 % relative to the flour mass); 3 - experimental sample with the addition of fructose (5 % relative to the flour mass); 4 - experimental sample with the addition of sorbitol (5 % relative to the mass of flour); A - dough made with the addition of high-grade wheat flour, B-dough made with the addition of whole-grain wheat and high-grade wheat flour in a 1:1 ratio, C - dough made with the addition of high-grade wheat flour and spelled flour in a 1:1 ratio

The obtained research results (Fig. 5) show that without the use of prebiotics (additional substrates) fermentation of the flour component takes place, which is confirmed by a sufficient LAB titer at the end of this process, which lasts 4 hours (the control sample has a LAB titer of 5×10^7 cells/g, which corresponds to the decimal logarithm of this number and is 7.2 for the test sample of the dough, which was made with wheat flour of the highest grade). When adding $\frac{1}{2}$ part of whole grain flour to the dough recipe, there is a slight decrease in the titer of LAB (the titer corresponds to the value of 1.3×10^7 cells/g, which is equal to the decimal logarithm 7.1). The titer of LAB

with the addition of $\frac{1}{2}$ spelled flour is also somewhat lower than in the sample with the addition of only high-grade wheat flour. This is because spelled and whole wheat flour contains more complex carbohydrates that are more slowly hydrolyzed. When lactulose is added to the dough recipe, a significant increase in the LAB titer is noted up to 7.3×10^{11} cells/g (for a dough sample made only on the basis of high-grade wheat flour, which corresponds to the decimal logarithm 11.9). For dough samples made with the addition of whole grain and spelled types of flour, this indicator is 7.2×10^{11} cells/g, which corresponds to the decimal logarithm of 11.9. When fructose is added, the titer of LAB for high-grade, whole-grain and spelled wheat flour increases to 7.6×10^9 ; 7.4×10^9 and 7.2×10^9 cells/g, respectively, which is equal to the decimal logarithm of 9.9; 9.86 and 9.8, respectively. For sorbitol, the LAB titer is 8.2×10^7 , 6.5×10^7 and 6.0×10^7 , which corresponds to the decimal logarithm of 9.7, 7.8×10^{11} , respectively, for wheat, whole grain and spelled types of flour.

That is, analyzing the research results, it can be seen that the addition of lactulose contributes to the fermentation processes of the flour component of such types of flour as whole wheat and spelled flour, which is confirmed by the gluten content and high titer of LAB in the dough. Analyzing the research results, it can be seen that the higher the LAB titers, the more active and deeper the process of gluten hydrolysis proceeds. Regarding the influence of fructose and sorbitol on the course of fermentation processes and the quality of bread products according to DSTU, it can be noted that due to the introduction of fructose there is an intensification of the growth of the LAB titer and an increase of hydrolysis by 30 % according to the control sample, while the use of sorbitol did not significantly affect these indicators, which suggests about the impracticality of its use in the bakery industry, as it also worsens the rheological properties of the dough. Sorbitol and fructose lead to liquefaction of the dough, and lactulose, due to its high-water absorption capacity, on the contrary, prevents the liquefaction of the dough and helps to maintain the shape of the dough blanks, which allows it to be used in the bakery industry, especially since it has lacto- and bifidogenic properties.

The value of oilseed meal also lies in the high content of various groups of carbohydrates. During grain fermentation at a moderate temperature, the metabolic activity of the present microorganisms interacts with the grain components. Lactic acid bacteria produce lactic and acetic acids, making the pH value generally lower than 5 and closer to 3.8. The changing conditions during fermentation favor the activation of the enzymes present, and the change in pH selectively increases the productivity of some enzymes, such as amylase, protease, hemicellulase, and phytase. Enzyme-induced changes together with microbial metabolites account for the technological and nutritional effects of fermented grain products. Sourdough fermentation can affect nutritional quality by decreasing or increasing levels of compounds and increasing or decreasing the bioavailability of nutrients. Endogenous phytase is found in flour, and active phytase is found in sourdough cultures of LAB. When using flour that does not contain gluten (soybean, chickpea, buckwheat, rice, coconut, etc.), in order to maintain the dough frame and improve the gasforming ability, it is necessary to add vegetable raw materials to it in the form of meal containing mucilaginous substances, for example, meal from oilseed crops, and to intensify the oxidation processes, it is necessary to add ascorbic acid, the effectiveness of which has been proven and confirmed by us in our own preliminary studies, the results of which were previously published.

Experiments have proven that during the storage of sourdough bread (within 7 days after baking), there is a gradual increase in the acidity of the bread product, as well as the titer of LAB, which indicates the viability of LAB after baking. For example, for a sample of bread (with the addition of lactulose), on the 5th day of storage, the LAB titer increased from 3.5×10^4 to 5.8×10^5 cells/g in the crumb of the bread¹⁵.

We conducted research on the fermentation process of sourdough, obtained on the basis of a consortium of LAB and a flour mixture with the addition of meal of oil crops (up to 5 %), which increase the titer of LAB and activate the fermentation processes of the flour component, which are accompanied by an increase in the LAB titer by 13 % compared to the reference sample.

When baking bread from types of flour that do not contain gluten, the organoleptic qualities of bread products deteriorate, especially this concerns the structure of bread and the preservation of the softness of bread products during their storage. The use of sourdough in bread baking with the addition of gluten-free types of flour proved to be effective in improving bread structure and organoleptic properties, especially when adding the prebiotic lactulose and vegetable meal from oilseed crops.

Fermentation of the flour component by sourdough microflora can affect intestinal health in several ways:

1) by modulation of the dietary fiber complex and its subsequent fermentation model;

2) by production of exopolysaccharides with prebiotic properties;

3) by potential provision with LAB fermentation metabolites, which affect the microflora.

¹⁵ Дробот В. І. Лабораторний практикум з технології хлібопекарського та макаранного виробництва : навчальний посібник. Київ : Центр навчальної літератури, 2006. 341 с.

3. Thermotolerance of lactic acid bacteria to high temperatures, the role of protective functions in bread baking

Biotechnologies of the use of probiotic cultures in the food industry are currently being intensively developed in order to correct and prevent microecological disorders in the human gastrointestinal tract at the expense of functional foods intended for daily use. The effectiveness of their use is determined by the set of biological properties of the strains included in their composition. Production strains of bacteria must possess a set of characteristics that allow them to compete with pathogenic and conditionally pathogenic microorganisms (MO).

One of the requirements for sourdough cultures in the production technology of functional products with probiotic properties is the ability of these MO to adhere.

During the fermentation processes of grain products, LAB are exposed to various stressful environmental conditions, such as increased temperature (during baking), excessive acidity build-up, pH change, high osmotic pressure (due to the addition of salt), and lack of readily available nutrients. But LAB, like other bacteria, have developed complex stress response systems that allow them to adapt to adverse conditions in order to survive. In recent years, stress responses of industrially important species of lacto– and bifidobacteria have attracted increased interest.

Therefore, the main indicators of the ability of LAB to take root in the human body are their resistance to temperature, bile, table salt, as well as the alkaline reaction of the environment, while further research is important in order to create highly active strains that have increased resistance to stress factors.

Given the relevance of the question, the presented work investigated and evaluated the effect of the main stress factor, namely, the effect of excessive temperatures (temperature resistance of the LAB), since the formed bread products are subject to baking. In general, each MO has a certain range of permissible temperatures for growth. Unlike pH, MO cannot regulate their temperature, i.e. their internal temperature corresponds to the ambient temperature. Any changes in temperature have the greatest effect on enzymes and their activity.

Depending on the optimal temperature for the development of MO, they are conditionally classified into several groups depending on the desired temperature range, but LAB can be classified as thermophiles, which is confirmed by a number of studies and by own research (Fig. 6).

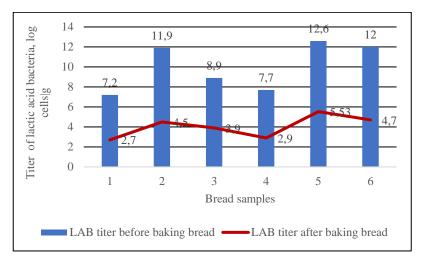


Fig. 6. The result of determination of the titer of viable LAB in samples of bread made on the basis of high-grade wheat flour:

1 - control sample of bread product (without the addition of functional components),
2 - test sample of bread product with the addition of lactulose in the amount of 5 % relative to the added flour, 3 - test sample of bread product with the addition of flax meal in the amount of 5 % relative to the flour, 4 - a test sample of a bread product with the addition of sesame meal in the amount of 5 % relative to the flour, 5 - a test sample of a bread product with the addition of lactulose and flax meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of lactulose and flax meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of a bread product with the addition of lactulose and sesame meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of lactulose and flax meal in the addition of lactulose and flax meal in the addition of lactulose and sesame meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of lactulose and flax meal in the addition of lactulose and sesame meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of lactulose and sesame meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of lactulose and sesame meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of lactulose and sesame meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of lactulose and sesame meal in the amount of 5 % relative to the flour, 6 - a test sample of a bread product with the addition of lactulose and sesame meal in the amount of 5 % relative to the flour.

Analyzing the presented research results, it can be seen that thanks to the introduction of functional components, especially lactulose, which is lactoand bifidogenic, there is an increase in the titer of LAB after fermentation (before and after baking). Due to the fact that lactulose has a high waterabsorbing capacity, when baking bread products at kl/g, it leads to limiting the influence of this stress factor on LAB. Since the temperature in the middle of the loaf during baking does not rise above 75 °C, the survival rate of LAB is fixed at the level of 3.5×10^4 cells/g, which is equal to the decimal logarithm of the number 4.5, as shown in the Fig. 6. When adding oilseed meal, the greatest increase in the LAB titer occurs in the presence of flax meal $(7.9 \times 10^8 \text{ cells/g or the logarithm of the number corresponding to the value}$ of 8.9), which has mucus that acts in the same way as lactulose on the LAB, therefore acting as a protective environment during baking. The greatest increase in the titer of LAB occurs with the simultaneous use of lactulose and flax meal, which makes it possible to increase this indicator compared to the control (before baking bread products from 1.5×10^7 to 3.9×10^{12} cells/g and after baking, the survival of LAB increased for the specified samples from 5.0×10^2 to 3.4×10^5 cells/g).

Analyzing the obtained data, it should be noted that lactobacilli are more resistant to high temperatures than bifidobacteria. Therefore, as a result of the influence of high temperatures on LAB, the regenerative properties in bread products during baking are enhanced, which leads to a decrease in redox potential and, according to literature data, to a decrease in the content of volatile substances.

Among the LAB, thermophiles include *Lactobacillus delbrueckii ssp bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus,* and *Lactobacterium helveticum.* Optimum temperature is a key characteristic of all LAB that helps distinguish them from one another, as temperature controls bacterial growth. Temperature affects the generation time of bacteria according to growth phase, as each species has a unique optimal growth. *L. lactis* quickly produces lactic acid and reduces production time. *S. cremoris* grows more slowly and adds flavor to products. Thus, the sourdough can consist of suitable paired strains of *L. lactis* and *S. cremoris.* Mixed mesophilic sourdoughs produce more acid than individual bacteria and significantly inhibit *E. coli.* Acid formation is slow or absent at temperatures below 20 °C. The growth temperature range is 10-42 °C, growth is inhibited at a temperature above 39°C, and the optimum growth range is 20-35°C.

Samples of baked bread were examined for the content of viable LAB and stained with methylene blue (Fig. 7). The evaluation of the morphological features of the test samples allows us to conclude that the presented cultures belong to LAB, and their viability was preserved during baking.

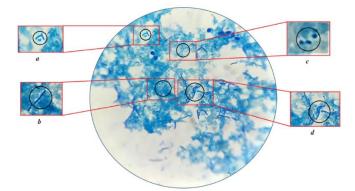


Fig. 7. The result of microscopic studies of samples of soft bread after baking for the detection of viable LAB:

a – B. lactis; b – L. acidophilus; c – S. thermophilus; d – L. bulgaricus; ×1000

Based on this, it is currently relevant to create new effective probiotics based on strains of probiotic microorganisms resistant to such a factor as temperature. Therefore, many foreign studies were conducted in which the influence of temperature on the growth of LAB was investigated.

Based on our own experience, LAB are widely used in sourdoughs in the production of improved quality bread, giving acidification and structuring of grain and crumb. In addition, the use of probiotic strains in breadmaking can slow the hardness and aging of bread. We found that the use of the LAB consortium as part of bread sourdough, thanks to active metabolites, allows to increase the shelf life of yeast-free bread, at the same time, preventing the growth of conditionally pathogenic culture *Bacillus subtilis* for up to 7 days at a temperature of 30° C.

In particular, the study presents evidence that bread was made from dough of a small mass (5, 30 and 60 g) with the addition of a lyophilized culture of the probiotic strain *B.lactis*. The number of viable cells was determined after baking for 0, 3, 6, 9, 12 min at 165, 185, 205 °C, respectively. Despite the fact that the viable amount of cells decreased significantly during baking in the core and middle part of the bread, some bacteria still survived in the bread even after baking for 12 minutes, their titer was not less than 1×10^4 cells/g. In addition, the specific volumes and moisture content of bread increased during baking, while the water activity of bread remained practically unchanged during baking¹⁶.

In general, such an endowed property of LAB, such as heat resistance, can be justified by the fact that their increased heat resistance is associated with the production of heat shock proteins under conditions of elevated temperature. Heat shock proteins act as molecular chaperones in protecting cells from heat damage by binding to cellular proteins in a way that preserves their original conformation and reduces denaturation. Therefore, thermal adaptation, also known as heat shock treatment, increases the heat resistance of bacterial cells during spray drying.

As we know, LAB are exposed to various environmental stress conditions. During stress, cells attempt to adapt through appropriate molecular responses, resulting in mitigation of adverse effects and restoration of growth or survival potential. These adaptive or stress responses are the main focus of LAB stress physiology research. Bacteria constantly monitor changes in the environment and react when necessary. Stress responses have been linked to specific phenotypes so that they can be induced in a controlled and reproducible manner.

¹⁶ Lu Zhang, Song Huang, Victoria Kristina Ananingsih. A study on *Bifidobacterium lactis* Bb12 viability in bread during baking. *Journal of food engineering*. 2014. Vol. 122. P. 33–37.

One such phenotype is the phenotype of an adapted cell. This type of adaptive response is usually triggered quickly, in the first minutes or hours of exposure to mild stress. The molecular mechanisms underlying temporary adaptation and habituation to a certain stress may overlap to some extent, but they are not completely identical. Alternatively, the cells temporarily adapt to the stress and the lethal challenge is performed with a different stress. This treatment often results in increased survival, a phenomenon known as crossprotection. The exact combination of the two stresses that results in crossprotection depends on the species or even subspecies. Cross-protection suggests the induction of molecular mechanisms upon exposure to a first stress that protects cells from a subsequent lethal challenge, and this may be particularly important for LAB, as they are often exposed to multiple stresses sequentially.

There are also phenotypes showing generalized resistance to multiple stresses simultaneously. One such phenotype is observed when cells enter stationary phase. The transition from the exponential to the stationary phase is accompanied by the induction of multiple regulons, resulting in the ability to cope with a number of different stresses. This phenotype increases the probability of survival until growth conditions are restored, and this is particularly important for LAB, which, unlike several other gram-positive bacteria, is unable to form spores.

There are many environmental or technological stress factors that LAB may encounter that directly affect their cell envelope. This is why it is very difficult to come up with a clear definition of cell membrane stress, since regulatory systems that respond to stresses acting on or sensed by the cell membrane often also play a role in normal cell physiology.

CONCLUSIONS

The concept of functional nutrition is now gaining particular popularity. First, this is caused by a change in the rhythm of people's lives, the deterioration of the ecological situation in the world, and an increase in the morbidity of all categories of the population. It was knowledge about the specifics of action of nutrients and probiotics at the cellular and molecular levels in the human body that gave impetus to the active study and implementation of the basics of functional nutrition.

As we can see, the countries with a high level of economic development were the first to take up the implementation of the so-called new field of preventive medicine and dietology, namely, functional nutrition. Today, most economically developed countries have their own documents that regulate the development and use of functional products, these are countries such as Japan, Canada, the USA, Holland, Great Britain, and China. Taking this into account, the implementation of foreign experience in preventive nutrition is a relevant and well-founded decision for Ukrainians in order to improve the health of the nation, especially in the post-war period, focusing on functional bakery products, which in terms of consumption rank 2nd.

Probiotic bread made on sourdough with the addition of lactulose and flax meal (in the amount of 5 % relative to the weight of flour) has a low glycemic index, contains less acrylamides, which are formed during baking, and phytic acid (by 62 %), which impairs iron absorption. zinc and calcium (baker's yeast compared to the LAB consortium reduces the content of phytic acid by 28 % from the initial concentration). Also, thanks to the intensive hydrolysis of gluten by the sourdough bread LAB consortium (experiments have established that the gluten content in bread products is reduced from 29.2 g/100g to 9 g/100g of dough in 4 hours of fermentation) the digestion of this product by the human body improves, as fermented bread products are better absorbed due to almost complete hydrolysis of gluten into simple sugars. Probiotic fermented cultures make it possible to extend the shelf life of bread products due to antagonism, synthesized bacteriocins and lactic acid.

Experiments have established that the consortium of sourdough LAB of the trademark "Vivo" as part of functional bread products has adaptive properties in relation to adverse (stressful) conditions, such as temperature. This consortium can be attributed to heat-resistant MO, since after baking bread products, the titer of LAB in the crumb of bread (made with the addition of lactulose and flax meal) is 3.4×10^5 cells/g, which is confirmed by our own research results. The viability of LAB cells in bread products is confirmed by microbiological research with the study of morphological features of LAB cultures, as well as by the increase in acidity during 7 days of storage of functional bread made on sourdough with the addition of 5 % lactulose and flax meal.

SUMMARY

The state of health and well-being of modern Ukrainians is influenced by many factors, such as improper nutrition, fast pace of life, age-related changes in organs and tissues, heredity, bad habits, and stressful situations. Since the average person cannot solve the range of these problematic issues, it is necessary to understand how important the composition of the diet is for the human body, as well as the quantity and quality of products that are consumed daily. And if it is impossible to influence most of the listed factors, then we can change the composition of the diet and the quality of products, since this factor is 100 % controlled by humans. Because bread is the second most consumed item in the daily diet of Ukrainians, there is a need to implement modern biotechnological solutions for the development of optimal recipes for

functional bread products. An improved technology for obtaining bread sourdough based on the probiotic preparation "Vivo" containing a heat-resistant consortium of lactic acid bacteria has been developed. Experiments have proven the role of probiotic cultures in fermentation processes, which are accompanied by a decrease in gluten from 29.2 g/100g to 9 g/100g of dough. To provide the functional characteristics of bread, as well as to improve the rheological properties of the dough in the technology of obtaining functional bread based on sourdough with the introduction of pure cultures of the lactic acid bacteria, we recommend the use of oilseed meal, namely, sesame and flax, as well as the introduction of a part of functional flour, derived from grain legumes such as soybeans and chickpeas, which contain valuable proteins. The titer of the viability of lactic acid bacteria after baking bread made with the addition of 5 % lactulose and flax meal was established (the titer of lactic acid bacteria is 3.4×10^5 cells/g), which is a confirmation that the pure cultures of lactic acid bacteria used are heat resistant.

References

1. Neposhyvaylenko N., Kornienko I. Current problems of individual health of adolescents and the use of modern food biotechnology to solve them. Collective Monograph: Actual problems of natural sciences: modern scientific disscusions. Lublin: Universiti of life sciences in Lublin, 2020. p. 391–409.

2. Смертність в Україні у 2020 році: офіційна статистика: вебсайт. URL: ttps://vyshneve-rada.gov.ua (дата звернення 17.12.22).

3. Martirosyan D., Brugger J., Bialow S. Functional food science: Differences and similarities with food science. *Functional Foods in Health and Disease*. 2021. Vol. 11, N_{2} 9. P. 408 430. URL: https://pdfs.semanticscholar.org.

4.European Commission. Functional foods: website. URL: http:// publications.europa.eu/resource/cellar (Accessed: 18.12.22)

5. Platkin, C., Cather, A., Butz, L., Garcia, I., Gallanter, M., Leung, MM. Food As Medicine: Overview and Report: How Food and Diet Impact the Treatment of Disease and Disease Management. *Center for Food As Medicine and Hunter College NYC Food Policy Center:* website. URL: https://www.nycfoodpolicy.org/wpcontent/uploads/2022/04/foodasmedicine (Accessed: 19.12.22).

6. Hill, C., Guarner, F., Reid, G. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol.* 2014. № 11. P. 506–514. URL: https://www.nature.com/articles/nrgastro.

7. Report of a Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food: website. URL: https://www.iqb.es/digestivo/pdfs/probioticos (Accessed: 19.20.22).

8. Benchimol E. I., Mack D. R. Probiotics in relapsing and chronic diarrhea. *Journal of pediatric hematology/oncology*. 2004. Vol. 26, №. 8. P. 515–517.

9. Aiba Y. et al. Lactic acid-mediated suppression of Helicobacter pylori by the oral administration of Lactobacillus salivarius as a probiotic in a gnotobiotic murine model. *The American journal of gastroenterology*.1998. Vol. 93, №. 11. P. 2097–2101.

10. Snigdha Misra, Debapriya Mohanty and Swati Mohapatra. Applications of Probiotics as a Functional Ingredient in Food and Gut Health. *Journal of Food and Nutrition Research*. 2019. Vol.7, № 3. P. 213–223. URL: http://www.sciepub.com/JFNR/abstract/10244

11. Iryna Korniienko, Oleh Lutsenko, Volodymyr Isaienko, Mykhailo Baranovskyi, Andrii Anatskyi, Lyudmila Laricheva. Optimization of technological parameters of nutrition mixture fermentation process with the use of spline interpolation. Chemistry and Technologies. 2021.Vol. 29, № 1. P.118–136.

12. Іваніщева, О., Пахомська, О. (2021). Тенденції формування якості хлібобулочних виробів функціонального призначення. Молодий вчений, 5 (93), 159–163. DOI.org/10.32839/2304-5809/2021-5-93-30.

13. Біофортифікація: вебсайт. URL: https://www.nas.gov.ua/legaltexts/ DocPublic/P-110608-189-1 (дата звернення: 19.12.22).

14. Пещук Л. В., Новеснко Т. Т. Біохімія та технологія оліє-жирової сировини : навчальний посібник. Київ : НУХТ, 2008. 296 с.

15. Дробот В. І. Лабораторний практикум з технології хлібопекарського та макаранного виробництва: навчальний посібник. Київ : Центр навчальної літератури, 2006. 341 с.

16. Lu Zhang, Song Huang, Victoria Kristina Ananingsih. A study on *Bifidobacterium lactis* Bb12 viability in bread during baking. *Journal of food engineering*. 2014. Vol. 122. P. 33–37.

Information about the authors:

Korniienko Iryna Mykhaylivna, Candidate of Technical Sciences, Associate Professor, Associate Professor at the Department of Biotechnology National Aviation University 1, Liubomyra Huzara ave., Kyiv, 03058, Ukraine

Kuznietsova Olena Oleksandrivna,

Candidate of Technical Sciences, Associate Professor, Associate Professor at the Department of Biotechnology National Aviation University 1, Liubomyra Huzara ave., Kyiv, 03058, Ukraine

Garkava Kateryna Hryhorivna,

Doctor of Biological Sciences, Professor, Head of the Department of Biotechnology National Aviation University 1, Liubomyra Huzara ave., Kyiv, 03058, Ukraine