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FUNCTIONAL FOOD ADDITIVES

Vesna Đurović[1,*], Monika Stojanova[2], Jasna Kureljušić[3], Jasmina Stojiljković[4], Leka Mandić[1], Dragutin Đukić[1]

[1]University of Kragujevac, Faculty of Agronomy, Čačak, Serbia
[2]Association for Scientific-Research, Educational and Cultural Activities 'Open Science', Ohrid, North Macedonia
[3]Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia
[4]The Academy of Applied Technical and Preschool Studies, Niš, Serbia
*Corresponding author: vesna.djurovic@kg.ac.rs

ABSTRACT

In this paper, the role of biologically active substances (additives) in functional food is briefly discussed. Prebiotics are functional food ingredients in the form of substances or dietary supplements, which are mostly not absorbed in the human intestine, but have a beneficial effect on the host organism through selective stimulation of growth and (or) activation of the metabolism of beneficial representatives of its intestinal microbial community, i.e. prebiotics are stimulators of probiotics. Probiotics are preparations or food products that include substances of microbial and non-microbial origin, which, when taken naturally, have a favorable effect on the physiological and biochemical reactions of the organism, optimizing its microbiological status (functioning of the normal microbial community). Symbiotics or multi-probiotics are preparations consisting of several probiotic strains. Symbiotic are preparations, biologically active supplements, and probiotic products of mixed composition - complexes of probiotics, including those with multiple strains, with various so-called prebiotic substances. Their effect is based on the synergism of the combination of pre-and probiotics. Parapharmaceuticals are biologically active food additives, which are used in prophylaxis, adjunctive therapy, and maintaining the functional activity of organs and organ systems within physiological limits.

Keywords: *Prebiotics, probiotics, symbiotics, pro pharmaceuticals, food.*

INTRODUCTION

The close reaction between health and the quality of food products caused the emergence of a new direction in the production of food products-the creation of functional food, which has a targeted effect on the body. Functional food makes it possible to achieve two goals: to supply the organism with the necessary amount of metabolically necessary food components and to protect it from possible diseases (Biliaderis, 2008; Siró et al., 2008). Because only non-toxic and non-pathogenic natural components are used in the production of new food products, there is a need to find suitable sources for their mass production. Using biotechnological methods (enzyme catalysis, cultivation of microorganisms, and plant cells), not only the problem of the mass production of food products can be quickly solved, but also the obtaining of various functionally important ingredients, such as, among others, pre-and probiotics, sym- and synbiotics and parapharmaceuticals (Đukić et al., 2003; Stojiljković et al., 2022; Ballini et al., 2023; Stojanova et al., 2024).

PREBIOTICS AND PROBIOTICS

The term ‘prebiotics’ is used to denote functional food ingredients in the form of substances or dietary, which are mostly not absorbed in the human intestine, but have a beneficial effect on the host organism by selectively stimulating the growth and (or) activating the metabolism of representatives of its intestinal microbial community, i.e. it can be said that prebiotics are stimulators or engines of probiotics.

Prebiotics include di- and trisaccharides, non-digestible oligosaccharides (NDO), and polysaccharides, with many preferring (give advantage to) fructooligosaccharides. Due to the absence of appropriate enzymes, NDO isnot hydrolyzed and absorbed in the small intestine, arriving in the large intestine in an unchanged form, where they are broken down under the influence of microbes (mainly bifidobacteria), which produce enzymes of the hydrolase type, and which use them as sources of energy, breaking them down to CO2 and organic acids. The latter, by lowering the pH of the intestinal environment, preventsthe development of harmful microorganisms and contributesto their elimination. Pathogenic microorganisms do not produce HCO processing enzymes that are not toxic to humans: they do not cause any side reactions when entering the body (Markowiak et al., 2017).

There are several classes of NDO: short- and medium-chain polymers (more precisely, oligomers) of fructose residues - fructooligosaccharides, fructans, including inulin; from glucose residues - glucooligosaccharides, glucans and dextrans; galactose - galactooligosaccharides, as well as oligosaccharides originating from plant cell walls, soybeans, milk whey. Natural NDOs serve as reserve carbohydrates in many higher plants, algae, bacteria, fungi, and yeasts. The influence of reserve carbohydrates of microorganisms on probiotic microorganisms has been known for a long time. So far, the most studied prebiotics are soluble fructooligosaccharides - FOS. According to the source of the enzymes involved in biosynthesis, FOS are divided into two groups: inulin (from plants such as asparagus, sugar beet, onion, and Jerusalem artichoke, which contain three types of fucosyltransferase, which act on sucrose), and fructan similar to inulin (of microbial origin, which is formed from sucrose and raffinose, thanks to the invertase activity of the transfructosylation system, which exists in representatives of various genera *Aspergillus*, *Aureobasidins*, *Arthobacter*, *Fusarium*, *Claviceps, Saccharomyces*). In the FOS molecule, fructose units are connected by a ß(2→1)-bond to the D-glucose molecule in the terminal position. The degree of polymerization of FOS varies from 2 to 60, and that of inulin from 20 to 60.

Biotechnological ways of obtaining FOS in industrial conditions are rated as the most economical. They are based on the action of the corresponding enzymes of different microorganisms on sucrose. In Korea, the production of FOS was successfully realized using immobilized cells of *Aureobasidium pullulans* producers. The extraction of FOS from plants is less efficient due to a large number of factors affecting the process, among which is its temperature dependence. However, the purification of FOS from natural sources has a wide application in the creation of symbiotics. Prebiotic compounds in a purified form can be used to enrich bread, pastries, cakes, soup concentrates, and the like because this way of achieving a probiotic effect is simpler than the use of probiotics and symbiotics (Filipović et al., 2016; 2017a, b). Important properties of FOS are their ability to reduce the absorption of carbohydrates and lipids in the intestine, thereby normalizing the level of serum lipids and glucose in the blood; and prevention of carbohydrate and lipid metabolism disorders in patients with diabetes. Thanks to this property of high sweetness and low caloric content (4.18–6.27 J/g), FOS can be used in the creation of products for diabetics.

Oligosaccharide lactulose, which stimulates the growth of bifidobacteria, has prebiotic properties. Lactulose has a positive effect in the treatment of dysbacteriosis and other diseases of the gastrointestinal tract. The applicationof foods with added lactulose is especially important for older people. When used as a food raw material for providing therapeutic-prophylactic properties to food products, lactulose can be added to the recipes of various food products, not hurting the technological process of production of those products, but sometimes improving it.

The technology of applying bifidogenic concentrate with lactulose in the recipe of traditional bakery products was developed. The positive influence of lactulose on their quality was determined. This bifidogenic supplement slightly improves the gasogenic ability of flour and has a relaxing effect on gluten. An increase in volume output, specific volume, and porosity of the final products is observed. Bakery products with bifidogenic concentrate retain all the properties that are valued in traditional products, which, together with new production technologies, allow mass production of these products to be established and affordable for the majority of the population. Microalgae (chlorella) and especially brown seaweed effectively increase the populations of bifidobacteria and lactobacilli in the intestine. Complex preparations of probiotics, microalgae, and cyanobacteria are becoming popular and are intensively advertised. The established prebiotic properties of algae are largely conditioned by the presence of fibrous oligosaccharides in them. It is known that polysaccharides and oligosaccharides of microorganisms can appear as antigenic complexes, which stimulate the induction of antibody production, and the production of interferon and lactoferrin. For example, haloderma, cordyceps, and shiitake mushrooms contain lentinan and ß-D-glucanreceptors, which condition the biological activity of those mushrooms. The given carbohydrate and its effect have been well studied, and the properties of these mushrooms have been described in detail, such as reducing the activity of natural antagonists.

Prebiotics can be certain vitamins and their products, for example, pantothenic acid and its products. Bifidobacteria are capable of transforming pantothenates into coenzyme A (CoA), thus ensuring the development of important metabolic processes of the bacterial cell. It is considered that biologically active immune proteins - lactoglobulins and glycopeptides, which are produced in the body of humans and mammals, are also prebiotics (Roškar et al., 2017). The bifidogenic effect of proteids is realized mainly at the expense of reducing (in different ways) the content of competing microorganisms in the intestine (for example, lactoferrin, an enzyme that binds iron, thus preventing pathogenic bacteria from absorbing iron, which they need for life; lactoperoxidase has a devastating effect on secondary microorganisms, forming an antibacterial system together with hydrogen peroxide, which is produced by catalase-positive microorganisms, and thiocyanates found in the tissue).

Prebiotics can be peptides and amino acids, polyatomic alcohols, organic acids, etc. The use of prebiotics and functional food products, without probiotics, can independently provide a probiotic effect organism, and besides that, according to specialists, the costs of production and storage of prebiotics are lower than analogous costs for probiotics. Probiotics - food preparations and products, the composition of which includes substances of microbial and non-microbial origin (living and dead microorganisms, their structural components, metabolites, and substances of other origin), which, when taken naturally (through the esophagus), have a beneficial effect on physiological and biochemical reactions of man through optimization of his microbiological status (functioning of the normal microbial community of human).

Most specialists classify probiotic bacteria, mainly into so-called eubiotics (representatives of the normal microbial community of intestines and other body cavities) and above all bifido- and lactic acid bacteria of the genus *Lactobacillus*, which are called classic probiotics. This is related to the fact that most of the bacteria that have a beneficial effect on human health are isolated from the human intestine, and it is these bacteria that colonize the gastrointestinal tract (GIT) and are constantly present in it, taking on a basic protective function, while other microorganisms are transit. However, there is sufficient factual data on the existence of probiotic properties in lactic acid bacteria, which are not found in the human intestine, as well as in other microorganisms, gram-positive (*Propionibacterium*, *Bacillus*) and gram-negative (*Escherichia coli*, *Citrobacter*) bacteria, yeasts (*Saccharomyces*, *Candida*) and fungus (*Aspergillus*, *Rhizopus*, *Cordiceps*).

The beneficial effect of probiotics on human health is manifested thanks to the overall positive effect, within which the following stand out: colonization of GIT with probiotic microorganisms, which show antagonism concerning conditionally pathogenic and pathogenic bacteria, viruses, fungi, and yeasts by creating a pH environment in their biotope that is unfavorable for non-native microbes, producing bacteriocides (antibiotic substances), preventing competing microorganisms from reaching feed and adhere; improving the disturbed balance of microorganisms in the intestine and removing dysbacteriosis and dysbiosis as a whole; useful metabolic activity (biosynthesis of vitamin K, biotin, niacin, pyridoxine, and folic acid; hydrolysis of bile salts and cholesterol, regulation of its level); control of toxin production by resident microbes in the intestine, proteolysis of endotoxins, allergens and antigens, and partially digested proteins, including those that contribute to the development of food intolerance and incidental skin diseases; optimization of food digestion and normalization of the motor function of the intestines through the production of substances that exhibit morpho-kinetic action; regulation of food transit time through GIG thanks to the participation of bile acids in metabolism; detoxification and protective role against adverse factors (radiation, chemical contamination of food, etc.), thanks to the stimulation of the immune response and the increase of non-specific immunoresistance (Bengmark, 2010; Manigandan et al., 2012). It is considered that lactic acid bacteria are the best carriers of immunogens and protective antigens. The development of effective immune preparations from probiotics is recognized as one of the most priority areas of modern vaccinology. At the same time, there are known successful attempts to use oral and rectal preparations of probiotics (in cases of immunodeficiency) - representatives of coccoid microorganisms: *Lactococcus*, *Micrococcus*, *Streptococcus* in high concentrations (Lee, 2009). Representatives of other genera (*Bifidobacterium*, *Propionibacterium*, *Eubacterium*, *Saccharomyces*, *Bacillus*), for which lactic acid is not the main metabolic product, exhibit immunostimulatory and antineoplastic effects as a result of increasing the non-specific-resistance-of-microorganisms. Based on the strains of representatives of these genera, biopreparations for clinical needs and lactic acid products with a probiotic effect were created (Bengmark, 2010).

Everything is still not clear regarding probiotics of representatives of genera and families, among which some microorganisms are pathogenic and conditionally pathogenic for humans. In contrast to the non-specific character of the influence of lactic acid bacteria, pathogenic and conditionally pathogenic microorganisms exert an immune effect on the host inanother way, which is closer to the mechanisms of specific and even cross-tolerance. In modern living conditions, probiotics serve as an important and necessary instrument of protection man from, first of all, GITdysbacteriosis, which iscaused by irrational antibiotic therapy, previous intestinal diseases, long-term use of non-steroidal anti-inflammatory drugs,

cytostatic therapy, improper diet and stress (Rioux et al., 2005) Now probiotics are used very often and the sources of their entry into the human body are different. These are primarily pharmaceutical forms of medical biological preparations and biologically active nutritional supplements (BAS). Secondly, they are natural food products based on milk or plants, which are produced by biotechnological methods with the use of various microorganisms, including classic probiotics as fermented or starter cultures or enriched with probiotics.

SYMBIOTICS AND SYNBIOTICS

Preparations and BAS contain microorganisms both in the form of pure monocultures (in protective nutrient media or without them), as well as in combinations, most often in a lyophilized state in capsules, in the form of powders, tablets for oral administration, rectal and vaginal suppositories, preparations for intraintestinal administration (dewing), chewing confetti and gum, and less often in liquid form. Combinations can also mean combinations of cultures (several strains of the same genus or species, different taxonomic groups). There is no single opinion regarding the optimal amount of strains in preparations. It is considered that preparations and products based on a single strain have an advantage over multi-component ones, in which one strain can start to dominate the storage process, while others are inactivated and the number of their living cells decreases sharply (Bengmark, 2010). Abroad are popular preparations consisting of 6-8 strains of probiotics. They are called symbiotics (from the Greek symbiosis - common life) and multiprobiotics. It is assumed that each strain of multiprobiotics seeks the best conditions for itself in the intestine and occupies a characteristic microecological niche for it. The search for promising strains for the creation of eubiotic preparations is limited by bifido- and lactobacteria. Isolation of such strains from other taxonomic groups, which are part of the representatives of the normal microbial community, makes it possible to create complex preparations containing microorganisms, which will complement each other with their effects and exhibit the most corrective effect (Sekhon and Jairath, 2010).

Along with symbiotics, synbiotics are also widely used - these are preparations, BAS, probiotic products of mixed composition - complexes of probiotics, including those with a large number of strains, with various so-called prebiotic substances (Bengmark, 2010; Pandey et al., 2015). The effect of synbioticsis based on the synergism of the combination (mixture) of probiotics and prebiotics, at the expense of which the most effective probiotic microorganisms are not only implanted,and introduced into the GIT of the host, but theirmicrobial community is stimulated. Dietary fibers, immunomodulators, enzymes, microelements, and herbal supplements can also be included in synbiotics, for example, the preparation ‘Bifiliz’ (‘Vigel’), in which the content of lysozyme and live bifidobacteria is balanced. Lysozyme, which is included in the composition of the preparation, improves the adhesive properties of bifido- and lactobacteria in the body, and manifests a number of its other biological properties - immunomodulating, anti-anemic, and the property of stimulating regeneration (Sekhon and Jairath, 2010; Pandey et al., 2015).

The synbiotic property also contains BAS ‘Vigerm PBI’, which is obtained during cold pressing and partial fermentation of sprouted wheat grains with lactic acid bacteria; it contributes to improving the condition of the human digestive system. Parapharmaceuticals - biologically active food additives, which are used for prophylaxis, adjunctive therapy, and maintaining the functional activity of organs and organ systems within physiological limits. The functional role of parapharmaceuticals is presented in Figure 1. Such nutrients include, first of all, proteins, enzymes, amino acids, etc. Scientists have created a large

number of protein concentrates, isolates, and pastes, which contain a whole set of amino acids, including essential ones, from dairy, vegetable, and microbial raw materials.

A pronounced deficit of iodine in the soil, water, and food causes a lack of iodine in the body, which leads to disruption of thyroid hormone synthesis.

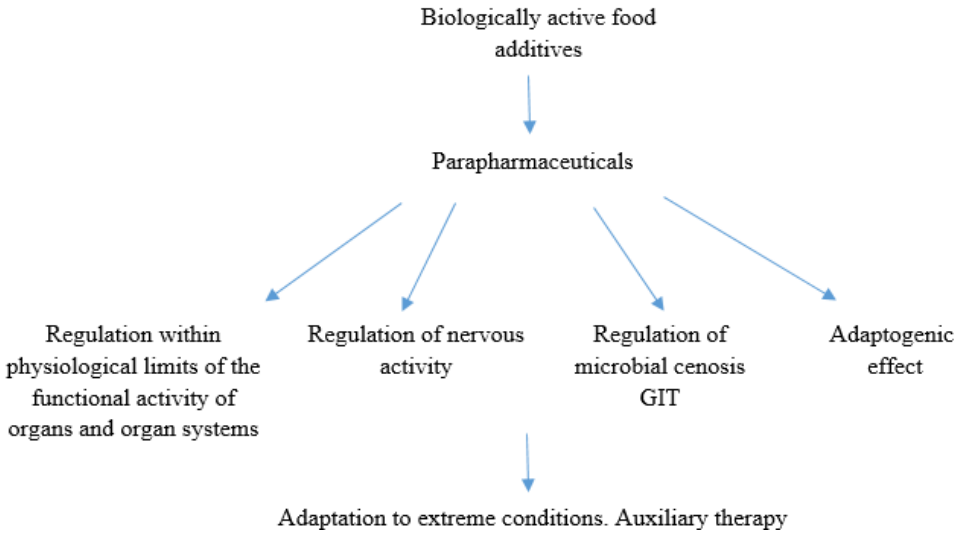

Figure 1. Functional role of BAS-parapharmaceuticals

As a result, deviation from the norm in newborns, fatigue, irritability, weakening of attention and memory, marked delay in mental and physical development in children, appearance of goiter and malignant formations of the thyroid gland in adults appear. Subsequent introduction of iodine into the body with products is effective if it is present in them in an organically bound form. Therefore, the creation and production of cheap and effective preparations, which contain a set of vitamins, microelements, free essential and non-essential amino acids in an organically bound form, is one of the most rational ways of solving the national task: prevention of iodine deficiency in the population.

A large number of food products enriched with iodine preparations have been developed, whereby the preference is given to potassium iodide and potassium iodate. Enrichment of bread and bakery products through the application of iodized baker's yeast has also been developed. Thanks to this, bread contains 25 μg of iodine in 100 g of bread and 35–50 μg of iodine in 100 g of bakery products. Conditions for enriching baker's yeast (*Sacharomoyces cerevisiae*) with selenium, as well as brewer's yeast (*Sacharomoyces carlsbergensis*) with selenium and iodine (Kieliszek at al., 2015) were developed. The results of the study of the growth of the yeast S. cerevisiae in aerobic conditions on a medium enriched with selenium and the accumulation of selenium in the biomass showed that with an increase in the content of selenium in the nutrient medium (from 6.8 to 193 mg/dm^3), the accumulation of selenium in the biomass decreases from 106 to 14, 9 mg/kg. The examination of the dependence of the growth of yeasts on the concentration of iodine in the (KJ) environment showed a practically complete absence of the influence of iodine on growth in concentrations from 0.05 to 45 g/dm^3; increasing the concentration of iodine up to 65 g/dm^3 leads to a decrease in growth activity by 10%; at an iodine concentration above 100 g/dm^3, yeast growth stops completely. These studies indicated the possibility of

cultivating brewer's yeasts in aerobic conditions on a medium enriched with iodine and selenium, with the aim of their application in BAS.

The vitamin-amino acid iodized concentrate 'Droželizin' is obtained through enzymatic hydrolysis of protein molecules of yeasts, grown on a nutrient medium with KJ, during double-cycle mixing during 12–24 hours in a diluted medium or ordinary conditions of iodized yeast milk at 50–55°C, with subsequent separation of the decomposed cell envelopes and drying of the target product to a moisture content of up to 5%. It contains iodine in an organically bound form, in the form of stable compounds with amino acids, at the following ratio of ingredients, % (m/m):

Table 1. Content of ingredients in Droželizin

Non-hydrolyzed proteins	10.3–23.5
Free amino acids	13.545–39.688
Carbohydrates	45.5–53
Lipids	1.4–5.5
Vitamins of group B	0.012–0.015
Microelements: Copper Zinc Iodine	5–8.5 0.0009–0.001 0.0028–0.003 0.0017–0.0042

Iodized yeast milk for hydrolysis can be obtained by preparing an aqueous suspension from pressed iodized yeasts. The high nutritional and healing properties of iodized 'Droželizin' are ensured by the presence of a wide range of necessary (for humans) substances, proteins, vitamins and microelements in its composition. The use of the vitamin-amino acid concentrate 'Droželizin', which contains the microelement iodine, contributes to the strengthening of the body's immune system, increases its protective functions, activates metabolic processes, and improves the work of the central and vegetative nervous system. The concentrate also has a positive effect when included in the diet of patients with brain vascular pathology, vegetative dysfunction, and asthmatic syndromes.

Biologically active food supplement 'Fervital'. In the countries of Southeast Asia and Oceania, three-stage fermentation of edible raw materials of plant origin (soy, rice, peanuts) with micellar fungi, yeasts, and bacteria is traditionally widely used. These products are the most important nutritional addition to the diet of the inhabitants of those countries. There is also sufficient data on the positive influence of yeasts on normal gut microbes. Yeasts enrich the fermented substrate with essential amino acids, vitamins, organic acids, and other biologically active substances, and the cell walls of yeasts, which contain glucose and mannose polymers, have lacto-, bifidogenic and sorption properties, and when they reach the intestine, they stimulate the growth of their microbial community and they bring out pathogenic bacteria and their toxins. Therefore, the production of yeast probiotic preparations and products is very promising and actual. Such BAS and products, with their low price and accessibility, can be widely used as a means of disease prophylaxis and as a supplement to medical therapy.

The BAS 'Fervital' probiotic technology was developed based on the solid-phase cultivation of yeasts on plant substrates. The most promising biomass producers are thermotolerant yeast-like fungi of the genera *Candida*, *Pichia*, *Clavispora*, and *Rhodotorula*, which grow intensively on solid-phase plant substrates. As a carbohydrate raw material for conversion by yeasts, plant substrates with a reaction of the environment

that is close to neutral are used: corn and products of its processing, tuberous and root vegetables. Yeasts are cultivated on solid substrates with stirring once every two hours and at a temperature of 28–30 ℃ for 24–48 hours. The finished culture is dried at a temperature of 60 ℃. The technological scheme of the process of obtaining BAS 'Fervital' is given in Figure 2.

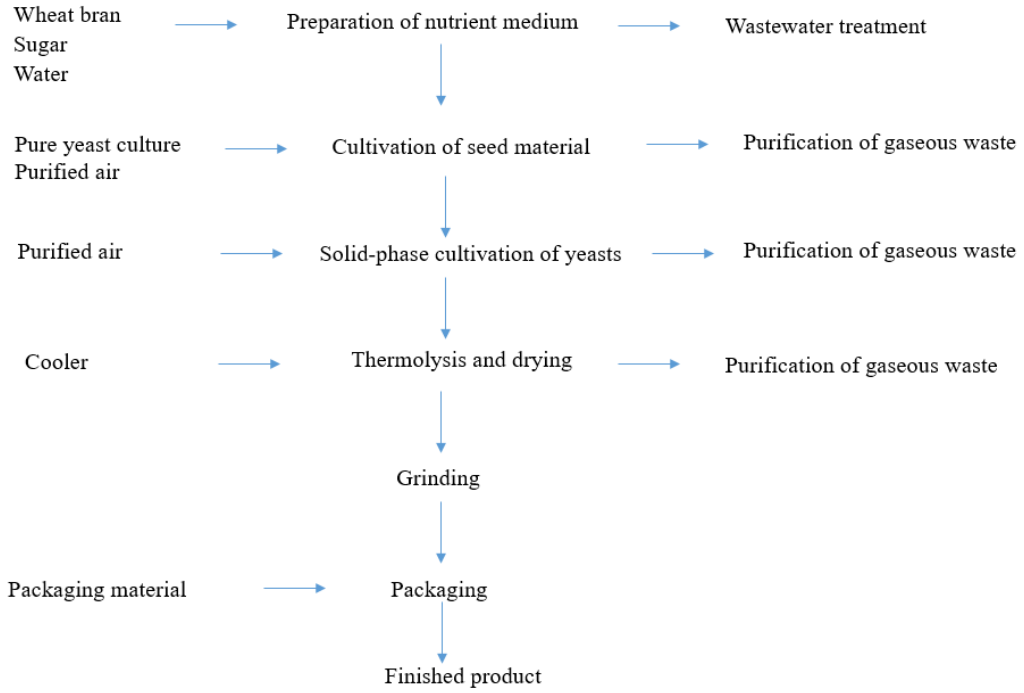

Figure 2. Technological scheme of the process of obtaining BAS 'Fervital'

Biochemical composition of BAS 'Fervital', % per 100 g: proteins 18–20, fats 4–5, carbohydrates 65–70. Protein accumulation in the bioconversion process amounts to a maximum of 4–5%, but the content of essential amino acids increases. BAS 'Fervital' is a source of vitamins B1, B2, and B6 (22.3–26.5% of the recommended intake). Thanks to the fact that it contains large amounts of iron, magnesium, as well as other microelements, it is successfully used in the therapy and prophylaxis of various types of alimentary diseases (Table 2).

To study the bifidogenic properties of BAS 'Fervital', an aqueous suspension of BAS 'Fervital' is introduced into the standard nutrient medium for the cultivation of probiotic microorganisms before the cultivation of the *B. bifidum* 791 strains. When adding 10% of the fervital suspension to the nutrient medium, the best effect on the growth stimulation of bifidobacteria is obtained.

'Fervital' has nutraceutical and para pharmaceutical properties. Its nutraceutical properties come from essential amino acids, vitamins, and micronutrients. The preparation is characterized as a parapharmaceutical due to its ability to stimulate the growth of probiotic microorganisms and to neutralize pathogenic and conditionally pathogenic microbes. The biologically active properties of the product determine the expediency of its application to BAS food, as well as its inclusion in products for therapeutic and prophylactic purposes.

Table 2. Content of vitamins and micronutrients in BAS 'Fervital'

Component	**Content in 100g supplement**	**Daily human needs**
Vitamin B1 (thiamine)	0.334 mg	1–1.4 mg
Vitamin B2 (riboflavin) 0	0.52 mg	1–3 mg
Vitamin B6 (pyridoxine) 0	0.53 mg	1–2 mg
Tocopherols (vitamin E) α-tocopherol β+γ-tocopherol	 not found 5.3 (1.6 mg)	10–12 mg
Selenium	6.28 μg	20100 μg
Molybdenum	21 μg	500 μg
Copper	0.946 μg	1–5 μg
Iron	68.9 mg	20–30 mg
Chromium	32.8 μg	100–200 μg
Magnesium	931.9 mg	500–750 mg
Manganese	26.4 mg	5–10 mg

Research on the application of BAS 'Fervital' in the complex therapy of gastric and duodenal ulcers showed the advantage of treatment with 'Fervital' for the removal (elimination) of heartburn, constipation, and reduction of the acidity of the stomach contents. Fervital combines the properties of an antacid (it can neutralize the excessive content of hydrochloric acid in the stomach) and a prokinetic (strengthens the motility of GIT). A large number of functional products have been created based on BAS 'Fervital'. Macaroni, bakery products, waffle bread and extruded breakfast cereals containing 10–15% BAS are produced under the 'Fervital' brand.

CONCLUSION

The health benefits of probiotics and prebiotics, as well as synbiotics, have been the subject of research for the past few decades. These dietary supplements, called functional foods, have been shown to alter, modify and re-establish pre-existing intestinal flora. The most commonly used probiotic strains are *Bifidobacterium*, *Lactobacilli*, and *S. boulardii*. Prebiotics such as inulin, fructans are the most commonly used fibers which, when used together with probiotics, are called synbiotics and can improve the viability of the probiotics. This review focuses on the composition and role of probiotics, prebiotics, and synbiotics in human health. Probiotics, prebiotics, and synbiotics have a systemic effect on the health metabolism and immune system of the host.

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