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# MICROBIAL FERMENTATION OF GRAIN RAW MATERIALS. PROSPECTS FOR FOOD TECHNOLOGY: AN ANALYTICAL REVIEW

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The development of technologies for deep processing of grain raw materials in order to obtain functional biological products is the most important economic aspect of the development of the agro-industrial complex. Grain is a strategic resource for food security of each country, which at the same time has a great potential of the components it contains, which determines its widespread use, both in food technologies and in the biotechnological industry. Particular attention is paid to the development of highly efficient processing technologies for this type of raw material, which can be traced in a significant amount of scientific research in this area. The purpose of the analytical review is to research current approaches to the microbial processing of grain raw materials, studying the prospects for the application of these technologies for the food industry. During the analysis of scientific data, three information blocks were identified, reflecting the main directions of research in the field of enzymatic processing of grain raw materials. First, numerous studies have proved that grain processing products have a significant biotechnological potential-they are characterized by a high content of nitrogenous and prebiotic components, essential micronutrients, and therefore are a favorable substrate for the accumulation of biomass of lactic acid microorganisms and the production of functional components with probiotic properties. Secondly, fermentation is considered as an aspect for improving the nutritional and functional characteristics of the grain. Throughout the enzymatic processing, the molecular structure of the protein changes and its digestibility improves, the synthesis and accumulation of biologically valuable components is ensured. Under the action of the microbial enzymatic pool, biopolymers are transformed, and a wide range of biologically active compounds are accumulated, such as peptides, oligosaccharides with prebiotic potential, enzymes, and polyphenolic components. And finally, during the processing of grain crops, a large number of by-products and waste products are formed, which are a source of natural polymers, dietary fibers, and micronutrients – and therefore can become a substrate for the growth of microbial biomass. By increasing the bioavailability of components and the decomposition of anti-nutrient substances, fermentation can improve the nutritional value of grain waste and by-products. Currently, effective technologies for deep processing of plant resources are associated with biotechnological methods, including microbiological fermentation of grain raw materials. The results of the analytical research formed the basis of the consolidated material, which reflects the biotechnological potential of grain raw materials.

**Keywords:** grain raw materials, microbial fermentation, probiotic products, grain processing by-products, biologically active substances.

In the context of the modern development of industrial biotechnological production aimed at creating innovative products, low-waste and non-waste technologies based on the full use of all components of plant resources are of great importance

The production of grain crops is a strategically important aspect of the state's economy, which ensures food security, both for an individual country and for the population of the whole world. According to the Food and Agriculture Organization of the United Nations (FAO), there

is a steady increase in the production of grain raw materials every year. Thus, the production of cereals worldwide has increased from 2,460 to 2,980 million tons over the past 10 years [3, 50, 51].

Grain, as a biological object, regardless of the type of culture, has a significant potential of the components it contains, which determines its widespread application both in food technology and in biotechnological industries [48].

Proteins of grain of different crops can differ significantly in their biological value and func-

tional properties; however, first of all, they are a valuable source of nitrogenous substances in the cultivation of microorganisms. Depending on the type, grain raw materials contain a different ratio of easily digestible and non-digestible carbohydrates [20]. It is established that cereals, legumes, and oilseeds are characterized by a significant content of both soluble and insoluble dietary fibers that have prebiotic properties in relation to microorganisms cultivated on a plant substrate. The biochemical components of grain raw materials are the activators of catalytic processes in the technologies of fermented products and target bioactive components.

In recent decades, the amount of scientific research related to the biotechnological aspects of the production of fermented foods has increased significantly. The main areas of research related to the enzymatic processing of grain raw materials by microorganisms include:

- increasing the bioavailability of valuable food components of grain raw materials, reducing anti-nutritional properties;
- production of fermented beverages based on grain extracts using industrial strains of microorganisms;
- accumulation of biomass of probiotic microorganisms on the grain substrate, production of probiotic additives;
- isolation of target components with functional activity during fermentation (soluble dietary fiber, bioactive peptides, etc.).

It is relevant to study the results of research in the field of microbial fermentation of grain raw materials to analyze the concept formed by the scientific community in this direction. Thus, the purpose of the analytical review is to research current approaches to the microbiological processing of grain raw materials, studying the prospects for using these technologies for the food industry.

The authors conducted a search for review scientific publications in the Scopus and Web of Science databases in the areas of scientific research in the field of fermentation of grain raw materials published over the past ten years. The query string was formed as follows: [TITLE-ABS-KEY (cereal AND fermentation), with the inclusion of open access articles with the search for publications since 2011 [ANDPUBYEAR > 2011]. Based on the results of the search for publications and their topics, the authors developed the structure and format of the review.

# Obtaining probiotic products during grain materials fermentation

For substrates containing processed grain raw materials, it is promising to use not individual microorganisms, but consortia, including both bacteria and yeast, selected according to the principle of synergy. To date, a number of studies have been conducted on the use of grain materials as potential nutrient media for the cultivation of probiotic microorganisms [7, 34, 39].

Charalampopoulos et al. [15] studied the growth kinetics of *L. fermentum*, *L. reuteri*, *L. acidophilus*, and *L. plantarum* on malt, barley, and wheat. nutrient media. It was noted that the growth of lactobacilli was higher in malt culture medium.

Radenkovs et al. [42] used wheat bran hydrolysates obtained using  $\beta$ -amylases and a complex of xylanases, hemicellulases, glucanases, and cellulases for the cultivation of bifidobacteria *B. lactis Bb-12* in order to obtain a functional product. The titer of active bifidobacteria in the final product was  $10^5$  CFU / g.

Novik et al. [37] studied the growth of lactoand bifidobacteria on nutrient media based on protein and polysaccharide fractions of barley pellets with the addition of various supplements, observed high biomass yields and the viability of microorganisms.

Salari et al. [46] investigated the physico-chemical characteristics of fermented cereal-based beverages. Changes in microbial population levels were determined during 48-hour fermentation of malt, barley and a mixture of barley and malt with probiotic strains *L. paracasei* and *L. delbrueckii*. The selected cultures grew well on single and mixed substrates without growth-stimulating additives. The highest growth potential was found in malt media after 15 hours of fermentation. Maltbased beverages, fermented with *L. delbrueckii* contained up to  $1.2 \times 10^6$  CFU / ml of Lactobacillus cells.

Angelov et al. [4] used a whole-grain oatmeal substrate that was fermented with lactic acid bacteria to develop a new probiotic beverage. The authors studied the influence of the concentration of the starter culture, the content of oat flour and sucrose on the fermentation process for 8 hours. The number of viable microbial cells at the end of the process reached 7.5×10<sup>10</sup> CFU / ml. The content of beta-glucan remained unchanged both during fermentation and during storage of the product. Martensson et al. [31] developed oat-based yogurt, which was fermented

using lactic acid bacteria. The high content of beta-glucan in the fermented grain product was noted

In the studies of Khromova et al. [26, 27], it was proved that the processing of grain raw materials with amyl- and proteolytic enzymes allows to obtain functional ingredients containing bifidobacteria or lactobacilli on its basis. Nutrient media based on hydrolysates are characterized by a high potential for the accumulation of probiotic microorganisms' biomass (up to  $10^8$ – $10^9$  CFU / ml). It was found that the use of wheat flour hydrolysate as a protective medium during the lyophilic drying of *B. adolescentis* allows us to obtain a product with a bifidobacterium content of at least  $10^{10}$  CFU / g with a survival rate of 90 %.

It is known that grain products contain xylooligosaccharides with prebiotic properties. Priscilla, et al. [41], studed the accumulation of xylooligosaccharides in the bio-processing of wheat flour, using *Bacillus subtilis*. It was found that the activity of xylanolytic enzymes continuously increased during 72 hours of bacterial growth. It has been proven that wheat grain is a potential substrate for the production of prebiotics, and *B. subtilis* is a source of xylanolytic enzymes.

Thus, the results of numerous studies prove that the products of grain processing are a favorable substrate for the growth and accumulation of biomass of probiotic cultures of microorganisms.

# Biologically valuable components of fermented grain

Numerous studies have proven that grain raw materials are the main source of bioactive compounds that have countless benefits for human health. Cereals, pseudo-cereals, oilseeds, legumes are characterized by a significant amount of polyphenols, rich in selenium, tocopherols, and have a well-balanced mineral composition [8]. They content a high amount of polyunsaturated fatty acids including essential linoleic and  $\alpha$ -linolenic acids. Furthermore, the considerable amount of phytosterol and antioxidants have positive health impacts such as lowering cholesterol, preventing cancer, modulating the immune system [17, 43].

During biotechnological processing of grain raw materials under the action of enzyme systems of microorganisms, there is an improvement in bioavailability, the accumulation of substances with physiological functions (Fig. 1).

Throughout fermentation, endogenous and bacterial enzymes are able to change the grain composition, which affects the structure, bioactivity and bioavailability of nutrients [22]. During microbial processing, proteases hydrolyze proteins in the food matrix of cereals, thereby improving the digestibility of proteins [33]. Due to their proteolytic activity, microorganisms transform the proteins into bioactive peptides which impart antihypertensive properties to the substrate.

Functional aspects of grain raw bio-

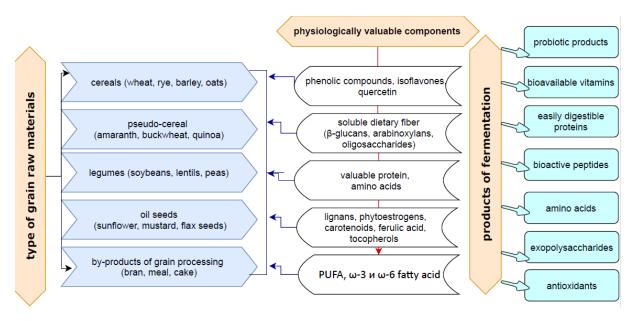


Fig. 1. Biotechnological potential of grain raw materials

fermentation are due to significant molecular changes in macronutrients. Through the action of a microbial enzymatic pool, lactic acid bacteria accumulate a broad range of volatile compounds, bioactive peptides and oligosaccharides with prebiotic potential [40].

To improve the functional characteristics, the grain compositions are fermented with lactic acid bacteria (LAB). During the fermentation LAB decrease the level of indigestible polysaccharides, synthesize low molecular organic components, like amino acids and B vitamins; essential fatty acids; improve the availability of micronutrients, such as iron, zinc and calcium [9, 16, 54]. Bacteriocins and antibacterial peptides, which reduce the growth of pathogenic and opportunistic microorganisms, are synthesized during fermentation by LAB [47].

The use of potentially probiotic yeast as a starter in the fermentation of cereal-based foods is a promising method for improving palatability and increasing the bioavailability of minerals [38].

Do Prado et al. [19] cultivated the *Rhizopus* on soybeans with the addition of various grains (brown rice, wheat, corn, and oats) to achieve high antioxidant activity. The biotransformation of soybeans and brown rice by *R. oligosporus* NRRL 2710c produced a product rich in isoflavone aglycones containing various antioxidant compounds such as trans-cinnamic acid, gallic acid, myricetin, quercetin and kaempferol. The fermented substrate has shown great potential for inhibiting the enzyme hyaluronidase and against the growth of CaCo-2 tumor cells.

In wheat germ, there are two quinones in physiologically inactive form that have cytotoxic activity against cancer cells – 2-methoxybenzo-quinone and 2,6-dimethoxybenzo-quinone. During wheat fermentation by *L. plantarum* LB1 and *L. rossiae* LB5 with high  $\beta$ -glucosidase activity, physiologically active forms of quinones were completely released, the concentration of these biologically active compounds increased up to 6 times [44].

The useful properties of phenolic compounds, which are present in cereals in significant quantities, include antidiabetic, anticancer, anti-inflammatory, antimicrobial, antioxidant, as well as neuro-, cardio- and hepatoprotective functions. Fermentation of millet with *L. sanfranciscensis*, *L. pentosus*, and yeast strains increased the total phenol content by 30 %, thus improving the functional value of this underuti-

lized cereal [10]. Korhola et al. [28], Tsafrakidou et al. [53], Xie et al. [58], noted the accumulation of vitamins (folic acid, vitamin B<sub>2</sub>, carotenoids, etc.) in cereal-based substrates during fermentation by various microorganisms.

The *in situ* synthesis of exopolysaccharides and oligosaccharides during the fermentation of cereal makes it possible to obtain a food substrate with increased functional properties. In fact, they can act as prebiotics and have been shown in *in vitro* experiments to have antitumor and immunomodulatory effects [2, 45].

#### Fermentation of grain processing byproducts

A large amount of by-product and waste is generated during the processing of cereals that is being the sources of various natural polymers such as lipids, carbohydrates, and proteins. By-products of the cereal industry have a high potential as substrates for fermentation and production of biologically active components [18].

It is scientifically proven that an integrated approach to the use of agricultural raw materials is highly effective [20, 24, 56]. The problem of converting by-products and waste from processing agricultural raw materials into a waste-free production cycle is based on the economic and ecological aspects [11, 25, 29, 32].

Fermentation is considered as an alternative option for improving the technological, sensory, functional and especially nutritional characteristics of the grain material. Fermentation can improve the nutritional value of cereal by-products by increasing the bioavailability of minerals, phenols and vitamins, the digestibility of proteins and the decomposition of anti-nutritional compounds in the form of the phytic acid.

A key factor in the successful use of cereal by-products in food products Verni et al. [55] noted the use of biotechnological processing, including fermentation with selected bacterial cultures. Since fermentation with lactic acid bacteria, yeast, or mycelial fungi is widely used for the production of cereal products for a healthy diet, its application to cereal by-products can increase the production of functional food [12, 13, 52].

Wheat bran is a rich source of proteins and amino acids, which have a high biological and nutritional value. However, the bioavailability of proteins in bran is limited due to the complex structure of the layers and the high content of substances such as phytate, which forms insoluble phytate-protein complexes [21]. During fermentation, the concentration of peptides and free amino

acids, including the functional non-protein gaminobutyric amino acid, increases due to the proteolytic activity of lactic acid bacteria and endogenous proteases activated by a decrease in pH.

Protein digestibility also increases *in vitro* [6, 30] (Table 1).

Wheat bran contains hydroxycinnamic acids, in particular, ferulic acid is the structural component of the cell walls of the aleurone and pericarp, which has a very low bioavailability. The potential health effects of ferulic acid are based on its antioxidant properties, in particular its ability to inhibit lipid peroxidation and the oxidation of low-density lipoproteins [5].

Fermentation *Lactobacillus*, *Leuconostoc*, and *Pediococcus*, as well as yeast, is effective in releasing ferulic acid – up to 82 % [30]. The release of ferulic acid is also produced during fermentation by the fungi *Hericium erinaceus*. A higher yield of the biologically active component was found in fermented bran (44 % higher than in unfermented bran) due to the combined action of cellulase and esterase, which can break down the cell walls of wheat bran [57].

Spaggiari et al. [49] were studied the phytic acid degradation and arabinoxylans solubilization properties during solid-state fermentation of wheat bran by *L. rhamnosus* strain. The treatment

Table 1

Fermentation technologies for cereal by-products

Cereal by- product Wheat bran	Preparation of raw materials for fermentation Milling and hydrolysis using β-amylases, xylanases, hemicellulases, glucanases and	Enzyme processing parameters  Cultivation temperature 38 °C, duration 72 h	Applied cultures of microor- ganisms  Bifidobacterium lactis Bb-12	Target fermentation product  Probiotic product with a titer of bifidobacteria 10 <sup>5</sup> CFU / g	References Radenkovs et al. (2013) [42]
	cellulases Grinding to 0.6–2 mm, drying the substrate at 65 °C until complete de- hydration	Cultivation temperature 25 °C, duration 72 h	Mucor subtillissimus UCP 1262	Fibrinolytic protease	Nascimento et al. (2015) [35]
	Hydration with water in the ratio 7:18	Cultivation temperature 18 °C, duration 13 days	Leuconostoc mesenteroides, L. acillus brevis, L. curvatus, L. sakei, L. plantarum, Pediococcus pentosaceus Pichia fermentans	Fermented bran with a high con- tent of biologi- cally active sub- stances	Manini et al. (2014) [30]
	Hydration with water in the ratio 1:4	Cultivation temperature 25 °C, duration 7 days	Proropioni- bacterium freudenreichii	Substrate enriched with vitamin B12	Xie et al. (2018) [58]
Barley grains	Extraction of protein and polysac- charide introduc- tion of growth- stimulating addi- tives	Cultivation temperature 37 °C, duration 24– 48 h	Lacto- and bifidobacteria	Biomass of probiotic microorganisms	Novik et al. (2007) [37]

used by the authors resulted in a 37 % decrease in the phytic acid content and a three-fold increase in the amount of arabinoxylans

Acin-Albiac et al. [1] determined the products of carbohydrate metabolism Lactiplantibacillus plantarum and Leuconostoc pseudomesenteroides when exposed to a plant matrix with a high content of phenol and fiber in the form of brewer's grains. The authors presented a view of the important role exerted by 6-phospho-β-glucosidases of Leuc. pesudomesenteroides DSM 20193 and L. plantarum PU1, as well as major metabolic pathways during plant-based fermentation. A positive effect on the technological and sensory properties of plant matrices, the production of dextrans by Leuc. pseudomesenteroides DSM 20193 has been established [59]. Žilić et al. [60] noted that *Lacto*bacillus spp. can produce caffeic acid starting from chlorogenic acids, presented in wheat.

Propionibacterium freudenreichii, one of the microorganisms recognized as producers of vitamin  $B_{12}$ , was used to ferment wheat bran. The content of the active form of vitamin  $B_{12}$  in fermented bran increased by about 5 times, and a higher content of riboflavin was also found, which proves that bran can be a potential substrate for vitamin synthesis [14].

Rye bran proved to be an excellent substrate for the synthesis of polyunsaturated fatty acids and beta-carotene during solid-phase fermentation of the *Mucor circinelloides* CCF-2617. Rye and wheat bran were used as substrates for the synthesis of exopolysaccharides in the fermentation of the *Lactobacillus reuteri*. During the fermentation of rye bran, a significant amount of glucan was produced. It was also found that rye bran is the optimal substrate for dextran production during fermentation of the *Weissella confusa* [23, 36].

#### **Conclusions**

Numerous studies have proven that grain raw materials have significant biotechnological potential, it is characterized by a high content of nitrogen-containing substances, important micronutrients, and therefore, such a substrate is favorable for growth and accumulation of probiotic microorganism's biomass.

In the process of grain bio-fermentation, the synthesis and accumulation of biologically valuable components, such as vitamins, minerals, exopolysaccharides, bioactive peptides, phenolic compounds and antioxidants occur.

The most important goal of biotechnology is to develop rational parameters for deep processing of grain raw materials using effective methods of microbiological fermentation.

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# МИКРОБНАЯ ФЕРМЕНТАЦИЯ ЗЕРНОВОГО СЫРЬЯ. ПЕРСПЕКТИВЫ ДЛЯ ПИЩЕВОЙ ТЕХНОЛОГИИ: АНАЛИТИЧЕСКИЙ ОБЗОР

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Разработка технологий глубокой переработки зернового сырья с целью получения функциональных биопродуктов – важнейший экономический аспект развития агропромышленного комплекса. Зерно – стратегический ресурс продовольственной безопасности каждой страны, который в то же время обладает большим потенциалом содержащихся в нем компонентов, что определяет его широкое применение как в пищевых технологиях, так и в биотехнологической промышленности. Особое внимание уделяется разработке высокоэффективных технологий переработки данного вида сырья, что прослеживается в значительном объеме научных исследований в этой области. Целью настоящего аналитического обзора является исследование современных подходов к микробной ферментации зернового сырья, изучение перспектив использования данных технологий для пищевой промышленности. При проведении анализа научных данных было выделено три информационных блока, отражающих основные направления исследований в области ферментативной переработки зернового сырья. Во-первых, многочисленные исследования доказали, что продукты переработки зерна обладают значительным биотехнологическим потенциалом – характеризуются высоким содержанием азотистых и преботических компонентов, эссенциальных микронутриентов и поэтому являются благоприятным субстратом для накопления биомассы молочнокислых микроорганизмов и производства функциональных компонентов с пробиотическими свойствами. Во-вторых, ферментация рассматривается как аспект для улучшения питательных и функциональных характеристик зерна. В процессе ферментативной обработки изменяется молекулярная структура белка и улучшается его усвояемость, обеспечивается синтез и накопление биологически ценных компонентов. Под действием микробного ферментативного пула происходит трансформация биополимеров и накопление широкого спектра биоактивных соединений, - пептидов, олигосахаридов с пребиотическим потенциалом, ферментов, полифенольных компонентов. И наконец, в процессе переработки зерновых культур образуется большое количество побочных продуктов и отходов, которые являются источником природных полимеров, пищевых волокон, микронутриентов, - поэтому могут служить субстратом для роста биомассы микроорганизмов. За счет увеличения биодоступности компонентов и разложения антипитательных соединений ферментация может улучшить питательную ценность отходов и побочных продуктов переработки зерна. В настоящее время эффективные технологии глубокой переработки растительных ресурсов связаны с биотехнологическими методами, в том числе с микробиологической ферментацией зернового сырья. Результаты аналитического исследования легли в основу сводного материала, в котором отражен биотехнологический потенциал зернового сырья.

**Ключевые слова:** зерновое сырье, микробная ферментация, пробиотические продукты, вторичные продукты переработки зерна, биологически активные вещества.

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