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DEVELOPMENT OF TECHNOLOGY FOR THE PRODUCTION OF FUNCTIONAL BEVERAGES FROM GERMINATED GRAIN

Abstract. The growth of the world's population has led to an increasing demand for functional food products that enrich the daily diet. Providing the population with environmentally friendly and high-quality products is an important prerequisite for promoting a healthy lifestyle. In this regard, determining the quantity and composition of microorganisms at the initial stage of grain processing is an essential preventive measure. During the study, the microflora of cereal grains, legumes, and oilseed crops was investigated. The research focused on the quality and safety of grain hydration during the development of a technology for producing beverages from germinated grains. It was established that the proliferation of microorganisms in grains and seeds depends on temperature and surface structure: the higher the temperature, the faster microorganisms multiply in moist materials. This relationship was observed only in hydrated materials, since microorganisms do not proliferate rapidly in dry grains and seeds regardless of temperature.

Keywords: cereal crops, legumes, oilseed crops, pathogenic microflora, germination period, nutrient medium, microbial colonies.

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Introduction. Grain and grain-based products constitute the foundation of the daily diet of the population, and their quality and safety directly affect the level of food security in the country. In this regard, extensive measures have been undertaken in recent years to promote healthy and balanced nutrition through the development of high-quality and safe grain-based products and the improvement of production technologies.

Finished food products obtained through the targeted modification of the technological, physicochemical, and biochemical properties of grain raw materials must comply with food safety requirements while enriching the daily diet with biologically active compounds [1,2].

At present, a general decline in the nutritional and biological value of manufactured food products is observed, resulting in deficiencies of essential macro- and micronutrients in the human diet [3,4]. Therefore, one of the priority directions of the modern food industry is the development and expansion of functional food products that contribute to maintaining human health and reducing the risk of various diseases. At the same time, there is a growing need to move away from the traditional approach of enriching foods with artificially added nutrients, known as fortification [5,6].

Domestic researchers have developed formulations and technologies for food products enriched with B vitamins, iron, calcium, iodine, and β-carotene. For these purposes, the production of iodine-containing additives and vitamin-mineral premixes with water- and fat-soluble β-carotene preparations has been established. Ready-to-eat breakfast cereals, corn flakes, and instant cereals are enriched with macro- and micronutrients of plant, animal, mineral, and synthetic origin. Rice and other cereal crops are treated with vitamins such as thiamine, riboflavin, and nicotinamide. Functional grain products contribute to reducing the risk of cardiovascular diseases, lowering cholesterol levels, and improving gastrointestinal health. Nevertheless, the raw material potential of grain crops remains underutilized [7,8].

In this context, it should be noted that virtually any cereal, legume, or oilseed crop, including wheat, rye, buckwheat, sunflower, sesame, lentils, soybeans, and others – can be germinated. Each of these crops possesses unique beneficial properties and contains a specific combination of vitamins, minerals, amino acids, and other biologically active compounds [9,10].

However, during the germination of grain raw materials, it is necessary to investigate the dynamics of enzymatic processes occurring within the studied materials, since profound biotechnological transformations take place during germination, actively modifying the chemical composition of plant raw materials.

According to published data, different groups of microorganisms develop in grains under varying conditions of moisture and temperature. For example, certain fungi can proliferate in wheat and maize grains at temperatures of 15-20°C and moisture contents of 14.5-15.0%, whereas bacterial growth in wheat grain begins when moisture reaches a critical level of approximately 17.5-18.0%. Each crop has its own critical moisture content at which microbial growth accelerates sharply. In legume and oilseed seeds, fungi develop at moisture levels of about 16% under the aforementioned temperature conditions, while in sunflower seeds fungal growth may occur at moisture contents as low as 7-9%. This phenomenon is determined by the amount of bound water in the seeds, which depends on their structural characteristics and chemical composition [11,12].

Microorganisms develop in grain only when free water becomes available, that is, when the moisture content exceeds the level of bound water.

Even sufficiently moist grain (18-19% moisture content) can be stored satisfactorily at a temperature of 10°C; however, at 15-20°C it rapidly becomes moldy and susceptible to bacterial spoilage. Therefore, successful grain storage requires a reduction in moisture content as storage temperature increases.

At the same degree of moisture content, the active development of microorganisms in the grain mass occurs at different rates depending on the crop

species. Wheat, rye, barley, peas, beans, and buckwheat are more resistant to microbial growth, whereas millet, maize, and sunflower seeds are characterized by more rapid and intensive microbial development [13].

In this regard, the aim of the study was to investigate the microbiological contamination of grain, legume, and oilseed crop samples.

Materials and methods. Grain, legume, and oilseed crops were used as the objects of the study. The microbiological contamination of the selected samples was investigated in the Laboratory of Microbiology and Biotechnology of the Almaty Branch of LLP "Kazakh Research Institute of Processing and Food Industry" (KazNIIPPI).

The total number and species composition of bacteria, fungi, and yeasts were determined by counting colonies grown on a dry nutrient agar medium. The composition of the dry nutrient agar (manufactured in India) per 1 L of distilled water was as follows: pancreatic digest of animal tissue – 5 g; sodium chloride – 5 g; beef extract – 1.5 g; yeast extract – 1.5 g; agar-agar – 15 g; pH 7.4 ± 0.2. The medium was sterilized at a pressure of 1 atm for 15 min [14].

The quantitative composition and structure of the microbial community were determined by inoculating diluted suspensions onto a solid nutrient medium. All procedures were carried out under strictly sterile conditions, including disinfection of instruments with alcohol, use of sterile Petri dishes, sterilized distilled water, and autoclaving.

At each stage of morphological development, bacterial isolates were obtained by inoculating the test material onto standard plate agar (SPA, dry nutrient agar). A dilution of $1:10^4$ was used, and the inoculum volume was 0.02 mL. The inoculated suspension was spread onto Petri dishes in duplicate. Sterile medium served as the control. The Petri dishes were incubated at 28-30°C for 5-6 h. After incubation, colony counts were determined taking the dilution factor into account.

The overall level of microbiological contamination was expressed as the number of colony-forming units (CFU) per milliliter. To investigate the morphological and tinctorial properties of the isolated microorganisms, preparations were made from the obtained colonies and stained using the Sinyev, Gram, and Ziehl–Neelsen methods, followed by microscopic examination.

Research results. Experimental studies were conducted to assess the food safety of cereal, legume, and oilseed grains in terms of microbiological contamination by pathogenic microflora. In addition, the cultural and morphological characteristics of the isolated strains were investigated (Table 1).

Table 1

Information on the Study Materials

№	Crop	Variety	Originator/Developer
1	Spring bread wheat	Almaken	LLP "Kazakh Research Institute of Agriculture and Plant Growing"
2	Triticale	Aziad	LLP "Kazakh Research Institute of Agriculture and Plant Growing"
3	Spring barley	KazSuffle-1	LLP "Kazakh Research Institute of Agriculture and Plant Growing"
4	Rice (grain crop)	Ai-Kerim	LLP "Kazakh Research Institute of Rice Growing named after I. Zhakhaev"

Seeds of leguminous crops are characterized by a high content of essential amino acids. Samples of domestically bred soybean, pea, and chickpea varieties were selected as research materials. These varieties have successfully passed variety testing and have been included in the State Register of Breeding Achievements of the Republic of Kazakhstan (Table 2).

Table 2

Information on the Study Materials

№	Crop	Variety	Originator/Developer
1	Soybean	Ivushka	LLP “Kazakh Research Institute of Agriculture and Plant Growing”, LLP “Agricultural Experimental Station ‘Zarechnoye’”
2	Pea	Aksary	LLP “Kazakh Research Institute of Agriculture and Plant Growing”
3	Chickpea	Satti	LLP “Kazakh Research Institute of Agriculture and Plant Growing”

Oilseed crops are a natural source of plant protein and valuable polyunsaturated fatty acids. Seeds of domestically bred oilseed crops, including sunflower, flax, safflower, and rapeseed, were selected as research materials (Table 3).

Table 3

Information on the Study Materials

№	Crop	Variety	Originator/Developer
1	Sunflower	Rauan	LLP “Kostanay Agricultural Research Institute”
2	Oilseed flax	Kostanayskiy	LLP “Agricultural Experimental Station Zarechnoye”
3	Safflower	Nika 80	LLP “Kazakh Research Institute of Agriculture and Plant Growing”
4	Spring rapeseed	Gulsary	LLP “Kostanay Agricultural Research Institute”, LLP “Kazakh Research Institute of Agriculture and Plant Growing”

Surface washings were prepared from the research samples and inoculated onto nutrient media. The cultural and morphological characteristics of the isolated strains were studied on a dry nutrient medium (NM). The following characteristics were evaluated: colony size, shape, profile, surface features, optical properties, color, structure, and bacterial consistency. The cultural and morphological characteristics of the bacterial strains are presented in (Table 4).

Table 4

Cultural and Morphological Characteristics of Bacterial Strains

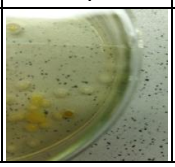
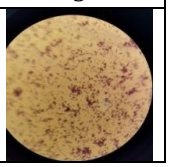
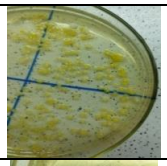
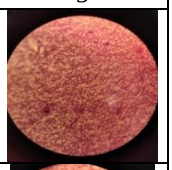
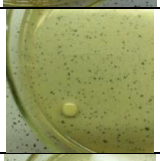
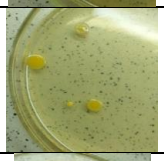
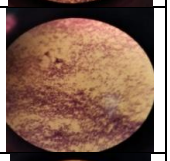
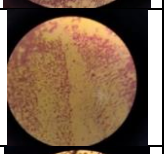
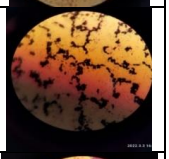
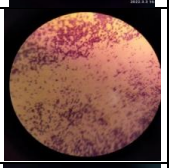
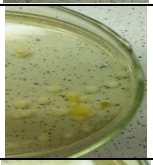
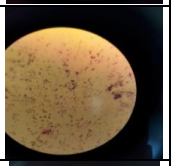
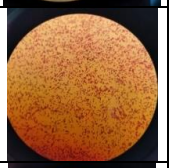
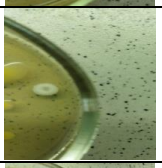
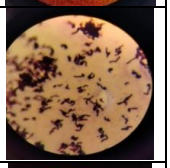
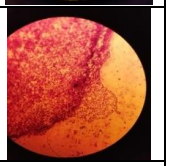
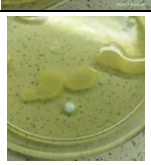
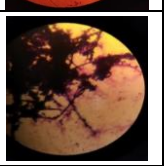
№	Crop (Sample)	Colony Morphological Characteristics	Plate	Microscopic Characteristics
1	2	3	4	5
1	Triticale (Sample No. 2)	Circular yellow colonies, slightly darker in the center, shiny, smooth surface, Ø2 mm Gram-negative short rods with rounded ends, occurring singly and in chains		

Table 4 (continued)

1	2	3	4	5
2	Triticale (Sample No. 2)	Yellow, circular, small, shiny colonies with smooth profile and convex center, Ø1-2 mm Gram-negative short rods with rounded ends, occurring singly and in chains		
3	Triticale (Sample No. 2)	Gray-white, circular, shiny colonies with smooth profile, Ø2-3 mm Gram-negative short rods with rounded ends, occurring singly and in chains		
4	Triticale (Sample No. 2)	Yellow, translucent, mucoid colonies spreading over the medium surface, Ø6 mm Gram-negative short rods with rounded ends, arranged in clusters		
5	Rice (Sample No. 3)	Yellow circular colonies with smooth edges, shiny surface, smooth profile, Ø5-6 mm Gram-negative short rods with rounded ends, arranged in clusters		
6	Rice (Sample No. 3)	Pink punctiform circular colonies, convex, smooth edges, shiny surface, Ø1 mm Gram-positive oval yeast cells, occurring singly or in close proximity		
7	Barley (Sample No. 4)	Circular colonies with smooth edges, convex profile, smooth shiny surface, cream-colored, Ø5 mm Gram-negative short rods with rounded ends, arranged in clusters		
8	Barley (Sample No. 4)	Circular colonies with smooth edges, flat profile, granular surface, cream-colored, Ø4 mm Gram-negative short rods with rounded ends, occurring singly and in chains		
9	Flax (Sample No. 9)	Circular colonies with smooth edges, convex profile, smooth and shiny surface, dirty white color, Ø5 mm Gram-negative short rods with rounded ends, occurring singly and in chains		
10	Safflower (Sample No. 10)	Circular colonies with slightly wavy edges, pale color, smooth, dull surface, paste-like consistency, Ø5 mm Gram-positive large, slightly curved rods, occurring singly, in chains, and clusters		
111	Spring rapeseed (Sample No. 11)	Yellow, translucent, mucoid colonies spreading across the medium surface, Ø15 mm Gram-negative short rods with rounded ends, occurring singly and in clusters		
11	Spring rapeseed (Sample No. 11), *Penicillium*	Circular colony, convex center, velvety texture, white with light green center and white margin Septate, colorless hyphae; numerous round conidia; long, narrow conidiophores observed		

As shown in Table 4, the colonies of bacteria isolated from the studied crop samples were characterized by circular shapes with irregular, wavy, or smooth margins; flat, convex-flat, or convex profiles; homogeneous structures; mucoid or dry consistency; smooth or wrinkled surfaces; and a wide range of colors, including apricot-yellow, brownish-orange, olive-yellow, pale, and light pink. The colony diameter varied from 25.5 ± 0.7 mm to 71.5 ± 2.1 mm. The studied microorganisms included both Gram-positive and Gram-negative bacteria.

The bacterial colonies were predominantly circular, festooned, irregular, or smooth-margined, with flat, slightly convex, or convex profiles. They exhibited homogeneous structures, mucoid or dry consistency, smooth or wrinkled surfaces, and various colors such as pale gray, pale, yellow, and sandy-yellow. The colony size ranged from 4.5 ± 0.7 mm to 31.7 ± 2.1 mm.

Conclusion. Based on the results obtained, it can be confidently stated that high-quality grain possesses a characteristic native microflora, which significantly changes under improper storage and spoilage conditions. Therefore, the species composition of microorganisms may serve as an indicator of the quality of germinated grain.

The dominant microflora of grain consists of bacteria. A major portion of the bacterial population is represented by non-spore-forming rods of the genus Pseudomonas, which actively proliferate on plant surfaces. Most commonly, Ps. herbicola is encountered, forming golden-yellow colonies on solid nutrient media. Microorganisms of the genus Pseudomonas, including Ps. fluorescens, which produces fluorescent colonies, are also frequently detected.

Among other bacterial groups, micrococci and lactic acid bacteria are present. Fatty acid bacteria and bacilli enter grains together with dust and insects and remain in a passive state without active proliferation on plant material. In total, up to 50 bacterial species may be identified on plant surfaces.

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ӨНГЕН АСТЫҚТАН ФУНКЦИОНАЛЬДЫ БАҒЫТТАҒЫ СУСЫНДАР ӨНДІРУ ТЕХНОЛОГИЯСЫН ӘЗІРЛЕУ

Аңдатпа. Жер халқының санының өсуі күнделікті рационды байытатын функционалдық өнімдерге сұраныстың артуына әкеледі. Халықты экологиялық таза, сапалы өнімдермен қамтамасыз ету салауатты өмір салтын қалыптастырудың маңызды шарты болып табылады. Бұл тұрғыдан алғанда, астықты өңдеудің бастапқы кезеңінде микроорганизмдер саны мен құрылымын анықтау маңызды алдын алу шарасы болып табылады. Зерттеу барысында астық, бұршақ тұқымдас және майлы дақылдар дәнінің микрофлорасы зерттелді, ал зерттеу нысаны өніп шыққан дәнді сусындарды дайындау технологиясын әзірлеу кезінде дәннің ылғалдану сапасы мен қауіпсіздігі болды. Дән мен тұқымдағы микроорганизмдердің көбеюі температура мен беттің құрылымына байланысты екені анықталды: температура неғұрлым жоғары болса, ылғалды материалда микроорганизмдер соғұрлым тез көбейеді. Бұл тәуелділік тек ылғалданған материалда байқалды, өйткені құрғақ дәнде және тұқымда микроорганизмдер температураға қарамастан тез көбеймейді.

Тірек сөздер: астық дақылдары, бұршақ дақылдары, майлы дақылдар, патогенді микрофлора, өну мерзімі, қоректік орта, микроорганизмдер колониясы.

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ИЗУЧЕНИЕ МИКРОФЛОРЫ ЗЕРНА ЗЛАКОВЫХ, ЗЕРНОБОБОВЫХ И МАСЛИЧНЫХ КУЛЬТУР ПРИ ИХ ПРОРАЩИВАНИИ НА ПИЩЕВЫЕ ЦЕЛИ

Аннотация. Рост численности населения Земли приводит к увеличению спроса на функциональные продукты питания, обогащающие ежедневный рацион. Обеспечение населения экологически чистыми и качественными продуктами является важным условием формирования здорового образа жизни. В этой связи определение количества и структуры микроорганизмов на начальном этапе

переработки зерна представляет собой важную профилактическую меру. В ходе исследования была изучена микрофлора зерна злаковых, зернобобовых и масличных культур. Объектом исследования являлись качество и безопасность увлажнения зерна при разработке технологии производства напитков на основе пророщенного зерна. Установлено, что размножение микроорганизмов на зерне и семенах зависит от температуры и структуры поверхности: чем выше температура, тем быстрее происходит развитие микроорганизмов во влажном материале. Данная зависимость наблюдалась только в увлажненном материале, поскольку в сухом зерне и семенах микроорганизмы не размножаются интенсивно независимо от температуры.

Ключевые слова: зерновые культуры, зернобобовые культуры, масличные культуры, патогенная микрофлора, продолжительность проращивания, питательная среда, колонии микроорганизмов.