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# DETERMINING THE INFLUENCE OF ENZYMES AND SOURDOUGH OF LACTIC ACID BACTERIA ON THE QUALITY OF WHEAT-HEMP BREAD

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*The object of this study is wheat bread with the addition of whole grain hemp flour. The principal issue related to using hemp flour in bread baking is its high oil and dietary fiber content. The inclusion of such raw materials in the recipe worsens the structural and mechanical properties of the dough and the quality of the bread.*

*It was established that hemp flour is a valuable raw material for increasing the nutritional value of wheat bread as it contains 22.3% protein, 34.4% oil, and 16.5% fiber. It has been established that the rational dosage of such raw materials in bread recipes is 5–10%. However, even this amount affects the quality of the dough and bread. The content of raw gluten decreases by 2.8% and its quality deteriorates, the color of the crust improves, the crumb darkens slightly, and notes of walnut appear in the aroma.*

*The effectiveness of using sourdough of lactic acid bacteria and enzymes α-amylase, lipase, cellulase, transglutaminase to improve the bread quality and its nutritional and physiological value, has been confirmed. In particular, the specific volume of bread increased from 4.3 to 39.1%. Its freshness improved, as evidenced by a decrease in crumbly texture, from 9.9 to 29.6%, and an increase in water absorption capacity from 16.9 to 44.5%, depending on the variant.*

*The results could be used in the technology of producing wheat-hemp bread for health and preventive purposes with increased nutritional and physiological value. The proposed technological solutions make it possible to expand the range of such products for mass production*

*Keywords: hemp flour, enzyme treatment, lactic acid bacteria, bread quality*

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## 1. Introduction

Wheat bread is one of the basic food products in the world [1]. Its production is more than 100 million tons per year and covers more than 20% of the daily energy needs of people [2]. However, such bread is characterized by high calorie content and glycemic index, low content of dietary fiber, vitamins, minerals, and can cause allergic reactions due to the presence of gluten [3].

Therefore, in recent decades, the production of bread with therapeutic and prophylactic properties has gained great popularity in the world. In such products, in order to



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increase their biological, physiological, and nutritional value, valuable plant raw materials are used. These are various types of grains and seeds [4], waste from the processing of fruit and vegetable raw materials [5], cereal crops, and such non-traditional raw materials as teff flour [6], spelt, amaranth, nuts, etc.

One of the promising additives for improving the quality of wheat bread is non-narcotic hemp seeds, which have a balanced chemical composition and contain up to 25–35% oil, 20–25% hypoallergenic proteins, 20–30% carbohydrates, 20–30% dietary fiber, 7% ash, vitamins, and minerals [7]. It should be noted separately that hemp seed oil has a high content of unsaturated omega-3 and omega-6 fatty acids in their optimal ratio, beneficial for human health [8].

However, hemp seeds, while improving nutritional, biological, and physiological value, worsen the organoleptic properties of such bread [9]. To eliminate the negative impact of this raw material on the dough system of bread, various additives (enzymes, sourdough of lactic acid bacteria, malt extracts, thickeners, etc.) or elements of manufacturing technology are used [10, 11].

In view of this, scientific research on this topic is important and relevant as it makes it possible to expand the segment of bread for health and preventive purposes with increased nutritional, physiological, and biological value. The use of specially selected additives in their correct combination could contribute to improving the quality of such products for mass consumption. The devised approaches to the methodology for selecting additives to improve the quality of bread based on the characteristics of the components used would be useful when applying other raw materials in the technology of manufacturing such products.

## 2. Literature review and problem statement

Industrial hemp (*Cannabis sativa subsp. sativa*) is a high-yielding annual technical crop that has been widely cultivated in many countries of the world since ancient times, due to its nutritional, medicinal properties, and as a raw material for technical processing [12, 13]. The evolution of science has contributed to the emergence of new areas of use of its raw materials and the development of technologies for deeper processing.

The entire hemp plant is a valuable source of biologically active components. Hemp seeds are rich in fats, proteins, amino acids, dietary fiber, antioxidants, minerals, and vitamins. The stems of the plant contain fibers for the textile industry, leaves and inflorescences are substances for medical purposes, and the roots are a powerful tool for the reclamation of lands contaminated with heavy metal salts [14, 15]. Of particular interest is the use of hemp seed-based products for food purposes, in particular in baking, given their unique composition and the development of processing technologies.

The prospects for the use of hemp seed products for making bakery products are beyond doubt. At the same time, issues related to the technological aspects of the production of wheat bread with these additives remain unresolved. The reason may be difficulties with the specificity of the chemical composition of the seeds, which are characterized by a high content of oil, and have an increased content of dietary fiber. Therefore, when it is included in the bread recipe, the structural and mechanical properties of the dough deteriorate and, consequently, the quality of the finished product. The results of studies on the choice of a certain type of hemp seed product, its dosage, and the assessment of the effect of the additive on the quality of the finished bread also differ.

In work [16], the authors used hemp meal (HM) in a dosage of 5–20%. The results obtained indicate a significant effect of HM on water absorption and dough formation time, and therefore on the volume, color, and structural and textural properties of the bread crumb. Adding HM at a dose of up to 10% had no significant effect on the quality of bread, while 20% led to its significant decrease. HM contains a large number of biopolymers that can negatively affect the organoleptic indicators of bread if they are not hydrolyzed. The authors did not conduct research on the development of elements of bread manufacturing technology that ensure the destruction of these compounds, their better incorporation into the dough system and, as a result, obtaining bread with high organoleptic indicators.

The results of using hemp oil, in particular, the technology of wheat bread production are reported in [17]. Its addition to the product formulation affected the rheological properties of the dough and the quality as a whole, increasing the nutritional and biological value. It was found that the rational dosage of hemp oil, which ensures the production of high-quality bread, is 5–10%.

In [18], 5%, 7.5%, and 10% HM was used to enrich wheat bread with nutrients. The inclusion of the additive in the formulation contributed to a significant increase in glutamic acid, tyrosine, proline, and essential amino acids such as leucine and isoleucine in the test samples. The results of the sensory analysis were good, demonstrating a high degree of consumer acceptance of the enriched bread, especially at high percentages of HM. In the experimental bread samples, a significant increase in the total polyphenol content from 0.73 to 1.73 mg GAE/g and antioxidant activity from 1.17 to 3.18 mmol TEAC/100 g was noted. Thus, the authors set the main objectives of their work to determine the effect of adding HM on the change in the biological value of bread and its sensory properties. However, studies on the effect of the amount of the additive on the rheological properties of the dough and the physicochemical parameters of the finished bread were not conducted.

Replacing 10–20% of wheat flour with hemp protein (HP) or crushed hemp kernels (HKs) accelerated dough ripening and reduced the total process time by 20–25 minutes [19]. The use of HP in an amount of 10 and 15% made it possible to obtain bread that was not significantly inferior to the control sample in terms of organoleptic and physicochemical parameters. However, the authors did not study the effect of these additives on the rheological properties of the dough and did not devise methods for hydrolysis of these additives, which would ensure the production of bread with high organoleptic parameters.

The addition of hemp flour (HF) to the wheat bread recipe had a significant effect on the rheological properties of the dough and the quality of the finished bread, in terms of its physical properties, texture and sensory characteristics [9]. The addition of HF worsened the consistency of the dough. The textural properties of bread samples showed that elasticity, plasticity, and cohesion improved when adding up to 5% HF, and then deteriorated. With increasing dose, the color of the bread became darker, the hardness of the bread increased, and the chewiness of the crumb decreased. In general, the authors found that according to the sensory assessment, the addition of HF up to 10% gives a positive correlation in terms of bread volume, porosity, cohesion, color, smell, and taste. The authors did not conduct studies to determine the effect of using LAB sourdough and enzymes on the quality of bread with HF.

In [20], the results of using various hemp seed products in bread making technology are reported: HK, HM, and HP. It was found that the addition of even 1% of the additive causes significant technological changes in the finished product: the hardness increases and the elasticity of the crumb decreases, the color darkens. The use of various products of hemp seed processing make it possible toed the authors to obtain valuable results regarding their influence on the quality indicators of both dough and finished bread. However, given the high content of biopolymers in the composition of hemp products, such studies would be desirable to conduct using bread making methods that affect the destruction of these structures. This could contribute to better incorporation of such substances into the dough system and obtaining bread of better quality.

In [7], the effect of using HF in bread making technology on its chemical composition, rheological properties, organoleptic characteristics, crumb color, crumb texture change, polyphenol profile, total polyphenol content, and furan derivatives was investigated. The authors claim that for industrial bread production, the share of using such flour should not exceed 30%. At the same time, the authors did not conduct research on solving the issues of eliminating the negative impact of HF on the quality indicators of bread and increasing the degree of its perception by consumers.

In [21], the results of using HF, HK, and hemp oil (HO) in the manufacture of wheat bread are reported. The test samples underwent sensory and physicochemical analysis (volume, crumb condition, porosity, moisture, and acidity) and were evaluated for the content of proteins, oil, dietary fiber, and minerals (Ca, Mg, K). Adding only HO affected the organoleptic and physicochemical parameters of bread but did not have a significant effect on the content of nutrients and minerals. The authors claim that 15% HF, 4% HK, and 8% HO are the optimal dosage for use in bread baking technology as they provide high organoleptic properties of bread with a significant increase in the content of valuable nutrients. The authors did not conduct research on the use of special additives to improve the quality of dough and bread with hemp.

In summary, we can conclude that hemp seeds and products of its processing (meal, oil, flour, hulled seeds, whole grain flour) are valuable raw materials for improving the quality of wheat bread to their balanced chemical composition. Partial replacement of the basic raw material with hemp could lead to an increase in the content of proteins, dietary fiber, unsaturated fatty acids, amino acids, and would make it possible to balance its dietary composition. In addition, its use will enrich the taste sensations when consuming such bread and will contribute to expanding the range of products for people with gluten intolerance.

However, despite the large body of research into the use of this raw material, there are significant discrepancies in the types and recommended doses of hemp raw materials, which vary from 1 to 30%. Given the significant impact of these additives on the quality indicators of products, the issues of the effect of the amount and type of hemp raw materials on the dough, the quality of bread, its nutritional, physiological, and biological value remain unresolved. It is necessary to conduct research on the production of wheat-hemp bread using specially selected enzymes and LAB sourdough, which would enable a high degree of hydrolysis of the biopolymers of the raw material, which could improve the organoleptic properties and quality of the finished product. That will make it possible to use such bread not only for therapeutic and prophylactic purposes but also for mass consumption.

## 3. The aim and objectives of the study

The aim of our was to determine the effect of enzyme complexes (ECs) of different action and the Biolight lactic acid bacteria sourdough on the quality of wheat bread made with the addition of whole-grain hemp flour (WGHF). This will make it possible to expand the range of bread with high nutritional and physiological value for mass production.

To solve this goal, the following tasks were set:

– to determine the quality indicators of WGHF obtained from hemp seeds;

– to establish a rational dosage of WGHF to the recipe and study its effect on the quality of dough and bread;

– to produce wheat-hemp bread using enzyme complexes (ECs) and Biolight LAB sourdough, examine its quality, and calculate its nutritional value.

## 4. The study materials and methods

### 4. 1. The object and hypothesis of the study

The object of our study was wheat bread with WGHF.

The principal hypothesis assumes that to increase the nutritional value of wheat bread, WGHF is to be added to the recipe, and to improve its quality, ECs of various effects and the Biolight lactic acid bacteria sourdough are to be used.

The hypothesis of our study assumed that an increase in the nutritional value of wheat bread could be achieved by partially replacing its flour with WGHF. Improving the quality of such a product was planned through the use of specially selected ECs of various effects and the Biolight lactic acid bacteria sourdough.

### 4. 2. Examined materials

*Raw materials*. Our research used hemp seeds of the Hlesiia variety from the Institute of Bast Crops, the NAAS of Ukraine; wheat flour (TM Zernari); pressed baker's yeast "Lvivski Drizdzhi" (Ukraine, PrAT "Enzym Company"); salt (DP Artemsil); sugar (Sarkara-Group LLC); water.

Enzyme preparations:

– food concentrated fungal *α-Amylase* SQzyme FAL (*Aspergillus oryzae*) (EC 3.2.1.1). Activity 30,000 units/ml (China, Suntaq company);

– food *Lipase SQfiltrase*. Activity 10,000 units/g (China, Suntaq company);

– food concentrated fungal *Cellulase* Grainzyme NL (*Trichoderma reesei*). Activity 5,000 units/ml (China, Suntaq company);

– food *Transglutaminase* Tranzim-100. Activity 100 units/g (Ukraine, Enzim Biotech company).

*Sourdough*. Baker's sourdough from pure cultures Biolight LAB starter, made from wheat flour and pure cultures (PCs) of LAB species *Lactobacillus plantarum, L. rhamnosus*, *L. fermentum* and *L. brevis* at the Department of Bread Technologies and Biotransformation of Grain Products, the Institute of Food Resources at the National Academy of Agrarian Sciences in Ukraine.

*Reagents*. Sodium hydroxide (TOV NVP Alfarus, Ukraine), phenolphthalein indicator, and methylene blue indicator (Bio-Optica, Italy).

Raw materials and reagents were stored under the conditions specified by the manufacturer.

**4. 3. Research methodology and methods**

Wheat bread was made according to a recipe that included high-grade wheat flour, pressed baker's yeast, sugar, table salt, and water [22].

The dough was made using the straight dough method. The duration of dough fermentation was 100 ± 2 min. at a temperature of 32 ± 2°C until the volume increased by 1.5 times. The dough was kneaded on a KVL4100S dough mixer (China) for 15 ± 1 min. The final shaping of the dough pieces was carried out manually. The dough proofing was carried out for 35 ± 2 min. at a temperature of 35 ± 2 ºC in an XLT 133-UNOX (Italy) cabinet. The mass of the dough pieces was 300 ± 10 g. The readiness of the dough pieces during the proofing process was determined by the increase in volume. Subsequently, the dough pieces were baked at a temperature of 185 °C in an Unox XFT133 oven (Italy) for 30 ± 2 min.

Organoleptic indicators of flour quality were determined according to the methodology from [22]. Flour acidity was determined by "flour-water slurry" method in the presence of phenolphthalein indicator [23]. Granulometric composition was determined by sieving on sieves with aperture sizes of 670 μm, 420 μm, 219 μm, and 144 μm. The mass fraction of protein was determined using the Kjeltec 8200 device (Foss company, Sweden) by the Kjeldahl method [24]. Ash content was determined by the ashing (combustion) method in a muffle furnace SNOL 8.2/1100 (Lithuania) using a magnesium acetate accelerator [24]. The mass fraction of fiber was determined by the Henneberg-Stomann method according to [24]. The activity of lactic acid bacteria of the sourdough was determined according to the methodology from [22]. The titrated acidity of the sourdough and dough was determined according to the methodology from [23]. The mass fraction of moisture in the sourdough and dough was determined by express drying on a Chizhova apparatus (TOV Olis, Ukraine) [23]. The elasticity, extensibility, P/L, and specific work of deformation of the dough were determined on an NG alveograph-consistograph (Chopin, France) [23]. The content of gluten was determined mechanically using the Glutomatic GM-2200 device (Perten company, Sweden). We determined the organoleptic indicators, physicochemical indicators of the quality of the finished bread (mass fraction of moisture, acidity, specific volume, bread weight, baking shrinkage, porosity) according to [24] 4 h after baking. The water absorption capacity of the crumb and friability – according to [22]. Bread moisture was determined by the standard method of drying the sample in a SESH-3M drying oven (TOV UkrAnalytika, Ukraine) at a temperature of 130°C [24]. Porosity was investigated using a Zhuravlev device (TOV NVF Standart-M, Ukraine) [22].

To establish a rational dose of enzymes, dough was prepared based on high-grade wheat flour with the addition of 10% WGHF to the recipe. The dough was prepared according to the following recipe: flour (mixture) – 100 g, yeast – 2.5 g, table salt – 1.5 g, sugar – 2.5 g, water – 55 ml. One enzyme was added to the dough recipe in doses recommended by manufacturers, units of activity per 1 g of flour: α-Amylase – 0.10; Lipase – 0.02; Cellulase – 0.30; Transglutaminase – 0.05, kept at a temperature of 32°C for 30 min; the samples were evaluated according to the following indicators: increase in volume, recovery as a result of mechanical pressure, plasticity, stickiness. As a result, the optimal doses of each enzyme were determined, unit of activity per 1 g of flour: α-Amylase – 0.075; Lipase – 0.030; Cellulase – 0.063; Transglutaminase – 0.055.

The dose of sourdough starter to the dough recipe was 20%, which was established based on the results of previous studies.

## 5. Results of investigating the influence of enzyme complexes and Biolight sourdough on the quality of wheat-hemp bread

**5. 1. Obtaining whole-grain hemp flour and examining its quality**

To obtain WGHF, hemp seeds of the Hlesiia variety from the Institute of Bast Crops, the NAAS of Ukraine, were used. The seeds were cleaned of impurities and used for whole-grain hemp flour (WGHF).

*Whole-grain hemp flour* was obtained by grinding whole uncrushed seeds in a laboratory mill with water cooling "SM-3C" (China) and sequential sieving on sieves with a hole diameter of 670 μm, 420 μm, 219 μm, and 144 μm. The quality indicators and chemical composition of the resulting WGHF are given in Table 1.

Table 1

Quality indicators of whole-grain hemp flour ($n = 5$, $p \leq 0.05$)

| Indicator ID | High-grade wheat flour | Whole grain hemp flour |
|---|---|---|
| Moisture mass fraction, % | 12.3 ± 0.1 | 11.4 ± 0.1 |
| Protein mass fraction, % on dry matter | 11.43 ± 0.1 | 22.3 ± 0.1 |
| Oil mass fraction, % on dry matter | 2.4 ± 0.1 | 34.4 ± 0.1 |
| Carbohydrate mass fraction, % on dry matter | 81.4 ± 0.1 | 15.1 ± 0.1 |
| Flour mass fraction, % on dry matter | 1.5 ± 0.1 | 16.5 ± 0.1 |
| Ash mass fraction, % on dry matter | 0.7 ± 0.1 | 4.7 ± 0.1 |
| Acidity, degree | 3.0 ± 0.2 | 4.8 ± 0.2 |
| Metal impurities mass fraction, mg per 1 kg of flour | 3.0 ± 0.2 | 2.0 ± 0.2 |

The results of the studies given in Table 1 show that WGHF is characterized by an increased content of valuable components: proteins (22.3 ± 0.1%), oil (34.4 ± 0.1%), and fiber (16.5 ± 0.1), which indicates the prospects for its use in the manufacture of wheat bread in order to improve the nutritional, biological, and physiological value of the product [16, 17]. Our results show that WGHF has a higher acidity, compared to wheat flour – 4.8 ± 0.2 degrees versus 3.0 ± 0.2 degrees (control), which must be taken into account when devising a recipe for wheat bread with its addition.

It was established that WGHF differs in its granulometric composition from ground and high-grade wheat flour (Table 2). In particular, ground wheat flour contains 70% of particles with a size close to 200 microns, and high-grade wheat flour contains 50% of particles with sizes less than 40–50 microns. In contrast, in WGHF, the bulk of particles has sizes over 420 microns, i.e., WGHF is close in composition to whole grain flour, which contains endosperm and peripheral particles that are heterogeneous in size.

Table 2

Granulometric composition of whole-grain hemp flour ($n = 3$, $p \geq 0.95$)

| Grinding fineness | Hole size, μm | Content, % |
|---|---|---|
| Residue on the sieve | | |
| No. 067 | 670 | 8.0 ± 0.1 |
| No. 045 | 420 | 65.4 ± 0.1 |
| No. 27PA:120 | 219 | 22.0 ± 0.1 |
| Passage through sieve No. 27PA:120 | 144 | 4.6 ± 0.1 |

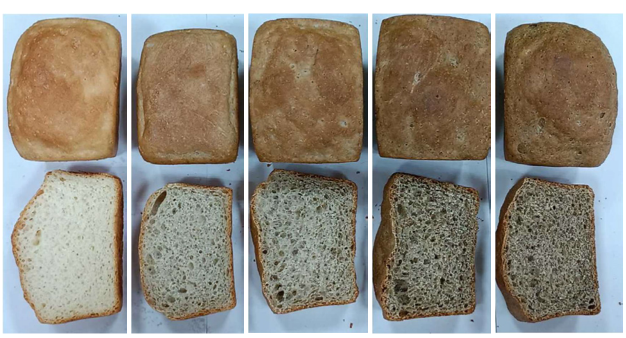
According to the organoleptic assessment, WGHF has a dark appearance and a brownish-greenish color of flour, with inclusions of crushed shells. The smell is typical of hemp flour, with a pleasant aroma of walnut, not musty, not moldy.

**5. 2. Determining hemp flour dosage and its effect on dough and bread quality**

The quantity and quality of gluten is of great importance for the formation of structural and mechanical properties of wheat flour dough. The gluten framework affects the rheological properties of dough: its plasticity, elasticity, gas-holding capacity, and dimensional stability of dough pieces during kneading and baking [25].

For the production of wheat bread with increased nutritional value, WGHF obtained from hemp seeds of the Hlesiia variety was used. WGHF was added to the recipe of wheat bread with partial replacement of wheat flour. The dosage of WGHF was selected based on the results of the literature review [21, 26]; the following ratios of WGHF to high-grade wheat flour were used: 5:95; 10:90; 20:80; 30:70. The control was wheat bread without the addition of WGHF.

As a result of baking and organoleptic evaluation of the bread, it was found that the addition of WGHF changes the color of the bread (Fig. 1) from light yellow (control) to dark brown (typical for rye) with green inclusions (at additive concentrations of 20–30%). Porosity deteriorated with increasing WGHF content and changed from fine in the control to coarse, uneven at the maximum dosage of the additive.



*a* *b* *c* *d* *e*

Fig. 1. Samples of wheat bread with the addition of whole-grain hemp flour (top view and cross-section): *a* – control; *b* – 5% whole-grain hemp flour; *c* – 10% whole-grain hemp flour; *d* – 20% whole-grain hemp flour; *e* – 30% whole-grain hemp flour

The smell also changed. At 5% WGHF, a light herbal smell was felt with barely perceptible notes of walnut. At 10%, the smell became more intense, not herbal, with a pronounced spicy aroma of walnut. Adding 20–30% WGHF gave the bread a very intense herbal smell.

The dosage of WGHF in amounts of 5–10% to the recipe did not significantly change the taste of the bread. A concentration of 20–30% of the additive made the taste oversaturated, the bread crumb became hard and rough, its elasticity deteriorated, and when chewing, a slight crunch of hemp shells was felt. That is, the rational dosage of WGHF to the recipe of wheat bread is within 5–10%, which is consistent with the results reported in [9]. For further research, the following ratio of components was used: 10% WGHF and 90% high-grade wheat flour.

The results of our determining the quantity and quality of raw gluten in the test and control dough samples are given in Table 3.

Table 3

Effect of whole-grain hemp flour on the quantity and quality of gluten in dough ($n = 5$, $p \leq 0.05$)

| Variant | Content of raw gluten, g | Elasticity, VDK device units | Plasticity | Stretchability, cm |
|---|---|---|---|---|
| Control | 25.9 ± 0.1 | 60 ± 1.0 | good | 14.5 ± 0.3 |
| WGHF 10% | 23.1 ± 0.1 | 85 ± 1.0 | good | 13.0 ± 0.3 |

Our results (Table 3) indicate that the addition of WGHF negatively affects the quality of the dough, which is also noted by other researchers [9]. As a result, a 2.8% decrease in the content of raw gluten was obtained. This is explained by the fact that hemp seeds mainly contain edestine and albumin proteins but do not have gluten proteins gliadin and glutenin. In addition, the dough became more spreading (elasticity – 85 units of VDK against 60 units of VDK in the control) and reduced its extensibility by 1.5 cm.

The effect of adding WGHF on the dough system was determined by using an NG alveograph-consistograph. The results of decoding the alveograms are given in Table 4.

Table 4

Properties of wheat dough with the addition of whole-grain hemp flour ($n = 5$, $p \leq 0.05$)

| Indicator ID | Control | WGHF 10% |
|---|---|---|
| Dough elasticity, *P*, mm | 55 | 30 |
| Dough stretchability, *L*, mm | 124 | 104 |
| *P*/*L* | 0.4 | 0,3 |
| Specific work of dough deformation, o.a. | 212 | 138 |

It was determined that the use of WGHF has a significant impact on the quality of the dough. Compared with the control, it is characterized by significantly lower elasticity (30 mm versus 55 mm) and extensibility (104 mm versus 124 mm). The elasticity to extensibility ratio (*P*/*L*) indicates significant changes in the mechanical properties of the dough with the addition of WGHF. Thus, the specific work of deformation of the dough in the experimental version was 138 o.a., which is significantly less than in the control sample (Table 4).

**5. 3. Production of wheat-hemp bread using enzymes and sourdough**

In order to improve the quality of wheat bread, various additives are introduced to its recipe [27], including HF. Such raw materials, due to their balanced chemical composition, increase its nutritional, biological, and physiological value, reduce the glycemic index, and improve hypoallergenic properties.

However, as a rule, all these additives worsen the rheological properties of the dough and, as a result, negatively affect the structure of the bread crumb, its porosity, volume, and other indicators [28].

In order to eliminate the negative impact of HF in the manufacture of wheat bread, ECs and Biolight LAB sourdough were added to the dough recipe.

The studies used enzyme the following preparations: α-Amylase, Lipase, Cellulase, and Transglutaminase, which were selected taking into account the characteristics of the chemical composition of the raw materials for bread production, their effect on the dough system, and the quality of the finished product.

Table 5 gives the substrates on which the selected enzymes act and their expected effect on the quality of bread, according to the recommendations from the manufacturers.

Table 5

Enzyme/substrate system, enzyme action, and expected effect from use

| Enzyme/substrate | Enzyme action | Expected impact |
|---|---|---|
| α-Amylase/starch | Hydrolyzes starch to dextrin and sugars | Intensification of fermentation by increasing nutrition for fermentation microflora, improving crust color, increasing volume |
| Lipase/fat | Hydrolyzes lipids, forming emulsifiers | Increased emulsification, improving texture, increasing volume, slower bread staleness |
| Cellulase /fiber | Hydrolyzes cellulose and partially hemicellulose | Reducing fiber stiffness, improving dough structure, and increasing its water absorption, loosening the dough |
| Transglutaminase/proteins | Crosslinks wheat flour proteins and WGHF proteins | Stabilization of the gluten network, improving dough structure, increasing bread volume, improving crumb texture |

Based on the expected effect of each enzyme on bread quality, three variants of their combinations were devised to improve the quality characteristics of bread (Table 6).

The effectiveness of using Biolight LAB sourdough has been proven by the results of previous studies in the technology of making wheat bread with the addition of teff flour [6], which indicates its potential for improving the quality of wheat bread with the addition of WGHF.

The characteristics of Biolight LAB sourdough are given in Table 7.

Table 7

Quality indicators of Biolight lactic acid bacteria sourdough

| Indicator | Value |
|---|---|
| Mass fraction of moisture, % | 65 ± 2.0 |
| Duration of fermentation, h | 12 |
| Final acidity, deg | 16.0 ± 1.0 |
| Activity of LAB, min. | 55–65 |

Using ECs and Biolight LAB sourdough, dough was prepared (Table 8), wheat bread was baked with the addition of 10% WGHF; its quality was assessed (Table 9, Fig. 2). Control is bread without the addition of WGHF, sourdough, and enzymes.

Evaluating the quality of wheat bread with WGHF by appearance, it can be seen that the addition of ECs and sourdough to the recipe affects the specific volume of products, porosity, color of the crust and crumb (Fig. 2). The use of sourdough and ECs stimulated an increase in the specific volume of bread from 0.1 $cm^3/g$ in the variant with sourdough, to 0.5–0.9 $cm^3/g$ in the variants with ECs (Table 9). The porosity of the experimental bread variants, compared to the control, is greater (especially in the variant with EC 1 and sourdough). The control bread sample was characterized by underdeveloped porosity, which negatively affected its quality (Fig. 2). The crust of bread in all experimental variants acquired an attractive brown color, which is explained by the increase in the amount of sugars that caramelized during baking. It should be noted that the addition of WGHF to the wheat bread recipe resulted in a darker color of the bread crumb, which is explained by the presence of a significant amount of polyphenols in hemp raw materials [18], which darken during oxidation [7]. The variant with sourdough formed a less dark crumb due to the fact that the bread had a high acid content – 3.6 degrees, which partially neutralized the course of such reactions. Variants with enzymes had similar values of this indicator – 2.4–2.8 degrees; and the control using only wheat flour only – 2.0 degrees. The bread moisture index in all variants, including the control, corresponded to optimal values.

Table 6

Enzyme complexes and their expected impact on bread quality

| Complex ID | Composition of the combination | Expected impact |
|---|---|---|
| EC 1 | Amylase + Lipase + Transglutaminase | Intensification of fermentation, increase in volume. Improvement of porosity, elasticity of dough, softness of dough structure due to emulsifying properties of lipids. Strengthening of gluten network, improvement of structure and stability of crumb |
| EC 2 | Amylase + Cellulase + Transglutaminase | Intensification of fermentation, increase in volume. Hydrolyzes of fibers, strengthening of gluten network, improvement of structure, elasticity, and stability of crumb |
| EC 3 | Amylase + Lipase + Cellulase + Transglutaminase | Complex action: partial hydrolyzing of fibers, active fermentation, strengthening of dough structure, stabilization of crumb texture, slowing down of staling |

Table 8

Dough quality indicators with the addition of whole-grain hemp flour; dough preparation parameters ($n = 3$, $p \leq 0.05$)

| Indicator | Variant | | | | |
|---|---|---|---|---|---|
| | Control | WGHF + starter | WGHF + EC 1 | WGHF + EC 2 | WGHF + EC 3 |
| Mass fraction of dough moisture, % | 41.7 ± 0.1 | 40.6 ± 0.1 | 40.6 ± 0.1 | 39.8 ± 0.1 | 40.1 ± 0.1 |
| Initial acidity of dough, deg. | 1.6 ± 0.1 | 2.6 ± 0.1 | 2.0 ± 0.1 | 1.8 ± 0.1 | 1.8 ± 0.1 |
| Final acidity of dough, deg. | 2.4 ± 0.1 | 4.2 ± 0.1 | 3.2 ± 0.1 | 2.8 ± 0.1 | 2.8 ± 0.1 |
| Lifting force, s | 55 | 50 | 53 | 52 | 56 |
| Duration of fermentation, min. | 90–100 | | | | |
| Duration of proofing, min. | 45 | 40 | 42 | 42 | 45 |

Table 9

Physicochemical quality indicators of wheat-hemp bread with the addition of ECs and Biolight LAB sourdough ($n = 5$, $p \leq 0.05$)

| Indicator | Variant | | | | |
|---|---|---|---|---|---|
| | Control | WGHF + sourdough | WGHF + EC 1 | WGHF + EC 2 | WGHF + EC 3 |
| Baking shrinkage, g/% | 12.7 ± 0.1 | 14.0 ± 0.1 | 14.6 ± 0.1 | 15.3 ± 0.1 | 15.3 ± 0.1 |
| Acidity, degrees | 2.0 ± 0.1 | 3.6 ± 0.1 | 2.8 ± 0.1 | 2.4 ± 0.1 | 2.4 ± 0.1 |
| Humidity, % | 40.8 ± 0.1 | 39.5 ± 0.1 | 39.8 ± 0.1 | 38.7 ± 0.1 | 39.0 ± 0.1 |
| Porosity, % | 69.0 ± 1 | 77.0 ± 1 | 80.0 ± 1 | 74.0 ± 1 | 73.0 ± 1 |
| Specific volume of bread, $cm^3/g$ | 2.29 ± 0.1 | 2.4 ± 0.1 | 3.2 ± 0.1 | 3.15 ± 0.1 | 2.83 ± 0.1 |
| Crumbly texture of bread after 72 hours, % | 9.2 ± 0.1 | 7.1 ± 0.1 | 8.5 ± 0.1 | 7.8 ± 0.1 | 9.0 ± 0.1 |
| Crumb water absorption capacity after 72 hours, % of DM | 277.9 ± 0.2 | 325.0 ± 0.2 | 383.7 ± 0.2 | 413.9 ± 0.2 | 401.7 ± 0.2 |

*a* *b* *c* *d* *e*

Fig. 2. Bread samples in the cross-section: *a* – control; *b* – whole-grain hemp flour + sourdough; *c* – whole-grain hemp flour + enzyme complex 1; *d* – whole-grain hemp flour + enzyme complex 2; *e* – whole-grain hemp flour + enzyme complex 3

The use of ECs and sourdough positively affected the freshness of the bread, ensuring its softness and attractive texture for a longer period of time, as evidenced by the indicators of water absorption capacity and bread crumbly in the experimental variants. The best water absorption capacity at the level of 413.9–401.7% was obtained in the variants with EC 2 and EC 3, where *Cellulase* was used. It breaks down cellulose and hemicellulose with the formation of oligosaccharides, which have a high hydrophilicity and actively bind water. Sourdough and EC 1, which break down the above-mentioned biopolymers worse, form a lower water absorption capacity, although in all experimental variants this indicator prevails over the control. The variant with sourdough provided the lowest bread crumbly – 7.1%, while the control sample provided the highest – 9.2%, and the variants with ECs – 7.8–9.0%.

The characteristics of the organoleptic properties of finished bread with WGHF and the addition of ECs or sourdough are given in Table 10.

Organoleptic assessment of the quality of wheat-hemp bread shows that the use of ECs and Biolight LAB starter sourdough positively affects the color of the crust, taste, aroma, chewiness. In general, according to the set of indicators, our technology enables the production of wheat-hemp bread with high organoleptic properties.

Calculations of the nutritional value according to the integral score of wheat-hemp bread for people of group I of labor intensity aged 40–59 years were carried out – scientists, workers of mainly mental labor, light physical activity. When consuming 277 g of such bread (daily norm), the need for protein is covered by 32.0% for men and 40.0% for women, in fats and fiber by 5.0 and 14.0%, respectively. It is worth noting that the starch content in this recipe is reduced by 10.1%, which helps reduce its glycemic index.

Table 10

Organoleptic quality indicators of wheat-hemp bread with the addition of ECs and Biolight LAB starter sourdough

| Indicator ID | Control | WGHF + starter | WGHF + EC 1 | WGHF + EC 2 | WGHF + EC 3 |
|---|---|---|---|---|---|
| Appearance | The shape is regular, the surface is smooth, without cracks | | | | |
| Crust color | Light yellow | Yellow-brown with spots | Light brown with a slight greenish tint | | |
| Crumb condition | White in color, without signs of underkneading. Elastic, flexible, does not wrinkle. Porosity is small, dense, not developed | Light gray color, without signs of underkneading. Elastic, flexible, does not wrinkle. Porosity is small, developed | Light gray color, developed, without signs of underkneading. Elastic, flexible, does not wrinkle. Porosity is medium, developed | | |
| Taste and aroma | Pleasant, inherent in wheat bread | Pleasant, inherent in wheat bread. Taste with a slight sourness. In the aroma there are notes of walnut | Pleasant, inherent in wheat bread. In the aroma there are notes of walnut | | |
| Crumb chewiness | Chews well, does not clump | | | | |

## 6. Discussion of results based on the influence of enzyme complexes and Biolight sourdough on the quality of wheat-hemp bread

The basis of most bakery products is high-grade wheat flour, which consists of starch, a small amount of high-quality proteins, and is characterized by a low content of vitamins, minerals, and dietary fiber [29]. Therefore, such products, although characterized by high organoleptic properties, require adjustment to improve their nutritional, physiological, and biological value. The optimal technique is considered to be the partial replacement of wheat flour with valuable plant raw materials [4, 5]. Among the large number of additives, HF is distinguished as one of the most promising for bakery products [16, 17], which has a high content of proteins, unsaturated fatty acids, dietary fiber, and minerals.

A comparative analysis of WGHF and premium wheat flour reveals that it significantly exceeds the latter in nutritional value as it has twice the protein content and 10 times the oil content (Table 1). WGHF is characterized by a reduced carbohydrate content – 15.1% versus 81.4% in wheat flour, which when used together make it possible to reduce the glycemic index of the finished bread and its calorie content. The acidity value of WGHF, 4.8 degrees, is slightly higher than that of wheat flour (3.0 degrees), which must be taken into account when devising a bread recipe with its addition. WGHF, compared to wheat flour, contains eleven times more fiber and nine times more ash (Table 1). This can negatively affect the structure of the dough, its quality, and the quality of wheat-hemp bread, which is also noted by researchers in a number of papers [7, 9, 16, 20]. Therefore, various additives are used to reduce their negative impact [6, 10, 11, 28].

Sieve analysis of WGHF (Table 2) revealed that it is close to ground wheat flour in terms of the ratio of coarse to fine particles since the former contains 73.4% coarse particles, and the latter – 70%. At the same time, coarse particles in WGHF are twice as large, and small ones are heterogeneous in size and vary from 140 microns to 219 microns. The larger size, heterogeneity of particles, and high fiber content in WGHF will worsen the course of the technological process and the quality of wheat-hemp bread, which must be taken into account in the technology of its manufacture.

Organoleptic evaluation of WGHF showed that it is significantly inferior to high-grade wheat flour. It has a dark, brownish-greenish color with inclusions of crushed shells.

All these shortcomings, regarding color, uneven particle grinding, and the presence of high fiber content (Table 1), will negatively affect both the organoleptic characteristics of wheat-hemp bread and the technology of its production, which requires improvement.

The smell of WGHF was characteristic of hemp flour, with a pleasant aroma of walnut, which will positively affect the aroma of bread, giving it original, new shades, as other authors also note [9].

The amount of WGHF added to the wheat dough recipe ranged from 5 to 30%. The control was wheat bread without the addition of WGHF. The optimal dosage of WGHF in the bread recipe was determined based on the results of organoleptic evaluation of the bread. It was found that the addition of WGHF to the recipe affects the change in bread color: from light yellow (control) to dark brown with green spots (at concentrations of 20–30%) (Fig. 1). The porosity of the bread at a dosage of more than 10% WGHF deteriorated to coarse and uneven.

The aroma of the bread also changed depending on the dosage of WGHF. At 5%, a light herbal smell with hints of walnut was felt. Increasing the dose to 10% contributed to an increase in its intensity, without herbal, with a spicy walnut aroma, and a dose of 20–30% – the appearance of an intense herbal smell.

The taste of bread with a dosage of WGHF in amounts of 5–10% to the recipe did not change significantly, compared to the control. The concentration of WGHF in amounts of 20–30% had a negative effect on perception: the crumb became hard and rough, its elasticity deteriorated, and when chewing, a slight crunch of hemp shells was felt. Based on our studies, the optimal dosage of WGHF to the recipe of wheat bread was established as 10%, which is consistent with the literature [9].

Studies on the effect of WGHF on the quantity and quality of raw gluten indicate a significant effect of the additive on these indicators (Table 3), which is also noted by other researchers [9]. Adding WGHF to the dough recipe contributed to a 2.8% decrease in the quantity of raw gluten and a deterioration in its quality. This is a consequence of the partial replacement of gluten proteins of wheat flour gliadin and glutenin, which are the basis of raw gluten, with hemp proteins – edestine and albumin. Although the latter have high digestibility, value, and increase the total protein content, they do not participate in the formation of the gluten framework. Therefore, the dough with the addition of WGHF became spreading (elasticity – 85 units of VDK versus 60 units of VDK in the control) and reduced its extensibility by 1.5 cm.

The study of the rheological properties of the dough showed that the variant with WGHF has 45.5% less elasticity compared to the control. This indicates a deterioration in the physical properties of the dough, in particular, its ability to restore its shape during mechanical deformations. The extensibility of the dough with WGHF also deteriorated by 16.1%, which indicates a decrease in its ability to stretch without breaking. Analysis of the elasticity to extensibility ratio ($P/L$) reveals significant changes in the mechanical properties of the dough. The specific work of deformation of the dough decreased by 34.9% compared to the control, which indicates a decrease in the total energy required for dough deformation and weakening of its structural and mechanical framework. Similar patterns of deterioration in the rheological properties of the dough were obtained when adding another valuable additive to the wheat bread recipe – teff flour [6].

The use of valuable plant raw materials, including WGHF, contributes to the increase of nutritional, biological, and physiological value of wheat bread, but negatively affects the dough system and the quality of the finished product [28]. Therefore, to reduce their negative impact, various additives are used, and elements of the manufacturing technology are improved [6, 10, 11]. In studies for this purpose, various ECs and Biolight LAB starter sourdough were used. The use of Biolight LAB starter sourdough for the manufacture of wheat bread with the addition of teff flour contributed to the intensification of biochemical, microbiological processes in the dough. This accelerated its ripening, reduced the duration of dough kneading by 5.4% and enabled the production of high-quality bread [6]. Our results confirmed the effectiveness of using Biolight LAB starter sourdough also in the technology of wheat-hemp bread.

For the research, enzyme complexes with $\alpha$-Amylase, Lipase, Cellulase, and Transglutaminase were devised, which were selected based on the characteristics of the chemical composition of the raw materials (Table 1), the expected effect of the enzymes on the dough system and the quality of the bread (Table 6).

The assessment of the bread obtained using ECs and Biolight LAB starter sourdough (Fig. 2 and Table 9) indicates their positive effect on its quality as it contributes to an increase in the specific volume of the products and an improvement in the color of the bread crust. The increase in the specific volume of the bread ranged from 4.3% in the variant with sourdough to 21.7–39.1% when using different ECs, which is explained by their strong hydrolytic effect on flour biopolymers. The substances that are formed in this process are a nutrient medium for fermentation microflora, which improves the rheological properties of the dough and contributes to the formation of a large volume of bread [30].

WGHF in the wheat bread recipe affects the formation of a darker-colored bread crumb, which is explained by the presence of a significant amount of polyphenols in hemp [18], which darken during oxidation [7]. At the same time, the variant with sourdough has a less dark crumb (Fig. 2) due to the higher acid content (3.6 degrees versus 2.4–2.8 degrees in variants with enzymes), which partially neutralized the course of such reactions (Table 9).

The use of enzymes and sourdough affected the porosity of the bread. This is especially true for the variant with EC 1, where the porosity is 80%, which indicates significant destruction of raw material biopolymers and the formation of "airy" bread.

The use of selected additives positively affected the freshness of the bread and its preservation, as evidenced by the indicators of bread crumbly and water absorption capacity. The variant with sourdough bread had the best bread crumbly. It was lower compared to the control by 29.6% and by 9.9–26.8% in variants with enzymes. EC 2 and EC 3 contributed to obtaining the highest water absorption capacity of bread – 413.9–401.7%. This can be explained by the presence of *Cellulase* in their composition, which breaks down cellulose and hemicellulose biopolymers to oligosaccharides, which are able to bind and retain a large amount of water due to their hydrophilic properties. Other variants (sourdough and EC 1) are less capable of the hydrolytic action of the above-mentioned biopolymers and therefore bread using them has a lower water absorption capacity (325.0–383.7%). However, in all experimental variants this indicator exceeded that of the control.

Organoleptic evaluation of bread with WGHF confirmed its high indicators (Table 10). The use of both ECs and sourdough contributed to the formation of a pleasant, characteristic wheat bread taste (with a slight sourness in the version with sourdough) and the appearance of piquant notes of "walnut" in the aroma.

It should be noted that our research is limited to the use of WGHF only, while there are many other products of hemp seed processing that can be used in bread baking. When using other raw hemp materials, the results of the research may differ from those reported in this paper.

Further research should involve the use of other products of hemp seed processing and the search for ways to reduce the negative impact of such additives on the quality of bread (shape, volume, porosity, color, etc.).

## 7. Conclusions

1. We have established that WGHF is characterized by a balanced chemical composition as it contains a large amount of valuable proteins (22.3%), vegetable oils (34.4%), and dietary fiber (16.5%). Therefore, WGHF is a promising additive for bakery products based on wheat flour of higher grades to improve their nutritional, biological, and physiological value. It has a higher acidity (4.8 versus 3.0 degrees), ash content (4.7 versus 0.7%), larger particle size, uneven distribution of their size and high content of dietary fiber (16.5 versus 1.5%), compared to wheat flour, which must be taken into account when devising the recipe and technology for making wheat-hemp bread.

2. It was determined that when using WGHF in the recipe of wheat bread, the rational dosage is 5–10%, as it contributes to the formation of bread with high volume, good porosity, with good taste and aroma with spicy walnut shades. However, WGHF slightly worsens the structural and mechanical properties of the dough: it reduces the content of raw gluten, its elasticity, extensibility, and specific work of deformation.

3. The results of our study on the use of ECs and Biolight LAB starter sourdough in the technology of wheat-hemp bread showed their high efficiency in improving its quality. The use of these additives contributed to an increase in the specific volume of bread from 4.3% to 39.1%, improved the color of the crust, and prolonged its freshness, as evidenced by a decrease in the bread crumbly index by 9.9–29.6%, compared to the control. A slowdown in bread staling was observed, as indicated by an increase in the water absorption capacity from 16.9 to 48.9%.

## Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study, as well as the results reported in this paper.

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## Data availability

All data are available, either in numerical or graphical form, in the main text of the manuscript.

## Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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