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DETERMINING THE EFFICACY OF USING WHEAT GERM MEAL AS AN ACTIVATOR OF FERMENTATION MICROFLORA FOR FERMENTED BEVERAGES

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This study investigates the process of fermenting apple and plum juices using kvass yeast and lactic acid bacteria, with modification of the medium with wheat germ meal. The task addressed relates to the insufficient intensity of fermentation and instability of the functioning of the microbial system at temperature fluctuations. This limits the efficiency of fermentation processes and requires using energy-intensive physical intensification methods.

It was established that the introduction of 5–7% of meal provides an increase in yeast biomass by 245–350%, an increase in the specific growth rate to 116%, and a reduction in the duration of cell generation by 48–53% compared to the control. At a temperature of 15°C, growth rates exceeded the control by 4–8 times, and at 4°C, the viability of the population increased by 6–7 times. At the same time, activation of lactic acid bacteria Lactobacillus plantarum was recorded. The discoloration of methylene blue in plum must with the addition of 3–7% wheat germ meal is accelerated by 38.5–100%, and in apple must by 33.3–177.8%, respectively, compared to the control. This indicates the formation of a synergistic fermentation ecosystem and is positive for defining the taste and aroma profile of beverages.

The results are attributed to the complex metabolic action of the meal, which optimizes the carbohydrate profile of the medium, eliminates catabolic limitations, provides cells with trace elements, and increases their resistance to temperature stress. It has been shown that nutrient modification of the medium makes it possible to achieve an increase in kinetic parameters of up to 400%, which exceeds the effectiveness of conventional physical intensification methods.

The results could be used in the production of non-alcoholic fermented beverages of functional purpose and the introduction of resource-saving technologies in the food industry

Keywords: soft drinks, sourdough yeast, lactobacilli, wheat germ meal, maltose

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1. Introduction

The spread of foodborne diseases has become a global problem. This is a prerequisite for the growth of demand for natural products with functional properties. There is also a popularization of fermented beverages and prebiotics. The advantage of fermented beverages over blended beverages is their positive effect on the intestinal microflora. It is fermented beverages that provide normalization of the entire digestive system because fermentation fortifies the finished product with probiotics, organic acids, and B vitamins. This, in turn, makes it possible to support immunity, detoxify the body, and improve overall well-being. Thus, according to studies conducted by the World Health Organization (WHO), the occurrence of diseases of the gastrointestinal tract and cardiovascular system is reduced with regular consumption of fermented products [1].

It is also known that the substances that are best absorbed in the human body are those that come in a liquid state, that is, with drinks. Given this, and the fact that trends are increasingly developing to promote a healthy lifestyle and reduce alcohol consumption, it is important to design new, beneficial for the human body, non-alcoholic fermented drinks. In this case, it should be noted that world farmers grow a significant number of garden crops, including apples and plums [2], which indicates the high potential of these fruits for use in the production of natural fermented drinks. However, a significant problem is the complexity of the fermentation process. The process can be slow, is long-lasting, and the resulting drinks have a short shelf life, which requires finding ways to improve fermentation in order to reduce the duration of the process and stabilize the resulting drink.

As an activator of fermentation microflora, it is proposed to consider the possibility of using wheat germ meal (WGM), which is a by-product from obtaining the corresponding oil by CO_2 extraction. Germ meal contains a wide range of vitamins and minerals that serve as additional nutrition for fermentation microflora. In addition, WGM has active amylolytic and proteolytic enzymes [3], due to which nutrients will be more easily released from the main raw material into the must, their availability for fermentation microflora will increase, and, as a result, the fermentation process will be accelerated. In this regard, scientific research aimed at finding ways to activate fermentation microflora for the production of fermented beverages by modifying the fermenting medium with wheat germ meal is relevant. In addition, this will make it possible to reduce the duration of the technological process without changing the equipment of enterprises.

The research reported here is of scientific interest for the complex processing of raw materials, reducing the number of by-products from oil production. This will make it possible to achieve sustainable development goals and, along with this, will have a positive impact on the fermentation microflora, as well as will make it possible to accelerate the technological process of producing fermented beverages. This, in turn, will increase the environmental friendliness and profitability of production, which is extremely important at present.

2. Literature review and problem statement

The increasing interest of people in a healthy lifestyle and consumption of natural products draws attention to fermented beverages. Among fermented products, the category of beverages represents a growing sector of the industry. This is due to the fact that consumers who care about their health position them as refreshing, convenient, and useful products. Thanks to the consumption of such beverages, well-being improves, and the risks of chronic and degenerative diseases are reduced [4]. The growth of demand contributes to the search for new solutions and improvement of the technological process in order to accelerate it and expand the range of such products. According to [4], it is proposed to reduce the technological process of producing fermented beverages through the newest equipment. The promising use of ohmic heating, moderate or pulsed electric fields, ultrasound, and high hydraulic pressure for this purpose has been proven. At the same time, the described solutions indicate the insufficient effectiveness of existing approaches to the intensification of fermentation processes, in particular at low temperatures. This is due to the limited metabolic activity of microorganisms and the need to use energy-intensive physical methods.

According to data from [5], apricots, plums, and cherries are subjected to fermentation in order to obtain fruit wines. At the same time, the high content of phenolic compounds in the listed raw materials and significant antioxidant potential make it possible to obtain high-quality fruit wines with functional properties. However, it should be noted that the above technology uses the process of alcoholic fermentation, which occurs with the introduction of the yeast strain *S. cerevisiae* and accumulates more than 1.2% vol. ethyl alcohol. This is the norm for fruit wines but will not satisfy the needs of consumers who strive for a healthy lifestyle and refuse alcohol. Therefore, research is promising to find technological solutions for obtaining fermented beverages with an alcohol content of up to 1.2% vol. Such beverages will be classified as non-alcoholic and, at the same time, have the full range of beneficial substances of fermented products.

In study [6], it was found that the use of secondary products of milk processing and cherry laurel using matsoni starter makes it possible to obtain a high-quality fermented milk drink. However, taking into account the prevalence of allergies to animal proteins and lactose, as well as the growing interest of people in veganism, the consumption of whey-based drinks will not be advisable. This issue is proposed to be solved using water-soluble pistachio extracts as a substrate for fermentation with kefir starters [7]. In order to activate the fermentation, sucrose, a mixture of cocoa and honey, pumpkin seed protein, and matcha were used. According to the data provided in [7], a mixture of sucrose, cocoa, and honey, as well as pumpkin seed protein, had a positive effect on the growth and development of kefir starter, while the introduction of matcha suppressed the process due to the significant content of catechins. Therefore, the composition of the raw materials used in the production of fermented drinks significantly affects the course of the technological process due to the effect on the fermentation microflora. However, such drinks contain vitamins and minerals that are only found in cereals. This narrows the spectrum of positive effects on the body and requires improvement. It is possible to expand the positive effect by using plant raw materials in the production of drinks.

One of the most common non-alcoholic fermented drinks is kombucha. Kombucha is made by fermenting black or green tea infusion using a symbiotic culture of bacteria and yeast [8]. Study [8] confirms the feasibility of aerating tea infusion during fermentation to obtain a positive organoleptic profile of the finished drink. In addition, it was found in [9] that high water hardness negatively affects the fermentation of must in the production of kombucha and worsens the organoleptic quality indicators of the drink. This requires additional water treatment with clinoptilolite, rock crystal, or activated carbon. Studies [8, 9] indicate the relevance of improving the technological process for the production of fermented drinks. However, the issue of shortening the technological process remains unresolved.

In [10], microbial metabolism and its functional role in fermentation were studied by analyzing the next-generation and meta-omics technologies. All of the above contributes to the reduction of the technological process and reduces energy consumption. However, the implementation of such solutions requires complete re-equipment of enterprises with the installation of expensive equipment. Therefore, it is advisable to consider the possibility of using fermentation activators of natural origin, which will accelerate fermentation without changing the technical equipment by directly interacting with the fermentation microflora. For this purpose, it is advisable to consider the possibility of using natural raw materials with a rich chemical composition, which will make it possible to activate the fermentation microflora during the fermentation of the beverage. Analysis of the data from [11] gives reason to assume that such raw materials will be wheat germ meal, since its addition to rye-wheat dough contributed to the improvement of the indicators of zymase and maltase activity of baker's yeast. The introduction of WGM also increased the activity of lactic acid bacteria in rye-wheat dough, and the alcoholic fermentation of the dough intensified by 39.0%. All of the above is an indicator of the positive effect of WGM on the fermentation microflora in the production of rye-wheat bread and is a prerequisite for reducing the technological process. Thus, it can be assumed that the proposed germ meal will have a similar positive effect on the fermentation microflora in the production of fermented beverages.

Considering the data above, it can be stated that existing approaches to the intensification of fermentation processes do not provide a simultaneous increase in the activity of the fermentation microflora and the stability of its functioning at low temperatures without the use of energy-intensive physical methods. This necessitates the search for effective ways of metabolic modification of the medium, which predetermines the feasibility of research in this area. The results will make it possible to shorten the technological process of producing high-quality fermented beverages without significant re-equipment of enterprises.

3. The aim and objectives of the study

The aim of our study is to determine the effectiveness of using wheat germ meal as an activator of fermentation microflora in the production of fermented beverages. This will make it possible to reduce the duration of the technological process, expand the range of functional beverages, and carry out production in accordance with the concepts of sustainable development.

To achieve this goal, the following tasks were set:

– to investigate the physicochemical quality indicators of raw materials that will be used for the production of fermented beverages;

– to determine the effect of wheat germ meal and diﬁ ferent temperatures on the fermentation microflora of fermented beverages.

4. The study materials and methods

4. 1. The object and hypothesis of the study

The object of our study is the fermentation process of apple and plum juices using kvass yeast *KVS-standart* and lactic acid bacteria *Lactobacillus plantarum* with modification of the medium with wheat germ meal.

The hypothesis of the study assumes that modification of the fermentation medium with wheat germ meal would contribute to increasing the activity of fermentative microflora and intensification of the fermentation process due to additional intake of nutrients.

The assumption adopted in the work is that the chemical composition of wheat germ meal, in particular the content of vitamins, minerals, and enzymes, can affect the metabolic activity of yeast and lactic acid bacteria in the fermentation medium.

The simplification accepted in the work is that the effect of wheat germ meal on the fermentation process is determined mainly by its nutrient composition without taking into account possible adverse interactions with raw material components and metabolic products of microorganisms.

4. 2. Methods for studying the quality indicators of raw materials to be used for the production of fermented beverages

The studies investigated plum and apple juice in accordance with DSTU 9125:2021. Drinking water complied with SanPiN 2.2.4-171-10 and DSTU 7525:2014, and wheat germ meal – with TUU 10.8-32062796-003:2008 developed and approved by TOV Zhytomyrbioproduct. Sourdough yeast *KVS-standart* and lactic acid bacteria *Lactobacillus plantarum* complied with the manufacturer's quality certificates. In the production of yeast and lactic acid bacteria, the international standards DSTU ISO 9001, DSTU ISO 22000 (HACCP), and DSTU ISO 14001 were taken into account.

The primary task for studying the activity of fermentative microflora is to investigate the medium used for fermentation. It is the composition of plum and apple juices that will have a significant impact on the fermentation process. In this regard, at the first stage of research, the physicochemical quality indicators of juices used as a basis for beverages were determined. Thus, titrated and active acidity, as well as the content of dry substances in juices, were determined according to generally accepted methodologies for fruits, vegetables, and products made from them [12]. The acidity of wheat germ meal was determined according to AOAS 943.02,981.12, moisture content – AACCI 44–15. The viscosity of juices was studied according to the methodology given in [13]. The determination of reducing sugars in juices was carried out by the Bertrand method [14], in wheat germ meal – by the Schorl iodometric method [15].

4. 3. Investigating the activity of fermentation mii croflora with the addition of wheat germ meal

In the production of non-alcoholic fermented beverages, the combination of lactic acid and alcoholic fermentation is important. The combination of these two types of fermentation is the reason for obtaining beverages with a pronounced taste and aroma profile, low alcohol content, and probiotic properties [16]. Therefore, the activity of fermentation microflora is important in the production of such beverages. Not only does the duration of the technological process depend on this, but also the formation of substances that affect the taste, aroma of the beverage, and form its functionality. That is why, at the next stage of research, the effect of WGM on the activity of fermentation microflora was investigated.

The effect of germ meal on the activity of lactic acid bacteria Lactobacillus plantarum was determined according to the methodology given in [11]. The effect on the physiological state of the kvass yeast *KVS-standart* was determined by microscopy at ×40 magnification with a ×10 or ×15 eyepiece in six fields of view. Microscopy was performed immediately after the yeast was added and every 24 hours thereafter using the "crushed drop" method. The age characteristics of the yeast were determined by adding a drop of Lugol's solution to a slide with the preparation [17]. In order to determine the effect of temperature on the fermentation process, the study was conducted at 4°C, 15°C, and 30°C. After that, the average gross and specific reproduction rates were calculated from formula (1)

$$X = X_{max} - X_0, \quad (1)$$

where $\boldsymbol{X}_{max}$ is the maximum number of yeasts during the stationary phase, million/g;

$\boldsymbol{X}_0$ is the initial number of yeasts, million/g.

The next step was to determine the average gross reproduction rate (V), which was calculated from formula (2)

$$V = \frac{X_t - X_0}{t - t_0}, \quad (2)$$

where X_t is the number of yeast at the end of the growth process, million/g;

X_0 is the number of yeast at the beginning of the growth process, million/g;

($t - t_0$) is the difference in the time of cultivation, hours;

t_0 is the beginning of the lag phase, hours.

The measure of the growth rate of cells in the exponential phase, or in other words, the specific growth rate (μ) was calculated as follows (3)

$$\mu = \frac{\lg X_t - \lg X_0}{\lg e\left(t - t_0\right)} = \frac{\ln X_t - \ln X_0}{t - t_0}, \quad (3)$$

where lg e = 0.43429

Therefore, the yeast growth rate (r) can be calculated as follows (4)

$$r = \frac{1}{t_d}, \quad (4)$$

where t_d is the average duration of cell generation, that is, doubling their number or mass.

4. 4. Statistical treatment of research results

The error for all studies was σ = 3–5%, the number of parallel experiments was n = 5, and the probability, $P \geq 0.95$. Experimental data were processed statistically using the Fisher-Student method at a reliability level of 0.95. The results were calculated as the arithmetic mean of at least five experiments. The MS Office 2016 application package, including MS Excel (USA), was used to process experimental data.

5. Results of determining the qualitative characteristics of raw materials and the activity of fermentation microflora for the production of beverages

5. 1. Research on quality indicators of raw materials used as a basis for fermentation

The quality indicators of the medium that will ferment are of great importance for the fermentation process. The results of research on physicochemical quality indicators of apple, plum juices, and wheat germ meal are given in Table 1.

According to the data in Table 1, it is possible to judge the possibility of creating a favorable medium for fermentation microflora by using apple and plum juices as a basis for fermentation. However, it is important to understand not only the quantitative characteristics of the indicators but also the qualitative ones as the composition of the sugars in the raw materials plays an important role in shaping the quality of fermented beverages. In addition, these values directly affect the fermentation microflora and, as a result, the course of the fermentation technological process. Therefore, for a more complete assessment of the medium, the qualitative composition of sugars was determined (Table 2).

Table 1

Physical-chemical quality indicators of raw materials (n = 5, $P \geq 0.95$, σ = 3–5%)

Indicator	Raw materials and indicator values		
	Apple juice	Plum juice	Wheat germ meal
Titrated acidity, deg	4.2	4.6	6.0
Active acidity (pH)	3.3	3.5	5.5
Dry solids content, %	12.0	17.0	–
Moisture mass fraction, %	–	–	12.4
Viscosity, MPa×s	1.8	3.2	–

Table 2

Qualitative composition of sugars in experimental raw materials (n = 5, $P \geq 0.95$, σ = 3–5%)

Sugar	Content in raw materials, g/100 g		
	Apple juice	Plum juice	Wheat germ meal
Fructose	5.8	3.1	23.2
Glucose	1.6	4.7	20.7
Saccharose	1.4	0.1	21.3
Maltose	0.003	–	33.7

According to the data given in Table 2, wheat germ meal has significantly more fructose, glucose, and sucrose in its composition. This serves as confirmation of the assumption regarding the possibility of using the proposed meal as an activator of fermentation microflora in the production of fermented beverages. An important factor in the fermentation process is the ratio of sugars, which determines the intensity of fermentation and the composition of metabolic products formed during the fermentation process. It has also been established that wheat germ meal contains a significant amount of maltose, which, as is known, is a fermentation sugar [18]. This feature of the proposed additive can help avoid fermentation attenuation. All of the above requires experimental verification. Therefore, the next stage was to study the effect of wheat germ meal on the fermentation microflora used in the production of fermented beverages.

5. 2. Results of determining the influence of wheat germ meal on fermentation microflora

An important component of the technological process of producing fermented beverages is lactic acid fermentation. It is this type of fermentation that is the biotechnological basis of production, as it ensures the formation of a characteristic taste and aroma of the finished beverage. Therefore, determining the influence of WGM on the activity of lactic acid bacteria is an important task. The results of the determination are shown in Fig. 1.

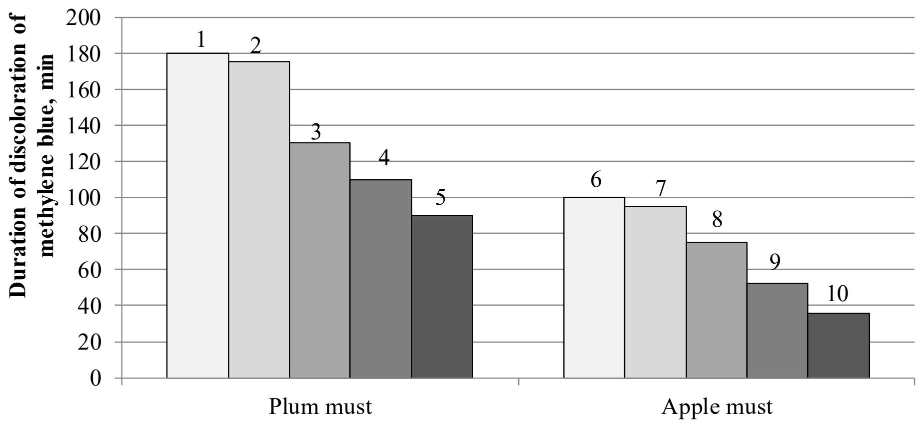
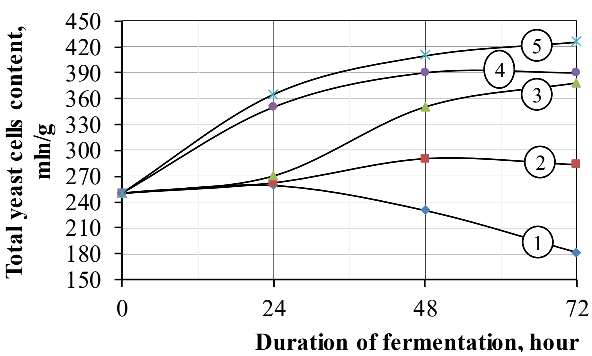
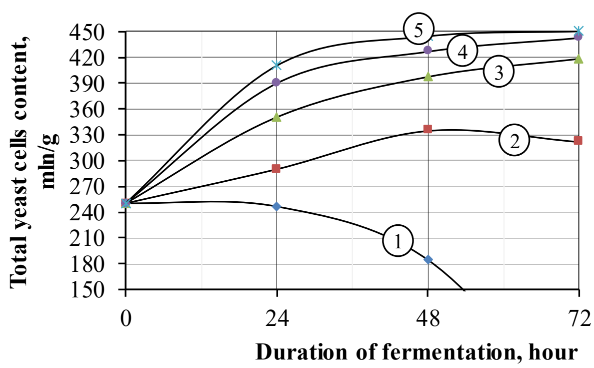


Fig. 1. Determining the lactic acid bacteria activity ($n = 5$, $P \geq 0.95$, $\sigma = 3–5\%$): 1 – plum must without adding meal (control), with the addition of wheat germ meal: 2 – 1%; 3 – 3%; 4 – 5%; 5 – 7%; 6 – apple must without adding meal (control); with the addition of wheat germ meal: 7 – 1%; 8 – 3%; 9 – 5%; 10 – 7%

According to the data illustrated in Fig. 1, the introduction of WGM contributes to an increase in the activity of lactic acid bacteria, as evidenced by a more intense discoloration of methylene blue in the test samples. This effect of the meal is characteristic of both the fermentation of plum must and apple must. The results correlate with the values of the sugar content in the raw materials (Table 2) and indicate the possibility of accelerating lactic acid fermentation during the production of fermented beverages.



a



b

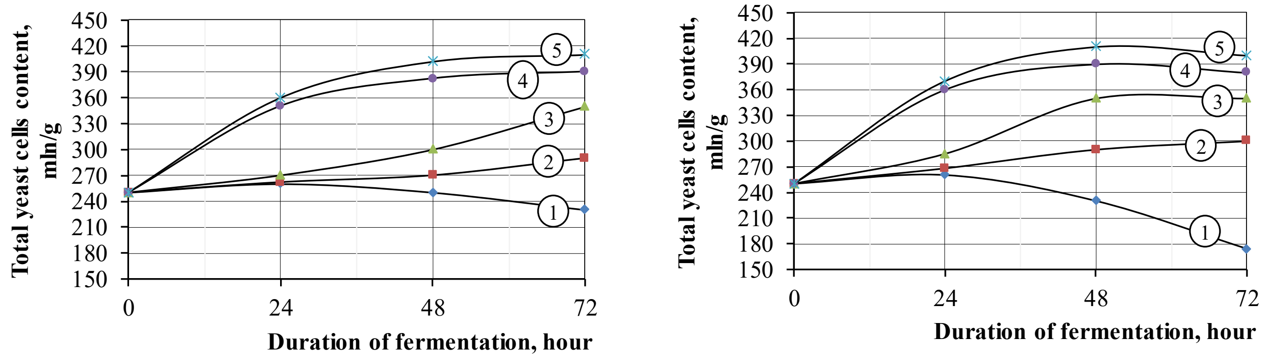
Fig. 2. Effect of wheat germ meal on the reproduction of kvass yeast depending on the medium and fermentation temperature ($n = 5$, $P \geq 0.95$, $\sigma = 3–5\%$): *a* – plum must, fermentation temperature 30°C; *b* – apple must, fermentation temperature 30°C; 1 – control (without the addition of meal); with the addition of meal: 2 – 1%; 3 – 3%; 4 – 5%; 5 – 7%

It should be noted that in the production of fermented beverages, along with lactic acid, alcoholic fermentation is of great importance [19]. It is the combination of lactic acid and alcoholic fermentation that is the key factor in the formation of characteristic soft drinks with high-quality indicators and substances useful for the human body. Therefore, at the next stage of research, the effect of germ meal on the activity of *KVS-standart* kvass yeast was analyzed. The results are shown in Fig. 2–4, *a, b*.

According to the data illustrated in Fig. 2, 3, *a*, *b*, in plum must at temperatures of 30°C and 15°C, fermentation occurred less intensively compared to apple. The process in both samples began to subside after 24–48 h, while the yeast did not gain the necessary biomass to form substances characteristic of fermented beverages. The introduction of WGM even in the smallest dosage (1%) allowed us to intensify the process and prevent the attenuation of fermentation in both plum and apple must. At the same time, the fermentation temperature of 4°C significantly suppressed the fermentation activity of yeast regardless of the fermenting medium. However, according to the data illustrated in Fig. 4, *a*, *b*, it was established that the death of the culture with the introduction of meal occurs more slowly, and, with its maximum amount (7%) after 24 h, even slight cell budding is observed. This indicates a positive effect of the introduction of WGM on kvass yeast and indicates the possibility of reducing the duration of the technological process and avoiding possible defects due to temperature fluctuations.

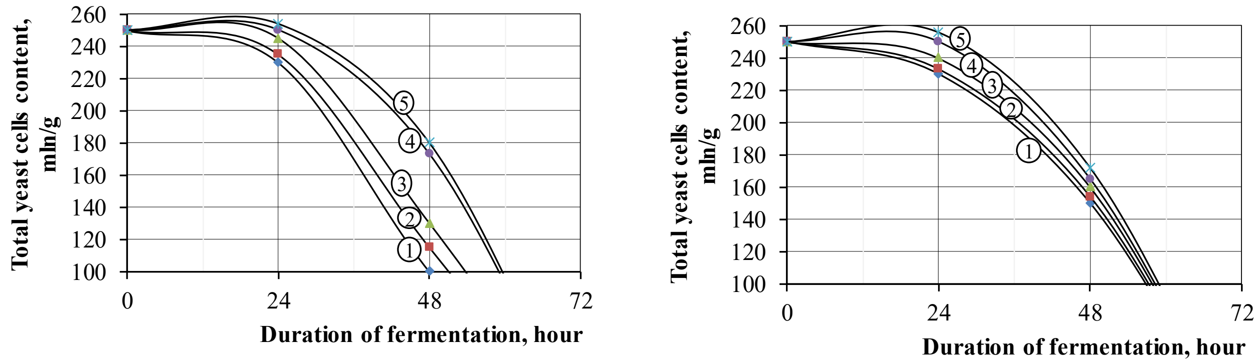
In order to study the possibility of shortening the technological process, the fermentation kinetics were calculated using formulas (1) to (4) and based on the results of studying the accumulation of biomass (Fig. 2–4, *a*, *b*). The results are given in Tables 3, 4.

The data in Tables 3, 4 show that at a temperature of 30°C and the addition of 5% of WGM, the maximum values of yeast biomass, specific growth rate, and minimum values of cell generation duration are observed compared to other studied regimes for both plum and apple must. Under the given conditions, increased values of fermentation kinetic parameters are recorded, which characterize a more intensive course of the process compared to the control and other variants of the experiment. At other temperatures and meal concentrations, the indicators of yeast biomass and specific growth rate have lower values or are characterized by less pronounced positive dynamics.



a *b*

Fig. 3. Effect of wheat germ meal on kvass yeast reproduction depending on the medium and fermentation temperature ($n = 5$, $P \geq 0.95$, $\sigma = 3–5\%$): *a* — plum must, fermentation temperature 30°C; *b* — apple must, fermentation temperature 15°C; 1 — control (without meal addition); with meal addition: 2 — 1%; 3 — 3%; 4 — 5%; 5 — 7%



a *b*

Fig. 4. Effect of wheat germ meal on the reproduction of kvass yeast depending on the medium and fermentation temperature ($n = 5$, $P \geq 0.95$, $\sigma = 3–5\%$): *a* — plum must, fermentation temperature 4°C; *b* — apple must, fermentation temperature 4°C; 1 — control (without the addition of meal); with the addition of meal: 2 — 1%; 3 — 3%; 4 — 5%; 5 — 7%

Table 3

Fermentation efficiency criteria of the kvass yeast *KVS-standart* for plum must

Growth parameter	Parameter value in test samples				
	Control	WGM introduced, %			
		1	3	5	7
At a temperature of 30°C					
Biomass of kvass yeast, million/g	40	90	160	180	185
Average gross reproduction rate	0.6	1.2	2.5	3.0	3.2
Specific growth rate, h^{-1}	0.006	0.009	0.012	0.013	0.014
Average duration of cell generation	150	100	75	70	70
Yeast growth rate, h	0.006	0.009	0.010	0.012	0.013
At a temperature of 15°C					
Biomass of kvass yeast, million/g	20	60	120	150	160
Average gross reproduction rate	–0.4	0.5	1.2	2.0	2.7
Specific growth rate, h^{-1}	–0.001	0.005	0.008	0.008	0.008
Average duration of cell generation	–690	138	86.3	86.3	86.3
Yeast growth rate, h	–0.001	0.007	0.01	0.01	0.01
At a temperature of 4°C					
Biomass of kvass yeast, million/g	5	15	25	30	35
Average gross reproduction rate	–0.5	0.1	0.2	0.3	0.4
Specific growth rate, h^{-1}	–0.001	0.002	0.004	0.005	0.006
Average duration of cell generation	–600	300	200	180	160
Yeast growth rate, h	–0.001	0.002	0.004	0.005	0.006

Table 4

Fermentation efficiency criteria of *KVS-standart* kvass yeast for apple must

Growth parameter	Parameter value in test samples				
	Control	WGM introduced, %			
		1	3	5	7
At a temperature of 30°C					
Biomass of kvass yeast, million/g	55	110	170	190	195
Average gross reproduction rate	1.0	1.8	2.8	3.3	3.5
Specific growth rate, h^{-1}	0.008	0.011	0.014	0.015	0.015
Average duration of cell generation	130	110	72	68	68
Yeast growth rate, h	0.006	0.009	0.010	0.012	0.013
At a temperature of 15°C					
Biomass of kvass yeast, million/g	30	80	140	160	170
Average gross reproduction rate	–0.2	0.7	1.5	2.3	2.8
Specific growth rate, h^{-1}	0.001	0.006	0.009	0.01	0.01
Average duration of cell generation	–400	120	85	86	75
Yeast growth rate, h	0.001	0.006	0.009	0.01	0.01
At a temperature of 4°C					
Biomass of kvass yeast, million/g	8	25	35	45	50
Average gross reproduction rate	–0.3	0.2	0.4	0.6	0.7
Specific growth rate, h^{-1}	–0.001	0.003	0.005	0.006	0.006
Average duration of cell generation	–550	280	200	170	150
Yeast growth rate, h	–0.001	0.003	0.005	0.005	0.005

6. Discussion of results based on investigating the activation of fermentation microflora by adding wheat germ meal

Analysis of the results from our studies reveals that the effect of activating fermentation microflora in the production of non-alcoholic fermented beverages is systemic. This is indicated by the values of the physicochemical properties of the medium, its carbohydrate profile, and nutrient modification by adding wheat germ meal. In addition, the temperature regime of the fermentation process has a significant impact. Thus, the physicochemical characteristics of apple and plum juices (Table 1) create generally favorable conditions for the development of both yeast and lactic acid bacteria since their pH is within 3.3–3.5. However, it is necessary to take into account different values of dry matter and viscosity (Table 1). These indicators are of fundamental importance for the kinetics of microbial growth. The increased viscosity of plum must potentially limits the mass transfer of substrates and oxygen, which can reduce the rate of diffusion of nutrients to cells. A similar effect of the rheological characteristics of the medium on microbial growth is described in [20], in which a slowdown in the metabolic activity of microorganisms in a medium with increased viscosity was demonstrated. This is consistent with the lower biomass values in the control plum must samples compared to apple must at the same temperature (Tables 3, 4) and the slower reproduction of yeast cells (Fig. 2–4, *a, b*).

When analyzing fermentation processes, it is important to note that the key factor for their successful progress is the qualitative composition of sugars in the medium. This directly determines the further dynamics of fermentation. It was established (Table 2) that fructose prevails in apple juice (5.8 g/100 g), while glucose dominates in plum juice (4.7 g/100 g). It is known that *Saccharomyces cerevisiae* yeast exhibits glucose repression and sequential use of carbohydrates, which determines the phase structure of growth [21, 22]. According to our research results (Table 2), WGM contains significantly higher concentrations of all major sugars, which radically changes the trophic profile of the medium. The presence of maltose as a "fermentation sugar" (33.7 g/100 g) creates a prolonged source of energy after the depletion of monosaccharides. This explains the avoidance of fermentation attenuation in the experimental samples (Fig. 2–4, *a*, *b*) and is logically consistent with the kinetic parameters given in Tables 3, 4.

Considering the research data, it should be noted that the most indicative evidence of the feasibility of using germ meal to activate fermentation is the growth kinetics of kvass yeast (Tables 3, 4). The increase in yeast biomass in plum must at 30°C and with the addition of 5% WGM was 3.5 times greater compared to the control sample under the same conditions, and in apple must, 2.45 times. At the same time, the average gross reproduction rate increased by 230–400%, the specific growth rate – by 87–116%, and the duration of cell generation was reduced by 48–53%. Such changes cannot be explained only by the additional supply of carbohydrates. Our results indicate an acceleration of the cell cycle with the introduction of additional nutrition. At the same time, the importance of the influence of the temperature factor cannot be avoided since it plays a decisive role in the fermentation process. Thus, at 30°C, which corresponds to the physiological optimum of *Saccharomyces cerevisiae*, a reduction in the period of cell adaptation was observed in the lag phase. This can be explained by a significant amount of macro- and microelements in the meal, which, in this case, act as cofactors of enzyme systems. In the exponential phase, an increased cell growth rate was established (Fig. 2, *a*, *b*, Tables 3, 4). This indicates an intensification of glycolysis and ATP synthesis, which is confirmed by the data reported in [21]. When analyzing the experimental data, it is important that the scale of biomass growth in wort samples with the introduction of meal exceeds the effect of the temperature optimum. This indicates a synergy between the optimal temperature and the improved nutrient medium.

Given the decisive role of temperature in the regulation of yeast metabolism, it is important to study the ability of yeast to maintain fermentation activity at low temperatures. Therefore, the next stage of the study was to investigate the fermentation activity of the kvass yeast *KVS-standart* at a temperature of 15°C. It should be noted that in control samples of plum and apple must at this temperature, a sharp slowdown in growth and prolongation of the lag phase were recorded (Fig. 3, *a, b*). This is typical of suboptimal temperatures when membrane fluidity and enzyme activity decrease [22]. The addition of WGM allowed us to transfer the indicators of gross and specific growth rate to the positive region (Tables 3, 4) and increase yeast biomass by 4–8 times (Fig. 3, *a, b*, Tables 3, 4). This indicates that the additional B vitamins, trace elements [11], and readily available sugars (Table 2) introduced with the meal compensate for the temperature-induced energy deficit.

Particularly indicative results indicating the feasibility of using WGM as a fermentation activator are the values for the reproduction of kvass yeast and fermentation kinetics at a temperature of 4°C (Fig. 4, *a*, *b*, Tables 3, 4). At the same time, in the control samples of plum and apple musts, the yeast biomass decreased to 5–8 million/g, which corresponds to the state of cold stress. However, when 7% WGM was introduced into both types of musts, the biomass increased by 6–7 times compared to the control sample. It is known that at low temperatures, yeasts rearrange the lipid composition of membranes and activate protective mechanisms [23]. The additional supply of nutrients and minerals increases the stability of membranes and ribosomes, reducing the effects of cold stress [24]. Thus, WGM performs not only the role of an activator but also a protective function, expanding the temperature range of stable fermentation.

Special attention was paid to the relationship between temperature and rheological characteristics of the medium, which is also a fundamental factor in the fermentation process. Thus, it was found that control samples of plum must at lower temperatures were characterized by a pronounced inhibition of the growth of kvass yeast biomass (Fig. 3, *a*, *b*). This is directly related to the increased viscosity of plum must (Table 1), which increases with decreasing temperature and, thus, increases diffusion limitations. The addition of WGM compensated for this effect by increasing the concentration of soluble substrates and stabilizing cellular metabolism.

In addition to the importance of yeast activation and alcoholic fermentation in the technological process of producing non-alcoholic fermented beverages, lactic acid bacteria and their activity are of great importance. According to research data (Fig. 1), the introduction of wheat germ meal promotes the activation of lactic acid bacteria *Lactobacillus plantarum*, as indicated by the faster discoloration of methylene blue in the experimental samples. Activation occurs even with a minimum addition of meal (1%) by 2.8% in plum must and by 5.3% in apple must; however, significant differences are observed with the addition of WGM in the amount of 3–7%. Thus, the discoloration of methylene blue in plum must is accelerated by 38.5–100% compared to the control, and in apple must, by 33.3–177.8%, respectively. The obtained data on the activation of lactic acid bacteria using germ meal correlate with data on the effect of non-conventional plant raw materials on lactic acid bacteria in other systems [11, 25, 26], which confirmed the positive effect of such raw materials on lactic acid fermentation. Since it is known that lactic acid bacteria require external sources of amino acids and vitamins [10], the positive effect of WGM on this group of microorganisms is obvious. In turn, this creates conditions for the synergy of lactic acid bacteria with yeast. Thus, the system moves from a competitive to a cooperative model of interaction of microorganisms [19]. This is very important for shaping the quality of non-alcoholic fermented beverages.

Our studies prove the effectiveness of using WGM as an activator of fermentation microflora in the production of fermented soft drinks. Comparing the proposed method with physical methods of intensification, such as pulsed fields, ohmic heating, and ultrasound [4], one can clearly see the fundamental difference in approaches. If instrumental methods mostly accelerate mass transfer, providing an increase in biomass by 20–60% [4], then in our study, an increase in kinetic parameters of up to 400% was achieved. This indicates that nutrient modification of the medium can be more effective than physical intensification. Such an effect may be associated with the effect of vitamin-mineral and protein complexes directly on the metabolic nodes of cells.

Our work demonstrates that wheat germ meal functions as a multicomponent metabolic regulator. The introduction of such an additive simultaneously optimizes the trophic structure, increases the stress resistance of microflora, and stabilizes microbial interaction. This approach opens up prospects for creating adaptive fermentation systems with an expanded temperature range and increased biotechnological efficiency.

However, the results have drawbacks that should be taken into account. First, our study did not investigate the effect of wheat germ meal on the quality indicators of the finished beverage and its stability during storage. This issue is important since the significant content of protein substances in the meal, along with the demonstrated activating effect on microflora, could potentially cause the formation of protein-polyphenol complexes and colloidal turbidity. In turn, this would significantly affect the consumer characteristics and presentation of the product. The lack of such data currently does not make it possible to fully assess the technological suitability of the proposed solution. Second, the analysis of volatile aromatic compounds present in wheat germ meal, as well as those formed or transformed during fermentation, was not performed. The interaction of meal components with the fruit juice matrix can significantly modify the metabolic pathways of yeast and lactic acid bacteria, affecting the formation of esters, higher alcohols, and organic acids, which determine the sensory profile of the beverage. Without studying these processes, it is impossible to draw comprehensive conclusions about the organoleptic attractiveness and competitiveness of the product.

Thus, further research is necessary not only to expand theoretical ideas about the mechanisms of action of wheat germ meal in fermentation systems but also for full technological validation of the proposed approach. A comprehensive assessment of colloidal stability, sensory profile, dynamics of changes during storage and reproducibility of results under laboratory and industrial conditions will minimize technological risks. It will also be possible to predict the behavior of the product throughout its life cycle and ensure its compliance with regulatory requirements and consumer expectations. In addition, a detailed analysis of the aromatic profile and stability of the system will create a basis for optimizing the formulation and controlled formation of organoleptic characteristics.

It is the integration of microbiological, physicochemical, sensory, and technological parameters that will make it possible to move from laboratory proof of effectiveness to a scalable, economically viable, and competitive solution. In this context, the outlined directions constitute a prospect for further study on the above systems and represent further vectors for advancing our work.

7. Conclusions

1. As a result of our study on the physicochemical parameaters of apple and plum juices, it was found that both types of raw materials are characterized by active acidity values favorable for the development of fermentative microflora (pH 3.3–3.5). They differ in the content of dry matter (12% and 17%, respectively), viscosity (1.8 and 3.2 MPa×s), and the qualitative composition of sugars. The predominance of fructose in apple juice and glucose in plum juice forms different conditions for the catabolite regulation of yeast. This affects the duration of the lag phase and the intensity of exponential growth. It was found that the increased viscosity of plum must potentially limits the mass transfer of substrates. This partially explains the lower biomass indicators in the control samples. Thus, the qualitative and quantitative composition of the raw materials determines the initial metabolic configuration of the fermentation system and the degree of its sensitivity to technological modifications.

2. It was established that the introduction of wheat germ meal in the amount of 5–7% in combination with the optimal temperature regime (30°C) provides a significant intensification of the development of fermentation microflora. At the same time, the biomass of kvass yeast increases by 245–350%, the specific growth rate – up to 116%, and the generation duration is reduced by almost 50% compared to the control. At suboptimal temperatures (15°C), the meal compensates for metabolic depression, increasing growth rates by 4–8 times. At 4°C, it performs a protective function, increasing the viability of population by 6–7 times. In parallel, activation of lactic acid bacteria was recorded. In plum must, when wheat germ meal is added in an amount of 3–7%, their activity increases by 38.5–100% compared to the control sample, and in apple must, by 33.3–177.8%. This indicates the formation of synergistic microbial interaction. Unlike conventional physical intensification methods, the obtained effect is of a metabolic nature and is due to the optimization of the carbohydrate profile of the medium, the removal of catabolite limitations, and the increase in the resistance of cells to temperature stress. This confirms the feasibility of using wheat germ meal as an effective biotechnological modifier of the fermentation process.

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 and the results reported in this paper.

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Data availability

All data are available in digital or graphical form in the main text of the manuscript.

Use of artificial intelligence

Tool used: ChatGPT (OpenAI, GPT-4 model).

Scope: entire paper, section 6, graphic annotation.

Using AI tools, the grammar in the paper was checked. A search was performed for literary sources that would strengthen the evidence base of the data obtained as a result of the research. A graphic annotation was visualized from the tables and figures given in the work.

Additional literary sources for section 6 were checked for reality, analyzed independently by the authors, and conclusions were drawn that the data reported in them really correlate or explain the data obtained during the research.

The use of AI tools did not in any way affect the results and conclusions described in the work. Thanks to the found literary data, the evidence base was only strengthened during the discussion.

Authors' contributions

Nadiia Lapytska: Conceptualization; Methodology; Investigation; Writing – original draft; Writing – review & editing; Supervision; **Oleksii Shkliaiev:** Resources, Data Curation, Visualization, Project administration; Anna Novik: Formal analysis, Data Curation, Writing – original draft; **Galyna Stepankova:** Writing – original draft, Writing – review & editing, Visualization; **Yuliia Voronina-Tuzovskykh:** Methodology, Investigation, Data Curation; **Nataliia Yasna:** Investigation, Writing – original draft.

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