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ASSESSING THE INFLUENCE OF LACTIC ACID FERMENTATION ON THE BIOCHEMICAL INDICATORS OF PLANT SUBSTRATES AND SUBSTANTIATING THE RATIONAL PROCESS DURATION

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This study investigates the lactic acid fermentation process involving plant substrates of various natures (spelt, flax seeds, kelp, oyster mushrooms) with the participation of Lactiplantibacillus plantarum. The task addressed relates to the insufficient certainty of the patterns in biochemical transformations in plant substrates of various natures in the lactic acid fermentation process. This complicates their targeted selection and use as fermented ingredients in food products.

Fermentation was carried out at a temperature of 32 ± 2°C for 24 hours with sampling every 6 hours. It was established that the pH dynamics have a phase character: the most intensive decrease occurs in the interval of 0–6 hours. The total pH decrease was 33.0% for spelt, 28.5% for oyster mushrooms, 20.2% for flax, and 12.2% for kelp. The average rate of pH decrease over 24 hours was from –0.088 units/h (spelt) to –0.034 units/h (kelp).

An increase in titrated acidity was found in all substrates, the most intense for spelt and oyster mushrooms: 9.35 and 9.5 times, respectively, with average rates of 0.042 and 0.040 g/100 g/h. For flax and kelp, the increase was lower – 0.48 and 0.28 g/100 g, respectively. A strong inverse correlation was found between pH and titrated acidity (r = –0.98 ± 0.03). The decrease in soluble carbohydrates was 46.2% in oyster mushrooms and 42.3% in spelt, while in kelp and flax – 6.7% and 13.8%. The content of soluble protein increased by 58% in kelp, 50% in flax, 43% in spelt and 33% in oyster mushrooms in the absence of significant changes in total protein.

It was found that after 18 hours of fermentation, the rate of change of all indicators is significantly reduced. It was substantiated that the rational duration of fermentation is 18 hours, which enables a balanced acid profile and effective transformation of the carbohydrate-protein complex without excessive acidification

Keywords: lactic acid fermentation, plant substrates, biochemical indicators of Lactiplantibacillus plantarum, fermentation duration

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1. Introduction

Fermentation of plant raw materials is one of the promising areas of modern food technology. The use of lactic acid bacteria makes it possible to change the composition and properties of plant substrates. The fermentation process results in the formation of organic acids, a decrease in the pH of the medium, partial hydrolysis of proteins and

carbohydrates, as well as the formation of new functional and technological properties of the product. Recent studies show that fermentation also improves many characteristics of food products that affect health, but mechanistic evidence is lacking [1].

Lactic acid fermentation is widely used to improve the nutritional value and digestibility of plant raw materials. As a result of biochemical transformations, the bioavailability of nutrients increases, the organoleptic properties and microbiological stability of products improve [2]. Plant substrates of various nature are of particular interest, in particular cereals, oilseeds, seaweeds, and edible mushrooms.

The efficiency of the fermentation process largely depends on the duration of its implementation. Thus, with a duration of lactic acid fermentation of less than 12 hours, the biotransformation of substrate components may be incomplete. On the other hand, prolonging fermentation to 48–72 hours may be accompanied by excessive accumulation of acids and destruction of the structure of the raw material.

Therefore, the issue of determining the dynamics of the main biochemical indicators in the lactic acid fermentation process acquires important scientific significance. Solving this issue is most relevant for plant substrates of different biochemical nature. This is due to the fact that it is the nature and depth of biochemical transformations that form the functional and technological potential of fermented raw materials. From this point of view, determining the patterns of the course of lactic acid fermentation in different plant systems is important both for the formation of theoretical ideas about the mechanisms behind this process and for further scientific substantiation of the use of fermented substrates in the production of food.

2. Literature review and problem statement

Plant substrates of various biochemical nature are increasingly considered as raw materials for lactic acid fermentation as this process makes it possible not only to preserve the material but also purposefully change its composition, acidity, degree of hydrolysis of macromolecules, as well as functional and technological properties. For grain, seed, algae, and fungal substrates, fermentation has different intensity due to differences in carbohydrate profile, availability of nutrients for lactic acid bacteria, buffer capacity, and structure of cell walls. That is why the choice of rational duration of the process should be based not on a single indicator but on a set of kinetic characteristics [3]. However, the cited paper is based on the analysis of lactic acid fermentation of the most common grain and pseudo-grain products by studying the microbiological and biochemical foundations of the process while other plant substrates were not considered.

Spelt (*Triticum spelta L.*) attracts attention as an ancient grain crop with a high content of protein, dietary fiber, minerals, and biologically active components compared to many modern varieties of soft wheat. Spelt flour and whole grain products have shown good functional and technological properties, which makes this raw material promising for the creation of combined food systems. At the same time, like other grain substrates, spelt is well amenable to lactic acid fermentation, during which a decrease in pH, an increase in titrated acidity, carbohydrate transformation and partial modification of the protein-polysaccharide complex occur. It is noted in papers [4, 5] that for cereal and pseudocereal systems fermentation is an effective way to increase nutritional value and improve sensory properties; however, for specific crops, in particular spelt, the issue of rational duration of the process is not yet sufficiently specified.

Flaxseed (*Linum usitatissimum L.*) is a special substrate due to its high content of lipids, proteins, mucilaginous polysaccharides, lignans, and insoluble dietary fiber [6]. The cited paper focuses on the technical and functional properties of individual components of flaxseed in food products, but lactic acid fermentation of flaxseed substrate was not investigated. It is the flax mucilage that has pronounced water-retaining, thickening, emulsifying, and structure-forming properties, which makes flax a valuable functional ingredient [7]. However, this very feature complicates the fermentation process as the high viscosity of the medium, significant moisture-binding capacity, and the presence of antimicrobially active compounds can affect the availability of substrates for lactic acid bacteria and the rate of acid formation. At the same time, it was shown in [8] that fermentation of flax products can improve antioxidant properties, change the protein and phenolic profile, reduce the content of undesirable compounds, and increase water retention capacity. It can also be used for dietary purposes for encapsulation of live probiotics or bioactive compounds or as a prebiotic agent. However, no data are provided on the study of the kinetics of the fermentation process.

The above indicates the feasibility of using flax in fermented systems but requires precise control over the process duration.

Laminaria (*Laminaria spp.*, in particular *L. digitata/L. japonica*) is a valuable marine raw material, rich in minerals, iodine-containing compounds, polyphenols and specific polysaccharides, primarily alginates, laminarin, and fucoidan. Due to this composition, kelp has significant functional potential, but its use in food technologies is limited by a pronounced marine taste, specific odor, high ash content, and complexity of the cell wall. Studies [9] show that fermentation of brown algae is a promising way to improve sensory characteristics, increase the bioavailability of components and form new metabolites. At the same time, the authors emphasize that the course of fermentation of seaweed significantly depends on the ability of microorganisms to assimilate mannitol and kelp oligosaccharides, as well as on the preliminary preparation of the substrate. Therefore, for kelp it is especially important to determine the duration of the process at which a sufficient level of biotransformation is achieved without excessive destruction of the structural matrix [9]. A decrease in pH to 3.8 in 7 days has been established, but the optimal duration of fermentation has not been substantiated.

In [10], most attention was paid to the development of fermented beverages from brown algae, the comparison of different cultures of microorganisms and the technological potential of substrates, while the temporal changes in biochemical parameters of algal raw materials remained only partially revealed. The authors directly indicate the need for further optimization in terms of strains, conditions, duration, and preliminary preparation of biomass.

The authors of [11] found that the fermentation of *Laminaria digitata* is accompanied by changes in the metabolome, the accumulation of organic acids, and the formation of valuable metabolites. However, the rational duration of the process according to biochemical criteria has not been substantiated.

Oyster mushroom (*Pleurotus ostreatus*) is considered a promising mushroom ingredient due to the content of pro-

tein, dietary fiber, β-glucans, chitin, minerals, and compounds with antioxidant activity. Unlike grain or seed substrates, oyster mushrooms contain fewer readily available sugars, but have a pronounced protein-polysaccharide complex, which makes them interesting from the point of view of controlled proteolysis and changes in water-binding properties during fermentation. In [12], it was shown that *Pleurotus ostreatus* has an important technological value as a source of structure-forming components, as well as a raw material with high functional value. However, the work does not provide data on the fermentation of oyster mushroom fruiting bodies substrate. Some studies indicate that fermentation treatment with the participation of *P. ostreatus* or using fungal systems significantly changes the protein composition, amino acid profile, and functional properties of ingredients [13]. However, data on the kinetics of lactic acid fermentation of oyster mushrooms as an independent plant-fungal substrate are currently insufficient, which emphasizes the scientific novelty of such studies.

The practical aspect of integrating plant components into meat products is reflected in work [14], in which the use of spelt flour and mushrooms in the minced mass made it possible to improve organoleptic indicators, reduce calorie content, and extend the shelf life of cooked sausage products. However, such studies are not accompanied by kinetic justification of the parameters of the preliminary fermentation preparation of raw materials, which limits the reproducibility of the functional effect.

Our review of the literature [4, 6, 9, 12] shows that spelt, flax, kelp, and oyster mushrooms differ significantly in the content of available carbohydrates, the nature of nitrogenous substances, the structure of cell walls, and water-binding properties. It is these differences that determine the substrate-specific course of lactic acid fermentation. Cereal systems are usually characterized by quite intense acid formation; for flax, mucous polysaccharides and high viscosity play an important role; for kelp, the limiting factor is the specificity of algal carbohydrates; for oyster mushrooms, the complex protein-chitin-glucan matrix. However, in most papers, these substrates are studied separately, and the assessment of the process is often limited only to pH or total acidity. Therefore, the use of a complex analysis that combines indicators of acid accumulation, carbohydrate metabolism, proteolysis is logical and necessary to substantiate the rational duration of fermentation.

Thus, the results of previous studies confirm the prospects of spelt, flax seeds, kelp, and oyster mushrooms as raw materials for fermentation modification [3]. At the same time, for these substrates there is no unified justification for the rational duration of lactic acid fermentation based on a complex of interconnected kinetic indicators. This justifies the need to apply a comprehensive analysis to the assessment of the process in order to determine the moment when the system reaches a conditionally steady state and forms stable functional and technological properties.

The above allows us to state that it is advisable to conduct a study on the kinetics of lactic acid fermentation of various plant substrates (spelt, flax, kelp, oyster mushrooms) to determine the rational duration of the process based on a set of biochemical indicators.

3. The aim and objectives of the study

The aim of our study is to determine the patterns of changes in biochemical parameters of plant substrates during lactic acid fermentation with the participation of *Lactiplantibacillus plantarum* and to justify the rational process duration. This will make it possible to establish the patterns of biochemical and functional-technological changes, justify the optimal duration of fermentation, and manage the quality of fermented plant products.

To achieve this goal, the following tasks are set:

– to establish the dynamics of changes in acidity parameters during fermentation of plant substrates of different chemical nature;

– to investigate the dynamics of soluble carbohydrates as the main substrate for lactic acid bacteria;

– to assess changes in protein complex parameters during fermentation;

4. The study materials and methods

The object of our research is the process of lactic acid fermentation of plant substrates of various nature, in particular spelt grains, flax seeds, kelp, and oyster mushroom fruiting bodies, with the participation of *Lactiplantibacillus plantarum*.

The principal hypothesis assumes that due to the metabolic activity of lactic acid bacteria during fermentation, the acidity of the medium increases, which initiates changes in the carbohydrate and protein complexes of plant substrates. Such changes significantly affect their functional and technological properties.

Adopted assumption: the study assumes that the starting concentration of the inoculum and the temperature regime are constant factors in the course of the lactic acid fermentation process. The influence of accompanying microflora is minimized by preliminary heat treatment of the raw materials.

Preparation of substrates for fermentation was carried out taking into account their physicochemical characteristics and the need to ensure optimal conditions for the development of lactic acid microflora.

Spelt and flax seeds were sorted, cleaned of impurities, and washed. For the purpose of surface sanitation, the grains were rinsed with a 0.5% solution of citric acid and dried to a surface-dry state. Then the seeds of both crops were crushed in a crusher to a fraction of 0.5–1 mm, after which an aqueous suspension was prepared. In this case, 3 parts of water were added to 1 part of crushed spelt seeds, and 8 parts of water were added to 1 part of crushed dry flax, thoroughly mixed until a homogeneous, and for flax – viscous dispersed mass.

Dried kelp was pre-hydrated in water for 35 ± 5 min. The ratio of kelp to water was 1:10. Then, in order to reduce excess salts and equalize the mineral profile of the substrate, the rehydrated kelp was soaked in water in two cycles with a duration of 10–15 min each. After the end of the second soaking cycle, the kelp was squeezed to a moisture content of 80–85% and homogenized to a puree-like state. Next, an aqueous dispersion system was prepared with a kelp to water ratio of 1:5.

Oyster mushroom fruit bodies were sorted by quality, specimens with signs of spoilage were removed, and mechanical impurities were removed. The prepared raw material was quickly washed with running water and dried to a surface-dry state, preventing an uncontrolled increase in humidity. Then they were ground to a homogeneous minced mass with a particle size of 3...5 mm. To prevent excessive

wateriness and the formation of an acidity gradient during the fermentation process, the ratio of crushed mushroom mass and water was taken at 1:0.3, respectively. This ensured the formation of a stable dispersed system without the need for further adjustment of humidity.

In order to destroy random foreign microflora and provide optimal conditions for the development of lactic acid bacteria, the prepared plant substrates were pasteurized. The pasteurization regime parameters were selected taking into account the characteristics of the substrates: the dispersed mass of spelt was pasteurized at 90 ± 2°C with a holding time of 12…15 min., flax seeds – 85…90°C, 8…10 min., kelp – at 80…85°C, 5 min., oyster mushrooms – 85…90°C for 5…7 min. After pasteurization, all substrates were rapidly cooled to the microorganism inoculation temperature of 32 ± 2°C.

Fermentation of the obtained dispersed systems of all plant substrates was performed with pure cultures of lactic acid bacteria of the species *Lactiplantibacillus plantarum* at a concentration of not less than $1 \cdot 10^7$ CFU/g. After adding the starter culture, the mixture was homogenized for 3 min at a centrifuge rotor shaft rotation speed of 3000 rpm. Fermentation was carried out in sealed containers filled to 70–80% of the volume under conditions of limited oxygen access at a temperature of 32 ± 2°C. The duration of fermentation was 24 hours. Sampling for determining biochemical parameters was carried out before the start of fermentation and every 6 hours during fermentation.

We determined the studied parameters according to standard methodologies.

Active acidity (pH) was determined by the potentiometric method using a Hanna Instruments pH meter (USA). The method is based on measuring the potential difference between the measuring electrode and the reference electrode immersed in the sample under study. To determine the titrated acidity, a titrimetric method was used, which is based on the neutralization of organic acids contained in the test samples with a 0.1 N alkali solution [15]. Titrations were performed until the solution transitioned from an acidic to an alkaline medium, which was visually recorded by the appearance of a pink color of the solution in the presence of the phenolphthalein indicator. The results of the content of titrated acids in lactic acid fermentation are expressed in terms of lactic acid.

The mass fraction of water-soluble carbohydrates in plant substrates was determined by photocolorimetry, based on the formation of colored substances due to the interaction of carbohydrates with anthrone reagent in an acidic environment [16].

Analysis of protein-nitrogen indicators included measurement of total and soluble nitrogen. Total nitrogen was determined by the Kjeldahl method: the sample was oxidized in concentrated sulfuric acid with a catalyst, alkalized, ammonia was steam distilled, captured with acid, titrated and the protein content was calculated by the conversion factor. The soluble nitrogen content was determined after extracting the sample with a buffer solution and its subsequent centrifugation. The nitrogen content in the resulting supernatant was determined by the Kjeldahl method. The mass fraction of soluble nitrogen was determined as the ratio of the supernatant nitrogen to the total nitrogen of the initial sample and was expressed in percent with subsequent conversion to protein by the appropriate coefficients. When converting the content of total and soluble nitrogen to protein, conversion factors specific to each substrate were used: for spelt substrate – 5.7, for flax substrate – 5.3, for kelp substrate – 5.0, for oyster substrate – 4.38 [17].

Methods of variational statistics were used to process experimental data. For each substrate and analyzed indicator, at least 5 independent measurements were performed ($n = 5$). The results are represented as the mean value and standard deviation ($M_{ean} \pm SD$). The significance of the results was assessed at the standard confidence level $\alpha = 0.05$. The mathematical description of changes in the studied indicators in dynamics was performed using regression analysis. The type of approximation model was selected individually for each research indicator, based on the best agreement with the obtained experimental data. The quality of the approximation was assessed using the coefficient of determination R^2. Calculations and construction of mathematical relationships were performed using Microsoft Excel 365 (Microsoft Corporation, USA).

5. Results of investigating patterns of changes in the biochemical indicators of plant substrates during lactic acid fermentation

5. 1. Studying the dynamics of acidity indicators during fermentation of plant substrates

The pH value quantitatively characterizes the active acidity of the medium and therefore can be used as the main criterion in analyzing the intensity of the course of lactic acid fermentation of plant substrates. The change in acidity is directly related to the metabolic activity of lactic acid bacteria and determines the conditions of structural and chemical transformations of biopolymers.

The dynamics of pH (Fig. 1) for all studied substrates are characterized by a sequential change in the rate of decrease. In this case, an intensive decrease in the indicator was established at the initial stage of fermentation, and a further gradual slowdown during the subsequent stages of the process. This nature of the obtained dependences indicates the phase dynamics of the course of fermentation and visualizes the change in the conditions of the functioning of lactic acid microflora over time.

The results of our regression analysis reveal that the mathematical model that most accurately describes the dynamics of the experimental data is a second-order polynomial. The values of the coefficient of determination were $R^2 > 0.99$, which states almost complete correspondence of the models to the actual pH change for all plant substrates during fermentation. The mathematical models built make it possible to predict the course of the fermentation process and determine the onset of the most intense changes or, conversely, the approach of the substrate to stabilization.

Among the substrates, spelt showed the greatest decrease. Over 24 hours of fermentation, the pH level of this substrate decreased by 33% compared to the initial value (Fig. 1). The maximum rate of decrease was observed at the initial stage – in the interval of 0–6 h (Table 1).

In the next 6 hours of fermentation, the rate of acid decrease was somewhat lower than at the beginning of the process but still remained at a high level. Subsequent periods of fermentation were characterized by a sharp decrease in pH – to 0.048 and 0.030 units/h in the intervals of 12–18 and 18–24 hours, respectively (Table 1). Thus, we can state that after 18 hours of fermentation, the rate of increase in active acidity of the substrate becomes minimal, which indicates the inhibition of the metabolic activity of microorganisms and the beginning of the stabilization phase.

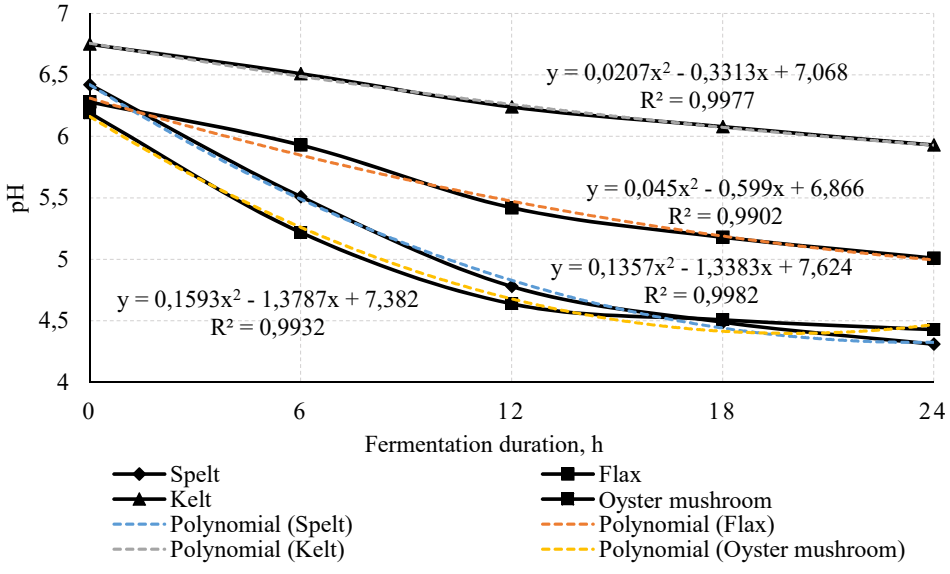

Fig. 1. pH dynamics during fermentation of plant raw materials

Table 1

Rate of pH change during fermentation of plant substrates

Substrate	Rate of pH change, υ, unit/h				
	0…6	6…12	12…18	18…24	0…24
Spelt	–0.152	–0.122	–0.048	–0.030	–0.088
Flax	–0.058	–0.085	–0.040	–0.028	–0.053
Kelt	–0.040	–0.045	–0.027	–0.025	–0.034
Oyster mushroom	–0.162	–0.097	–0.022	–0.013	–0.073

Note: a minus sign indicates a decrease in the indicator.

The oyster mushroom substrate was characterized by the maximum rate of pH decrease at the initial stage. The rate of decrease was 0.162 units/h. The high initial rate may be due to the presence of easily accessible water-soluble carbohydrates and the porous structure of the fungal tissue, which facilitates the mass transfer of metabolites [18]. In the subsequent stages of fermentation, the rate of pH decrease tended to decrease – initially more intensively, then more slowly (Table 1). After 18 hours, the value reached the minimum value among all the studied substrates, which indicates the achievement of the stabilization phase [19]. The total decrease in the active acidity of the fungal substrate was 28.5% of the initial value.

During the fermentation of flax, the decrease in acidity was 20.2% and occurred at a slower rate compared to other substrates, without a sharp initial decrease. Such dynamics may be associated with the ability of flax mucilaginous polysaccharides to significantly increase the proportion of bound moisture and form a viscous consistency of the substrate, which complicates the diffusion of metabolites and enhances its buffer properties [8]. The level of active acidity after 24 hours of fermentation indicates the incompleteness of the process of lactic acid accumulation. However, the introduction of such ingredients into the formulations of meat systems can provide moderate acidification and excellent rheological properties.

The minimum decrease in pH was recorded during the fermentation of the kelp substrate – by 12.2% of the initial value. And, accordingly, the rate of decrease was also at a low level. This is due to the biological characteristics of algae, in particular the high content of minerals and the presence of specific polysaccharides, which contribute to increasing the buffer capacity of the substrate and inhibiting the process of acid formation [20].

Thus, based on our results, we can rank the studied substrates by the rate of pH decrease during 24 hours of fermentation:

spelt (υ = –0.088 units/h) → oyster mushroom (υ = –0.073 units/h) → flax (υ = –0.053 units/h) → kelp (υ = –0.034 units/h).

It should be noted that for all plant substrates after 18 hours of fermentation, a significant decrease in the metabolic activity of lactic acid microorganisms is characteristic, which is manifested in a significant slowdown in the growth of pH and the beginning of the stabilization of the process.

Titrated acidity (TA) is considered one of the main markers that characterizes the metabolic activity of lactic acid bacteria during the fermentation of plant substrates. Unlike pH, which records the concentration of free hydrogen ions and characterizes active acidity, TA makes it possible to estimate the total amount of organic acids that are formed by pure cultures of lactic acid bacteria of the species *Lactiplantibacillus plantarum*. Organic acids accumulated during fermentation, and primarily lactic acid, play a leading role in the formation of the taste and aroma of future finished products and affect the duration of their storage.

The dynamics of titrated acidity (Fig. 2) demonstrate an increase in all studied substrates; however, the intensity and nature of changes depend on the nature of the raw materials.

The initial content of titrated acids in plant substrates was determined by their biological characteristics and ranged from 0.083 g/100 g in the kelp substrate to 0.120 g/100 g in the spelt substrate.

The maximum rates of accumulation of organic acids during fermentation were characterized by spelt and oyster substrates (Table 2).

Table 2

Rate of change in TA during fermentation of plant substrates

Substrate	Rate of change in TA, υ, g/100g/h				
	0…6	6…12	12…18	18…24	0…24
Spelt	+0.043	+0.060	+0.037	+0.027	+0.042
Flax	+0.013	+0.027	+0.023	+0.017	+0.020
Kelt	+0.007	+0.013	+0.013	+0.013	+0.012
Oyster mushrooms	+0.055	+0.063	+0.027	+0.013	+0.040

Note: the plus sign shows an increase in the indicator.

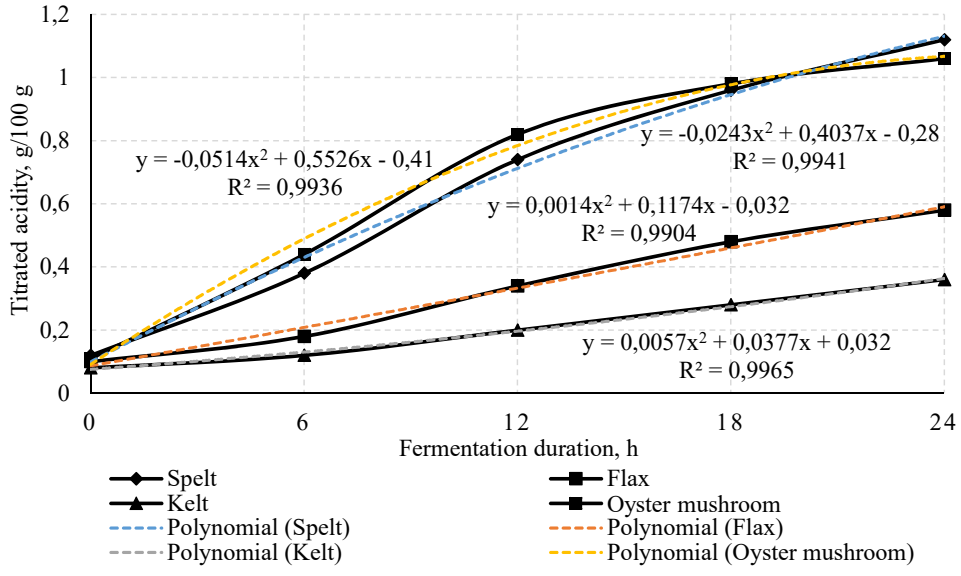

Fig. 2. Dynamics of titrated acidity during fermentation of plant substrates (in terms of lactic acid, g/100 g)

The dynamics of TA during fermentation deserve special attention. Thus, at the initial stage, oyster substrates were characterized by maximum rates. The rate of TA growth in spelt substrates was lower. After 12 hours of fermentation, the dynamics changed significantly. The rate of acid formation in mushroom substrates began to decrease rapidly, and after 18 hours it reached a minimum level. In spelt substrates, a decrease in the rate of TA growth was also noted, but its values were 1.4...2 times higher than in the mushroom substrate. In general, over the entire fermentation period, the content of titrated acids in the spelt substrate increased by 9.35 times with an average rate of 0.042 g/100 g/h over 24 hours, and in the mushroom substrate – 9.5 times with an average rate of 0.040 g/100 g/h.

Flax and kelp are characterized by a more moderate and even nature of the accumulation of titrated acids. The total growth of TA for 24 hours of fermentation was 0.48 g/100 g for flax and 0.28 g/100 g for kelp. As for the growth rate, for these substrates its growth was recorded in the period from 6 to 12 hours of fermentation. However, both in this period and in all other periods, the values of the rate of acid formation process were significantly lower compared to the spelt and oyster substrates. Such kinetics are associated with the pronounced buffer stability of flax and kelp substrates, which is due to the polysaccharide and mineral composition of the raw materials.

Correlation analysis revealed a strong inverse relationship between changes in titrated and active acidity of plant substrates during fermentation with a correlation coefficient of $r = -0.98 \pm 0.03$.

Thus, the fermentation period over 12–18 hours is characterized by a significant decrease in the rate of accumulation of titrated acids in spelt and oyster substrates, while in flax and kelp substrates a more moderate, but relatively uniform increase in TA was established.

Experimental data were approximated by second-order polynomial equations (Fig. 2). High values of the coefficients of determination ($R^2 = 0.9904–0.9965$) confirm the adequacy of the models and the possibility of their use for quantitative description of the kinetics of accumulation of organic acids and determination of the time of transition of the process to the stabilization stage.

5. 2. Studying the dynamics of soluble carbohydrates during fermentation of plant substrates

Along with acidity indicators, another important marker of the course of fermentation processes is the content of soluble carbohydrates. It should be noted that soluble carbohydrates are the primary energy substrates for lactic acid microflora. Changes in their ratio and quantitative content characterize the kinetics of two opposite processes. The first process is the hydrolytic transformation of complex carbohydrates in the middle of the substrates, as a result of which the total amount of simple carbohydrates increases. And the second is the consumption of easily accessible simple carbohydrates by lactic acid microflora, which is accompanied by their significant quantitative decrease. The greatest intensity of carbohydrate transformation is observed in the first 24 hours of fermentation, when the metabolic activity of lactic acid microflora is maximum.

The dynamics of the content of soluble carbohydrates in plant substrates during fermentation are shown in Fig. 3; the kinetics of their change are in Table 3. Our results indicate that during 24 hours of fermentation, a significant decrease in the amount of soluble carbohydrates was observed; however, the speed of this process was determined by the specific characteristics of plant substrates (Fig. 3).

Table 3

Rate of change in soluble carbohydrates during fermentation of plant substrates

Substrate	Rate of change in carbohydrates, υ, g/100g/h				
	0...6	6...12	12...18	18...24	0...24
Spelt	–0.084	–0.065	–0.035	–0.009	–0.048
Flax	+0.025	–0.038	–0.018	–0.009	–0.010
Kelt	+0.034	–0.066	–0.018	–0.008	–0.014
Oyster mushroom	–0.199	–0.140	–0.034	–0.008	–0.095

Note: the plus sign shows an increase in the indicator, the minus sign shows a decrease in the indicator.

Thus, the initial content of soluble carbohydrates in the substrates was within the biologically determined values. It was maximum in the kelp substrate (Fig. 3), somewhat lower in the oyster substrate. The minimum values were set for the flax substrate.

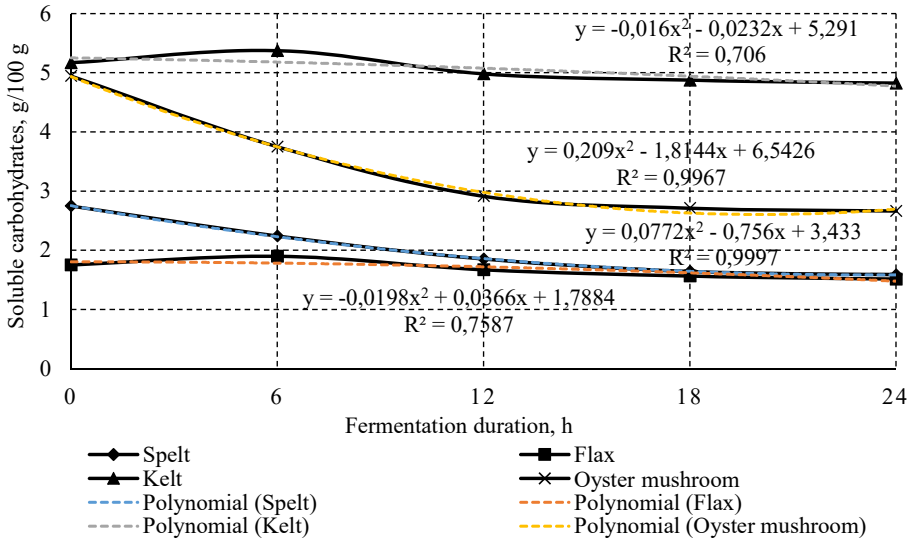

Fig. 3. Dynamics of soluble carbohydrates during fermentation of plant substrates (g/100 g)

During 24 hours of lactic acid fermentation, the most significant changes in the content of soluble carbohydrates were recorded in the oyster substrate. At the same time, their decrease was noted throughout the process with an average rate of 0.095 g/100 g per hour. The total percentage of decrease was at the level of 46.2% of the initial value. Analyzing the dynamics, it should be noted that the maximum rates were in the first 6 hours of fermentation (Table 3). Later, the process began to slow down, and after 18 hours of fermentation, the rate of decrease in total carbohydrates reached minimum values – 0.008 g/100 g/h. The established dynamics are evidence of the high availability of carbohydrate components of the mushroom substrate for lactic acid bacteria at the initial stages of fermentation. As they were consumed, the metabolic activity of microorganisms decreased, and the fermentation process entered the stabilization stage.

Similar dynamics were established for soluble carbohydrates in the spelt substrate. The overall decrease in this indicator over 24 hours of fermentation was 42.3% with an average rate of 0.048 g/100 g/h. The highest rate was observed in the first 0–6 h of fermentation, then from 6 to 18 h it decreased and after 18 h it reached 0.009 g/100 g/h, which indicates stabilization of the process, similar to the mushroom substrate.

The dynamics of soluble carbohydrates during the fermentation of kelp and flax were similar to each other but differed from oyster mushrooms and spelt. In the kelp and flax substrates, an increase in their content was recorded in the first 6 hours of fermentation, which indicates the predominance of the hydrolysis of complex carbohydrates over the consumption of water-soluble sugars by lactic acid microflora. In the following stages of fermentation, the content of water-soluble carbohydrates gradually decreased, reaching a minimum after 18 hours of fermentation, which indicates the beginning of stabilization of the system similarly to the oyster mushrooms and spelt substrates. However, the rate of decrease was significantly lower. The total decrease in the content of soluble carbohydrates over 24 hours of fermentation in the kelp substrate was 6.7% with an average rate of 0.014 g/100 g/h, in the flax substrate – 13.8% with an average rate of 0.010 g/100 g/h.

Our approximation of the experimental data established that the most adequate model is a second-order polynomial. At the same time, the maximum accuracy of the models was established for the substrates of spelt ($R^2 = 0.9997$) and oyster mushrooms ($R^2 = 0.9967$). For the substrates of flax and kelp, the relationship was somewhat lower ($R^2 = 0.7603$ and ($R^2 = 0.704$, respectively) but was within the interval that is quite acceptable for interpreting the trend of changes and predicting the onset of stabilization of the system.

5. 3. Studying the dynamics of protein content during fermentation of plant substrates

The next most important marker that characterizes the course of metabolic processes during lactic acid fermentation is the protein composition of plant substrates. It should be noted that when analyzing the protein composition of substrates, special attention should be paid not only to the content of total protein but also to the proportion of soluble protein and its changes during fermentation. It is the changes in the proportion of soluble protein that are considered the most sensitive indicator that characterizes the degree of conversion of high-molecular compounds and the increase in the availability of nitrogenous substances during the fermentation process.

According to the results of our studies, insignificant changes in the content of total protein in all plant substrates were found during 24 hours of lactic acid fermentation (Table 4). All fluctuations in this indicator were within the limits of statistical error (HIP_{05} = 0.278...0.446 depending on the substrate). The obtained data are fully consistent with those reported in [21, 22], which state that during enzymatic biological transformation, there are not quantitative changes in the total protein, but a redistribution of its fractional structure.

Thus, the data given in Table 4 indicate a statistically significant increase (HIP_{05} = 0.053...0.433 depending on the substrate) in the content of soluble protein in all plant substrates during fermentation. The maximum increase was for the kelp substrate (by 58% of the initial value), then flax (by 50% of the initial value), spelt (by 43% of the initial value), and the minimum – for the oyster substrate (by 33% of the initial value). Such a ranking is primarily due to the biological characteristics of plant substrates. Thus, according to data from [23], during the fermentation of brown algae, the bioavailability of proteins associated with the polysaccharide complex increases, which is accompanied by an intensive increase in the soluble protein fraction. In contrast, in oyster mushroom fruit bodies, the initial proportion of readily available protein fractions is higher, which is the reason for the less pronounced relative growth [24].

Table 4

Dynamics of total and soluble protein in plant substrates during fermentation

Substrate	Fermentation control stages, hours				
	Before fermentation	6	12	18	24
Total protein, % CP					
Spelt	15.605 ± 0.243	15.603 ± 0.281	15.601 ± 0.299	15.596 ± 0.282	15.770 ± 0.514
Flax	21.394 ± 0.240	21.399 ± 0.220	21.410 ± 0.246	21.416 ± 0.252	21.418 ± 0.253
Kelt	13.782 ± 0.453	13.814 ± 0.434	13.822 ± 0.454	13.829 ± 0.455	13.833 ± 0.451
Oyster mushroom	23.985 ± 0.212	23.988 ± 0.210	23.992 ± 0.209	23.995 ± 0.211	23.997 ± 0.213
Soluble protein, % CP					
Spelt	4.000 ± 0.073	4.742 ± 0.038	5.343 ± 0.026	5.698 ± 0.022	5.721 ± 0.033
Flax	5.199 ± 0.149	6.464 ± 0.166	7.367 ± 0.128	7.800 ± 0.156	7.813 ± 0.161
Kelt	3.736 ± 0.415	4.713 ± 0.212	5.483 ± 0.444	5.905 ± 0.133	5.909 ± 0.327
Oyster mushroom	6.241 ± 0.142	7.302 ± 0.085	7.891 ± 0.109	8.331 ± 0.112	8.334 ± 0.119

Analyzing the growth rates of soluble protein fractions during fermentation, we should note similar dynamics for all plant substrates (Table 5). In the first six hours of fermentation, the growth rate was maximum ($\upsilon = 0.124...0.177$ depending on the substrate), during the subsequent stages its noticeable decrease was established. In the period from 6 to 12 hours, although it still remained quite high, it was lower compared to the initial value. In the period from 12 to 18 hours, an even greater inhibition of the process of redistribution of the protein fraction with the formation of soluble protein was recorded ($\upsilon = 0.059...0.073$ depending on the substrate). After 18 hours, the growth rates of the soluble protein fraction were minimal and approached zero (Table 5), which is evidence of the completion of the main processes of biological transformation of substrates and the approach of the system to the stabilization stage.

Table 5

Rate of change in soluble protein content during fermentation of plant substrates

Substrate	Soluble protein change rate, υ, % CP/h				
	0...6	6...12	12...18	18...24	0...24
Spelt	+0.124	+0.100	+0.059	+0.004	+0.072
Flax	+0.211	+0.151	+0.072	+0.002	+0.109
Kelt	+0.163	+0.128	+0.070	+0.001	+0.091
Oyster mushroom	+0.177	+0.098	+0.073	+0.001	+0.087

Note: the plus sign shows an increase in the indicator, the minus sign shows a decrease in the indicator.

Among the substrates, the maximum average growth rate of the soluble protein fraction was characterized by flax, the minimum by spelt. Such a gradation indicates that the protein fractions of flax showed the highest sensitivity to enzymatic metabolism, while in other substrates it occurred at a slower rate. Along with this, in terms of the absolute quantitative content of soluble protein after 24 hours of fermentation, the maximum values were recorded for oyster mushroom and flax substrates, which indicates their powerful potential as sources of easily accessible nitrogenous substances.

Thus, based on our results, we can conclude that the main biological transformation of protein substances in all plant substrates occurs in the first 18 hours of fermentation. This period is characterized by the active formation of soluble protein fractions and therefore has the greatest technological importance for the formation of the necessary properties of substrates. A further fermentation period of 18–24 hours does not provide an increase in the technological efficiency of the process, which makes it possible to determine 18 hours as the most rational duration of the process for all studied plant substrates.

6. Discussion of results based on studies assessing the impact of lactic acid fermentation on changes in the biochemical parameters of plant substrates

Our dependences of pH dynamics (Fig. 1, Table 1) and titrated acidity (Fig. 2, Table 2) are consistent with the classical kinetics of lactic acid fermentation, which states an intensive increase in the acid content at the initial stages of fermentation. At the subsequent stages of the process, the rate of acid growth decreases. This is due to the depletion of available substrates and the inhibition of the metabolic activity of lactic acid microflora by accumulated metabolic products. Similar phase dynamics for plant systems are described in works on the fermentation of vegetable, grain, and legume substrates, in which the active phase is accompanied by a rapid decrease in pH and the accumulation of organic acids with subsequent stabilization of the parameters [25–27].

The revealed features of the dynamics of acidity indicators are logically related to changes in the content of soluble sugars of plant substrates (Fig. 3, Table 3), which had a distinct substrate-specific character. Thus, in the substrates of spelt grain and oyster mushroom fruiting bodies, the average rate of decrease in the content of soluble carbohydrates during 24 hours of fermentation was 3.4–9.5 times higher compared to other substrates. This is evidence of their active use by lactic acid bacteria during enzymatic metabolism. Our results are fully consistent with those reported elsewhere, which indicate that soluble carbohydrates play an active part in the enzymatic metabolism of LAB, thereby regulating acid accumulation and other biochemical transformations [3, 28].

Along with this, in the kelp substrate, changes in the content of soluble sugars were less pronounced (Fig. 3, Table 3). It can be assumed that this is due to the features of the kelp polysaccharide complex. The dominant share of carbohydrates in this substrate is represented by structural and reserve polysaccharides, which are less accessible to enzymatic metabolism without prior hydrolytic cleavage, which occurs at the first stage of fermentation. The presence of such features determines the lower average rate of change in the content of soluble sugars compared to the substrate of spelt grain and oyster fruit bodies (Table 3). Similar features of the enzymatic metabolism of macroalgae are described in papers tackling lactic acid fermentation of brown algae [9, 29].

Lactic acid fermentation had a significant impact not only on the carbohydrate but also on the nitrogen composition of plant substrates, regardless of their nature. Our results (Tables 4, 5) indicate a significant increase in the content of soluble protein, while changes in total protein fluctuated within the limits of statistical error. Such dynamics can be explained by partial hydrolytic changes in proteins, which are accompanied by the accumulation of low-molecular nitrogen compounds. A similar trend has been established in studies dealing with the fermentation of plant substrates using bacteria of the genus *Lactobacillus* [30, 31].

The rational duration of fermentation was established based on the results of a generalized analysis of changes in pH, titrated acidity, soluble carbohydrate content, as well as total and soluble protein. The benchmark was the achievement of a state under which the system enters a relatively stable mode, accompanied by a slowdown in biocatalytic transformations and reproducibility of basic biochemical indicators.

For all studied substrates, the initial interval of 0–18 hours corresponded to the greatest intensity of fermentation processes. During this period, a significant decrease in pH and active accumulation of titrated acids were noted, and maximum rates of soluble carbohydrate utilization were also observed. At the same time, the greatest increase in soluble nitrogen was recorded. After 18 hours of fermentation, a sharp decrease in the rates of change of all studied biochemical indicators was observed, which is evidence of the approach of plant systems to a technologically stable state.

Thus, the duration of fermentation at the level of 18 hours can be considered the most rational for spelt grain, flax seeds, kelp, and oyster mushroom fruiting bodies as it provides a balanced acid profile and controlled transformation of carbohydrate and protein complexes.

Our results of experimental studies are consistent with data on the fermentation of grain, mushroom, and algae substrates, but differ in a unified comparison of four systems under the same conditions according to a set of kinetic criteria. This approach makes it possible to substantiate 18 hours as a technologically feasible point of slowing down the process for further integration of fermented substrates into protein-fat systems.

The limitations of our study are related to the use of a single combination of starter cultures and a fixed temperature range, which does not make it possible to extrapolate the results to other strain compositions or fermentation regimes. The shortcomings of the work include the lack of assessment of changes in the number of lactic acid bacteria during fermentation, which makes it difficult to establish a reliably confirmed relationship between changes in biochemical parameters and metabolic activity of the microflora.

Further studies should be aimed at testing the effect of 18 hour fermentation on the emulsifying ability and stability of the systems, optimizing inoculation and temperature, as well as expanding the analysis with rheological, microstructural, and microbiological methods.

7. Conclusions

1. Our experimental studies have established a statistically significant decrease in pH and an increase in titrated acidity during lactic acid fermentation of all plant substrates. The highest rate of change in these indicators was characterized by substrates of spelt grain and oyster mushroom fruiting bodies, while in substrates of flax seeds and kelp, the process of acid formation occurred at a slower pace. After 18 hours of lactic acid fermentation, the rate of change in acidity indicators significantly decreased, which demonstrated that plant systems were approaching the stage of biological stabilization.

2. A decrease in the content of water-soluble carbohydrates was established for all plant substrates during lactic acid fermentation. The maximum rate of decrease in this indicator was established for substrates of spelt grain and oyster mushroom fruiting bodies. For substrates of flax seeds and kelp, a significant short-term increase in the content of soluble carbohydrates was recorded in the first 6 hours of fermentation, with a further decrease during the process. After 18 hours of fermentation, the rate of decrease in all plant substrates decreased.

3. The assessment of changes in the protein complex indicators showed that changes in the total protein content of all plant substrates remained within the statistical error, which indicates the absence of their losses during lactic acid fermentation. At the same time, a significant increase in the content of soluble protein was recorded in all plant substrates. The maximum rates of increase in the content of soluble protein were established in the first hours of fermentation but, after 18 hours, they significantly decreased.

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

The data will be provided upon reasonable request.

Use of artificial intelligence

The authors used the artificial intelligence assistant Perplexity (Grok 4.1, Perplexity AI) to search for literature sources by keywords and criteria. The authors bear full responsibility for the final version of the manuscript.

Authors’ contributions

Bal-Prylipko Larysa: Conceptualization, Management, Project administration; **Nikolaienko Mykola**: Conceptualization, Methodology, Investigation; **Serdyuk Marina**: Methodology, Validation, Data curation; **Bandura Valentyna**: Formal analysis, Visualization; **Svitlana Danylenko**: Writing – review & editing, Validation; **Olena Sydorenko**: Writing – original draft, Visualization; **Inna Kurbatova**: Formal analysis; Resources; **Natalia Nesterenko**: Formal analysis.

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