ANIMAL SCIENCE AND FOOD TECHNOLOGY

Founder:
National University of Life and Environmental Sciences of Ukraine

Year of foundation: 2010

Recommended for printing and distribution
via the Internet by the Academic Council
of National University of Life and Environmental Sciences of Ukraine
(Minutes No. 5 of November 27, 2025)

Decision of the National Council of Television and Radio Broadcasting of Ukraine
No. 1721, Minutes No. 30, dated 11.12.2023
Media identifier – R30-02314.

The journal is included in the list
of Professional Scientific Publications of Ukraine
Category "B". Branch of sciences: Agricultural. Specialty: 0721 – Food processing,
0811 – Crop and livestock production, 0831 – Fisheries.
(order of the Ministry of Education and Science of Ukraine
of December 11, 2025, No. 1618)

The journal is presented international scientometric databases, repositories and scientific systems: Google Scholar, Vernadsky National Library of Ukraine, BASE, AGRIS, Ulrichsweb, Dimensions, University of Oslo Library, University of Hull Library, SOLO – Search Oxford Libraries Online, European University Institute, Leipzig University Library, Cambridge University Library, UCSB Library, OUCI (Open Ukrainian Citation Index),
WorldCat, EuroPub, Professional publications of Ukraine, CORE, EBSCO,
Litmaps, Cosmos Impact Factor

Editors office address:
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
E-mail: as@animalscience.com.ua
https://animalscience.com.ua/en

ТВАРИННИЦТВО ТА ТЕХНОЛОГІЇ ХАРЧОВИХ ПРОДУКТІВ

Засновник:
Національний університет біоресурсів і природокористування України

Рік заснування: 2010

Рекомендовано до друку та поширення
через мережу Інтернет Вченою радою
Національного університету біоресурсів і природокористування України
(протокол № 5 від 27 листопада 2025 р.)

Рішення Національної Ради України з питань телебачення і радіомовлення
№ 1721, протокол № 30 від 11.12.2023 р.
Ідентифікатор медіа – R30-02314.

Журнал входить до переліку
наукових фахових видань України
Категорія «Б». Галузь знань: Сільськогосподарські науки,
спеціальності – 181 «Харчові технології»,
204 «Технологія виробництва і переробки продукції тваринництва»,
207 «Водні біоресурси та аквакультура»
(Наказ Міністерства освіти і науки України від 11 грудня 2025 р. № 1618)

Журнал представлено у міжнародних наукометричних базах даних,
репозитаріях та пошукових системах: Google Scholar, Національна бібліотека України імені В. І. Вернадського, BASE, AGRIS, Ulrichsweb, Dimensions, University of Oslo Library, University of Hull Library, SOLO – Search Oxford Libraries Online, European University Institute,
Leipzig University Library, Cambridge University Library, UCSB Library,
OUCI (Open Ukrainian Citation Index), WorldCat, EuroPub, Фахові видання України,
CORE, EBSCO, Litmaps, Cosmos Impact Factor

Адреса редакції:
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
E-mail: as@animalscience.com.ua
https://animalscience.com.ua/uk

Editorial Board

CONTENTS

ЗМІСТ

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 16(4), 9-27
Received 01.07.2025 Revised 30.10.2025 Accepted 27.11.2025

UDC 636.2.034.082.2:575.113
DOI: 10.31548/animal.4.2025.9

Latent phenotype potential: Modelling response to selection for dairy cattle productivity traits considering genetic correlations

Sergiy Ruban*

Doctor of Agricultural Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-8114-3665

Viktor Danshyn

PhD in Agricultural Sciences
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0001-9012-6835

Oleksandr Borshch

Doctor of Agricultural Sciences, Professor
Bila Tserkva National Agrarian University
09100, 8/1 Soborna Sq., Bila Tserkva, Ukraine
https://orcid.org/0000-0002-8450-2109

Mykola Zbroi

Postgraduate Student
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0009-0006-6078-7513

Olena Fedota

Doctor of Biological Sciences, Professor
LLC "AMS"
61037, 247 Heroiv of Kharkiv Ave., Kharkiv, Ukraine
https://orcid.org/0000-0001-9659-383X

Suggested Citation:
Ruban, S., Danshyn, V., Borshch, O., Zbroi, M., & Fedota, O. (2025). Latent phenotype potential: Modelling response to selection for dairy cattle productivity traits considering genetic correlations. *Animal Science and Food Technology*, 16(4), 9-27. doi: 10.31548/animal.4.2025.9.

**Corresponding author*

Abstract. The aim of the study was to analyse the dynamics of changes in economically important traits of dairy cattle: milk fat and protein content, daughter pregnancy rate, productive longevity, residual feed intake, live weight, somatic cell score, when modelling selection for dairy productivity traits such as milk yield, milk fat and protein content. The research material consisted of data from a reference herd of Holstein cows at the "Terezyne" dairy farm (Kyiv region, Ukraine). The total number of cows observed was 14,712 across seven lactations. MRS software was used for modelling. When selecting for milk yield at selection differentials of 100 kg, 300 kg and 500 kg, the genetic response of the next generation for milk yield was 31.59 kg, 94.77 kg and 157.97 kg, respectively. At the same time, there was an increase in such traits as productive longevity, from 0.59 months to 2.94 months, live weight – from 0.36 to 1.79 kg, and the score for somatic cell content in milk – from 0.03 to 0.13. Genetic changes in milk fat and protein content, signs of daughter fertility and residual feed intake had reverse values. Significant phenotypic correlations were obtained between the milk yield of first-calf heifers and milk fat content (-0.2985), milk fat content (+0.9631), milk protein content (-0.2642), milk protein (+0.9924), open days period (+0.0989), productive longevity (+0.0989) and live weight (+0.2199). There were mixed levels of correlation in the daughters of sires between milk yield and milk protein content from -0.1229 to +0.1708, and between milk yield and open days from -0.0726 to +0.1836. Modelling of possible changes in genetic correlations in the range from -0.10 to -0.95 between milk yield and daughter pregnancy rate (DPR) was performed. The rates of correlated response when selecting for milk yield, depending on the values of genetic correlation between these traits, affected the reduction in the level of pregnancy in cows from -0.51 to almost -5.0 points. The impact on the strength and direction of the established relationships can contribute to the stabilisation or deterioration of reproductive traits when selecting for milk yield. The proposed modelling method makes it possible to predict changes in the main traits used for selection, depending on the genetic correlations between them

Keywords: genetic parameters; phenotypic correlations; dairy productivity; reproduction

Introduction

An important aspect of breeding work in dairy cattle farming is modelling the response to the selection of a set of economically important traits, taking into account the genetic links (correlations) between them. P. VanRaden (2020) pointed out that the global trend in dairy cattle selection programmes is to increase the number of control traits, due to their impact on production profitability. For a comprehensive assessment, indices are used to determine the selection rank of individual animals within a breed. According to official data from the United States Department of Agriculture (USDA), several methods are used to evaluate dairy cattle, the main one being NM$ – Lifetime Net Merit (VanRaden *et al.*, 2021). This is the official evaluation for Holstein animals, and the NM$ value is considered a measure of the expected additional net profit in US dollars that the sire's daughters can earn during their lifetime. P. VanRaden *et al.* (2021) note that the total NM$ value is based on the Predicted Transmitting Ability (PTA), which is equal to half of the breeding value for 17 traits with the corresponding economic weight of each. Most traits are negatively correlated, but at the same time, predicted PTA values remain one of the main tools for genetic evaluation. PTA allows comparing sires for a specific trait that can be observed in 50% of offspring. The remaining 50% of "success" will depend on the successful selection of pairs, sires and brood stock, according to the ranks of such evaluation, which also affects the level of genetic correlations between traits in the next generation.

The Holstein Association USA uses its own productivity index, TPI (Type-Production Index), whose formula has a slightly different weighting of traits compared to the PTA index, and the overall performance is aimed at obtaining a profitable cattle herd. It should be noted that similar or even identical results of a comprehensive assessment can be obtained with different values for individual traits included in the index, which are characterised by different directions of genetic relationships between these traits. However, changes in some traits in the index do not always lead to positive changes in others, which requires constant verification of this effect over several generations based on phenotypic or genetic correlations. According to A. Caballero (2020) and S. Xu (2022), the genetic correlation between two quantitative traits is defined as the relationship between the breeding values of animals for these traits, and their significance lies in the ability to predict genetic changes when selecting for one of them.

M. Satoh (2024) used three methods for predicting response to truncated selection based on BLUP (Best Linear Unbiased Prediction), where only a certain percentage of fit individuals are selected from the population for reproduction and the rest are culled: (1) based on the mean value of the estimated breeding values (EBV) in the candidate population for selection (Δg1); (2) based on the variance of EBV in the candidate population for selection (Δg2); (3) based on the diagonal elements of the inverse matrix on the left side of the mixed model equation (Δg3). The author concluded that it is desirable to use Δg1 or Δg2 to predict the response to truncated selection based on BLUP BV. However, in populations where selection is ongoing, the accuracy of predicting the selection response is likely to depend on distribution bias and the Balmer effect for Δg2, when generalised descriptions of a specific situation are applicable to many others. L. Brito *et al*. (2021) noted that for the development of milk production, it is necessary to improve current breeding indices and breeding goals, paying more attention to traits related to animal welfare, health, longevity, environmental performance (e.g., methane emissions and feed efficiency) and overall resilience. This should be done by identifying selection criteria (traits) that accurately reflect the biological mechanisms underlying the relevant phenotypes, are heritable, and can be measured quickly and cost-effectively in large numbers of animals.

N. Lopez-Villalobos *et al*. (2024) assessed the value of continuous genetic improvement using a model covering all dairy producers in an industry where dairy prices were determined by supply and demand curves for a specific product. Over 10 years of genetic improvement, the present value of the benefits was estimated at USD 123,000 per farm. The corresponding benefit, when assuming that markets had fixed commodity prices, was USD 183,000 per farm. The model showed that systematic genetic gains have a limited duration, during which additional benefits gradually decline and eventually disappear. T. Niehoff *et al*. (2024) proposed a new selection criterion that describes the genetic level of selected grandprogenitors produced by the offspring of a given cross. The authors compared this criterion with other published criteria in a stochastic simulation of the current breeding programme over 21 generations to validate the concept. The proposed result showed better results than all other tested criteria. Thus, the new selection option offers a tool for accelerating genetic progress for modern genomic breeding programmes. It preserves more genetic variation than previously published criteria. Taking into account future gametic Mendelian sampling variations in the selection process also appears promising for maintaining greater genetic variation.

The aim of this study was to establish the nature of correlated genetic changes in a complex of economically valuable traits in Holstein cattle under conditions of targeted selection for increased milk productivity of the herd.

Literature Review

B. Cuyabano *et al*. (2024) suggested considering genetic correlations not only as one of the genetic parameters used in assessing the breeding value of animals based on a set of traits, but also as a certain "latent phenotype", based on the fact that the values of genetic correlations may vary in the offspring of different sires. If genetic correlations are specific values inherent in each individual, then they can be considered as a phenotype of a hidden regulatory trait that controls the relationship between selection traits. For such antagonistic traits in dairy cattle as productivity – fertility, somatic cell count, live weight – residual feed intake, it can be assumed that animals at the extreme ends of the rank distribution are likely to represent different breeding values for a particular hidden regulatory trait and, especially, when generations change. B. Olasege *et al*. (2024), using the "correlation scanning" method, identified regions of the Australian Holstein cattle genome that determine antagonistic genetic correlations between traits. Several such regions were identified, most of which were related to muscle development, body weight, and milk quality traits. Future studies may identify such "antagonistic regions" as potential genomic areas to alter unfavourable correlations and improve milk quality, milk production, fertility traits, and milk urea levels.

Between 1930 and 2015, dairy cattle selection criteria focused on increasing milk production, which led to a decline in health and fertility and triggered metabolic diseases (Egger-Danner *et al*., 2015). As noted by O. Fedota *et al*. (2020), growing concern for animal welfare, consumer demands for food quality and product composition, and reduced exposure to veterinary drugs have prompted changes in breeding strategies. The health and production efficiency of different breeds and species of animals are becoming increasingly important due to growing competition for high-quality plant sources of energy and protein. Agro-economic and climatic characteristics of milk production and genetic heterogeneity of breeds influence the target function of the selection index, which will be constantly aimed at total profit and minimisation of losses. Thus, according to V. Danshin *et al.* (2017), these estimates make it possible to determine the approximate expected effect of breeding stock. At the same time, it is important to take into account the productive and adaptive qualities of animals in different natural and climatic zones, especially when using interbreeding (Nogoev *et al*., 2025). The assessment of genetic correlations requires the use of highly accurate methods, such as REML (Residual Maximum Likelihood) – a method of limited maximum likelihood. The method involves the use of model fitting techniques in which all observations are expressed additively through fixed and random effects (Misztal *et al*., 2024).

A negative effect of genomic selection may be unfavourable genetic correlations between the performance traits for which selection is carried out and the so-called secondary traits, which include adaptation traits. This phenomenon was noted by I. Misztal & D. Lourenco (2024), who pointed out that such adaptation traits in dairy cattle include heat stress resistance, which should also be included in selection indices. An important advantage of genomic selection is the possibility of genetic improvement of traits that are difficult or costly to measure. Such traits can be measured in small numbers of animals in reference populations designated specifically for this purpose. One such trait is feed efficiency, i.e. the ability of cows to produce the same amount of milk with less feed (Brito *et al*., 2020). Recently, this trait has received a lot of attention, which, according to M. Madilindi *et al*. (2022), is due to its economic and environmental significance.

As a modern mainstream, it is worth noting software solutions based on artificial intelligence (AI) that are used in dairy farming for various purposes (De Vries *et al*., 2023; Neethirajan, 2023). H. Monteiro *et al*. (2024) proved that the composition of the rumen microbiome describes a significant part of the variability in residual feed intake by dairy cows. According to the scientists' conclusion, the application of AI and the development of sensor technologies have radically changed traditional sectors of the economy, including dairy farming (Akash *et al*., 2022). Precision livestock farming using AI and sensor technologies offers innovative solutions in the field of milk production. According to G. Koutouzidou *et al*. (2022), such approaches have enabled real-time monitoring and management decisions to improve animal welfare and increase their productivity. Measuring individual animal characteristics, which, according to B. Martins *et al.* (2020), is important for assessing their health and productivity, remains a challenging task that takes time when using conventional routine methods. That is why artificial intelligence and sensor technologies automate individual accounting based on cow behaviour during feeding, including time spent at the feeder, feeding frequency and amount of feed consumed (Lee & Seo, 2021). According to A. De Vries *et al*. (2023), the main areas of application for artificial intelligence technologies in dairy farming are: (1) building expert systems to improve feeding, culling, mastitis control, and individual selection of breeding bulls; (2) literature reviews on management areas; (3) disease diagnosis; (4) detection of cows in oestrus and prediction of the probability of successful insemination; (5) prediction of future milk productivity of cows; (6) assessment of individual feed consumption by cows based on data on productivity, behaviour and metabolic characteristics; (7) voluntary milking systems or milking robots. Thus, modelling and predicting probable changes in dairy cattle populations can be based on genetic correlations between traits.

Materials and Methods

The research was based on individual records of a reference herd of Holstein cows at the "Terezyne" dairy farm in the Kyiv region for the period from January 2020 to May 2025. The study did not involve any experimental interventions in animals; all data were obtained from the farm's routine accounting system and collected in accordance with ICAR (2014) requirements. The herd consists of 1,350 cows descended from sires of the Chief 1427381.62, Elevation 1491007.65, Starbuck 352790.79, Bell 1667366.74, Montfretch 91779b72, Hanover Red 1629391.72, Marshall 2290977.95, and J. Besne 5694028588.94 lines. The average annual productivity of cows is within 10,000 kg. The dry matter ration for the dairy cows consists of 9.55 kg of maize silage, 1.97 kg of lucerne haylage, 3.08 kg of soya bean meal, 3.05 kg of maize grain, 1.87 kg of sunflower meal, and 1.8 kg of wheat and barley grain, as well as mineral and vitamin supplements. The total weight of the ration was 45.8 kg at 23 kg of dry matter, with a protein content of 18%, a Neutral Detergent Fibre (NDF) level of 28%, and an Acid Detergent Fibre (ADF) level of 16.2%. Milking of cows (n = 500) was carried out on VMS DeLaval robotic milking systems, and the second part (n = 850) – in a 2 × 16 Parallel milking parlour. Table 1 shows descriptive statistics of the studied traits of cows.

Table 1. *Descriptive statistics of the studied traits of cows for standard lactation, 305 days*

Trait	N	M ± m	σ^2	σ	Cv, %
First lactation (5,105 head)					
Milk yield, kg	5,105	7,133.43 ± 24.63	3,097,643.56	1,760.01	24.7
Milk fat content, %	5,105	4.01 ± 0.01	0.10	0.31	7.8

Table 1. Continued

Trait	N	M ± m	σ²	σ	Cv, %
First lactation (5,105 head)					
Milk fat amount, kg	5,105	287.90±1.14	6,621.95	81.38	28.3
Milk protein content, %	5,026	3.33±0.02	0.01	0.12	3.6
Milk protein amount, kg	5,026	239.71±0.87	3,767.58	61.38	25.6
Second lactation (4,032 head)					
Milk yield, kg	4,032	7,818.71±33.64	4,561,996.43	2,135.88	27.3
Milk fat content, %	4,032	4.07±0.01	0.10	0.31	7.6
Milk fat amount, kg	4,032	320.29±1.56	9,792.74	98.96	30.9
Milk protein content, %	3,991	3.35±0.01	0.02	0.14	4.1
Milk protein amount, kg	3,991	263.90±1.19	5,643.08	75.12	28.5
Third lactation (2,628 head)					
Milk yield, kg	2,628	7,461.29±47.33	5,886,521.69	2,426.22	32.5
Milk fat content, %	2,628	4.05±0.01	0.10	0.31	7.8
Milk fat amount, kg	2,628	304.86±2.15	12,188.14	110.40	36.2
Milk protein content, %	2,612	3.35±0.01	0.02	0.14	4.2
Milk protein amount, kg	2,612	251.09±1.66	7,178.99	84.73	33.7
Fourth lactation (1,542 head)					
Milk yield, kg	1,542	7,180.17±63.97	6,310,879.79	2,512.15	35.0
Milk fat content, %	1,542	4.05±0.01	0.10	0.32	8.0
Milk fat amount, kg	1,542	293.29±2.89	12,882.52	113.50	38.7
Milk protein content, %	1,531	3.35±0.01	0.02	0.14	4.3
Milk protein amount, kg	1,531	241.72±2.24	7,689.99	87.69	36.3
Fifth lactation (805 head)					
Milk yield, kg	805	6,775.39 ± 90.14	6,541,314.99	2,557.60	37.7
Milk fat content, %	805	4.04 ± 0.01	0.10	0.32	7.9
Milk fat amount, kg	805	276.48 ± 4.05	13,223.79	114.99	41.6
Milk protein content, %	799	3.34 ± 0.01	0.02	0.16	4.7
Milk protein amount, kg	799	227.38 ± 3.15	7,930.42	89.05	39.2
Sixth lactation (408 head)					
Milk yield, kg	408	6,478.12 ± 123.83	6,256,262.35	2,501.25	38.6
Milk fat content, %	408	4.01 ± 0.02	0.10	0.32	7.9
Milk fat amount, kg	408	262.76 ± 5.53	12,482.83	111.73	42.5
Milk protein content, %	404	3.33 ± 0.01	0.03	0.17	5.1
Milk protein amount, kg	404	216.99 ± 4.32	7,555.89	86.92	40.1
Seventh lactation (192 head)					
Milk yield, kg	192	6,137.05 ± 175.72	5,928,770.24	2,434.91	39.7
Milk fat content, %	192	3.97 ± 0.02	0.10	0.32	8.1
Milk fat amount, kg	192	245.96 ± 7.74	11,495.00	107.21	43.6
Milk protein content, %	191	3.30 ± 0.01	0.03	0.16	4.9
Milk protein amount, kg	191	203.74 ± 6.08	7,071.25	84.09	41.3
Open days period	2,201	233.22 ± 4.04	35,905.42	189.49	81.2
Live weight at first calving, kg	3,692	578.33 ± 0.74	1,998.61	44.71	7.7
Productive longevity, months	5,604	83.20 ± 4.21	99,165.23	314.91	3.8

Note: N – number of observations; M ± m – mean value of the trait and its standard error; σ2 – variance of the trait; σ – standard deviation of the trait; Cv – coefficient of variation of the trait

Source: developed by the authors

The general algorithm for calculating the traits included in the test set is presented below. Daughter Pregnancy Rate (DPR) was calculated in points using formula (1):

$$DPR = \frac{21}{(SP - RWP) + 11} \times 100, \quad (1)$$

where 21 – the length of the cows' sexual cycle; SP – the service period; RWP – the required waiting period (at least 60 days); 11 – the correction factor.

The Somatic Cell Score (SCS) in milk was calculated in scores using formula (2):

$$SCS = \log 2\ (NSC/100{,}000) + 3, \quad (2)$$

where NSC – the number of somatic cells per 1 ml of milk.

The somatic cell count indicators were assessed in scores: 100,000 cells/ml – 3 points; 400,000 cells/ml – 5 points; 200,000 cells/ml – 4 points; 500,000 cells/ml – 5.3 points; 300,000 cells/ml – 4.6 points. Residual Feed Intake (RFI) was defined as the difference between actual and expected feed intake in terms of dry matter – Dry Matter Intake (DMI). The expected daily dry matter intake of a dairy cow was calculated based on its live body weight using a linear regression equation: DMI*e* = -38.09 + 0.106 × (live weight). Residual feed intake was equal to: RFI = DMI*a* – DMI*e*, where DMI*a* is the actual dry matter intake.

Animals with lower and, especially, negative residual feed intake are considered more economically efficient. According to I. Berro *et al*. (2019), the effectiveness of breeding programmes, including genomic ones, depends on the quality of recording and the volume of phenotypic data, the number and type of markers, and the size and composition of the Training Population (TR), in which a sufficient amount of such data has been accumulated. In addition, the structure and diversity of the population play a key role in compiling optimal sets for specific testing populations (TE). Phenotypic and genetic correlations between the traits studied that are characteristic of the Holstein breed were considered as TR (Tables 2, 3).

Table 2. *Phenotypic correlations between investigated traits in the Holstein breed*

Trait	1	2	3	4	5	6	7	8
1. Milk yield	1.0							
2. Milk fat	+0.619	1.0						
3. Milk protein	+0.901	+0.723	1.0					
4. Fertility rate	-0.342	-0.245	-0.307	1.0				
5. Productive longevity	+0.175	+0.144	+0.179	+0.544	1.0			
6. Residual feed intake	+0.040	+0.030	+0.030	+0.010	0.0	1.0		
7. Live weight	-0.066	-0.045	-0.055	-0.009	-0.041	0.0	1.0	
8. Somatic Cell Score	-0.159	-0.167	-0.148	-0.019	-0.163	0.0	+0.005	1.0

Source: P. VanRaden *et al*. (2021)

Table 3. *Genetic correlations between the traits studied in the Holstein breed*

Trait	1	2	3	4	5	6	7	8
1. Milk yield	1.0							
2. Milk fat	+0.399	1.0						

Table 3. Continued

Trait	1	2	3	4	5	6	7	8
3. Milk protein	+0.835	+0.591	1.0					
4. Fertility rate	-0.094	-0.075	-0.078	1.0				
5. Productive longevity	+0.113	+0.090	+0.128	+0.102	1.0			
6. Residual feed intake	+0.010	-0.070	+0.080	+0.020	-0.010	1.0		
7. Live weight	-0.131	-0.115	-0.100	-0.024	-0.221	+0.143	1.0	
8. Somatic Cell Score	+0.179	+0.080	+0.168	-0.313	-0.456	-0.124	-0.192	1.0

Source: P. VanRaden *et al*. (2021)

Multivariate Response to Selection (R) was calculated using the formula by B. Walsh & M. Lynch (2018) (3):

$$R = GP^{-1}s, \quad (3)$$

where G – the matrix of additive genetic variances and covariances between traits; P – the matrix of phenotypic variances and covariances between traits; s – the vector of selection differentials.

The calculations were performed using proprietary software – MRS (Multivariate Response to Selection), where, when modelling selection based on two traits, the system of equations has the form (4):

$$MRS = \begin{bmatrix} R_1 \\ R_2 \end{bmatrix} = GP^{-1}s = \begin{bmatrix} G_{11} & G_{12} \\ G_{12} & G_{22} \end{bmatrix} \begin{bmatrix} P_{11} & P_{12} \\ P_{12} & P_{22} \end{bmatrix}^{-1} \begin{bmatrix} S_1 \\ S_2 \end{bmatrix}, \quad (4)$$

where R_1 and R_2 – responses to the selection of the first and second traits; G_{11} and G_{12} – genetic variances of the first and second traits; G_{12} – genetic covariance between traits; P_{11} and P_{12} – phenotypic variances of the first and second traits; P_{12} – the phenotypic covariance between traits; S_1 and S_2 – the selection differentials of the first and second traits. Covariance $P_{12} = \rho_z (P_{11} P_{22})^{½}$, where ρ_z – the phenotypic correlation between two traits. Then:

$$P^{-1}s = \frac{1}{P_{11}P_{22} - P^2_{12}} \begin{bmatrix} P_{22} & -P_{12} \\ -P_{12} & P_{11} \end{bmatrix} \begin{bmatrix} S_1 \\ S_2 \end{bmatrix}$$

$$= \frac{1}{P_{11}P_{22}(1 - \rho^2_z)} \begin{bmatrix} S_1P_{22} - S_2P_{12} \\ - S_1P_{12} + S_2P_{11} \end{bmatrix}. \quad (5)$$

Thus, the response to selection of the first trait is obtained from the above derivation. The final form of this response is given in equation (6):

$$R_1 = \frac{h^2_1}{(1 - \rho^2_z)} \left[S_1 - S_2 \frac{P_{12}}{P_{22}} \right] + \frac{G_{12}(- S_1P_{12} + S_2P_{11})}{P_{11}P_{22}(1 - \rho^2_z)}, \quad (6)$$

where h^2_1 – the heritability coefficient of the first trait.

The correlated response of trait Y to selection for trait X was calculated using the formula by A. Caballero (2020) (7):

$$R_{YX} = i_X . r_{Gxy} . h_Y . h_X . \sigma_X, \quad (7)$$

where i_X – the intensity of selection for trait X; r_{Gxy} – the genetic correlation between traits X and Y; h_Y – the heritability coefficient of trait Y; h_{DPR} – the heritability coefficient of trait X; σ_X – the standard phenotypic deviation of trait X.

Results and Discussion

Tables 4-6 show the results of modelling genetic changes in the studied traits when selecting for milk productivity indicators such as milk yield, milk fat and protein content, depending on the values of the corresponding selection differentials. When selecting for milk yield with corresponding selection differential values of 100 kg, 300 kg and 500 kg, the genetic response of the next generation for milk yield will be 31.59 kg, 94.77 kg and 157.97 kg, respectively. At the same time, the amount of milk fat will increase by 0.98 kg, 2.94 kg and 4.91 kg, and the amount of milk protein – by 0.29 kg, 0.88 kg and 1.46 kg. At the same time, there is an increase in indicators such as productive longevity – from 0.59 months to 2.94 months, live weight – from 0.36 to 1.79 kg, and the somatic cell score content in milk – from 0.03 to 0.13. At the same time, genetically determined changes in the fat and protein content of milk, signs of the fertility level of daughters and residual feed consumption had negative values (Table 4).

***Table 4.** Genetic changes in investigated traits under selection for milk yield*

Traits	Selection differential for milk yield, kg		
	100	300	500
Milk yield, kg	31.59	94.77	157.95
Milk fat amount, kg	0.98	2.94	4.91
Milk protein amount, kg	0.29	0.88	1.46
Milk fat content, %	-0.005	-0.007	-0.009
Milk protein content, %	-0.003	-0.004	-0.006
Daughter pregnancy rate, scores	-0.19	-0.58	-0.97
Productive longevity, months	0.59	1.76	2.94
Residual feed intake, kg	-0.03	-0.08	-0.14
Live weight, kg	0.36	1.08	1.79
Somatic cell score content in milk	0.03	0.09	0.13

Source: developed by the authors

Similar patterns were observed when selecting by milk fat amount (Table 5), where milk fat amount at selection differentials of 10 kg, 30 kg and 50 kg was +2.46 kg, +7.37 kg and +12.29 kg, respectively. Genetic changes in all traits show a positive trend towards an increase with a decrease in the level of daughter fertility (-0.02 – -0.11) and residual feed intake (-0.01 kg – -0.07 kg).

The level of milk protein has become one of the main indicators in determining the price of milk in many countries around the world, which prompts to model the consequences of selection based on this trait (Table 6).

Table 5. *Genetically determined changes in the studied traits when selecting by milk fat amount*

Traits	Selection differential for milk fat amount, kg		
	10	**30**	**50**
Milk yield, kg	3.24	9.71	16.18
Milk fat amount, kg	2.46	7.37	12.29
Milk protein amount, kg	0.32	0.97	1.62
Milk fat content, %	0.004	0.005	0.007
Milk protein content, %	0.002	0.003	0.005
Daughter pregnancy rate, scores	-0.02	-0.06	-0.11
Productive longevity, months	0.13	0.40	0.67
Residual feed intake, kg	-0.01	-0.04	-0.07
Live weight, kg	0.21	0.64	1.06
Somatic cell score content in milk	0.003	0.009	0.015

Source: developed by the authors

Table 6. *Genetically determined changes in the studied traits when selecting for milk protein amount*

Traits	Selection differential for milk fat amount, kg		
	10	**30**	**50**
Milk yield, kg	1.46	4.38	7.29
Milk fat amount, kg	0.49	1.46	2.44
Milk protein amount, kg	3.12	9.35	15.58
Milk fat content, %	0.002	0.003	0.004
Milk protein content, %	0.003	0.005	0.008
Daughter pregnancy rate, scores	-0.14	-0.41	-0.68
Productive longevity, months	0.21	0.61	1.02
Residual feed intake, kg	-0.04	-0.11	-0.19
Live weight, kg	0.47	1.40	2.33
Somatic cell score content in milk	0.003	0.009	0.015

Source: developed by the authors

When selecting for milk protein amount with selection differentials of 10 kg, 30 kg and 50 kg, genetic changes in milk protein amount were +3.12 kg, +9.35 kg and +15.58 kg, respectively. The level of fertility of daughters decreased by 0.14, 0.41 and 0.68 points. Residual feed consumption decreased by 0.04 kg, 0.11 kg and 0.19 kg. An increase in the absolute values of fat and protein content in milk was noted. Productive longevity increased by 0.21 months, 0.61 months and 1.02 months, and live weight increased by 0.47 kg, 1.40 kg and 2.33 kg. At the same time, the somatic cell concentration score increased by 0.003, 0.009 and 0.015 points. Thus, selection based on milk productivity traits contributes to an increase in productive longevity and live weight of cows while reducing residual feed intake. At the same time, there is a deterioration in indicators such as the level of pregnancy in daughters and the concentration of somatic cells in milk.

The results obtained regarding the rate of genetic change are consistent with current trends in dairy cattle breeding. According to P. VanRaden (2020), genomic selection has been used in dairy cattle breeding since 2009, which has led to a significant reduction in generation intervals. Across four genetic improvement pathways, the average generation interval has decreased from 6-6.5 years to 2.5-3 years, i.e. by a factor of 2. According to G. Wiggans & J. Carrillo (2022), the largest reduction in the generation interval occurred in the category of bull sires – from 8 to 2-3 years, which led to accelerated genetic progress in the main selection pathways. Genetic progress in milk fat content in Holstein bulls in the United States increased sixfold between 2009 and 2023, and the overall level of genetic progress nearly doubled. This explains the need for constant monitoring of correlated changes in fitness traits during intensive selection for productivity.

Assuming the existence of the “latent phenotype” phenomenon, modelling of possible changes in genetic correlations during selection for milk yield and correlated response for DPR was performed. Changes in the values of genetic correlation between these traits in the range from -0.10 to -0.95 (Fig. 1) cause a decrease in the correlated response of the fertility level of cows from -0.51 to almost -5.0 points. Thus, the strength and direction of these relationships can influence the improvement or deterioration of reproductive traits when selecting for milk yield.

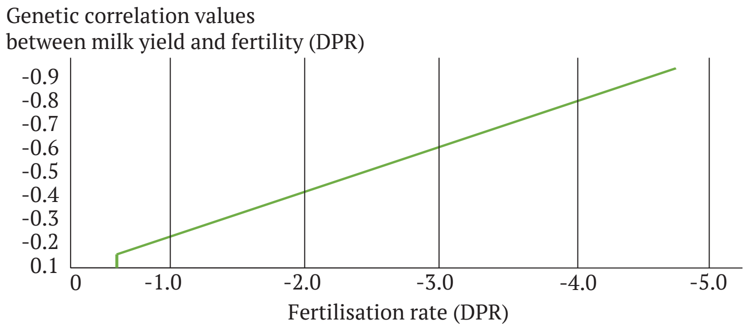

Figure 1. Correlated response of DPR change to selection for milk yield when genetic correlation values change

Source: developed by the authors

Table 7 shows phenotypic correlations between milk productivity traits, service period, productive longevity and live weight at first calving at the “Terezyne” farm.

***Table 7.** Phenotypic correlations between milk productivity traits, service period, productive longevity and live weight at first calving at the “Terezyne” farm (7,128 heads)*

Traits	1	2	3	4	5	6	7	8
1. Milk yield	1.00							
2. Milk fat content	-0.2985 ***							
3. Milk fat amount	+0.9631 ***	+0.5363 ***						
4. Milk protein content	-0.2642 ***	+0.6039 ***	+0.3860 ***					
5. Milk protein amount	+0.9924 ***	+0.3557 ***	+0.9740 ***	+0.3749 ***				
6. Service period	+0.0166 *	+0.2953 ***	-0.0714 ***	-0.1918 ***	-0.0050			
7. Productive longevity	+0.0989 ***	-0.1230 ***	+0.0559 ***	-0.0546 ***	+0.0901 ***	+0.8466 ***		
8. Live weight	+0.2199 ***	+0.1447 ***	+0.2233 ***	+0.2019 ***	+0.2095 ***	+0.0485 ***	+0.0875 ***	

Note: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$

Source: developed by the authors

At the "Terezyne" farm, reliability correlates positively with the amount of milk fat and protein, the length of the service period, productive longevity and live weight, and negatively with the fat and protein content of milk. The fat content in milk is positively correlated with all traits except productive longevity. The protein content in milk is negatively correlated with the service period and productive longevity. The amount of milk fat is positively correlated with the service period and live weight and negatively correlated with productive longevity, while the amount of milk protein is negatively correlated with the service period and positively correlated with productive longevity and live weight. The service period is characterised by a high positive correlation with productive longevity. Within the framework of the assumption of "latent phenotype" possibilities, an assessment of phenotypic correlations between the studied traits in cows depending on their paternal origin was carried out (Table 8).

Table 8. *Phenotypic correlations between milk productivity traits, open days period, productive longevity and live weight at first calving of different sires*

Traits	Sires				
	CA 106627797 D. Kickball Et	CA 7746123 S. Stady Et	CA 9969495 V. Sophomore Et	NL 493726366 O. Gigant	NL 520913639 N. Saltie
Number of animals	69	105	34	22	29
Milk fat content	-0.0258	+0.0238	+0.0279	+0.2603***	+0.0730***
Milk fat amount	+0.9993***	+0.9948***	+0.9981***	+0.9535***	+0.9625***
Milk protein content	-0.0973**	+0.0345	+0.1708***	+0.0872***	-0.1229***
Milk protein amount	+0.9986***	+0.9971***	+0.9992***	+0.9962***	+0.9948***
Open days period	-0.0726	+0.1836***	+0.0512	+0.0529	+0.1332***
Productive longevity	+0.2441***	+0.1974***	+0.2014***	+0.0339	+0.0709***
Live weight	-0.1850*	-0.0014	-0.3563***	+0.2389***	+0.2378***

Note: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$
Source: developed by the authors

There are significant differences between the daughters of bulls of different origins in terms of phenotypic correlations between the above traits. In the offspring of the breeding bull CA 106627797 D. Kickball Et, the correlation between milk yield and milk protein content was -0.0973**, and in the offspring of the bull CA 9969495 V. Sophomore Et, it was +0.1708***, and in the offspring of the bull NL 520913639 N. Saltie, it was -0.1229***. Similar results were obtained for other traits. In the offspring of the breeding bull CA 9969495 V. Sophomore Et, the correlation between milk yield and live weight of cows was -0.3563***, and in the offspring of the breeding bull NL 520913639 N. Saltie, it was +0.2378***. The results obtained indicate the possibility of a "latent phenotyp", i.e. cows of different origins are characterised not only by different values of selection traits, but also by the values and direction of the relationships between them.

The reduction in residual feed intake obtained when selecting for productivity is of great practical importance. Iranian scientists S. Nadri *et al.* (2023) concluded that including feed efficiency in the breeding goals for dairy cattle is desirable in terms of increasing sustainability and profitability. A study of selection indices that include feed efficiency showed that this allows for the greatest economic effect.

At the same time, it is possible to use indicators such as certain characteristics of the cows' body structure, namely fatness, milk type, etc. A number of studies have shown that feed efficiency in dairy cows is related to their behaviour and the response of certain genotypes to environmental conditions (Fedota *et al*., 2022). In a study by B. Nascimento *et al*. (2024), behavioural indicators such as chewing duration, lying duration and motor activity of cows were studied. Heritability was 0.19 for chewing and activity and 0.37 for lying duration. According to the authors, cows that spend more time lying down and are less active in movement are characterised by higher feed efficiency. This confirms the advisability of including feed efficiency indicators in breeding programmes.

It should be noted that modelling the response to selection is quite often used in modern breeding studies. J. Weller *et al*. (2022) used modelling to assess the effects of selecting Israeli dairy cattle based on reproduction and milk production indicators. In another study, J. Weller *et al*. (2023) used modelling to study the possibility of selecting cows based on viability, which was defined as the inverse of the probability of death. The viability of 523,954 cows was established for the period from 2000 to 2026, which was 89.6%, and according to this model, pregnancy reduced viability by 15%. The viability index increased with each calving, but was associated with an increase in the duration of lactation. The heritability coefficient of viability was 0.0082, and the phenotypic and genetic trends in viability over 14 years were -0.042% and -0.22% per year, respectively. A. Bouquet *et al*. (2022) presented a new methodology for predicting the response of selection for feed efficiency in dairy cattle. This approach combines genetic and mechanistic modelling to describe the biological mechanisms underlying these traits. A dairy cattle breeding scheme was modelled, taking into account an unrestricted feeding environment and two different selection goals, focused either on milk production or feed efficiency. The selection response was predicted in an unrestricted environment as well as for a promising low-cost system. The predictions obtained using traditional and mechanistic methods were consistent for milk production, body weight and feed efficiency in an unrestricted environment.

The current study revealed a decrease in reproduction rate, or daughter pregnancy rate, when selecting for milk productivity traits, indicating genetic antagonism between these traits. The genetic basis of antagonism is evidenced by the results of studies by L. Ma *et al.* (2019). The authors conducted a genomic comparison of two lines of Holstein cows, where one line was selected for milk productivity for 40 years since 1964, and the other line was a control line without selection. The results showed that selection for milk productivity resulted in a deterioration in reproduction and fertility rates. Thanks to this work, 198 genes associated with milk productivity and reproduction rates in cows were identified.

Thus, the analysis of predictive traits that may differ within certain genetic groups and the improvement of tools for their assessment are promising. Existing contradictions in the objectives and selection criteria between traits call into question the relatively rapid improvement in those traits that are related to health, reproduction, feed efficiency, or that reduce methanogenesis in ruminants. Modelling processes based on mathematical patterns that describe the relationships between such traits and the use of accurate and predictive data makes it possible to optimise selection and breeding programmes by identifying economically important traits for each group of animals.

Conclusions

The proposed algorithm for modelling the correlated response to selection makes it possible to predict the dynamics of genetic changes for a complex of traits when selecting for individual traits. When selecting for milk yield with corresponding selection differentials of 100 kg, 300 kg and 500 kg, the genetic response of the next generation for milk yield will be 31.59 kg, 94.77 kg and 157.97 kg, respectively. At the same time, the amount of milk fat will increase by 0.98 kg, 2.94 kg and 4.91 kg, and the amount of milk protein by 0.29 kg, 0.88 kg and 1.46 kg. At the same time, there is an increase in such characteristics as productive longevity – from 0.59 months to 2.94 months, live weight – from 0.36 to 1.79 kg, and the score for somatic cell content in milk – from 0.03 to 0.13. A decrease in the reproduction rate, or the level of pregnancy in daughters, was established when selecting for milk productivity traits, which indicates the presence of genetic antagonism between these indicators. When selecting for milk fat amount with selection differentials of 10 kg, 30 kg and 50 kg, the additional milk fat yield was +2.46 kg, +7.37 kg and +12.29 kg, respectively. Genetic changes in all traits have a positive dynamic of increase with a decrease in the level of daughter fertility (-0.02 – -0.11) and residual feed intake (-0.01 kg – -0.07 kg). When selecting for milk protein amount with selection differentials of 10 kg, 30 kg and 50 kg, genetic changes in milk protein amount were +3.12 kg, +9.35 kg and +15.58 kg, respectively. The daughter pregnancy rate decreased by 0.14, 0.41 and 0.68 points. Residual feed intake decreased by 0.04 kg, 0.11 kg and 0.19 kg. At the same time, an increase in the absolute values of fat and protein content in milk was observed. In the offspring of bulls of different origins, there are significant differences in phenotypic correlations between selection traits. In the daughters of the breeding bull CA 106627797 D. Kickball Et, the correlation between milk yield and milk protein content was -0.0973**, and in the offspring of the bull CA 9969495 V. Sophomore Et, it was +0.1708***, and in the offspring of the bull NL 520913639 N. Saltie, it was -0.1229***. Similar results were obtained for other traits. In the offspring of the breeding bull CA 9969495 V. Sophomore Et, the correlation between milk yield and live weight of cows was -0.3563***, and in the offspring of the breeding bull NL 520913639 N. Saltie, it was +0.2378***.

Selection for milk productivity traits contributes to an increase in productive longevity and live weight of cows while reducing residual feed intake. The values of phenotypic correlations between the studied traits in cows descended from different breeding bulls show significant differences. Further research should be directed towards identifying genomic regions that determine antagonistic genetic correlations between productivity and reproduction traits, as well as on the development of selection indices that take into account the individual variability of genetic correlations in the offspring of different sires to optimise genetic progress for a complex of economically valuable traits in dairy cattle.

Acknowledgements

The authors acknowledge National University of Life and Environmental Sciences of Ukraine for financial support.

Funding

At the expense of the National University of Life Resources and Environmental Management of Ukraine

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Akash, Hoque, M., Mondal, S., & Adusumilli, S. (2022). Sustainable livestock production and food security. In S. Mondal & R.L. Singh (Eds.), *Emerging issues in climate smart livestock production* (pp. 71-90). Cambridge, MA: Academic Press. doi: 10.1016/B978-0-12-822265-2.00011-9.

[2] Berro, I., Lado, B., Nalin, R.S., Quincke, M., & Gutiérrez, L. (2019). Training population optimization for genomic selection. *Plant Genome*, 12(3), 1-14. doi: 10.3835/plantgenome2019.04.0028.

[3] Bouquet, A., Slagboom, M., Thomasen, J., Friggens, N.N.C., Kargo, M., & Puillet, L. (2022). Mechanistic-based prediction of selection response on resilience and feed efficiency traits in dairy cattle. In *Proceedings of 12th world congress on genetics applied to livestock production* (pp. 268-271). Rotterdam: HAL. doi: 10.3920/978-90-8686-940-4_55.

[4] Brito, L.F., Bedere, N., Douhard, F., Oliveira, H.R., Arnal, M., Peñagaricano, F., Schinckel, A.P., Baes, C.F., & Miglior, F. (2021). Review: Genetic selection of high-yielding dairy cattle toward sustainable farming systems in a rapidly changing world. *Animal*, 15(1), article number 100292. doi: 10.1016/j.animal.2021.100292.

[5] Brito, L.F., *et al*. (2020). Genetic mechanisms underlying feed utilization and implementation of genomic selection for improved feed efficiency in dairy cattle. *Canadian Journal of Animal Science*, 100(4), 587-604. doi: 10.1139/cjas-2019-0193.

[6] Caballero, A. (2020). *Quantitative genetics*. Cambridge: Cambridge University Press. doi: 10.1017/9781108630542.

[7] Cuyabano, B.C.D., Croiseau, P., Shokor, F., Motta, M.R., Aguerre, S., & Mattalia, S. (2024). Genetic correlations: A parameter or a latent phenotype in genetic evaluations? *Interbull Bulletin*, 60, 179-186.

[8] Danshin, V.O., Ruban, S.Y., & Afanasenko, V.Y. (2017). Evaluation of breeding values of sires and cows in dairy breeds. *The Animal Biology*, 19(1), 44-53. doi: 10.15407/animbiol19.01.044.

[9] De Vries, A., Bliznyuk, N., & Pinedo, P. (2023). Invited review: Examples and opportunities for artificial intelligence (AI) in dairy farms. *Applied Animal Science*, 39(1), 14-22. doi: 10.15232/aas.2022-02345.

[10] Egger-Danner, C., Cole, J.B., Pryce, J.E., Gengler, N., Heringstad, B., Bradley, A., & Stock, K.F. (2015). Invited review: Overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. *Animal*, 9(2), 191-207. doi: 10.1017/S1751731114002614.

[11] Fedota, O., Babalian, V., Ryndenko, V., Belyaev, S., & Belozorov, I. (2020). Lactose tolerance and risk of multifactorial diseases on the example of gastrointestinal tract and bone tissue pathologies. *Georgian Med News*, 303, 109-113.

[12] Fedota, O., Puzik, N., Skrypkina, I., Babalyan, V., Mitiohlo, L., Ruban, S., Belyaev, S., Borshch, O.O., & Borshch, O.V. (2022). Single nucleotide polymorphism C994g of the cytochrome P450 gene possess pleiotropic effects in *Bos taurus*, L. *Acta Biologica Szegediensis*, 66(1), 7-15. doi: 10.14232/abs.2022.1.7-15.

[13] International Committee for Animal Recording (ICAR). (2014). *ICAR recording guidelines*. Retrieved from https://pecuaria.pt/docs/Guidelines_2014.pdf.

[14] Koutouzidou, G., Ragkos, A., & Melfou, K. (2022). Evolution of the structure and economic management of the dairy cow sector. *Sustainability*, 14(18), article number 11602. doi: 10.3390/su141811602.

[15] Lee, M., & Seo, S. (2021). Wearable wireless biosensor technology for monitoring cattle: A review. *Animals*, 11(10), article number 2779. doi: 10.3390/ani11102779.

[16] Lopez-Villalobos, N., Wiles, P., & Udy, G. (2024). The value of genetic improvement evaluated using a whole of enterprise market model. *Dairy*, 5(3), 372-383. doi: 10.3390/dairy5030030.

[17] Ma, L., Sonstegard, T.S., Cole, J.B., VanTassell, C.P., Wiggans, G.R., Crooker, B.A., Tan, C., Prakapenka, D., Liu, G.E., & Da, Y. (2019). Genome changes due to artificial selection in U.S. Holstein cattle. *BMC Genomics*, 20, article number 128. doi: 10.1186/s12864-019-5459-x.

[18] Madilindi, M.A., Zishiri, O.T., Dube, B., & Banga, C.B. (2022). Technological advances in genetic improvement of feed efficiency in dairy cattle: A review. *Livestock Science*, 258, article number 104871. doi: 10.1016/j.livsci.2022.104871.

[19] Martins, B.M., Mendes, A.L.C., Silva, L.F., Moreira, T.R., Costa, J.H.C., Rotta, P.P., Chizzotti, M.L., & Marcondes, M.I. (2020). Estimating body weight, body condition score, and type traits in dairy cows using three dimensional cameras and manual body measurements. *Livestock Science*, 236, article number 104054. doi: 10.1016/j.livsci.2020.104054.

[20] Misztal, I., & Lourenco, D. (2024). Potential negative effects of genomic selection. *Journal of Animal Science*, 102, article number skae155. doi: 10.1093/jas/skae155.

[21] Misztal, I., Tsuruta, S., Lourenco, D., Masuda, Y., Aguilar, I., Legarra, A., & Vitezica, Z. (2024). *Manual for BLUPF90 family of programs*. Athens, USA: University of Georgia.

[22] Monteiro, H.F., *et al.* (2024). An artificial intelligence approach of feature engineering and ensemble methods depicts the rumen microbiome contribution to feed efficiency in dairy cows. *Animal Microbiome*, 6, article number 5. doi: 10.1186/s42523-024-00289-5.

[23] Nadri, S., Sadeghi-Sefidmazgi, A., Zamani, P., Ghorbani, G.R., & Toghiani, S. (2023). Implementation of feed efficiency in Iranian Holstein breeding program. *Animals*, 13(7), article number 1216. doi: 10.3390/ani13071216.

[24] Nascimento, B.M., Cavani, L., Caputo, M.J., Marinho, M.N., Borchers, M.R., Wallace, R.L., Santos, J.E.P., White, H.M., Penagaricano, F., & Weigel, K. (2024). Genetic relationships between behavioral traits and feed efficiency traits in lactating Holstein cows. *Journal of Dairy Science*, 107(10), 8141-8149. doi: 10.3168/jds.2023-24526.

[25] Neethirajan, S. (2023). Artificial intelligence and sensor technologies in dairy livestock export: Charting a digital transformation. *Sensors*, 23(16), article number 7045. doi: 10.3390/s23167045.

[26] Niehoff, T.A.M., ten Napel, J., Bijma, P., Pook, T., Wientjes, Y.C.J., Hegedűs, B., & Calus, M.P.L. (2024). Improving selection decisions with mating information by accounting for Mendelian sampling variances looking two generations ahead. *Genetics Selection Evolution*, 56, article number 41. doi: 10.1186/s12711-024-00899-2.

[27] Nogoev, A., Azhibekov, A., Derkenbaev, S., & Ilyaz kyzy, Zh. (2025). Crossbreeding – the main method of increasing beef production in Kyrgyzstan. *Bulletin of the Kyrgyz National Agrarian University*, 23(3), 10-20. doi: 10.63621/bknau./3.2025.10.

[28] Olasege, B.S., van den Berg, I., Haile-Mariam, M., Ho, P.N., Oh, Z.Y., Porto-Neto, L.R., Hayes, B.J., Price, J.E., & Fortes, M.R.S. (2024). Dissecting loci that underpin the genetic correlations between production, fertility, and urea traits in Australian Holstein cattle. *Animal Genetics*, 55(4), 540-558. doi: 10.1111/age.13455.

[29] Satoh, M. (2024). Prediction of response to truncated selection based on BLUP of breeding values and its prediction accuracy. *Animal Science Journal*, 95(1), article number e13928. doi: 10.1111/asj.13928.

[30] VanRaden, P.M. (2020). Symposium review: How to implement genomic selection. *Journal of Dairy Science*, 103(6), 5291-5301. doi: 10.3168/jds.2019-17684.

[31] VanRaden, P.M., Cole, J.B., Neupane, M., Toghiani, S., Gaddis, K.L., & Tempelman, R.J. (2021). *Net merit as a measure of lifetime profit: 2021 revision*. Washington, D.C.: USDA.

[32] Walsh, B., & Lynch, M. (2018). *Evolution and selection of quantitative traits*. Oxford: Oxford Academic. doi: 10.1093/oso/9780198830870.001.0001.

[33] Weller, J.I., Ezra, E., Seroussi, E., & Gershoni, M. (2023). Genetic and genomic analysis of cow mortality in the Israeli Holstein population. *Genes*, 14(3), article number 588. doi: 10.3390/genes14030588.

[34] Weller, J.I., Gershoni, M., & Ezra, E. (2022). Breeding dairy cattle for female fertility and production in the age of genomics. *Veterinary Sciences*, 9(8), article number 434. doi: 10.3390/vetsci9080434.

[35] Wiggans, G.R., & Carrillo, J.A. (2022). Genomic selection in United States dairy cattle. *Frontiers in Genetics*, 13, article number 994466. doi: 10.3389/fgene.2022.994466.

[36] Xu, S. (2022). *Quantitative genetics*. Cham: Springer. doi: 10.1007/978-3-030-83940-6.

Можливості латентного фенотипу: моделювання відповіді на відбір за ознаками продуктивності молочної худоби з урахуванням генетичних кореляцій

Сергій Рубан
Доктор сільськогосподарських наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-8114-3665

Віктор Даншин
Кандидат сільськогосподарських наук
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0001-9012-6835

Олександр Борщ
Доктор сільськогосподарських наук, професор
Білоцерківський національний аграрний університет
09100, пл. Соборна, 8/1, м. Біла Церква, Україна
https://orcid.org/0000-0002-8450-2109

Микола Згрой
Аспірант
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0009-0006-6078-7513

Олена Федота
Доктор біологічних наук, професор
ТОВ «AMS»
61037, просп. Героїв Харкова, 247, м. Харків, Україна
https://orcid.org/0000-0001-9659-383X

Анотація. Метою досліджень був аналіз динаміки змін економічно важливих ознак молочної худоби: вмісту жиру та білка в молоці, рівня тільності дочок, продуктивного довголіття, залишкового споживання корму, живої маси, балу за вмістом соматичних клітин, при моделюванні відбору за такими ознаками молочної продуктивності, як надій, кількість молочного жиру та білка. Матеріалом для досліджень були дані референтного стада голштинських корів молочної ферми «Терезине» (Київська область, Україна). Загальна кількість спостережень корів склала 14 712 в розрізі семи лактацій. Для моделювання використане програмне забезпечення «MRS». При відборі за надоєм на рівні селекційних диференціалів 100 кг, 300 кг та 500 кг генетична відповідь наступного покоління по надою склала 31,59 кг, 94,77 кг та 157,97 кг відповідно. Одночасно спостерігалось збільшення таких ознак, як продуктивне довголіття, з 0,59 міс. до 2,94 міс., жива маса – з 0,36 до 1,79 кг, та бал за вміст соматичних клітин в молоці – з 0,03 до 0,13. Генетичні зміни вмісту жиру та білка в молоці, ознаки рівня тільності дочок та залишкового споживання корму мали зворотні значення. Отримано значущі показники фенотипових кореляцій між надоєм первісток та вмістом жиру в молоці (-0,2985), кількістю молочного жиру (+0,9631), білка в молоці (-0,2642), молочного білка (+0,9924), періоду відкритих днів (+0,0989), продуктивним довголіттям (+0,0989) та живою масою (+0,2199). Відмічено різноспрямований рівень зв'язків у дочок плідників між надоєм та вмістом білка в молоці від -0,1229 до +0,1708, надоєм та періодом відкритих днів – від -0,0726 до +0,1836. Проведено моделювання можливих змін генетичних кореляцій в діапазоні від -0,10 до -0,95 між надоєм та рівнем тільності (DPR). Темпи корельованої відповіді при відборі за надоєм залежно від значень генетичної кореляції між цими ознаками вплинули на зменшення рівня тільності корів з -0,51 до майже -5,0 балів. Вплив на силу та спрямованість встановлених зв'язків може сприяти стабілізації або погіршенню ознак відтворення при відборі за надоєм. Запропонований метод моделювання дає змогу прогнозувати зміни основних ознак, за якими проводиться відбір, в залежності від генетичних кореляцій між ними

Ключові слова: генетичні параметри; фенотипічні кореляції; молочна продуктивність; розмноження

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 16(4), 28-46
Received 24.06.2025 Revised 03.11.2025 Accepted 27.11.2025

UDC 639.3.043.13:636.087.7
DOI: 10.31548/animal.4.2025.28

Optimising growth and physiological performance of carp in polyculture within an integrated multitrophic aquaculture system

Olena Honcharova[*]
PhD in Agricultural Sciences, Associate Professor
Kherson State Agrarian and Economic University
73006, 23 Stritenska Str., Kherson, Ukraine
https://orcid.org/0000-0002-9702-7458

Vitaliy Bekh
Doctor of Agricultural Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-4254-815X

Iryna Kononenko
PhD in Agricultural Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-3906-3650

Olesia Okhrimenko
PhD in Agricultural Sciences
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0001-9867-0595

Abstract. The study aimed to determine the impact and evaluate the effectiveness of technological factors in producing a synergistic effect during the feeding and rearing of juvenile cyprinids. The research was conducted using theoretical, experimental, and laboratory methods in fisheries science. The findings indicated that the inclusion of alternative protein sources (*Spirulina platensis*, *Artemia*, and *Hermetia illucens*) within an Integrated Multitrophic Aquaculture (IMTA)

Suggested Citation:
Honcharova, O., Bekh, V., Kononenko, I., & Okhrimenko, O. (2025). Optimising growth and physiological performance of carp in polyculture within an integrated multitrophic aquaculture system. *Animal Science and Food Technology*, 16(4), 28-46. doi: 10.31548/animal.4.2025.28.

[*]*Corresponding author*

system significantly enhanced growth performance and reduced dependence on conventional feeds. The cultivation of all components within a single IMTA framework ensured optimal control over both qualitative and quantitative parameters throughout the production cycle. The results demonstrated improved viability in juvenile cyprinids and enhancement of blood composition in experimental Groups A and B. However, Group B exhibited the most pronounced improvements in both qualitative and quantitative parameters, characterised by increased homeostatic regulation and enhanced haematopoiesis. Improvements in haematological profiles, body weight, and muscle tissue composition were closely correlated with elevated protein and carbohydrate metabolism and accelerated ontogenetic development. Fish in the experimental groups displayed superior indicators of mass accumulation and metabolic processes (WG, FCR, HSI, VSI). A strong positive correlation was recorded in experimental Groups A and B: body weight ↔ protein in the muscle tissue (R^2 = 0.8-0.9); cholesterol in the blood ↔ fat in the muscle tissue (R^2 = 0.8-0.7). In experimental Group A: a higher red blood cell count (RBC) and mean corpuscular haemoglobin (MCH) were recorded in the blood of carp *Cyprinus carpio* (Linnaeus, 1758) ($p \leqslant 0.05$). In the blood of carp *Cyprinus carpio* (Linnaeus, 1758) from Group B: RBC, MCH, total protein and creatinine content ($p \leqslant 0.05$) and haemoglobin (Hb) ($p \leqslant 0.01$) exceeded those of the control group. In the blood of silver carp hybrid *Hypophthalmichthys* molitrix / *Hypophthalmichthys* nobilis of Group B: RBC, Hb, total protein and creatinine content ($p \leqslant 0.05$) also exceeded the control group. The most favourable biochemical composition of muscle tissue was recorded in fish from experimental Groups A and B

Keywords: feeding; cultivation; cyprinids; physiological and biochemical processes; multitrophicity; recirculation systems

Introduction

Aquaculture contributed to the development of high-quality, strategic programmes aimed at ensuring food security in various countries. The priority was to maintain a balance between the ecosystem and each country's globalised food system. In intensive aquaculture systems, inexpensive aquafeed and high-quality water were regarded as critical requirements; therefore, the issue of rationalising resource use became both relevant and practical. In addition, high-quality fish feed was considered essential for aquafarmers, as feeding represented a continuous process in aquaculture technology, and the quality of the feed directly influenced the quality of aquaculture products. At the global level, modern concepts and strategic programmes focused on the sustainable development of aquaculture. Simultaneously, the dominant approach emphasised the efficient use of resources with minimal negative impact on the ecosystem (FAO, 2024). Numerous studies confirmed that the application of chemicals, hormones, and antibiotics remained one of the most urgent problems in fish farming and aquaculture production. Such technological interventions contributed to water pollution and the disruption of ecosystems, as reported by O. Honcharova & V. Bekh (2023), V. Sakharnatsky (2024).

Research within this area revealed and confirmed the positive effects of phytopreparations, essential oils, and probiotics on the general physiological state of the organisms (Silva *et al.*, 2024; Taşcı *et al.*, 2025). Scientific works increasingly substantiated the relevance of developing plant-based feed additives. It was noted that the environmental friendliness of such natural components had become a research focus and was regarded as being of great importance for the sustainable development of aquaculture (Gusmão, 2024; Wang *et* *al*., 2024). The literature contained positive evidence regarding the use of adaptogens, hepatoprotectors, growth stimulants in aquaculture, etc. (Fraijo-Valenzuela *et al*., 2024). The rapid development of technologies implied the need for innovative solutions to correspond with the physiology of aquatic organisms. Therefore, the optimisation of technological schemes for rearing aquatic species and producing aquaculture products remained an open question. At the same time, relatively few combined systems existed in aquaculture. Consequently, the multitrophic aquaculture model was considered a promising approach for addressing the identified challenges. An increasing number of studies from different countries confirmed the rationale and trend towards the use of recirculating and integrated aquaculture systems (Land-based aquaculture report, 2023; Gupta *et al*., 2024). Most authors emphasised the environmentally safe direction of multitrophic aquaculture models, which acquires practical significance and formed prerequisites for the sustainable development of the industry (Alam *et al*., 2024; Meitei *et. al*., 2025). Thus, the multitrophic aquaculture system offered several advantages; however, it required comprehensive and in-depth research in the future. The purpose of the study was to conduct a multicomponent analysis and comparative experiment assessing the impact of several ecologically oriented technological solutions on fish physiological functionality.

Literature Review

Considering the trends and current state of the aquaculture industry, the dominant issue in most scientific works was the search for and development of alternative protein sources for aquatic organisms with an emphasis on their rationality and environmental friendliness (Belluco *et al*., 2013; Honcharova *et al*., 2020; FAO, 2024). Studies by various researchers reflected the possibility of optimising the overall diet of fish through alternative protein sources (Henry *et al*., 2015; Bekh *et al*., 2020). The focus of such research was directed towards achieving less expensive production and reduced environmental impact. At the same time, it was experimentally established that the use of natural additives was aimed at replacing fishmeal. In the scientific works of A. Fraijo-Valenzuela *et al*. (2024) a synergistic effect was observed between the growth of aquaculture production and consumer demand for such products. At the same time, attention was drawn to the importance of comprehensive investigation of each component, taking into account both its beneficial and potentially adverse effects. For instance, in aquaculture feeds, the inclusion of crickets was recommended for study while considering the biological characteristics of aquatic organisms, since their bodies could absorb individual components of such protein supplements differently.

According to O. Deren & M. Fedorenko (2023), the introduction of non-traditional feed components for Ukrainian aquaculture was considered relevant. Under conditions involving the use of the black soldier fly (*Hermetia illucens*) in aquaculture, improvements in fishery parameters, feed conversion, and the overall quality and safety of the resulting products were reported. The positive outcomes of using *Tenebrio molitor* were justified by its antioxidant and immunological properties (Shafique *et al*., 2021). Similarly, the incorporation of naked oats as a feed component has demonstrated beneficial effects on growth performance and haematological parameters in carp, with optimal results achieved at 30% inclusion rates (Syrovatka, 2021). In scientific works by H. Wang *et al*. (2011), M. Henry *et al*. (2015) the prospects for utilising biological waste as protein alternatives for aquaculture were presented. Furthermore, increases in the developmental parameters of hydrobionts during ontogenesis were experimentally confirmed. It was emphasised that modular combined aquaculture systems had lower competition for land resources compared with the production of other feed components and additives.

According to R. Chaklader *et al*. (2019) and F. Melenchón *et al*. (2021), the inclusion of mealworm, black soldier fly, and poultry by-product protein in aquaculture diets contributed to enhanced functional-active parameters and histomorphology in fish. At the same time, A. Józefiak & R. Engberg (2017) and S. Nogales-Mérida *et al*. (2019) focused on comprehensive analyses, particularly of chitins and antimicrobial peptides, which not only improved growth but also strengthened the defence mechanisms, immunity, and antimicrobial properties of fish receiving such protein alternatives. A. Gopalakannan & V. Arul (2006) reported that the application of crustacean chitin in low quantities when feeding fish stimulated immune responses and enhanced resistance to pathogens. After analysing available sources and scientific developments, it was noted that the use of natural components and protein alternatives was highly promising. It was also considered advisable to pay attention to technological conditions, as optimisation and adaptation to modern standards ensured the sustainable development of aquaculture.

Furthermore, the hydrochemical regime, particularly within recirculating aquaculture systems (RAS), was recognised as a critical factor (Hrynevych *et al*., 2019). According to E. Can & Ş. Seyhaneyildiz Can (2023), the improvement of technological schemes in aquaculture, for example, through elements of aquaponics and multitrophic aquaculture, was developing dynamically and showed a tendency towards global implementation. Thus, modern directions of optimisation measures in aquaculture were oriented towards the rational use of resources, the maximum environmental sustainability of production, and the high quality of aquaculture products, with particular emphasis on the multitrophic aquaculture model. However, this issue remained open and required further justification and comprehensive evaluation of the effectiveness of such models.

Materials and Methods

The experimental study was carried out at the Scientific Research Laboratory and the "Physiological and Biochemical Research" Laboratory of Kherson State Agrarian and Economic University (KSAEU) and in the conditions of production of Kherson fish enterprise (Ukraine) in 2025. Experiments lasted 120 days. Scientific and research activities were conducted in accordance with generally accepted requirements and standards for the organisation of experiments in fish farming. Compliance with the Directive 2010/63/EU (2010) was ensured. All manipulations involving carp and silver carp hybrids in both experimental and control groups were performed in accordance with the European Convention (1986). All scientific and practical research was based on theoretical (analysis, synthesis, comparison and modelling) experimental, laboratory methods, taking into account the specialisation of the fisheries industry. The functional status of the fish organism was evaluated according to the qualitative and quantitative parameters of biological material in the laboratory of the Department of Aquatic Bioresources and Aquaculture of the KSAEU on the Humalyzer 3000 analyser (Germany) using unified Human GmbH kits.

The study focused on *Cyprinus carpio* (Linnaeus, 1758) and silver carp hybrid *Hypophthalmichthys molitrix / Hypophthalmichthys nobilis*. Fish were reared under recirculating aquaculture system conditions, which included standard water filtration, temperature regulation, and oxygenation systems. The experimental setup incorporated a modular integrated multitrophic aquaculture system, that included the cultivation of natural feed components (Fig. 1). The modular system was constructed on a mobile platform equipped with an environmental monitoring unit and microcrystalline solar panels for supplementary energy. Each module was functionally organised to enable resource recycling, improve energy efficiency, and promote synergistic interactions among aquatic and agricultural components.

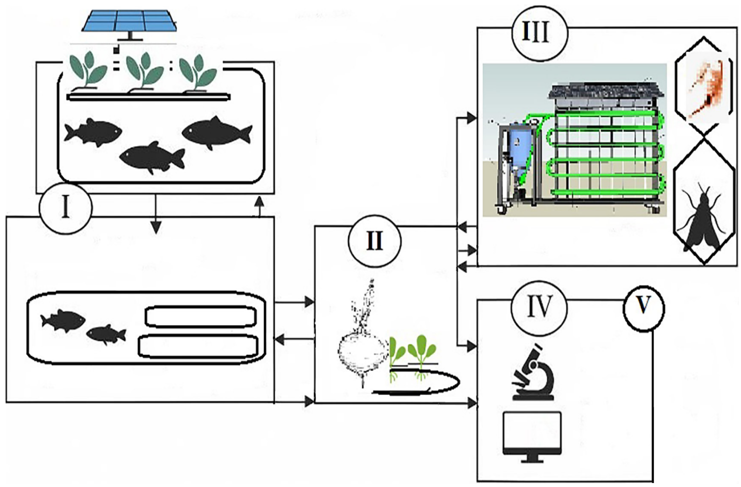

Figure 1. Schematic representation of the modular multitrophic aquaculture system

Note: I – RAS tanks and ponds, combined with elements of biological filtration; II – cultivation units for agricultural raw materials used in feed production; III – cultivation chambers for *Hermetia illucens* (black soldier fly), *Artemia* and *Spirulina*; IV – technological management units for equipment and process control; V – mini-laboratories for rapid hydrochemical and process diagnostics

Source: authors' development

Cyprinus carpio (Linnaeus, 1758) and *Hypophthalmichthys molitrix / Hypophthalmichthys nobilis* were used in the experiment. The initial average body weight of fish was 1.5-1.6 mg, and each tank contained 260 individuals. The volume of the rearing tanks was gradually increased as the fish developed through ontogenesis from 150 dm^3 and 250 dm^3 to 500 dm^3. It was taken into account that during days 10-15 of rearing, the carp's body undergoes an active transformation of metabolic processes. The initial daily ration was set at 2% of body weight and was increased to 5% from day 10. The stocking density corresponded to carp culture standards and averaged 25-30 kg/m^3 (FAO *et al.*, 2025).

Feed factor. The diet of carp fish was optimised in accordance to generally accepted recommendations (Yevtushenko & Khyzhniak, 2019; Yevtushenko *et al.*, 2022; The International Aquaculture Feed Formulation Database, n.d.). At the beginning of early ontogenesis, cyprinids were fed with finely dispersed flour (0.1-0.2 mm) in accordance with the recommendations of the standard diet in fish farming. Feeding was provided at a rate of 2% of body weight; after 14 days, the feeding rate was increased to 4.8-5%. The main oriented periods of transition to another diet were identified: initial with an active phase for 10-15 days, transitional and growth. In the experimental groups, the fish received a general diet and supplements in accordance with the study scheme. The composition of the base diet, %, was as follows: fish meal (13), meat and bone meal (5), grass meal (3), wheat (9.5), sunflower meal (40), peas (10), feed yeast (18.5), premix (1). Natural feed components were obtained from in-house production within the autonomous sectors of the modular multitrophic aquaculture system. The technological scheme of cultivation provided appropriate equipment and optimal conditions for each sector (Fig. 1). The design of the experimental groups is presented in Figures 2 and 3.

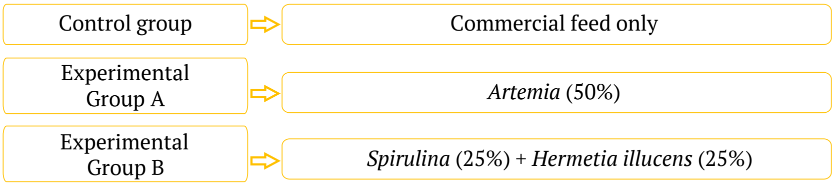

Figure 2. Scheme for introducing natural components to the fish diet

Source: authors' development

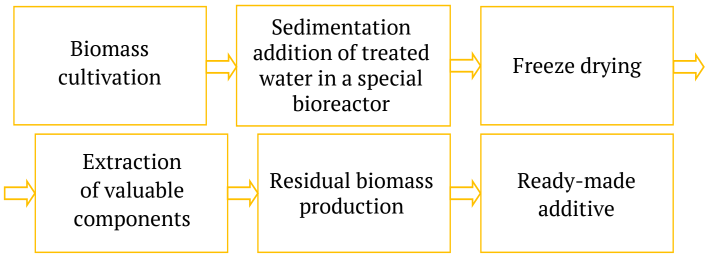

Figure 3. Technological aspects of the preparation of feed ingredients for addition during feeding to the experimental group

Source: authors' development

The day before the experiment, the optimal ratio of natural components was experimentally established and subsequently used in the present study:

➤ *Hermetia illucens* were cultured using cereal bran and fruit/vegetable residues. Cereal bran was selected as the optimal substrate based on prior trials. Rearing conditions (temperature, humidity, light) were maintained in accordance with standard rec ommendations. Composition (%): moisture – 68.4; protein – 43.8; dry matter – 31.6; fat – 25.9; ash – 6.5.

➤ *Spirulina* biomass was cultivated in a closed-loop bioreactor using an energy-saving technology developed by the authors. The harvested *Spirulina* biomass was green and mildly saline. Composition (%): moisture – 10; dry matter – 90; protein – 62; carbohydrates – 14.7; fat – 4; fibre – 3; ash – 6.3.

➤ *Artemia nauplii* were reared under standard hydrochemical conditions (temperature: 25-28°C; salinity: 30-80‰. *Nauplii* measured 0.3-0.6 mm in length and weighed approximately 0.01-0.06 mg per individual). Composition (%): moisture – 12; dry matter – 88; protein – 53; carbohydrates – 10; fat – 13.2; ash – 4.0. *Artemia* were used primarily during the early feeding stages of carp larvae. The feeding substrate for *Artemia* consisted of *Spirulina* and *Chlorella* cultures obtained from the multitrophic aquaculture system. To obtain *nauplii*, cysts were hydrated at a density of approximately 3 g L^{-1}. After hatching, aeration was discontinued and the *nauplii* were allowed to settle for 5 minutes before being filtered. Stocking density was maintained at 10-12 *nauplii* mL^{-1} in the rearing tanks.

Study of blood composition. Blood samples were collected from the caudal vein using Pasteur needle and heparinised syringes. Morpho-functional and biochemical parameters were determined for cyprinid blood: red blood cell count (RBC), haematocrit (Ht), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH). Biochemical indices in plasma: total protein, albumin, cholesterol, creatinine and glucose, were quantified photometrically using a Humalyzer 3000 biochemical analyser (Germany, 2010) with commercial kits (Human GmbH, Germany).

Study of ontogenesis. Growth performance was assessed through regular biometric monitoring of a representative subsample of 40 fish per group (measurements were taken on: 1 – day 0; 2 – day 30; 3 – day 60; 4 – day 90; 5 – day 120). For each specimen, total length and body weight were recorded, and the following indices were calculated: weight gain (WG), specific growth rate (SGR), Fulton's condition factor (FC), feed conversion ratio (FCR), and survival rate. Furthermore, the liver and viscera of 12 randomly selected fish per group were excised and weighed to compute the hepatosomatic index (HSI) and viscerosomatic index (VSI). After dissection, fillets were skinned, homogenised, and analysed to determine muscle composition.

$$\text{WG, g} = \text{Final body weight (g)} - \text{Initial body weight (g)}. \quad (1)$$

$$\text{Fulton's Condition Factor} = [\text{body weight (g)} / \text{total length (cm)}^3] \times 100. \quad (2)$$

$$\text{SGR, \%} = \left[\left(\frac{\log(\text{final body weight,g}) - \log(\text{intial body weight,g})}{\text{number of feeding days}}\right)\right] \times 100 \quad (3)$$

$$\text{FCR} = \text{total feed consumed per tank biomass cyprinids (g)} / \text{weight gain (g)}. \quad (4)$$

$$\text{Survival, \%} = (\text{number of fish initial} / \text{number of fish final}) \times 100. \quad (5)$$

The liver and viscera of *Cyprinus carpio* (Linnaeus, 1758) and *Hypophthalmichthys molitrix / Hypophthalmichthys nobilis* from each group (12 specimens per tank) were separated and their weights were measured. HSI and VSI (%) were calculated:

$$\text{HSI, \%} = [(\text{liver weight (g)} / \text{body weight (g)}] \times 100. \quad (6)$$

$$\text{VSI, \%} = [(\text{viscera weight (g)} / \text{body weight (g)}] \times 100. \quad (7)$$

Study of the chemical composition of carp muscle tissue. Biological samples were transported using a ThermoMix unit (USA). Fillets were skinned, homogenised, and prepared for the determination of chemical composition. The analysis of muscle tissue was performed in triplicate. Moisture content was determined by drying samples at 105°C to constant weight. Crude protein was quantified using the Kjeldahl method, while ash content was measured by standard incineration. The fat content in muscle tissue was determined using the classical gravimetric extraction method with organic solvents.

Study of hydrochemical parameters. During the entire period of the experiment, the hydrochemical regime corresponded to generally accepted standards for carp with appropriate age characteristics of cyprinids. Water quality was monitored throughout the experimental period using a Palintest 7500 photometer (UK) and corresponding reagent kits, following aquaculture guidelines (Yevtushenko & Khyzhniak, 2019). Dissolved oxygen, pH, and temperature were measured daily, while ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) concentrations were analysed three times per week. The rearing environment was maintained within optimal ranges: temperature (22.1-24.2°C), dissolved oxygen (5.8-6.1 mg/L), pH (7.2-7.4), NO_3^- (1.0-1.2 mg/L), and NO_2^- (0.08-0.012 mg/L). Measurements were performed using both rapid on-site tests and laboratory analyses to ensure the reliability of data.

Statistical analysis. All data were expressed as mean ± standard deviation (SD). Statistical comparisons were conducted using one-way analysis of variance (ANOVA). Differences between treatments were considered statistically significant at $p \leqslant 0.05$ with comparisons made relative to the control group.

Results and Discussion

The study of the growth and development of cyprinids in ontogenesis demonstrated that under the conditions of using elements of multitrophic aquaculture in two experimental groups (A and B), the parameters were higher than the control values (Fig. 4).

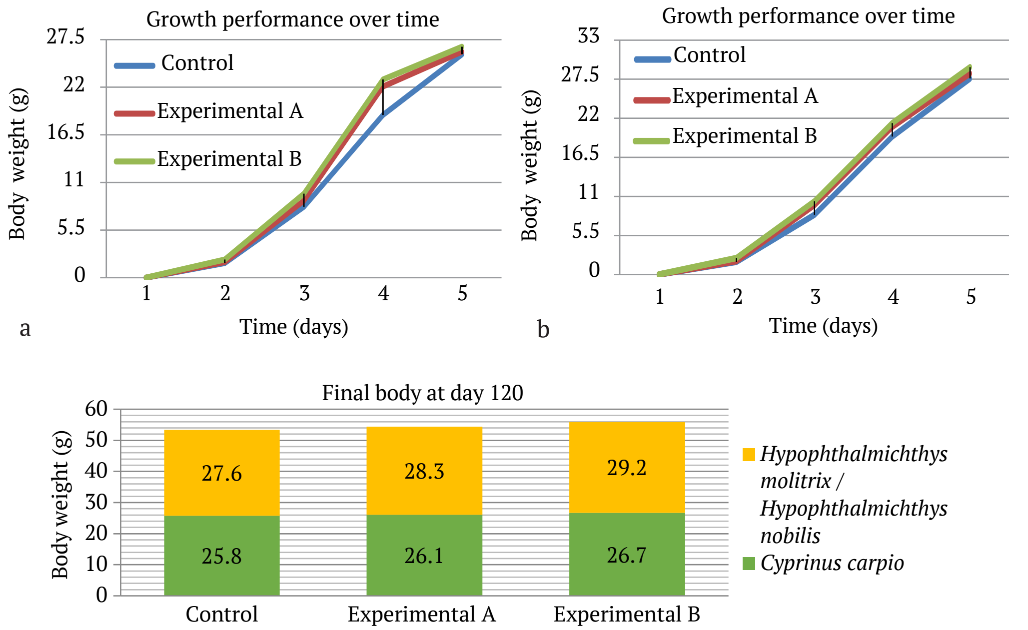

Figure 4. Study of the growth rate of *Cyprinus carpio* (a) and *Hypophthalmichthys molitrix / Hypophthalmichthys nobilis* (b) in ontogenesis under the influence of technological factors ($x \pm SD$)
Note: 1 – day 0; 2 – day 30; 3 – day 60; 4 – day 90; 5 – day 120
Source: authors' development

In experimental Group B, compared with the control group, significantly higher values of metabolic activity were observed than in the other groups. This was reflected in several key parameters, including Fulton's condition factor, average body weight, weight gain, and feed conversion ratio. At the end of the experimental period, the average body weight of carp in Group B exceeded that of the control group by 3.4%, WG by 6.9%, and FCR by 14.6% ($p \leqslant 0.05$). In experimental Group A, the average body weight of carp surpassed the control group by 1.21%, WG by 2.9%, and FCR by 9.2%. Overall, carp and silver carp in experimental Group B demonstrated more active weight accumulation and a more efficient utilisation of physiological potential compared with Group A and the control group (Fig. 5).

At the end of the experiment, the growth and development rate indicators in silver carp demonstrated higher values in experimental Group B compared with both the control and experimental Group A. Specifically, the average body weight exceeded that of the control by 7.0% ($p \leqslant 0.001$) and that of Group A by 3.3% ($p < 0.01$). Weight gain was higher by 6.8%

($p \leq 0.05$) and 5.2%, while the feed conversion ratio improved by 7.6% ($p \leq 0.001$) and 4.9%, respectively. Parameters such as the specific growth rate, viscerosomatic index, and hepatosomatic index in carp and silver carp were also higher in both experimental groups compared with the control group, correlating positively with mass accumulation indicators.

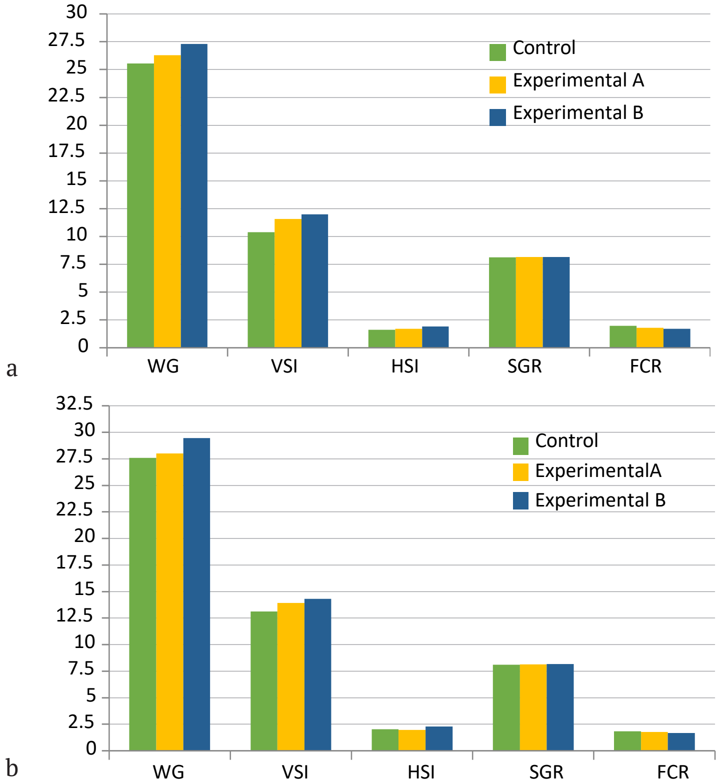

Figure 5. Study of the growth rate of *Cyprinus carpio* (a) and *Hypophthalmichthys molitrix*/*Hypophthalmichthys nobilis* (b)
Source: authors' development

Given that the activity and efficiency of physiological and biochemical processes in fish are synergistically dependent on the parameters of homeostatic balance, the morpho-functional composition of blood in experimental fish was analysed (Fig. 6). The total erythrocyte count and haemoglobin content were higher in both experimental groups than in the control, remaining within the physiological range for cyprinids. The obtained results suggest that redox reactions and oxygen-synthesising processes in the organism of experimental fish occurred more actively. In carp blood, the erythrocyte content in experimental Group A exceeded the control value by 30.4% ($p \leq 0.05$), while in Group B the difference reached 34.8% ($p \leq 0.05$). The haemoglobin concentration and MCH followed a similar trend, indicating enhanced oxygen transport efficiency and metabolic activity in the experimental groups. In carp from this group, haemoglobin levels exceeded those of the control by 6.7% ($p \leq 0.01$), while MCH values were higher by 22.9% ($p \leq 0.05$) (Fig. 6).

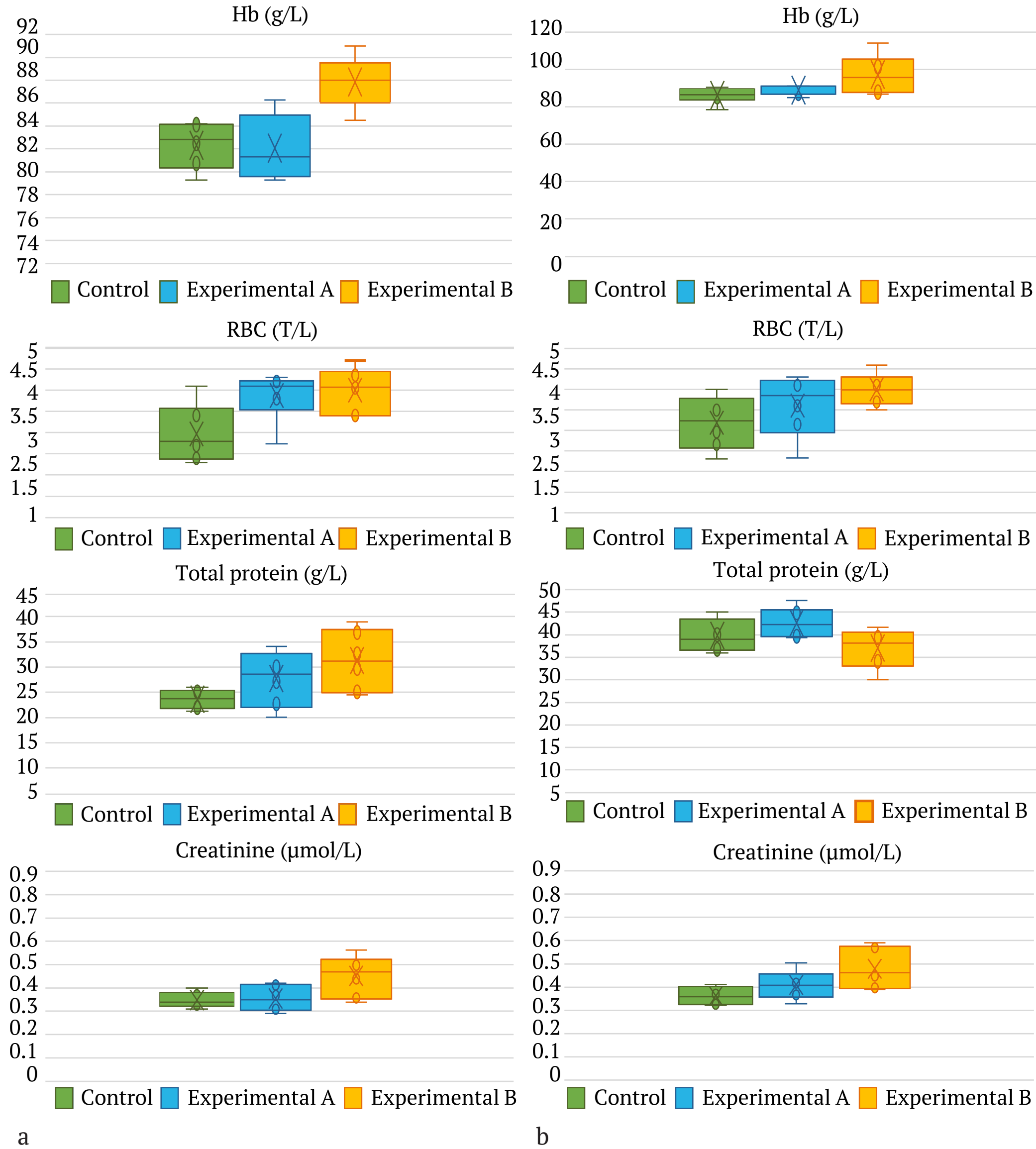

Figure 6. Study of the growth rate of *Cyprinus carpio* (a) and *Hypophthalmichthys molitrix* / *Hypophthalmichthys nobilis* (b) in ontogenesis under the influence of technological factors (x ± SD)
Source: authors' development

In experimental Group B, the erythrocyte and haemoglobin contents in the blood of silver carp exceeded those in the control group by 25.3% ($p \leq 0.05$) and 12.9% ($p \leq 0.05$), respectively. In experimental Group A, the average erythrocyte count and haemoglobin content were also higher than in the control group; however, the differences were not statistically significant. The concentrations of total protein and creatinine, which are indicative of protein synthesis, amino acid catabolism, and energy metabolism, were higher in all experimental groups compared with the control. The total protein content in the serum of carp and silver carp from experimental Group A exceeded that of the control by 17.1% and 4.5%, respectively. In experimental Group B, these parameters in silver carp and carp were higher than in the control by 15.4% ($p \leqslant 0.05$) and 32.3% ($p \leqslant 0.05$), respectively. Correlation analysis of parameters with the highest R^2 values indicated a synergistic effect of the technological factor (natural feed components) on the physiological status of carp and silver carp in both experimental groups. However, the values recorded in Group B were higher while remaining within the physiological norm for cyprinids (Tables 1, 2) A high level of correlation was established with the specified parameters, which was within the limits of $R^2 = 0.6\text{-}0.9$ (A) and $R^2 = 0.8\text{-}0.9$ (B) (Table 1) and $R^2 = 0.8\text{-}0.9$ (A) and $R^2 = 0.7\text{-}0.9$ (B) (Table 2).

Table 1. *Coefficient of determination of mass accumulation and blood parameters of carp in polyculture*

Parameters	Experiment A	Experiment B
	Cyprinus carpio	
creatinine	$R^2 = 0.7771$	$R^2 = 0.8499$
	$y = 11.588\ln(x) + 38.17$	$y = 8.5236\ln(x) + 33.635$
total protein	$R^2 = 0.7954$	$R^2 = 0.8974$
	$y = 8.6543\ln(x) - 2.5546$	$y = 9.2401\ln(x) - 4.9808$
red blood cell count (RBC)	$R^2 = 0.5797$	$R^2 = 0.863$
	$y = 9.0266\ln(x) + 13.935$	$y = 13.333\ln(x) + 8.307$
haemoglobin (Hb)	$R^2 = 0.8661$	$R^2 = 0.9112$
	$y = 54.353\ln(x) - 213.49$	$y = 68.903\ln(x) - 281.65$
	Hypophthalmichthys molitrix / Hypophthalmichthys nobilis	
creatinine	$R^2 = 0.8151$	$R^2 = 0.9468$
	$y = 9.9406\ln(x) + 37.235$	$y = 5.72\ln(x) + 33.25$
total protein	$R^2 = 0.7127$	$R^2 = 0.9169$
	$y = 15.566\ln(x) - 29.031$	$y = 13.652\ln(x) - 22.257$
red blood cell count (RBC)	$R^2 = 0.8934$	$R^2 = 0.8787$
	$y = 6.5648\ln(x) + 19.968$	$y = 10.044\ln(x) + 15.077$
haemoglobin (Hb)	$R^2 = 0.9396$	$R^2 = 0.7785$
	$y = 57.569\ln(x) - 230.12$	$y = 8.4743\ln(x) - 9.7891$

Source: developed by the authors

Table 2. *Coefficient of determination of parameters of chemical composition of muscle tissue and indicators of mass accumulation and blood of carp in polyculture*

Parameters	Experiment A	Experiment B
	Cyprinus carpio	
Body weight ↔ protein in muscle tissue	$y = 82.287\ln(x) + 201.74$	$y = 31.112\ln(x) + 60.316$
	$R^2 = 0.7697$	$R^2 = 0.9376$
Moisture ↔ fat in muscle tissue	$y = 34.912\ln(x) + 145.98$	$y = 11.483\ln(x) + 44.467$
	$R^2 = 0.7517$	$R^2 = 0.7026$
Cholesterol in the blood ↔ fat in muscle tissue	$y = 4.2002\ln(x) + 15.481$	$y = 3.2918\ln(x) + 10.727$
	$R^2 = 0.7855$	$R^2 = 0.6591$
	Hypophthalmichthys molitrix / Hypophthalmichthys nobilis	
Body weight ↔ protein in muscle tissue	$y = 54.587\ln(x) + 131.24$	$y = 28.289\ln(x) + 54.84$
	$R^2 = 0.8991$	$R^2 = 0.8748$

Table 2. Continued

Parameters	Experiment A	Experiment B
	Cyprinus carpio	
Moisture ↔ fat in muscle tissue	$y = 11.028\ln(x) + 44.954$	$y = 21.7\ln(x) + 91.032$
	$R^2 = 0.9532$	$R^2 = 0.8625$
Cholesterol in the blood ↔ fat in muscle tissue	$y = 1.6325\ln(x) + 3.9733$	$y = 2.6439\ln(x) + 7.9951$
	$R^2 = 0.9762$	$R^2 = 0.9029$

Source: developed by the authors

After analysing the leading parameters of protein and carbohydrate metabolism, was noted the positive effect of the technological factor with elements of multitrophic aquaculture on the blood composition of cyprinids. At the same time, in the blood of carp and silver carp, the creatinine concentration in experimental Group B significantly exceeded the value in the control by 30% $p \leq 0.05$ and by 32% $p \leq 0.05$, respectively (Tables 3, 4).

Table 3. *Functional status of the Cyprinus carpio organism under the influence of the feed factor under the conditions a modular multitrophic aquaculture system, (x ± SD)*

Parameters	Groups		
	Control	Experiment A	Experiment B
Red blood cell count, T/L	2.967 ± 0.680	3.870 ± 0.583*	4.001 ± 0.5195*
Haemoglobin content, g/L	82.333 ± 1.962	82.073 ± 2.848	87.833 ± 2.295**
MCV, mkm^3	124.649 ± 36.541	104.453 ± 30.058	102.903 ± 19.819
MCH, pg	28.912 ± 6.278	21.715 ± 4.159*	22.294 ± 3.1900*
MCHC, %	23.839 ± 3.810	21.493 ± 4.117	22.048 ± 3.675
Glucose, µmol/L	97.983 ± 12.001	102.117 ± 13.206	110.708 ± 12.4708
Total protein, g/L	23.667 ± 1.838	27.733 ± 5.495	31.317 ± 5.9975*
Creatinine, µmol/L	0.347 ± 0.033	0.355 ± 0.054	0.451 ± 0.0890*
Cholesterol, mg/dL	114.983 ± 5.115	112.400 ± 4.767	113.967 ± 2.658

Note: *0.05 ($p \leq 0.05$); **0.01 ($p \leq 0.01$); ***0.01 ($p \leq 0.001$); MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration
Source: developed by the authors

Table 4. *Functional status of the organism Hypophthalmichthys molitrix / Hypophthalmichthys nobilis under the influence of the feed factor under the conditions a modular multitrophic aquaculture system, (x ± SD)*

Parameters	Groups		
	Control	Experiment A	Experiment B
Red blood cell count, T/L	3.190 ± 0.655	3.613 ± 0.763	3.998 ± 0.3907*
Haemoglobin content, g/L	86.117 ± 4.144	88.980 ± 2.428	97.233 ± 10.7306*
MCV, mkm^3	122.231 ± 33.935	114.652 ± 24.598	105.798 ± 21.2466
MCH, pg	27.841 ± 4.984	25.680 ± 6.050	24.347 ± 1.7034
MCHC, %	23.547 ± 4.512	22.771 ± 4.588	23.692 ± 4.3003
Glucose, µmol/L	102.745 ± 7.820	111.750 ± 11.401	115.398 ± 12.7321
Total protein, g/L	37.033 ± 4.371	38.717 ± 8.007	42.750 ± 4.0412*
Creatinine, µmol/L	0.363 ± 0.038	0.409 ± 0.061	0.478 ± 0.0856*
Cholesterol, mg/dL	91.300 ± 4.839	90.883 ± 3.311	88.533 ± 3.168

Note: *0.05 ($p \leq 0.05$); **0.01 ($p \leq 0.01$); ***0.01 ($p \leq 0.001$)
Source: developed by the authors

In carp that received natural feed components within the multitrophic aquaculture system in experimental Group A and Group B, the concentrations of glucose, creatinine, and cholesterol in the blood differed from those observed in the control group. At the same time, the correlation between the biochemical composition of the muscle tissue and that of the blood exhibited a high R^2 value (Tables 1, 2). From a physiological and biochemical perspective, these findings were consistent with the activation of compensatory mechanisms within the cyprinid organism.

Modern scientific and applied research increasingly demonstrated the relevance of combined systems in aquaculture, with particular emphasis on their environmental friendliness (Dyudyaeva, 2021). Experimental findings published by various researchers highlighted the growing recognition of the multitrophic approach in aquaculture (Can & Seyhaneyildiz Can, 2023). The importance of reducing nutrient emissions into the environment and rationally utilising the potential of such systems was emphasised. However, investigations in this area, particularly those concerning marine bioremediation, remained largely at the pilot stage. Analysis of the available publications indicated that the majority of multitrophic systems were developed under conditions of predominantly marine aquaculture, whereas land-based systems of this type exhibited greater efficiency compared with open-water systems (Batır *et al.*, 2025).

A combined approach in aquaculture met the modern requirements of the industry. One of its notable advantages was the potential to reduce the use of fishmeal and fish oil in aquaculture diets (Cao *et al.*, 2018; Harmantepe & Yılmaz, 2025). Previous research confirmed the positive effects of incorporating *Spirulina* and *Chlorella* (for example, replacing up to 25% of fishmeal) in *Cyprinus carpio* diets, resulting in enhanced growth, nutrient conversion, and digestive enzyme activity. General physiological parameters of the blood, as well as the immunity and resistance of juveniles, improved accordingly. The studies of H. Sabetmand *et al.* (2024) also demonstrated an increase in growth rate and immune response ($p < 0.05$), alongside a reduction in the feed conversion ratio, when *Spirulina* sp. and citric acid were used in carp diets. The influence of natural components on these parameters was similarly confirmed experimentally in the present study.

According to A. van Huis (2020), increasing attention in aquaculture was directed towards the use of the black soldier fly (*Hermetia illucens*) as a component capable of converting organic waste into nutritionally valuable feed ingredients, thereby enhancing the organism's metabolic activity. In the present experiment, the total protein level in the blood of cyprinids in the experimental groups increased simultaneously with protein accumulation in muscle tissues. This could be attributed to elevated protein synthesis and stimulated amino acid metabolism in fish receiving natural feed supplements. Meanwhile, cholesterol levels in the blood of the experimental fish were lower than those in the control and other experimental groups, which was also reflected in the reduced lipid content of the muscle tissue. A positive correlation was therefore established between body weight and blood composition parameters of cyprinids. Similar results were reported by P. Bryant & A. Matty (1980) and V. Serra *et al.* (2024), who demonstrated improved health, welfare, growth performance, and fillet quality in fish. These findings were further supported by research demonstrating enhanced product quality and sustainable protein utilisation in aquaculture diets (D'Souza *et al.*, 2006; Hua *et al.*, 2019). S. Maiolo *et al.* (2020) highlighted the practical significance of using natural protein-enriched components as alternative energy sources within environmentally sustainable production systems. Such approaches facilitated the development of aquaculture while simultaneously reducing environmental pressure, thereby contributing to the sustainable development of the industry. The findings of R. Chaklader *et al.* (2019) further substantiated that metabolic processes in aquatic organisms could be modified through dietary optimisation. The rational use of resources in aquaculture, alongside consideration for ecosystem balance and biodiversity, was confirmed by both scientific and applied studies in this field (Samarathunga *et al.*, 2023; Parsa Khanghah & Can, 2024). In addition to optimising technological processes in aquaculture, particular attention was devoted to feeding strategies and multitrophic production models.

Recent studies revealed positive effects of herbal preparations, beneficial bacteria, and probiotics on the general physiological condition of aquatic organisms. Positive findings in aquaculture and biotechnology literature emphasised the synergistic potential of such natural components. Furthermore, the implementation of integrated aquaculture management systems substantially increased production efficiency compared with traditional approaches and farming models.

Conclusions

The application of the proposed modular system incorporating elements of multitrophic aquaculture contributed to the production of aquaculture outputs that were as close as possible to environmentally friendly and safe standards. This effect was attributed to the use of natural components cultivated autonomously within a single modular system, which allowed full control of cultivation conditions and quality parameters throughout the entire production cycle. The integration of components with different trophic levels within the system provided a synergistic effect that promoted the rational use of resources. The inclusion of carp-based components as supplementary feed in experimental Group B resulted in higher developmental rates during ontogenesis. Overall, improvements in physiological and biochemical processes were observed in comparison with other experimental groups. The multitrophic system model demonstrated a high potential for stimulating crustacean development. Compared with the control group, the actual performance indicators in experimental Group A and Group B were higher, with Group B showing the most pronounced improvement. Increases relative to the control were recorded as follows: average body weight (1.21-7%), weight gain (2.9-6.9%), and feed conversion ratio (4.9-14.6%). In parallel, the blood composition of fish in the experimental groups improved, particularly in haematopoietic and protein-synthesising functions. The total number of erythrocytes and haemoglobin content increased by 13-36% and 3-13%, respectively, compared with the control. Total protein content increased by 5-32%. Correlation analysis confirmed strong relationships among the studied parameters (R^2 = 0.7-0.9). Future research should focus on a more detailed analysis of the biochemical composition of fish muscle tissue under the influence of technological factors, along with a comprehensive histomorphological evaluation.

Acknowledgements

The authors express their gratitude to the Armed Forces of Ukraine for the opportunity to carry out scientific research and support Ukrainian science during this difficult time for Ukraine.

Funding

This research did not receive any specific grant from funding institutions in the public, commercial, or non-profit sectors.

Conflict of Interest

The authors declare that they have no potential conflict of interest regarding the authorship or publication of this article.

References

[1] Alam, M., *et al*. (2024). Potential of integrated multitrophic aquaculture (IMTA) to make prawn farming sustainable in Bangladesh. *Frontiers in Sustainable Food Systems*, 8, article number 1412919. doi: 10.3389/fsufs.2024.1412919.

[2] Batır, E., Aydın, İ., Theodorou, J.A., & Rakaj, A. (2025). *Mytilus galloprovincialis*'s role in Integrated Multi-Trophic Aquaculture (IMTA): A comprehensive review. *Journal of the World Aquaculture Society*, 56 (2), article number e70013. doi: 10.1111/jwas.70013.

[3] Bekh, V.V., Martseniuk, V.P., & Tushnytska, N.I. (2020). Prospects for the use of protein components of non-traditional origin in compound feed for aquaculture (review). *Fisheries Science of Ukraine*, 2(52), 53-64. doi: 10.15407/fsu2020.02.053.

[4] Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C.C., Paoletti, M.G., & Ricci, A. (2013). Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive Reviews in Food Science and Food Safety*, 12(3), 296-313. doi: 10.1111/1541-4337.12014.

[5] Bryant, P.L., & Matty, A.J. (1980). Optimisation of *Artemia* feeding rate for carp larvae (*Cyprinus carpio* L.). *Aquaculture*, 21(3), 203-212. doi: 10.1016/0044-8486(80)90131-3.

[6] Can, E., & Seyhaneyildiz Can, Ş. (2023). Species combinations; polyculture, integrated multi-trophic aquaculture, and aquaponics as the sustainable aquaculture practice. *Aquatic Animal Reports*, 1(1), 27-33. doi: 10.5281/zenodo.7660399.

[7] Cao, S., Zhang, P., Zou, T., Fei, S., Han, D., Jin, J., Liu, H., Yang, Y., Zhu, X., & Xie, S. (2018). Replacement of fishmeal by spirulina *Arthrospira platensis* affects growth, immune related-gene expression in gibel carp (*Carassius auratus gibelio* var. CAS III), and its challenge against *Aeromonas hydrophila* infection. *Fish & Shellfish Immunology*, 79, 265- 273. doi: 10.1016/j.fsi.2018.05.022.

[8] Chaklader, R., Siddik, M.A.B., Fotedar, R., & Howieson, J. (2019). Insect larvae, *Hermetia illucens* in poultry by-product meal for barramundi, *Lates calcarifer* modulates histomorphology, immunity and resistance to *Vibrio harveyi*. *Scientific Reports*, 9, article number 16703. doi: 10.1038/s41598-019-53018-3.

[9] Deren, O.V., & Fedorenko, M.O. (2023). Substantiation and prospects of using insects as a source of protein in fish feeds (a review). *Fisheries Science of Ukraine*, 4(66), 114-140. doi: 10.61976/fsu2023.04.114.

[10] Directive 2010/63/EU of the European Parliament and of the Council "On the Protection of Animals Used for Scientific Purposes". (2010, September). Retrieved from https://eur-lex.europa.eu/eli/dir/2010/63/oj.

[11] D'Souza, N., Skonberg, D.I., Stone, D.A.J., & Brown, P.B. (2006). Effect of soybean meal-based diets on the product quality of rainbow trout fillets. *Journal of Food Science*, 71(4), 337-342. doi: 10.1111/j.1750-3841.2006.00018.x.

[12] Dyudyaeva, O.A. (2021). The state of harmonization of Ukrainian legislation in the sphere production of organic aquaculture products with European norms. *Aquatic Bioresources and Aquaculture*, 9, 62-85. doi: 10.32851/wba.2021.1.6.

[13] European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. (1986, March). Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A21999A0824%2801%29.

[14] FAO, IFAD, UNICEF, WFP & WHO. (2025). *The state of food security and nutrition in the world 2025 – addressing high food price inflation for food security and nutrition*. Rome: FAO, IFAD, UNICEF, WFP, WHO. doi: 10.4060/cd6008en.

[15] FAO. (2024). *The state of world fisheries and aquaculture 2024 blue transformation in action*. Retrieved from https://surl.lu/cqhjbs.
[16] Fraijo-Valenzuela, A., Arias-Moscoso, J.L., García-Pérez, O.D., Rodriguez-Anaya, L.Z., & Gonzalez-Galaviz, J.R. (2024). The biotechnological potential of crickets as a sustainable protein source for fishmeal replacement in aquafeed. *BioTech*, 13(4), article number 51. doi: 10.3390/biotech13040051.
[17] Gopalakannan, A., & Arul, V. (2006). Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. *Aquaculture*, 255(1-4), 179-187. doi: 10.1016/j.aquaculture.2006.01.012.
[18] Gupta, S., *et al*. (2024). Recent developments in recirculating aquaculture systems: A review. *Aquaculture Research*, 2024, article number 6096671. doi: 10.1155/are/6096671.
[19] Gusmão, C.T.P. (2024). Evaluation of the effects of essential oils on the reduction of stress: A rapid narrative review. *Brazilian Journal of Health Aromatherapy and Essential Oil*, 1(1), article number bjhae4. doi: 10.62435/2965-7253.bjhae.2024.bjhae4.
[20] Harmantepe, B., & Yılmaz, E. (2025). Effects of *Spirulina* and *Chlorella* used as protein source on growth and digestion enzymes of common carp (*Cyprinus carpio*, L., 1758). *Turkish Journal of Agriculture – Food Science and Technology*, 13(3), 787-793. doi: 10.24925/turjaf.v13i3.787-793.7254.
[21] Henry, M., Gasco, L., Piccolo, G., & Fountoulaki, E. (2015). Review on the use of insects in the diet of farmed fish: Past and future. *Animal Feed Science and Technology*, 203, 1-22. doi: 10.1016/j.anifeedsci.2015.03.001.
[22] Honcharova, O., & Bekh, V. (2023). Adaptive solutions in aquaculture under the influence of transformation of abiotic and biotic factors. *European Science*, 3, 58-64. doi: 10.30890/2709-2313.2023-16-03-006.
[23] Honcharova, O., Kutishchev, P., & Korzhov, Ye. (2020). A method to increase the viability of *Cyprinus carpio* (Linnaeus, 1758) stocking of the aquatories under the influence advanced biotechnologies. *Aquaculture Studies*, 21(4), 139-148. doi: 10.4194/2618-6381-v21_4_01.
[24] Hrynevych, N.E., Khomyak, O.A., Prysiazhniuk, N.M., & Mykhalskyi, O.R. (2019). Analysis of the hydrotechnological component of industrial aqua farms with a closed water supply. *Water Bioresources and Aquaculture*, 59-76. doi: 10.32851/wba.2019.2.5.
[25] Hua, K., *et al*. (2019). The future of aquatic protein: Implications for protein sources in aquaculture diets. *One Earth*, 1(3), 316-329. doi: 10.1016/j.oneear.2019.10.018.
[26] Józefiak, A., & Engberg, R.M. (2017). Insect proteins as a potential source of antimicrobial peptides in livestock production. A review. *Journal of Animal and Feed Sciences*, 26(2), 87-99. doi: 10.22358/jafs/69998/2017.
[27] Land-based aquaculture report 2023 (3rd ed.) (2023). Retrieved from https://surl.li/axnrgp.
[28] Maiolo, S., Parisi, G., Biondi, N., Lunelli, F., Tibaldi, E., & Pastres, R. (2020). Fishmeal partial substitution within aquafeed formulations: Life cycle assessment of four alternative protein sources. *International Journal of Life Cycle Assessment*, 25, 1455-1471. doi: 10.1007/s11367-020-01759-z.
[29] Meitei, M.M., Muralidhar, A.P., Syamala, K., Sureesh, S., Biswas, G., Megarajan, S., & Munilkumar, S. (2025). Assessment of filtration capacity of different bivalve species suitable for integrated multi-trophic aquaculture (IMTA) systems vis-à-vis waste valorization for sustainable environment. *Discover Sustainability*, 6, article number 240. doi: 10.1007/s43621-025-01104-0.

[30] Melenchón, F., Larrán, A.M., de Mercado, E., Hidalgo, M.C., Cardenete, G., Barroso, F.G., Fabrikov, D., Lourenço, H.M., Pessoa, M.F., & Tomás-Almenar, C. (2021). Potential use of black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) insectmeals in diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, 27, 491-505. doi: 10.1111/anu.13201.

[31] Nogales-Mérida, S., Gobbi, P., Józefiak, D., Mazurkiewicz, J., Dudek, K., Rawski, M., Kierończyk, B., & Józefiak, A. (2019). Insect meals in fish nutrition. *Reviews in Aquaculture*, 11, 1080-1103. doi: 10.1111/raq.12281.

[32] Parsa Khanghah, A., & Can, E. (2024). Effect of vermicompost supplement on *Rainbow trout* performance. *Marine Science and Technology Bulletin*, 13(3), 234-238. doi: 10.33714/masteb.1524629.

[33] Sabetmand, H., Langarudi, H.F., Zamini, A., & Tizkar, B. (2024). Influence of *Spirulina* sp. and citric acid dietary supplements on the growth performance and immune parameters of common carp (*Cyprinus carpio*). *International Aquatic Research*, 16(1), 91-99. doi: 10.22034/iar.2023.1972999.1358.

[34] Sakharnatsky, V.V. (2024). Multi-vector nature of ecological and economic assessment of water resources of Ukraine. *Agroecological Journal*, 1, 165-176. doi: 10.33730/2077-4893.1.2024.299953.

[35] Samarathunga, J., Wijesekara, I., & Jayasinghe, M. (2023). Seaweed proteins as a novel protein alternative: Types, extractions, and functional food applications. *Food Reviews International*, 39(7), 4236-4261. doi: 10.1080/87559129.2021.2023564.

[36] Serra, V., Pastorelli, G., Tedesco, D.E.A., Turin, L., & Guerrini, A. (2024). Alternative protein sources in aquafeed: Current scenario and future perspectives. *Veterinary and Animal Science*, 25, article number 100381. doi: 10.1016/j.vas.2024.100381.

[37] Shafique, L., Abdel-Latif, H.M.R., Hassan, F., Alagawany, M., Naiel, M.A.E., Dawood, M.A.O., Yilmaz, S., & Liu, Q. (2021). The feasibility of using yellow mealworms (*Tenebrio molitor*): Towards a sustainable aquafeed industry. *Animals*, 11(3), article number 811. doi: 10.3390/ani11030811.

[38] Silva, F.R.O., Ripardo, N.A., Jesus, R.A., & Camargo, J.O.B.S. (2024). Copaiba essential oil: Composition, therapeutic actions, and methods of use for health and well-being. *Brazilian Journal of Health Aromatherapy and Essential Oil*, 1(1), article number bjhae18. doi: 10.62435/2965-7253.bjhae.2024.bjhae18.

[39] Syrovatka, N. (2021). Pisciculture, biological and physiological indicators of age–1+ carp while adding naked oats into main feeding. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 17(5), 115-128. doi: 10.31548/dopovidi2021.05.011.

[40] Taşcı, B., Maita, M., Futami, K., Haga, Y., Sakai, Y., & Katagiri, T. (2025). Evaluating the effects of dietary bergamot essential oil supplementation on growth performance, blood parameters, and antioxidant responses in common carp *Cyprinus carpio* juveniles. *Fisheries Scince*, 91, 557-566. doi: 10.1007/s12562-025-01858-6.

[41] The International Aquaculture Feed Formulation Database (IAFFD). (n.d.). Retrieved from https://www.iaffd.com/about.html.

[42] van Huis, A. (2020). Insects as food and feed, a new emerging agricultural sector: A review. *Journal of Insects as Food and Feed*, 6(1), 27-44. doi: 10.3920/JIFF2019.0017.

[43] Wang, H.C., Liao, H.Y., & Chen, H.L. (2011). Tenebrio small-scale ecological farming feasibility study. In *Advanced materials research* (Vols. 356-360, pp. 267-270). Baech: Trans Tech Publications. doi: 10.4028/www.scientific.net/amr.356-360.267.

[44] Wang, J., Deng, L., Chen, M., Che, Y., Li, L., Zhu, L., Chen, G., & Feng, T. (2024). Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade. *Animal Nutrition*, 17, 244-264. doi: 10.1016/j.aninu.2024.01.012.
[45] Yevtushenko, M.Yu., & Khyzhniak, M.I. (2019). *Methodology of scientific research in fish farming*. Kyiv: Center for Educational Literature.
[46] Yevtushenko, M.Yu., Dudnyk, S.V., Rudyk-Leuska, N.Ya., & Khyzhniak, M.I. (2022). *Physiology and biochemistry of hydrobionts. Part 1*. Kyiv: FOP Yamchynskyi O.V.

Оптимізація розвитку та фізіологічного статусу коропа в полікультурі за умов впровадження елементів мультитрофічної аквакультури

Олена Гончарова
Кандидат сільськогосподарських наук, доцент
Херсонський державний аграрно-економічний університет
73006, вул. Стрітенська, 23, м. Херсон, Україна
https://orcid.org/0000-0002-9702-7458

Віталій Бех
Доктор сільськогосподарських наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-4254-815X

Ірина Кононенко
Кандидат сільськогосподарських наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-3906-3650

Олеся Охріменко
Кандидат сільськогосподарських наук
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0001-9867-0595

Анотація. Метою дослідження було визначення впливу та аналіз ефективності використання технологічних факторів для формування їх синергічного ефекту при годівлі та вирощуванні молоді коропових риб. Дослідження базувалося на теоретичних та експериментальних лабораторних методах у рибництві. Результати дослідження продемонстрували, що альтернативні джерела білка (*Spirulina platensis*, *Artemia* та *Hermetia illucens*), включені до інтегрованої мультитрофічної системи аквакультури (МТА), значно покращують темпи росту та зменшують залежність від традиційних кормів. Культивування всіх компонентів в єдиній системі МТА забезпечувало максимальний контроль над якісними та кількісними характеристиками протягом усього циклу. Результати продемонстрували підвищення життєздатності організму молоді коропових риб, покращення складу їхньої крові в дослідних групах А та В. Однак, група В продемонструвала вищі якісні та кількісні параметри, що характеризувалося активацією гомеостатичної регуляції та процесів кровотворення. Покращення гематологічного профілю, маси тіла та складу м'язової тканини тісно корелювало з параметрами активації білкового та вуглеводного обміну та прискоренням онтогенезу. Риби в дослідних групах відрізнялися кращими параметрами, що визначають накопичення маси, метаболічні процеси (WG, FCR, HSI, VSI). Позитивна висока кореляція була зафіксована в дослідних групах А та В: маса тіла ↔ білок у м'язовій тканині (R^2 = 0,8-0,9); холестерин у крові ↔ жир у м'язовій тканині (R^2 = 0,8-0,7). В дослідній групі А: у крові коропа *Cyprinus carpio* (Linnaeus, 1758) зафіксовано вищий вміст еритроцитів та загального білка ($p \leqslant 0{,}05$). У крові коропа *Cyprinus carpio* (Linnaeus, 1758) групи В: вміст еритроцитів, MCH, загального білка, креатиніну ($p \leqslant 0{,}05$) та гемоглобіну (Hb) ($p \leqslant 0{,}01$) перевищував контрольну групу. У крові товстолобика *Hypophthalmichthys molitrix* / *Hypophthalmichthys nobilis* групи В: вміст еритроцитів, Hb, загального білка та креатиніну ($p \leqslant 0{,}05$) перевищував контрольну групу. Найкращий біохімічний склад м’язової тканини зафіксовано у риб з дослідних груп А та В

Ключові слова: годівля; вирощування; коропові; фізіолого-біохімічні процеси; мультитрофність; рециркуляційні системи

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 16(4), 47-58
Received 29.05.2025 Revised 28.10.2025 Accepted 27.11.2025

UDC 636.3.084
DOI: 10.31548/animal.4.2025.47

Rumen methane production, microbial population, and blood metabolites in sheep fed jengkol (*Archidendron jiringa*) peel as a substitute for native grass

Nur Hidayah*
Master of Animal Sciences, Assistant Professor
Tidar University
59155, 1 Barito Str., Magelang, Indonesia
https://orcid.org/0000-0002-9290-3578

Komang Gede Wiryawan
Doctor of Animal Sciences, Professor
IPB University
16680, IPB Darmaga Campus, Agatis Str., Bogor, Indonesia
https://orcid.org/0000-0002-0593-9653

Sri Suharti
Doctor of Animal Sciences, Professor
IPB University
16680, IPB Darmaga Campus, Agatis Str., Bogor, Indonesia
https://orcid.org/0000-0002-0542-4086

Abstract. Agro-industrial and agricultural by-products can be converted into high-quality feed by ruminants. Jengkol (*Archidendron jiringa*) peel is an agricultural by-product characterised by a high crude fibre content. The aim of this study was to evaluate whether jengkol peel could be used as a substitute for native grass as a fibre source for ruminants. The variables investigated were rumen methane prediction, microbial population, and blood metabolites in sheep. The experiment used 15 male sheep with body weights ranging from 15 to 27 kg in a randomised complete block design with three treatments (0.0%, 15.0%, and 22.5% jengkol peel substitution) and five replications. Substituting native grass with up to 22.5% jengkol peel did not adversely affect ($p > 0.05$) the total bacterial population, nor did it alter ($p > 0.05$) methane prediction, but it reduced ($p < 0.05$) the

Suggested Citation:
Hidayah, N., Wiryawan, K. G., & Suharti, S. (2025). Rumen methane production, microbial population, and blood metabolites in sheep fed jengkol (*Archidendron jiringa*) peel as a substitute for native grass. *Animal Science and Food Technology*, 16(4), 47-58. doi: 10.31548/animal.4.2025.47.

**Corresponding author*

protozoal population by 13.95% compared with the control, likely due to the saponin content of the peel. Moreover, substitution up to 22.5% did not negatively influence ($p > 0.05$) key blood metabolites such as glucose, cholesterol, triglycerides, and high-density lipoproteins (HDL). It was concluded that jengkol peel can be substituted for native grass at levels up to 22.5% without detrimental effects on blood health or rumen bacterial populations, while reducing protozoal numbers

Keywords: *in vivo* study; jengkol by-product; native grass; rumen ecosystem; ruminants

Introduction

The utilisation of agricultural by-products, including crop residues, agro-industrial by-products, and other fibrous materials as livestock feed, represents a sustainable strategy to address the limited availability of conventional feed resources. These by-products, which are typically indigestible by non-ruminants, often retain considerable nutritional value and can be effectively digested by ruminants due to their unique rumen microbial fermentation system (Kazemi, 2025). According to E. Ungerfeld *et al.* (2023), the rumen harbours a complex community of bacteria and protozoa, which digest and ferment the different chemical fractions in agricultural by-products into volatile fatty acids as an energy source. N. Hidayah *et al.* (2019) reported that one such underutilised by-product is jengkol (*Archidendron jiringa*) peel, which contains high levels of crude fibre (3.07-35.28%) and total digestible nutrients (TDN) (15.17-19.26% and 63.87-65.82%), demonstrating its potential as ruminant feed.

The *Archidendron jiringa* (Jack) I.C. Nielsen plant, commonly known as jengkol, is a species in the *Archidendron* genus of the Fabaceae family. This plant contains biologically active secondary metabolites that can be used to treat cancer, kill bacteria and fungi, and act as antioxidants (Noviany *et al.*, 2022). The plant typically reaches a height of 25 metres with smooth, grey bark, and usually grows in communal gardens and forests. The coiled pods that hang from the tree's branches contain three to nine beans per pod. Phytochemical screening has revealed the presence of alkaloids, steroids, triterpenoids, glycosides, saponins, flavonoids, and tannins in jengkol (Noviany *et al.*, 2021). According to A. Singh *et al.* (2024), saponins improve rumen fermentation by enhancing microbial protein synthesis and nutrient digestibility. K. Loregian *et al.* (2023) stated that tannins bind to dietary proteins, reducing protein degradation in the rumen and improving protein utilisation.

The availability of native grasses, which serve as a primary fibre source for ruminants, is often inconsistent, particularly during dry seasons. This has led to an increasing need for alternative fibre sources that are locally available, cost-effective, and nutritionally suitable. A. Onyango *et al.* (2019) stated that utilising local supplementary feedstuffs can help compensate for nutritional deficiencies during dry seasons. Jengkol peel, commonly discarded as waste, holds potential as a substitute for traditional fibre sources in ruminant diets. Previous *in vitro* evaluations have shown that incorporating jengkol peel can enhance total volatile fatty acid (VFA) production and increase NH_3 concentration, both of which are important for energy supply and microbial protein synthesis, ultimately supporting livestock productivity. However, scientific data on the effects of jengkol peel substitution on rumen fermentation, methane prediction, microbial populations, and blood metabolites in *in vivo* studies remain limited. This study aimed to evaluate the potential of jengkol peel as a substitute for native grass in sheep diets by examining its effects on methane prediction, rumen microbial populations, and blood metabolites. The findings were expected to contribute to a scientific basis for the sustainable use of jengkol peel as an alternative fibre source in ruminant nutrition.

Materials and Methods

This study was conducted at the Animal Science Faculty, IPB University, Bogor farm from September to November 2019. The experiment included 15 male sheep weighing 15-27 kg. These sheep were allocated into three treatment groups (n = 5 per group) based on their live weight (LW) and arranged in a randomised complete block design. The sheep were housed in individual pens with a temperature of 25-27°C and humidity of 70-72%. Pen lamps were operated on a 12-hour light/dark cycle. The treatments were as follows: T1 = 40% concentrate (C) + 60% native grass (NG), T2 = 40% C + 45% NG + 15% jengkol peel (JP), and T3 = 40% C + 37.5% NG + 22.5% JP. The concentrate contained 10-11% crude protein and 56-60% total digestible nutrients, and was composed of rice bran, tapioca industry by-product, molasses, copra meal, NaCl, $CaCO_3$, urea, and premix (Tables 1, 2). The nutrient content of the concentrate was determined according to AOAC (2000) methods. The jengkol peel powder was prepared by sun-drying fresh jengkol peel for 3-4 days until its weight stabilised, after which it was ground into powder. Jengkol peel was collected from market waste. Based on dry matter (DM), this peel powder contained ash (3.32%), crude protein (8.16%), ether extract (0.72%), crude fibre (34.18%), nitrogen-free extract (53.64%), total digestible nutrients (52.19%), saponin (35.13%), and tannin (1.43%). Each sheep received 3.5-4.0% of its body weight as daily feed ration, provided twice daily: concentrate at 08:00 and 13:00, and chopped native grass (5-8 cm) at 10:00 and 15:00. Clean water was provided *ad libitum*. The *in vivo* experiment lasted 45 days in total, comprising 10 days for adaptation, 35 days for the treatment period, and 14 days for total collection at the end of the treatment period.

Table 1. *Feed formulation (% dry matter)*

Treatments	T1	T2	T3
Native grass	60	45	37.5
Jengkol peel	0	15	22.5
Rice brand	5	6	9
Cassava by-product	8	9	9
Molasses	8	8	8
Copra meal	15	13	10
NaCl	1	1	1
CaCO3	1.5	1.5	1.5
Urea	1	1	1
Premix	0.5	0.5	0.5
Total (%)	100	100	100

Note: T1 = 40% concentrate (C) + 60% native grass (NG); T2 = 40% C + 45% NG + 15% jengkol peel (JP); T3 = 40% C + 37.5% NG + 22.5% JP

Source: compiled by the authors

Table 2. *Nutrition content native grass substitution with jengkol (A. jiringa) peel (% DM)*

Ingredient (%)	T1	T2	T3
Ash	10.54	8.41	7.63
Ether extract	3.08	1.84	2.19
Crude protein	11.12	10.74	11.11
Crude fibre	22.93	24.32	23.58

Table 2. Continued

Ingredient (%)	T1	T2	T3
Nitrogen-free extract	52.32	54.70	55.49
Total digestible nutrient	60.24	56.81	59.07

Note: T1 = 40% concentrate (C) + 60% native grass (NG); T2 = 40% C + 45% NG + 15% jengkol peel (JP); T3 = 40% C + 37.5% NG + 22.5% JP
Source: compiled by the authors

Before morning feeding on the final days of the entire collection period, rumen fluid was collected from each sheep using a vacuum pump for volatile fatty acid and rumen microbial analysis. The molar proportion of VFA was determined using gas chromatography (GC 8A, Shimadzu Corp., Kyoto, Japan; capillary column containing 10% SP-1200, 1% H_3PO_4 on 80/100 Chromosorb WAW, with nitrogen as the carrier gas) according to the method described by A. Moss *et al.* (2000). Methane production was estimated using the following formula based on molar proportions of VFA: CH_4 = 0.45 acetate – 0.275 propionate + 0.40 butyrate. The protozoa population was quantified under a 40 × microscope using a Fuchs-Rosenthal counting chamber (4 mm × 4 mm × 0.2 mm) from a rumen liquid sample (0.5 mL) mixed with 2 mL methyl green formaldehyde saline solution. The total bacterial population was enumerated using the method described by K. Ogimoto & S. Imai (1981) with BHI media and the roller tube technique.

Blood samples were collected the day before rumen fluid sampling from the jugular vein using a Venoject needle and sterile tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (3.00 mL, BD Vacutainer, Plymouth, Devon, UK) before morning feeding. Blood metabolite analysis included glucose (mg/dL), cholesterol (mg/dL), triglycerides (mg/dL), and high-density lipoprotein (HDL) (mg/dL) levels, determined using photometry techniques with a blood haematology analyser (Photometer Microlab 300, Vital Scientific, USA). Data were statistically analysed using analysis of variance (ANOVA), and Duncan's multiple range test was employed to examine differences between treatment means. Statistical analysis was conducted using IBM SPSS Statistics version 26. The study was approved by the Ethics Committee of the Institute of Research and Community Service, IPB University, Bogor (approval number: 155-2019 IPB). All experimental procedures were conducted in accordance with the principles outlined by FASS (2010).

Results and Discussion

The substitution of native grass with jengkol peel at levels up to 22.5% did not adversely affect the total bacterial population or predicted methane production, yet it significantly reduced ($p < 0.05$) the protozoal population by 13.95% compared with the control (Table 3). This indicates that the inclusion of jengkol peel did not impair overall rumen microbial fermentation activity, which is essential for maintaining normal rumen function and nutrient digestibility.

Table 3. *Correlation coefficients between ECM and other studied characteristics (n = 595)*

Treatments	Methane production (%)	Protozoa (log cell/mL)	Total bacteria (log cell/mL)
T1	6.06±0.24	5.95^b**±0.06**	7.79±1.68
T2	6.20±0.16	5.21^a**±0.14**	8.48±1.16
T3	6.24±0.23	5.12^a**±0.16**	6.85±1.18

Note: T1 = 40% concentrate (C) + 60% native grass (NG); T2= 40% C + 45% NG + 15% jengkol peel (JP); T3 = 40% C + 37.5% NG + 22.5% JP
Source: compiled by the authors

Notably, the observed 13.95% reduction in protozoal numbers is consistent with the known defaunating action of saponins. N. Hidayah *et al*. (2019) reported that jengkol peel contains 26.52% saponin, a level considerably higher than that found in the leaves (13.79%). Saponins form complexes with sterols in protozoal cell membranes, thereby increasing membrane permeability, disrupting cellular homeostasis, damaging cell walls, and ultimately causing lysis (Wallace *et al*., 2002). M. Chen *et al*. (2019) demonstrated that the steroidal saponin 1688-1 interacts with cholesterol-containing lipid membranes composed of mixed liquid-ordered (Lo) and liquid-disordered (Ld) phases, inducing membrane defects and the formation of small aggregates on the membrane surface. A. Kholif (2023) further noted that, in the rumen, saponins act as membrane-permeabilising, immunostimulating, hypocholesterolaemic, and defaunating agents that modulate ruminal fermentation. It is also well documented that saponins can provoke cell rupture, selectively eliminate ruminal protozoa, and interact with cholesterol in cell membranes, thereby improving nitrogen-use efficiency and potentially enhancing overall ruminant performance.

Protozoa are known to contribute to enteric methane production in the rumen owing to their symbiotic relationship with methanogenic archaea. Nevertheless, the observed reduction in protozoal numbers did not lead to a corresponding decrease in predicted methane emissions. This suggests that, at the inclusion levels tested, jengkol peel did not contain sufficient concentrations of active compounds to suppress methanogenesis directly, that compensatory microbial pathways maintained methane output, or that saponins are more effective against protozoa than against methanogenic archaea. Consistent with this, an in vitro study by A. Patra *et al*. (2012) showed that supplementation with Quillaja (QSP) or Yucca (YSP) saponins at 0-0.6 g/L did not alter the archaeal community. Similarly, A. Patra & Z. Yu (2014) reported that saponins (0.6 g/L), either alone or combined with nitrate (5 mM) and sulphate (5 mM), failed to reduce populations of total bacteria, *Ruminococcus albus*, or methanogenic archaea. A meta-analysis by M. Ridla *et al*. (2021) indicated that saponins generally reduce methane emissions, but their effects on methanogen populations are inconsistent across studies. Combinations of saponins with other compounds, such as nitrate, have been shown to produce substantially greater reductions in both methane production and methanogen abundance than saponins alone (Wu *et al*., 2019).

The absence of methane mitigation in the present study may also be attributable to the low tannin concentration in jengkol peel (1.43% DM), which was insufficient to inhibit methanogenic archaea or redirect metabolic pathways away from methanogenesis. Methanogenic archaea (commonly termed methanogens) are a unique group of anaerobic microorganisms that produce methane as a by-product of their energy metabolism (Wagner *et al*., 2025). A. Jayanegara *et al*. (2018) reported that tannins can directly reduce methane production by inhibiting methanogenic archaea. A. Scalbert (1991) showed that tannin monomers such as pyrogallol, gallic acid, and tannic acid are toxic to methanogens. However, G. Wischer *et al*. (2013), evaluating ten tannin sources (chestnut, mimosa, myrabolan, quebracho, sumach, tara, valonea, oak, cocoa, and grape seed), found that only extracts with very high total tannin content (chestnut ⩾ 76%, sumach ⩾ 62%, valonea ⩾ 67%) significantly lowered methane production, whereas others did not. Similarly, A. Cieslak *et al*. (2016) observed that *Sanguisorba officinalis* tannin extract (451 mg g^{-1} total tannins) reduced methane only at the highest inclusion level (100 mg per feed portion of 60% meadow hay and 40% barley meal). These findings demonstrate that the efficacy of tannins in suppressing methane varies considerably with concentration, source, chemical structure, and molecular weight.

Other rumen fermentation characteristics reported by N. Hidayah *et al*. (2020) showed that the treatments had no effect on ruminal pH (6.06 vs. 6.24) or NH_3 concentration (14.99 vs. 18.35 mM) but reduced total VFA concentration (103.23 vs. 68.90 mM) compared with the control. The lower total VFA is likely explained by the higher acid-detergent fibre (ADF) content of jengkol peel (40.84-43.78% DM) relative to native grass (30.12-41.08% DM). X. Xie *et al*. (2018) reported that diets containing corn stover with higher ADF (37.2% DM) produced lower total VFA (62.0, 61.2, and 57.0 mM at 3, 6, and 24 h post-feeding, respectively) than diets based on corn silage and alfalfa with lower ADF (19.6% DM; total VFA 89.7, 93.8, and 63.4 mM) in Hu sheep. Similarly, B. Ma *et al*. (2022) found that increasing dietary ADF from 16.65% to 29.17% DM significantly decreased total VFA from 146.61 to 130.43 mM in Chinese black Tibetan sheep. Despite the reduction in total VFA, substitution of jengkol peel up to 22.5% did not impair sheep performance (Hidayah *et al*., 2020); dry matter intake actually increased (746.74 vs. 610.42 g/head/day), whereas average daily gain (36.67 vs. 48.00 g/head/day), feed efficiency (5.06 vs. 7.62%), and final body weight (21.20 vs. 21.04 kg) remained comparable with the control.

Moreover, substitution up to 22.5% did not adversely affect the levels of key blood metabolites, including glucose, cholesterol, triglycerides, and HDL (Table 4). This indicates that jengkol peel can be used as a partial native grass replacement without disrupting the animals' metabolic health. The stability of glucose levels suggests that energy metabolism remained unaffected, whilst consistent cholesterol and triglyceride levels reflect maintained lipid metabolism. The unchanged HDL levels further support the notion that the dietary intervention did not elevate lipid transport and cardiovascular risk.

Table 4. *Blood metabolite with jengkol peel substitution*

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)
T1	66.49 ± 4.02	38.12 ± 5.08	42.70 ± 12.53	43.29 ± 5.24
T2	58.38 ± 2.71	36.65 ± 7.04	40.64 ± 14.58	40.18 ± 6.26
T3	59.21 ± 9.50	30.18 ± 1.45	32.38 ± 16.41	38.99 ± 6.22

Note: T1 = 40% concentrate (C) + 60% native grass (NG); T2 = 40% C + 45% NG + 15% jengkol peel (JP); T3 = 40% C + 37.5% NG + 22.5% JP

Source: developed by the authors

These data are consistent with those reported by N. Hidayah *et al*. (2020), where jengkol peel substitution up to 22.5% did not disturb sheep performance. Although this treatment increased dry matter intake, average daily gain, feed efficiency, and final body weight remained comparable to the control treatment. This indicated that jengkol peel inclusion did not cause feed refusal by sheep and consequently did not decrease performance. The maintenance of normal blood metabolite profiles, similar to observations by M. Koshchavka *et al*. (2020) in cattle under comfortable conditions, suggests that sheep can tolerate the tannin content in jengkol peel without compromising palatability. S. Soares *et al*. (2020) stated that tannin compounds are widely associated with the organoleptic properties of astringency and bitterness. C. Ginane *et al*. (2011) further explained that lingual receptors in ruminants, such as sheep, cattle, and goats, detect all five basic tastes (sweet, bitter, salty, sour, and umami), with bitter taste appearing to have a negative hedonic value. Studies by J. Carulla *et al*. (2005) and R. Eckard *et al*. (2010) reported that adding tannin ⩾ 50 g/kg dry matter of feed decreased dry matter intake and weight gains in sheep and cows. In the present research, sheep tolerance to this treatment was likely due to the low tannin level in jengkol peel (1.43%). S. Ibrahim & A. Hassen (2022) reported that a higher tannin level than in the present treatment – 2% DM unencapsulated Mimosa tannin (containing 67.76% total tannin) added to total mixed ration – did not decrease dry matter intake of South African mutton Merino ram lambs. A. Pineiro-Vazquez *et al*. (2015) stated that the antinutritional effect of tannins is strongly correlated with their level of inclusion. Sheep can neutralise tannin astringency by secreting salivary proteins rich in proline (Frutos *et al*., 2004). C. Ginane *et al*. (2011) also reported that cows possess fewer genes coding for bitter receptors than other mammals, rendering them less tolerant of this taste.

The blood metabolite results in this study are similar to findings reported by A. Oni *et al*. (2017), where supplementing cassava peels with cassava leaves and cowpea haulms at different ratios (700:100:175 (T1); 500:200:275 (T2); 300:300:375 (T3); and 100:400:475 (T4) g/kg DM) did not alter blood metabolites of West African dwarf goats. This study suggests that cassava peel can be a viable feed component without adverse effects on blood health. In another study, S. Shilwant *et al*. (2023) investigated the impact of a saponin- and polyphenolic-rich composite plant extract (CPE) (seed, root, bark, and peel from different plants) at 20 g/kg diet on blood profiles in lactating Beetal goats. The results showed that CPE supplementation did not significantly affect plasma concentrations of glucose, triglycerides, cholesterol, total protein, albumin, or the albumin-to-globulin ratio. In this regard, it can be stated that the substitution of native grass with jengkol peel up to 22.5% did not negatively affect blood health or the rumen microbial community of sheep. Sheep performance also remained within safe and satisfactory parameters. This indicates that jengkol peel up to 22.5% has the potential to be used as a fibre source to substitute native grass in sheep diets. Future research should examine the same treatment in other ruminants and assess meat quality variables. Furthermore, continued research into jengkol peel as an extract product is warranted, as this material contains bioactive compounds that could potentially be used as a ruminant feed additive to decrease methane emissions and increase ruminant productivity.

Conclusions

The results obtained from this study demonstrate the effects of substituting native grass with jengkol (*Archidendron jiringa*) peel as a fibre source for ruminants. The findings showed that the substitution of native grass with jengkol peel up to 22.5% did not adversely affect ($p > 0.05$) the total bacterial population, whilst significantly decreasing ($p < 0.05$) the protozoa population by 13.95% compared with the control, although it did not alter ($p > 0.05$) methane prediction. The reduction in protozoa may be attributed to the saponin content in jengkol peel. Moreover, substitution up to 22.5% did not adversely affect ($p > 0.05$) the levels of key blood metabolites, including glucose, cholesterol, triglycerides, and high-density lipoprotein. These findings indicate that jengkol peel substitution up to 22.5% maintained blood metabolite concentrations and total bacterial populations whilst selectively reducing protozoal numbers by 13.95%, without affecting methane production. The selective antiprotozoal effect observed, combined with the maintained rumen bacterial populations and normal blood metabolite profiles, suggests that jengkol peel represents a viable alternative fibre source that does not compromise animal health or rumen function. It is concluded that jengkol peel can be used at levels up to 22.5% as a substitute for native grass in sheep diets without negatively affecting blood health or rumen bacterial communities. Future research should examine the same treatment in other ruminant species and assess meat quality parameters. Furthermore, continued investigation into jengkol peel as an extract product is warranted, as this material contains bioactive compounds that could potentially be utilised as a ruminant feed additive to mitigate methane emissions and enhance ruminant productivity.

Acknowledgements

The authors would like to express their gratitude to the Ministry of Research, Technology and Higher Education of Indonesia for supporting this research; the Nutrition Science and Feed Technology Department, Animal Science Faculty, IPB University – for supporting this study through the provision of laboratory facilities.

Funding

PKPT grant No. 108/SP2H/LT//K2/KM/2019, Ministry of Research, Technology and Higher Education of Indonesia.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] AOAC. (2000). *Official methods of analysis* (17th ed.). Maryland: Association of Official Analytical Chemists.

[2] Carulla, J.E., Kreuzer, M., Machmüller, A., & Hess, H.D. (2005). Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Australian Journal of Agricultural Research*, 56(9), 961-970. doi: 10.1071/AR05022.

[3] Chen, M., Balhara, V., Castillo, A.M.J., Balsevich, J., & Johnston, L.J. (2019). Interaction of saponin 1688 with phase separated lipid bilayers. *Biochimica et Biophysica Acta*, 1859(7), 1263-1272. doi: 10.1016/j.bbamem.2017.03.024.

[4] Cieslak, A., Zmora, P., Matkowski, A., Nawrot-Hadzik, I., Pers-Kamczyc, E., El-Sherbiny, M., Bryszak, M., & Szumacher-Strabel, M. (2016). Tannins from *Sanguisorba officinalis* affect *in vitro* rumen methane production and fermentation. *The Journal of Animal & Plant Sciences*, 26(1), 54-62.

[5] Eckard, R.J., Grainger, C., & de Klein, C.A.M. (2010). Options for the abatement of methane and nitrous oxide from ruminant production: A review. *Livestock Science*, 130(1-3), 47-56. doi: 10.1016/j.livsci.2010.02.010.

[6] FASS. (2010). *Guide for the care and use of agricultural animals in research and teaching*. Retrieved from https://www.fass.org/images/science-policy/Ag_Guide_3rd_ed.pdf.

[7] Frutos, P., Hervas, G., Giradez, F.J., & Mantecón, A.R. (2004). Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research*, 2(2), 191-202. doi: 10.5424/sjar/2004022-73.

[8] Ginane, C., Baumont, R., & Favreau-Peigne, A. (2011). Perception and hedonic value of basic tastes in domestic ruminants. *Physiology and Behaviour*, 104(5), 666-674. doi: 10.1016/j.physbeh.2011.07.011.

[9] Hidayah, N., Lubis, R., Wiryawan, K.G., & Suharti, S. (2019). Phenotypic identification, nutrients content, bioactive compounds of two jengkol (*Archidendron jiringa*) varieties from Bengkulu, Indonesia and their potentials as ruminant feed. *Biodiversitas Journal of Biological Diversity*, 20(6), 1671-1680. doi: 10.13057/biodiv/d200624.

[10] Hidayah, N., Lubis, R., Wiryawan, K.G., Suharti, S., Rita, W., & Nurhaita. (2020). Effect of native grass substitution with Jengkol (*Archidendron jiringa*) peel on sheep performance. *IOP Conference Series: Earth and Environmental Science*, 465, article number 012021. doi: 10.1088/1755-1315/465/1/012021.

[11] Ibrahim, S.L., & Hassen, A. (2022). Effect of non-encapsulated and encapsulated mimosa (*Acacia mearnsii*) tannins on growth performance, nutrient digestibility, methane and rumen fermentation of South African mutton Merino ram lambs. *Animal Feed Science Technology*, 294, article number 115502. doi: 10.1016/j.anifeedsci.2022.115502.

[12] Jayanegara, A., Ridla, M., Laconi, E.B., & Nahrowi, N. (2018). Tannin as a feed additive for mitigating enteric methane emission from livestock: Meta-analysis from RUSITEC experiments. *IOP Conference Series: Materials Science and Engineering*, 434, article number 012108. doi: 10.1088/1757-899X/434/1/012108.

[13] Kazemi, M. (2025). Recycling agricultural waste: Sustainable solutions for enhancing livestock nutrition. *Veterinary Medicine and Science*, 11(3), article number e70321. doi: 10.1002/vms3.70321.

[14] Kholif, A.E. (2023). A review of effect of saponins on ruminal fermentation, health and performance of ruminants. *Veterinary Sciences*, 10(7), article number 450. doi: 10.3390/vetsci10070450.

[15] Koshchavka, M., Boyko, N., & Tzvilikhovsky, M. (2020). The results of morphological examination of blood of cows under heat stress depending on the stages of temperature-humidity index. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 16(6). doi: 10.31548/dopovidi2020.06.018.

[16] Loregian, K.E., Pereira, D.A.B., Rigon, F., Magnani, E., Marcondes, M.I., Baumel, E.A., Branco, R.H., Del Bianco Benedeti, P., & Paula, E.M. (2023). Effect of tannin inclusion on the enhancement of rumen undegradable protein of different protein sources. *Ruminants*, 3(4), 413-424. doi: 10.3390/ruminants3040034.

[17] Ma, B., Zhang, C., Raza, S.H.A., Yang, B., Aloufi, B.H., Alshammari, A.M., AlGabbani, Q., Khan, R., Hou, S., & Gui, L. (2022). Effects of dietary non-fibrous carbohydrate (NFC) to neutral detergent fiber (NDF) ratio change on rumen bacterial community and ruminal fermentation parameters in Chinese black Tibetan sheep (*Ovis aries*). *Small Ruminant Research*, 216, article number 10679. doi: 10.1016/j.smallrumres.2022.106793.

[18] Moss, A., Jouany, J.P., & Newbold, J. (2000). Methane production by ruminants: Its contribution to global warming. *Annales de Zootechnie*, 49(3), 231-253. doi: 10.1051/animres:2000119.

[19] Noviany, N., Hasanah, U., Dewi Lotulung, P., & Hadi, S. (2022). 10 antibacterial, antioxidant and cytotoxic activities of the stem bark of *Archidendron jiringa* (Jack) I.C. Nielsen. In P. Ramasami (Ed.), *Volume 1: Organic and natural product synthesis* (pp. 123-134). Berlin, Boston: De Gruyter. doi: 10.1515/9783110752601-010.

[20] Noviany, N., Sialdian, D., Setiawan, A., Irawan, B., Azmi, M.N., & Hadi, S. (2021). Bioassay-guided separation approach for characterization of new antibacterial fractions from the stem roots extracts of *Archidendron jiringa*. *Journal of The Turkish Chemical Society*, 8(2), 391-402. doi: 10.18596/jotcsa.831054.

[21] Ogimoto, K., & Imai, S. (1981). *Atlas of rumen microbiology*. Tokyo: Japan Scientific Societes.

[22] Oni, A.O., Oluwayemisi, A., Adebayo, K., Sowande, O.S., Iposu, S., & Onwuka, C.F.I. (2017). Effects of supplementing cassava peels with cassava leaves and cowpea haulmson the rumen environment and blood profile parameters of West African dwarf goats. *Archivos de Zootecnia*, 66(255), article number 395. doi: 10.21071/az.v66i255.2516.

[23] Onyango, A.A., Dichoefer, U., Rufino, M.C., Butterbach-Bahl, K., & Goopy, J.P. (2019). Temporal and spatial variability in the nutritive value of pasture vegetation and supplement feedstuffs for domestic ruminants in Western Kenya. *Asian-Australasian Journal of Animal Sciences*, 32(5), 637-647. doi: 10.5713/ajas.18.0114.

[24] Patra, A.K., & Yu, Z. (2014). Combinations of nitrate, saponin, and sulfate additively reduce methane production by rumen cultures in vitro while not adversely affecting feed digestion, fermentation or microbial communities. *Bioresource Technology*, 155, 129-135. doi: 10.1016/j.biortech.2013.12.099.

[25] Patra, A.K., Stiverson, J., & Yu, Z. (2012). Effects of Quillaja and Yucca saponins on communities and select populations of rumen bacteria and archaea, and fermentation *in vitro*. *Journal of Applied Microbiology*, 113(6), 1329-1340. doi: 10.1111/j.1365-2672.2012.05440.x.

[26] Pineiro-Vazquez, A., Canul-Solís, J., Alayon-Gamboa, J., Chay-Canul, A., Ayala-Burgos, A., Aguilar-Perez, C., Solorio-Sanchez, F., & Ku-Vera, J. (2015). Potential of condensed tannins for the reduction of emissions of enteric methane and their effect on ruminant productivity. *Archivos de Medicina Veterinaria*, 47(3), 263-272. doi: 10.4067/S0301-732X2015000300002.

[27] Ridla, M., Laconi, E.B., Nahrowi, & Jayanegara, A. (2021). Effects of saponin on enteric methane emission and nutrient digestibility of ruminants: An in vivo meta-analysis. *IOP Conference Series: Earth and Environmental Science*, 788, article number 012028. doi: 10.1088/1755-1315/788/1/012028.

[28] Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*, 30(12), 3875-3883. doi: 10.1016/0031-9422(91)83426-L.

[29] Shilwant, S., Hundal, J.S., Singla, M., & Patra, A.K. (2023). Ruminal fermentation and methane production *in vitro*, milk production, nutrient utilization, blood profile, and immune responses of lactating goats fed polyphenolic and saponin-rich plant extracts. *Environmental Science and Pollution Research*, 30(4), 10901-10913. doi: 10.1007/s11356-022-22931-y.

[30] Singh, A.K., Ojha, L., Kumari, P., Choubey, M., & Chaudhary, S.K. (2024). Phytochemicals as natural feed additivies for ruminants. In M.S. Mahesh & V.K. Yata (Eds.), *Feed additives and supplements for ruminants* (pp. 167-196). Singapore: Springer. doi: 10.1007/978-981-97-0794-2_8.

[31] Soares, S., Brandao, E., Guerreiro, C., Soares, S., Mateus, N., & de Freitas, V. (2020). Tannins in food: Insights into the molecular perception of astringency and bitter taste. *Molecules*, 25(11), article number 2590. doi: 10.3390/molecules25112590.

[32] Ungerfeld, E.M., Cancino-Padilla, N., & Vera-Aguilera, N. (2023). Fermentation in the rumen. In C.J. Hurst (Ed.), *Microbial fermentations in nature and as designed processes* (Chapter 4). New Jersey: John Wiley & Sons. doi: 10.1002/9781119850007.ch4.

[33] Wagner, T., Toffin, L., & Borrel, G. (2025). Methanogenic archae. In B. Clouet-d'Orval, B. Franzetti & P. Oger (Eds.), *Biology of archaea 1: Discovery, evolution and diversity of archaea* (Chapter 5). Hoboken: Wiley. doi: 10.1002/9781394351848.ch5.

[34] Wallace, J., Mcewan, N., McIntosh, F., Teferedegne, B., & Newbold, C. (2002). Natural products as manipulators of rumen fermentation. *Asian-Australasian Journal of Animal Sciences*, 15(10), 1458-1468. doi: 10.5713/ajas.2002.1458.

[35] Wischer, G., Boguhn, J., Steingaß, H., Schollenberger, M., & Rodehutscord, M. (2013). Effects of different tannin-rich extracts and rapeseed tannin monomers on methane formation and microbial protein synthesis in vitro. *Animal*, 7(11), 1796-1805. doi: 10.1017/S1751731113001481.

[36] Wu, H., Meng, Q., Zhou, Z., & Yu, Z. (2019). Ferric citrate, nitrate, saponin and their combinations affect in vitro ruminal fermentation, production of sulphide and methane and abundance of select microbial populations. *Journal of Applied Microbiology*, 127(1), 150-158. doi: 10.1111/jam.14286.

[37] Xie, X., Wang, J., Guan, L., & Liu, J.-X. (2018). Effect of changing forage on the dynamic variation in rumen fermentation in sheep. *Animal Science Journal*, 89(1), 122-131. doi: 10.1111/asj.12915.

Вироблення метану в рубці, мікробна популяція та метаболіти крові овець за годівлі шкіркою дженголу (*Archidendron jiringa*) як замінником природної трави

Нур Хідаях
Магістр наук у галузі тваринництва, асистент
Університет Тідар
59155, вул. Баріто, 1, м. Магеланг, Індонезія
https://orcid.org/0000-0002-9290-3578

Команг Геде Віряван
Доктор наук у галузі тваринництва, професор
Університет IPB
16680, вул. Агатіс, кампус IPB Дармага, м. Богор, Індонезія
https://orcid.org/0000-0002-0593-9653

Срі Сухарті
Доктор наук у галузі тваринництва, професор
Університет IPB
16680, вул. Агатіс, кампус IPB Дармага, м. Богор, Індонезія
https://orcid.org/0000-0002-0542-4086

Анотація. Агропромислові та сільськогосподарські побічні продукти можуть бути перетворені жуйними тваринами на високоякісний корм. Шкірка дженголу (*Archidendron jiringa*) є сільськогосподарським побічним продуктом із високим вмістом сирої клітковини. Метою цього дослідження було оцінити, чи може шкірка дженголу використовуватися як замінник природної трави як джерело клітковини для жуйних тварин. Досліджуваними змінними були прогноз утворення метану в рубці, мікробна популяція та метаболіти крові овець. В експерименті використовували 15 баранів масою тіла від 15 до 27 кг у рандомізованому блочному дизайні з трьома варіантами годівлі (0,0 %, 15,0 % і 22,5 % заміни шкіркою дженголу) та п'ятьма повтореннями. Заміна природної трави шкіркою дженголу до рівня 22,5 % не впливала негативно ($p > 0,05$) на загальну бактеріальну популяцію та не змінювала ($p > 0,05$) прогноз утворення метану, але зменшувала ($p < 0,05$) чисельність найпростіших на 13,95 % порівняно з контролем, ймовірно, завдяки наявності сапонінів у шкірці. Крім того, заміна до 22,5 % не мала негативного впливу ($p > 0,05$) на ключові метаболіти крові, такі як глюкоза, холестерин, тригліцериди та ліпопротеїни високої щільності (HDL). Було зроблено висновок, що шкірка дженголу може замінювати природну траву до рівня 22,5 % без негативного впливу на стан крові чи бактеріальні популяції рубця, одночасно зменшуючи чисельність найпростіших

Ключові слова: дослідження *in vivo*; побічний продукт дженколу; природні трави; рубцева екосистема; жуйні тварини

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 16(4), 59-69
Received 30.06.2025 Revised 04.11.2025 Accepted 27.11.2025

UDC 663.5:661.7
DOI: 10.31548/animal.4.2025.59

Specific features of fermentation of higher gravity wort from starch-containing raw materials with a mixed culture of microorganisms

Oleksiy Oliynichuk
Postgraduate Student
Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine
02002, 4-A Ye. Sverstiuk Str., Kyiv, Ukraine
https://orcid.org/0009-0009-8014-1703

Liubomyr Khomichak*
Doctor of Technical Sciences, Professor, Corresponding Member of NAAS
Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine
02002, 4-A Ye. Sverstiuk Str., Kyiv, Ukraine
https://orcid.org/0000-0001-9003-0315

Olga Koval
PhD in Technical Sciences
Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine
02002, 4-A Ye. Sverstiuk Str., Kyiv, Ukraine
https://orcid.org/0000-0003-1035-5895

Abstract. The modern technological process of starch hydrolysis to fermentable sugars in the ethanol production from starch-containing raw materials is based on the use of amylolytic commercial enzyme preparations and the application of a low-temperature liquefaction stage for greater process efficiency, which significantly affects the final product's cost. Under such conditions, the search for alternative ways to reduce the costs of these technological process' components and, accordingly, the cost of the manufactured product is relevant. The purpose of the study was to investigate the use of a commercial mixture of microorganisms, namely alcohol yeast *Saccharomyces cerevisiae* and mould fungus *Rhyzopus*, as components of the commercial product Angel Leaven for a single-stage process of starch hydrolysis and fermentation of higher gravity wort based on starch-containing raw materials. The methods and processes for preparing raw materials generally accepted in ethyl alcohol

---

Suggested Citation:
Oliynichuk, O., Khomichak, L., & Koval, O. (2025). Specific features of fermentation of higher gravity wort from starch-containing raw materials with a mixed culture of microorganisms. *Animal Science and Food Technology*, 16(4), 59-69. doi: 10.31548/animal.4.2025.59.

**Corresponding author*

production were used in the studies. The technological mode of single-stage starch hydrolysis and sugar fermentation in higher gravity wort by an association of mould fungus and alcohol yeast has been experimentally substantiated. It has been established that saccharification and starch fermentation by a mixed culture occurs in one stage and almost simultaneously at the temperature of 30-32°C, which creates prerequisites for energy saving and technological process simplification. The energy saving effect is enhanced by fermentation of higher gravity wort, obtained without the stage of low-temperature liquefaction, but with the stage of exposure with a proteolytic enzyme for 1 hour at the temperature of 50°C. This technological method is effective for a medium with a concentration of up to 306.6 g/dm^3 of dry matter, which allows getting a wort with an ethanol content of up to 14.2% v/v. Increasing the concentration of the medium to 350 g/dm^3 of dry matter does not allow getting the calculated ethanol content in mature wort in a single-stage process and requires further research to optimise the fermentation conditions. A set of technological solutions for optimising of starch hydrolysis and fermentation process with a mixed culture creates conditions for increasing the efficiency of raw materials and improving the energy efficiency of production process

Keywords: amylolytic enzymes; starch hydrolysis; mature mash; *Saccharomyces cerevisiae*; association of microorganisms

Introduction

Contemporary biotechnological methods for producing ethanol from starch-containing raw materials are based on the use of exo- and endoamylases for the starch hydrolysis to fermentable sugars. The use of enzyme preparations in practice helped to develop and introduce into production low-temperature methods of water-heat treatment of starch-containing raw materials, which allows saving heat resources, although even under such conditions, the costs of cooking make up to 10% of the costs of energy and fuel resources of production, according to G. Shmatkova & N. Hubenko (2013). However, according to V. Prybylskyi *et al.* (2024), the use of enzyme preparations (EP) for liquefaction and saccharification of starch entails certain financial costs, which can amount to up to 3% of the cost of produced ethanol. An alternative to the use of commercial enzyme preparations is the organisation of a single-stage technological process of hydrolysis and fermentation of starch under conditions of joint cultivation of amylolytic enzyme producers and *Saccharomycetes* yeast.

One variant for co-cultivation is to use a microbial complex, the predominant components of which are mould fungi such as *Aspergillus* and *Rhyzopus*. Such complexes are a traditional fermenting product in countries of Asia, produced based on raw materials rich in starch (wheat, soybeans, rice) inoculated with strains cultivated under controlled temperature and humidity. As mentioned by J. Zhang *et al.* (2023), they are mainly formed into spherical or square granules after drying and used as a starter for fermentation after grinding. The most common component of the mould starter of koji, particularly in Japan, is the fungus *Aspergillus oryzae*. *A. oryzae* is considered particularly suitable for fermentation given its ability to overexpress hydrolytic enzymes and degrade complex molecules such as polysaccharides and proteins (Allwood *et al.*, 2021; Daba *et al.*, 2021). It was also confirmed by N. Watarai *et al.* (2019) that of all the studies on microbial pairs for symbiotic cultivation, the most effective pair was *Aspergillus oryzae* and *Saccharomyces cerevisiae*.

The basic principles for improving of starch fermentation efficiency by direct conversion through the symbiotic cultivation of amylolytic and fermenting organisms were elucidated by the co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* by I. Han & M. Steinberg (1987), which were further developed by many scientists, in particular A. Drosos *et al*. (2021). In South Korea, nuruk starter is widely used in the production of traditional rice wine. The microbes in the nuruk depend on the grain source and the fermentation environment, but the main of them essential for the fermentation are *Saccharomyces cerevisiae*, *Aspergillus* spp., *Lactobacillus* spp., *Rhizopus* spp., and *Penicillium* spp. The α-amylase, β-amylase, glucoamylase enzymes produced by *Aspergillus* spp. and *Rhizopus* spp. are responsible for the saccharification of starch, as noted in research by B. Wong *et al*. (2023). *Rhizopus* and *Mucor* fungi can be used as an alternative to *Aspergillus* in the production of fermented products, as stated in research by M. Kim & J. Seo (2021) and S. Heo *et al*. (2023).

The purpose of this study was to investigate the technological regimes of the single-stage process of saccharification and fermentation of starch in increased concentration wort with components of microorganisms' mixed culture in the commercial product Angel Leaven in comparison with the parameters of processes operating in the alcohol industry.

Materials and Methods

The experimental work was carried out from March to July 2025 in the Department of Technologies of Fermentation Products of Institute of Food Resources of National Academy of Agrarian Sciences of Ukraine. Physical and chemical analyses, including determination of alcohol content in wort and content of unfermented sugars, were carried out using the equipment of the institute's laboratory certified according to DSTU EN ISO/IEC 17025:2019 (2021). Grain waste from corn processing obtained from the State Enterprise Zarubinsky Distillery, Ternopil region, Ukraine, was used as a raw material for fermentation. The raw material was characterised by the following parameters: dry matter content – 85.6 ± 0.24%, starch content – 59.5 ± 0.10%, moisture content – 14.4 ± 0.1%. The commercial product Angel Leaven (Angel Yeast Co., Ltd, China), containing *Saccharomyces cerevisiae* alcohol yeast, *Rhizopus* mould fungi enriched with an additional enzyme complex, was used as a producer for single-stage starch hydrolysis and simultaneous fermentation into ethyl alcohol. The product dosage is 4-4.8 g/dm^3 in dry form. Before application, the product was activated by mixing with water in a ratio of 1:10 and exposure for 30 minutes at the temperature of 28-30°C.

The following enzyme preparations were used: thermostable α-amylase (Tegamyl BLHL, Tegaferm Holding GmbH, Austria), glucoamylase (TEGAMYL AG90L, Tegaferm Holding GmbH, Austria), protease (Tegalase AP75L, Tegaferm Holding GmbH, Austria). The dosage of α-amylase was based on the amylolytic activity of EP at the rate of 2 units of activity per 1 g of starch, the dosage of glucoamylase was based on the amylolytic activity of EP at the rate of 8 units of activity per 1 g of starch, protease was added according to the manufacturer's recommendation. The preparation Baktrilon (LLC TMA Tristan, Ukraine) was used as an antiseptic, in the dosage recommended by the manufacturer, for effective adherence of microbiological purity for use in alcohol production.

Grain waste from corn processing was ground in a laboratory mill to obtain a grinding of 99-100% of which passes through a sieve with the apertures of diameter 1 mm. The grinding was mixed with water in a ratio necessary to obtain a dry matter content in the medium at the level of 263.5-350 g/dm^3, which corresponds to the content of fermentable sugars and the estimated ethanol content in mature wort at the level of 183.2-243.3 g/dm^3 and 12 ± 0.2-16.0 ± 0.2% v/v, respectively. The grinding weight was calculated according to the expected ethanol content in the mature wort, considering the starch content in the raw material, the regulated alcohol yield from a tonne of the raw material's conditional starch, and the amount of wort required for the fermentation test. The grinding mixed with water was acidified to pH 5.0-5.1 to achieve the optimal value for the complex of amylo- and proteolytic enzymes action.

With the classic low-temperature liquefaction (variants 1-12, 3-12, 1-14, 1-16), recommended in production, mashing was carried out at a temperature of 88-90°C, after adding heat-stable α-amylase EP for three hours. Then the mixture was cooled to a temperature of 32-35°C, glucoamylase and protease EP, antiseptic were added, and inoculated with rehydrated Angel Leaven. The vessels with wort were kept for 96 hours at a temperature of 30 ± 1°C. In variant 3-12, no additional enzyme preparations were added. For a single-stage process of hydrolysis and fermentation of the formed sugars, a protease solution was added to the prepared wort to destroy protein-starch complexes and exposed in a thermostat at a temperature of 50 ± 1°C for 1 hour (variants 2-12, 2-14, 2-16). After exposing, the mixture was cooled to a temperature of 32-35°C, an antiseptic was added and inoculated with rehydrated Angel Leaven. In variant 3-14, exposing with protease was not performed. The duration of the single-stage process at a temperature of 30 ± 1°C was 96 hours. Differences in wort preparation by every variant are presented in Table 1.

Table 1. *Research variants according to the processes of wort preparation and concentration*

Stage of the process of preparing the wort for fermentation	Medium concentration, g/dm³							
	263.5 g/dm³						350.0 g/dm³	
	Variant							
	1-12	2-12	3-12	1-14	2-14	3-14	1-16	2-16
Liquefaction with adding heat-stable α-amylase (88-90°C, 3 hours)	+			+			+	
Adding glucoamylase to fermentation (30 ± 1°C)	+			+			+	
Adding protease to fermentation (30 ± 1°C)	+			+			+	
Exposing with protease (50 ± 1°C, 1 hour)		+			+			+

Source: developed by the authors

The moisture content of the starch-containing raw materials was determined by the gravimetric method in accordance with DSTU 4864:2007 (2009), the dry matter content – by the calculation method, the starch content – by the fermentation test method in accordance with GSTU 46.045.2003 (2003). The fermentation activity of the microorganism association was determined by the gravimetric method, determining the amount of carbon dioxide that formed during the time the wort was in the thermostat. The samples were weighted after 24, 48, 72, 96 hours. The alcohol content in mature wort was determined by the areometric method according to DSTU 7457:2013 (2014), the content of unfermented sugars – by the photoelectrocolorimetric method with anthrone reagent according to DSTU 4854:2007 (2009). The content of unfermented starch and dextrins was determined by the calculation method. In a series of studies, the samples were cultivated in 0.5 dm³ biological flasks equipped with air locks; each variant was cultivated in three replicates. The paper presents the average values of the research results. Comparison of means was performed using one method in SAS software. Statistical significance was determined at the level of $p < 0.05$.

Results and Discussion

An effective method for monitoring the enzymatic activity of microorganisms was the intensity of carbon dioxide emission during wort fermentation. The results for this indicator for all research variants were shown in Figure 1. It was found that with dry matter content in wort at the level of 263.5 g/dm^3 (variants 1-12 – 3-12) intensity of CO_2 emission in the first day of fermentation was high and changed insignificantly depending on the variation of wort preparation, amounting to 57.87-64.77% of the total amount of carbon dioxide released, i.e., the consumption of sugars during this period was about 60% of the input. As can be seen from Figure 1, CO_2 emission for these variants stopped after 72 hours from the start of fermentation, which allows confirming the completion of the sugar conversion process. Removal of the low-temperature liquefaction stage in case preprocessing by protease did not reduce the intensity of carbon dioxide emission during the fermentation process. Removing the stage of adding glucoamylase and proteolytic enzyme preparations after the liquefaction stage (variant 3-12) did not affect the intensity of carbon dioxide emission during the fermentation period.

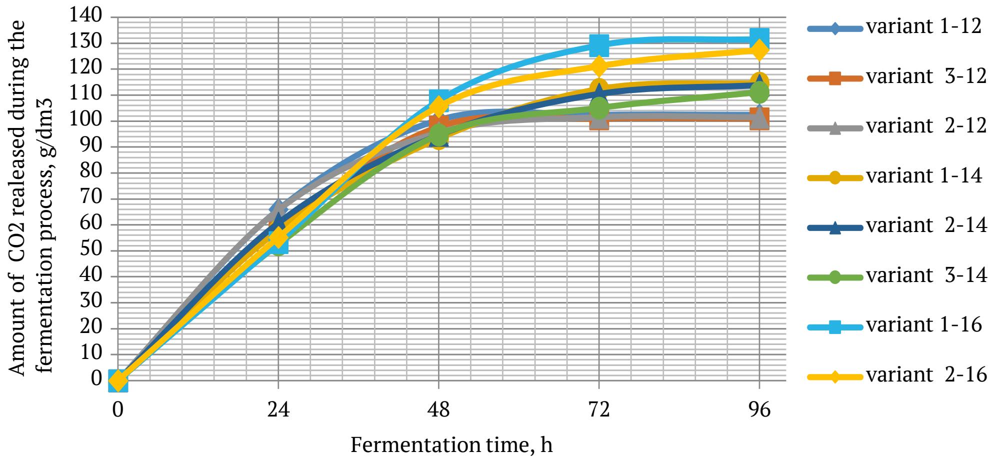

Figure 1. Intensity of carbon dioxide emission during the fermentation of wort based on starch-containing raw materials under various pre-processing conditions

Note: dry matter content in the wort – 263.5 g/dm^3 (variants 1-12 – 3-12), 306.6 g/dm^3 (variants 1-14 – 3-14) and 350 g/dm^3 (variants 1-16 and 2-16)

Source: developed by the authors

In case of increasing the dry matter content to obtain the ethanol content in the mature wort at the level of 14.0-14.2% v/v, 3 variants for raw material pre-processing before fermentation were carried out: classic liquefaction, extraction of the liquefaction stage with the introduction of additional preprocessing with protease, and extraction of all preprocessing stages. According to the research results, it was found that the extraction of all pre-processing stages (variant 3-14) entails some decrease in the intensity of carbon dioxide formation both in the first day of fermentation and in the process as a whole. The duration of the fermentation process in a case an increase of the medium concentration rose for the Angel Leaven product both with classic liquefaction and under conditions of its use without high-temperature processing. Probably, further prolongation of the fermentation period in variant 3-14 can lead to an increase in the alcohol content in the mature wort to the estimated one; however, excessive fermentation duration increases the risks of the medium contamination and, accordingly, deterioration of the final product quality. Fermentation of higher gravity wort with a dry matter content of 350 g/dm^3 showed that the removal of the liquefaction phase from the technological process negatively affects the total amount of released carbon dioxide, reducing it by 3.2% compared to variant 1-16. However, in the first day, the amount of CO_2 was higher in variant 2-16, which can be explained by the lower rate of starch breakdown to fermentable sugars and, accordingly, a reduced negative impact on the yeast cell functionality.

Research by J. Li *et al*. (2021), using *Rhizopus nigricans* as an enzyme producer for the saccharification stage of starch-containing raw materials followed by fermentation with *Saccharomyces cerevisiae* showed that the highest alcohol-forming capacity and conversion rate were with 16-23% of raw materials concentration. Based on the results of the intensity of CO_2 emission during the fermentation process, it can be argued that the starch conversion into fermentable sugars by a mixture of microorganisms in the product Angel Leaven at a concentration of 263.5 g/dm^3 in a single-stage process of liquefaction and fermentation of wort from starch-containing raw materials was effective and, under certain conditions, did not lose efficiency when the concentration of dry substances rises to 306.6 g/dm^3. For a more accurate understanding of the progress in the single-stage process in mature wort, the content of ethanol and residual fermentable sugars was determined; the research results are shown in Table 2.

Table 2. *Physical and chemical characteristics of mature wort*

Indicators	Estimated ethanol content in mature wort, % v/v							
	12.0-12.2			14.0-14.2			16.0-16.2	
	Variants							
	1-12	2-12	3-12	1-14	2-14	3-14	1-16	2-16
Ethanol content in mature wort, % v/v	12.18 ± 0.04	12.17 ± 0.03	12.18 ± 0.03	14.17 ± 0.03	14.17 ± 0.03	13.77 ± 0.10	16.18 ± 0.03	15.78 ± 0.03
Content of total unfermented carbohydrates, g/100 cm^3	0.304 ± 0.030	0.293 ± 0.007	0.341 ± 0.003	0.262 ± 0.007	0.534 ± 0.007	0.872 ± 0.005	0.345 ± 0.005	0.875 ± 0.004
Content of water-soluble unfermented carbohydrates, g/100 cm^3	0.285 ± 0.035	0.202 ± 0.004	0.302 ± 0.003	0.243 ± 0.003	0.525 ± 0.005	0.817 ± 0.003	0.283 ± 0.003	0.820 ± 0.005
Content of unfermented starch, g/100 cm^3	0.017 ± 0.004	0.082 ± 0.005	0.035 ± 0.002	0.017 ± 0.004	0.008 ± 0.003	0.049 ± 0.003	0.056 ± 0.003	0.014 ± 0.001
Content of alcohol-soluble unfermented carbohydrates, g/100 cm^3	0.057 ± 0.006	0.071 ± 0.003	0.112 ± 0.003	0.021 ± 0.004	0.040 ± 0.002	0.042 ± 0.002	0.068 ± 0.002	0.137 ± 0.006
Content of dextrins, g/100 cm^3	0.205 ± 0.027	0.118 ± 0.004	0.171 ± 0.004	0.200 ± 0.005	0.437 ± 0.004	0.698 ± 0.002	0.193 ± 0.002	0.614 ± 0.004
Yield of ethanol from a ton of conditional starch, dm^3	664.8	664.3	664.8	664.9	664.9	646.2	665.0	648.6

Source: developed by the authors

According to the data of Table 2, it was established that with a dry matter content in the wort from starch-containing raw materials at the level of 263.5 g/dm^3, the commercial product Angel Leaven allowed obtaining an alcohol yield from the mash at the level of 664.3-664.8 dm^3 from a tonne of conventional starch, regardless of the raw material preprocessing method used in the study. Accordingly, processing of raw materials at such medium concentration allows the costs reducing of using additional enzyme preparations and heat treatment of the wort without deteriorating of the process quality. The content of undissolved starch fluctuated slightly depending on the preprocessing variant from 0.017 to 0.082 g/100 cm^3, however, remaining within the indicator typical for high-quality liquefaction process. The content of total unfermented carbohydrates, which characterises the overall efficiency of raw material fermentation, for variants 1-12 – 3-12 was within 0.293-0.341 g/100 cm^3, which was 1.6-1.9% of the starch content introduced into fermentation. Accordingly, this indicator did not go beyond the standards established for Ukrainian production. The low content of alcohol-soluble carbohydrates in mature mash – no more than 0.112 g/100 cm^3 – characterised the high efficiency of fermentation of the medium by yeast cells. The low content of dextrins – no more than 0.205 g/100 cm^3 – is an indicator that the amount of enzymes with glucoamylase activity is sufficient for effective saccharification of the wort. The findings correlate with the data from C. Zhao *et al*. (2023) on the effect of *Rhizopus* in the composition of the microorganism mixture on the amount of reducing sugars and the alcohol content in the final product.

Increasing the dry matter content in the wort to 306.6 g/dm^3 when using Angel Leaven in the process with standard raw material preprocessing allowed achieving the estimated alcohol content in the wort, which helped to obtain a regulated yield from a tonne of conditional starch of raw materials. Removing the stage of separate liquefaction and adding amylolytic enzymes with the replacement of processing with protease did not have a negative effect upon the course of fermentation and the alcohol content in the mature wort. However, the content of the spectrum of unfermented sugars was higher than the control variant 1-14, which, together with achieving the normative alcohol yield, may indicate that the gradual release of sugars during the destruction of starch by the fungal component enzymes reduced the stress effect of excess nutrients on yeast cells and, probably, reduced the amount of osmoprotectors formed by yeast (Saito & Posas, 2012; Auesukaree, 2017). However, according to P. Puligundla *et al*. (2011), long-term exposure to both higher gravity wort and increasing ethanol stress prevented yeast from efficiently metabolising residual sugars.

The variant in which protease was not added and liquefaction was not applied was characterised by a decrease in the ethanol content in the mature wort to 3% compared to the variant with classical processing. Accordingly, the ethanol yield from a tonne of conditional starch decreased by 18.7 dm^3, and, therefore, the use of Angel Leaven on an industrial scale for wort with a concentration of 306.6 g/dm^3 and higher without pre-processing is impractical. When the concentration of the wort increased to 350 g/dm^3, 2 processing variants were compared: classical (variant 1-16) and the exposure with protease (variant 2-16). It was found that with such a concentration of dry substances in the medium, the extraction of the stage of the temperature treatment from the process negatively affected the course of fermentation, reducing the alcohol content in the mature wort by 2.5% compared to the classical technology, respectively, the ethanol yield from a tonne of conditional starch also did not meet the normative one.

A detailed analysis of the spectrum of unfermented sugars in the mature wort showed that the liquefaction process in both variants occurs within the normal range, as evidenced by the low content of undissolved starch. However, the total content of unfermented sugars in variant 2-16 was significantly higher, 0.835 g/100 cm^3 versus 0.345 g/100 cm^3 in the variant with classic liquefaction. In addition, the content of alcohol-soluble carbohydrates in both variants was low, so it means that the yeast cells were able to consume the sugars available to them to the maximum. High content of dextrins in variants 2-16 can indicate the lack of glycoamylase enzymes. It is obvious that some enzymes present in the commercial product, and those created by *Rhyzopus*, were not sufficient for dextrins to be converted into sugars available to yeast with a required speed. Probably, a high starting concentration of the medium, an increase in the amount of acids as a fermentation byproducts and an increase in the amount of ethanol in wort negatively affected the growth and cultivation of *Rhizopus* as enzyme producents, which, accordingly, delayed the overall process of converting sugars into the final product. These data need to be confirmed, since studies by E. Uyar *et al*. (2010) in this area were focused on the accumulation of trehalose under the influence of individual stress factors and did not consider the specifics of the use of the producent in the fermentation process of starch-containing raw materials.

One of the options of the problem solution may be to increase the fermentation time, which would allow the glucoamylase enzymes to complete the transformation process. However, such process duration is not advisable when using the technology in the production process. Moreover, one of the variants for adjusting the fermentation efficiency may be to increase the amount of Angel Leaven inoculum, since it is known that increasing the amount of *Rhyzopus* inoculum can have a positive effect on the amount of alcohol in the mature wort, as mentioned by A. Büyükkileci *et al*. (2006). Considering the above-mentioned results of research using the commercial product Angel Leaven as a producent of a mixed culture of microorganisms during wort fermentation at a concentration that ensures the alcohol content in the mature wort not exceeding 14% vol., the effectiveness of replacing the stage of low-temperature wort liquefaction by an exposure with a proteolytic enzyme for 1 hour at a temperature of 50°C was confirmed.

Conclusions

Studies have shown that the efficiency of single-stage fermentation of wort based on starch-containing raw materials with a mixed culture of alcohol yeast *Saccharomyces cerevisiae* and mould fungus *Rhyzopus*, enriched with additional amylolytic enzymes contained in the Angel Leaven product complex, depends on the concentration of the medium. With an increase in the initial concentration of the medium, the duration of the process of converting starch into the final product can be extended to 96 hours. On the condition that the calculated alcohol content in the mature wort is obtained up to 14.2% vol., the Angel Leaven product can effectively ferment the wort without the low-temperature liquefaction stage; however, it requires a stage of exposing with a proteolytic enzyme for 1 hour at a temperature of 50°C. The content of total unfermented sugars is higher in comparison with the current technology (0.534 ± 0.007 versus 0.262 ± 0.007 g/100 cm^3, respectively) but does not exceed the regulated values. The content of alcohol-soluble carbohydrates is low regardless of the characteristics of the process – no more than 0.137 ± 0.006 g/100 cm^3, which indicates the efficiency of the process of assimilation of the obtained fermentable sugars by yeast cells. Increasing the concentration of the medium to 350 g/dm^3 of dry matter does not allow obtaining the calculated alcohol content in the mature wort in a single-stage process and requires further research on optimising fermentation conditions, in particular, the efficiency of increasing the amount of the initial mass of producent. The obtained results provide a basis for developing a regime of single-stage starch hydrolysis and fermentation of the obtained sugars in larger-scale, in particular plant, conditions. The implementation of the proposed technological regime will help not only to reduce the costs of commercial enzyme preparations, but also to reduce the energy intensity of the liquefaction process.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

References

[1] Allwood, J.G., Wakeling, L.T., & Bean, D.C. (2021). Fermentation and the microbial community of Japanese koji and miso: A review. *Journal of Food Science*, 86(6), 2194-2207. doi: 10.1111/1750-3841.15773.

[2] Auesukaree, C. (2017). Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation. *Journal of Bioscience and Bioengineering*, 124(2), 133-142. doi: 10.1016/j.jbiosc.2017.03.009.

[3] Büyükkileci, A.O., Hamamcı, H., & Yucel, M. (2006). Lactate and ethanol productions by *Rhizopus oryzae* ATCC 9363 and activities of related pyruvate branch point enzymes. *Journal of Bioscience and Bioengineering*, 102(5), 464-466. doi: 10.1263/jbb.102.464.

[4] Daba, G.M., Mostafa, F.A., & Elkhateeb, W.A. (2021). The ancient koji mold (*Aspergillus oryzae*) as a modern biotechnological tool. *Bioresources and Bioprocessing*, 8, article number 52. doi: 10.1186/s40643-021-00408-z.

[5] Drosos, A., Boura, K., Dima, A., Soupioni, M., Nigam, P.S., Kanellaki, M., & Koutinas, A.A. (2021). A cell-factory model of *Saccharomyces cerevisiae* based on bacterial cellulose without GMO for consolidated bioprocessing of starch. *Food and Bioproducts Processing*, 128, 202-214. doi: 10.1016/j.fbp.2021.05.006.

[6] DSTU 4854:2007. (2009). *Starch-containing raw materials for alcohol production. Determination of mass concentration of fermentable carbohydrates by photoelectrocolorimetric method*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=85091.

[7] DSTU 4864:2007. (2009). *Starch-containing raw materials for alcohol production. Methods for determining moisture content*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=85099.

[8] DSTU 7457:2013. (2014). *Aqueous-alcoholic solutions. Methods for determining the content of ethyl alcohol*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=61147.

[9] DSTU EN ISO/IEC 17025:2019. (2021). *General requirements for the competence of testing and calibration laboratories*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=88724.

[10] GSTU 46.045.2003. (2003). *Grain. Methods for determining conditional starch content*. Retrieved from https://zakon.rada.gov.ua/rada/show/v0250555-03#Text.

[11] Han, I.Y., & Steinberg, M.P. (1987). Amylolysis of raw corn by *Aspergillus niger* for simultaneous ethanol fermentation. *Biotechnology and Bioengineering*, 30(2), 225-232. doi: 10.1002/bit.260300212.

[12] Heo, S., Park, J., Lee, K.G., Lee, J.H., & Jeong, D.W. (2023). Quality characteristics of soybean fermented by *Mucor*, *Rhizopus*, and *Aspergillus* from *meju*. *Heliyon*, 9(3), article number e14092. doi: 10.1016/j.heliyon.2023.e14092.

[13] Kim, M., & Seo, J.A. (2021). Fermentation profiling of rice wine produced by *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 newly isolated from Korean fermentation starter. *Applied Biological Chemistry*, 64(1), article number 25. doi: 10.1186/s13765-020-00582-2.

[14] Li, J., Tang, X., Qian, H., Yang, Y., Zhu, X., Wu, Q., Mu, Y., & Huang, Z. (2021). Analysis of saccharification products of high-concentration glutinous rice fermentation by *Rhizopus nigricans* Q3 and alcoholic fermentation of *Saccharomyces cerevisiae* GY-1. *ACS Omega*, 6(12), 8038-8044. doi: 10.1021/acsomega.0c05452.

[15] Prybylskyi, V.L., Kuts, A.M., Boiarchuk, Ya.A., & Dulka, O.S. (2024). The impact of enzyme preparations and growth activators on yeast in the technology of alcohol fermentation. *Journal of Chemistry and Technologies*, 32(2), 333-342. doi: 10.15421/jchemtech.v32i2.299359.

[16] Puligundla, P., Smogrovicova, D., Obulam, V.S.R., & Ko, S. (2011). Very high gravity (VHG) ethanolic brewing and fermentation: A research update. *Journal of Industrial Microbiology and Biotechnology*, 38(9), 1133-1144. doi: 10.1007/s10295-011-0999-3.

[17] Saito, H., & Posas, F. (2012). Response to hyperosmotic stress. *Genetics*, 192(2), 289-318. doi: 10.1534/genetics.112.140863.

[18] Shmatkova, G.K., & Hubenko, N.Yu. (2013). Formation of the cost price of ethanol production in the context of innovative development. *Scientific Works of the National University of Food Technologies*, 48, 169-174.

[19] Uyar, E.O., Hamamci, H., & Türkel, S. (2010). Effect of different stresses on trehalose levels in *Rhizopus oryzae*. *Journal of Basic Microbiology*, 50(4), 368-372. doi: 10.1002/jobm.200900339.

[20] Watarai, N., Yamamoto, N., Sawada, K., & Yamada, T. (2019). Evolution of *Aspergillus oryzae* before and after domestication inferred by large-scale comparative genomic analysis. *DNA Research*, 26(6), 465-472. doi: 10.1093/dnares/dsz024.

[21] Wong, B., Muchangi, K., Quach, E., Chen, T., Owens, A., Otter, D., Phillips, M., & Kam, R. (2023). Characterisation of Korean rice wine (*makgeolli*) prepared by different processing methods. *Current Research in Food Science*, 6, article number 100420. doi: 10.1016/j.crfs.2022.100420.

[22] Zhang, J., Liu, K., Duan, X., Wang, X., Ge, W., & Jin, W. (2023). Restoration of Choujiu Koji and evaluation of its brewing performance. *LWT*, 183, article number 114933. doi: 10.1016/j.lwt.2023.114933.

[23] Zhao, C., Su, W., Mu, Y., Luo, L., Zhao, M., Qiu, S., Su, G., & Jiang, L. (2023). Effects of Jiuqu inoculating *Rhizopus oryzae* Q303 and *Saccharomyces cerevisiae* on chemical components and microbiota during black glutinous rice wine fermentation. *International Journal of Food Microbiology*, 385, article number 110012. doi: 10.1016/j.ijfoodmicro.2022.110012.

Особливості зброджування сусла підвищеної концентрації з крохмалевмісної сировини змішаною культурою мікроорганізмів

Олексій Олійнічук

Аспірант
Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
https://orcid.org/0009-0009-8014-1703

Любомир Хомічак

Доктор технічних наук, професор, член-кореспондент НААН
Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
https://orcid.org/0000-0001-9003-0315

Ольга Коваль

Кандидат технічних наук
Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
https://orcid.org/0000-0003-1035-5895

Анотація. Сучасний технологічний процес гідролізу крохмалю до зброджуваних цукрів у виробництві етанолу з крохмалевмісної сировини базується на використанні комерційних ферментних препаратів амілолітичної дії та застосуванні стадії низькотемпературного розрідження для більшої ефективності процесу, що суттєво впливає на собівартість готового продукту. За таких умов пошук альтернативних шляхів зменшення витрат на дані компоненти технологічного процесу і, відповідно, собівартості виробленої продукції є актуальним. Метою роботи було дослідження використання комерційної суміші мікроорганізмів, а саме спиртових дріжджів *Saccharomyces cerevisiae* та плісеневого грибу *Rhyzopus* як складових комерційного продукту «Angel Leaven» для одностадійного процесу гідролізу крохмалю та зброджування сусла підвищеної концентрації на основі крохмалевмісної сировини на етанол. В дослідженнях використовували методи та процеси підготовки сировини, що є загальноприйнятими у виробництві спирту етилового. Експериментально обґрунтовано технологічний режим одностадійного гідролізу крохмалю та зброджування цукрів в суслі підвищеної концентрації асоціацією плісеневого гриба та спиртових дріжджів. Встановлено, що оцукрювання та зброджування крохмалю змішаною культурою відбувається в одну стадію і практично одночасно за температури 30-32 °C, що створює передумови для енергозбереження та спрощення перебігу технологічного процесу. Ефект енергозбереження посилюється за рахунок зброджування сусла підвищеної концентрації, отриманого без стадії низькотемпературного розрідження, однак зі стадією витримки з протеолітичним ферментом впродовж 1 години за температури 50 °C. Даний технологічний прийом був ефективним для середовища з концентрацією до 306,6 г/дм^3 сухих речовин, що дозволило отримати бражку зі вмістом спирту до 14,2 % об. Підвищення концентрації середовища до 350 г/дм^3 сухих речовин не дозволяє отримати розрахунковий вміст спирту в зрілій бражці за одностадійного процесу і потребує подальших досліджень щодо оптимізації умов зброджування. Сукупність технологічних рішень оптимізації процесу гідролізу і зброджування крохмалю змішаною культурою створює умови для підвищення ефективності використання сировини та зростання енергоефективності виробничого процесу

Ключові слова: амілолітичні ферменти; гідроліз крохмалю; зріла бражка; *Saccharomyces cerevisiae*; асоціація мікроорганізмів

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 16(4), 70-88
Received 29.06.2025 Revised 02.11.2025 Accepted 27.11.2025

UDC 637.146.34:637.044
DOI: 10.31548/animal.4.2025.70

Study of technological parameters of the fermentation process in the technology of low-lactose yoghurts based on buttermilk

Tetiana Yudina
Doctor of Technical Sciences, Professor
State University of Trade and Economics
02156, 19 Kyoto Str., Kyiv, Ukraine
https://orcid.org/0000-0002-7407-4534

Anton Serenko
PhD in Technical Sciences, Senior Lecturer
State University of Trade and Economics
02156, 19 Kyoto Str., Kyiv, Ukraine
https://orcid.org/0000-0002-0390-369X

Oksana Vitriak
PhD in Technical Sciences, Associate Professor
State University of Trade and Economics
02156, 19 Kyoto Str., Kyiv, Ukraine
https://orcid.org/0000-0002-6614-1928

Lyubov Tkachenko
PhD in Technical Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-2731-1178

Alona Altanova*
PhD in Pedagogical Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-2783-4932

Suggested Citation:
Yudina, T., Serenko, A., Vitriak, O., Tkachenko, L.,& Altanova, A. (2025). Study of technological parameters of the fermentation process in the technology of low-lactose yoghurts based on buttermilk. *Animal Science and Food Technology*, 16(4), 70-88. doi: 10.31548/animal.4.2025.70.

**Corresponding author*

Abstract. Expanding the range of low-lactose dairy products, including yoghurts, is a relevant direction and task for the food industry in the context of the growing number of people suffering from lactase deficiency. Research into the technological aspects of the fermentation process was an important step in the development of such products. The main objective of this work was to analyse the technological conditions of the fermentation process in the production of low-lactose yoghurts using whey. The studies used milk mixtures based on buttermilk, normalised for dry matter content and hydrolysed lactose, and starter cultures containing the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The effect of fermentation temperature, starter culture dose and fermentation duration on the organoleptic and physicochemical properties of the finished product was investigated. It was established that the rational parameters for obtaining sour milk curd and reducing the lactose content are: fermentation temperature 40-45°C, fermentation agent dose 2.6-2.8 mg/100 g, and fermentation duration 180-240 minutes. The results showed that during fermentation, the lactose content in the finished product decreased by 45%, which allowed it to be classified as a low-lactose dairy product. At the same time, an increase in the concentration of vitamins, in particular B vitamins, indicated an increase in the nutritional value of the product. The resulting low-lactose yoghurt based on buttermilk had a thick consistency, a pleasant sour milk taste and sweetness due to the accumulation of monosaccharides – glucose and galactose – as a result of lactose hydrolysis. The technological parameters of the fermentation process determined in this work can be used in the production of low-lactose yoghurts for people with lactase deficiency and will contribute to the expansion of the range of low-lactose dairy products produced in Ukraine

Keywords: secondary milk raw materials; lactase deficiency; starter culture; fermentolysis; fermented products; fermented milk drinks; nutritional value

Introduction

Modern scientific programmes and development priorities in the food industry within the European Union are focused on creating innovative products with proven nutritional value and health benefits. In this context, considerable attention was paid to establishing requirements for the correct use of nutritional and medical-biological claims, as well as developing technologies that combine traditional ingredients with low-cost technological solutions. This approach enables the production of affordable, balanced, and nutritious products aimed at supporting the health of broad population groups (Faienza *et al.*, 2024). Special importance is placed on research in the field of specialised food products designed for individuals with increased sensitivity to specific nutrients. These include consumers with chronic diseases, fermentopathies, and food allergies who require dietary adjustments through the replacement or exclusion of certain components. According to M. Essa *et al.* (2023), the development of such technologies not only improves the quality of life of these groups, but also corresponds to global trends in functional and therapeutic-prophylactic nutrition.

As noted by Yu. Honchar & V. Gnitsevych (2024), the number of individuals in the population with health problems related to lactase deficiency, i.e., the inability to digest lactose contained in dairy products, increases annually. One of the most effective methods for treating lactase deficiency is diet therapy, which includes either the complete exclusion of dairy products from the diet or the consumption of fermented dairy products such as sour cream, cottage cheese, kefir, ryazhanka, and yoghurts, where lactose is partially converted into lactic acid. In milk, the lactose content is 4.5-5.2%; in cottage cheese, it is 1.8-2.0%; in sour cream, 2.7-3.2%; in kefir, about 4.0%; and in ryazhanka and drinking yoghurts, about 3.5%. Thus, fermentation of dairy raw material using starter cultures based on lactic acid bacteria strains can reduce only 25-30% of the initial lactose content (Ryzhkova *et al.*, 2024). The final lactose level in such products remains quite high, which makes their regular use in the diets of people suffering from lactase deficiency impossible.

However, milk and dairy products are an important source of complete proteins, vitamins, and mineral substances, especially calcium. Their exclusion from the diet can lead to a deficiency of essential nutrients and, as a consequence, to a decrease in work capacity and the body's resistance to diseases and negative environmental factors. This is why one of the promising directions for solving this problem is the development of technologies for producing dairy products, particularly yoghurts, that are lactose-free or have a reduced lactose content. Research by T. Yudina & A. Serenko (2021) established that the assortment of lactose-free and low-lactose dairy products of Ukrainian origin is quite limited. It is proven that yoghurts are highly popular among consumers. However, the volume of low-lactose and lactose-free yoghurts on the country's dairy market constitutes only 36.4%, which is almost 2 times less compared to imported products.

Thus, the development of scientifically substantiated technologies for yoghurts with a regulated carbohydrate composition for individuals with lactase deficiency, in the context of state policy regarding resource conservation and the increase of high-quality Ukrainian-produced goods, is a topical and timely task. The aim of this study was to establish the optimal technological parameters of the fermentation process for the production of low-lactose yoghurts based on buttermilk.

Literature Review

The fermentation process is one of the most important stages in yoghurt production. It is at this stage that the sour milk clot is formed, and the structural and mechanical properties and characteristic organoleptic indicators of yoghurt are formed. A key factor in the fermentation process is the use of starter cultures, which carry out biochemical reactions that lead to the formation of curds and the desired organoleptic properties (Savaiano & Hutkins, 2021). According to DSTU 4343:2004 (2005), in order for a fermented milk product to be labelled "yoghurt", the enzyme preparation used in its production must contain cultures of the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii bulgaricus*. The strains *Str. thermophilus* and *L. bulgaricus* are used as starting material for the creation of symbiotic starters for yoghurt. The main condition for the selection of these strains is their symbiotic relationship. Milk fermented under the action of a single strain of *Str. thermophilus* or *L. bulgaricus* has a different consistency than milk fermented by a combination of these microorganisms (Ibrahim *et al.*, 2021). When strains are used together, the finished product has a thicker consistency with a pronounced taste and aroma. During milk fermentation, Bulgarian bacillus strains produce acetaldehyde, which gives the products a characteristic taste and aroma, as well as antibiotic substances that suppress negative intestinal microbiota. When *Str. thermophilus* and *L. bulgaricus* strains are used together, both microorganisms have higher acid resistance. Thus, in the process of fermenting milk raw materials when *Str. thermophilus* is used separately, the maximum acidity for it is 110...120°C, while in combination with *L. bulgaricus*, it can withstand an acidity of 180-190°T, which plays a significant role in the production of fermented milk products (Asiimwe *et al.*, 2021; Hussein *et al.*, 2021).

According to E. Yamamoto *et al.* (2021), in the production of fermented dairy products, including yoghurts, for people suffering from lactase deficiency, the characteristic of microorganisms in terms of their β-galactosidase activity is of particular importance. Most strains of microorganisms included in starter cultures have selective enzymatic activity towards lactose. During the life cycle of these microorganisms, a small amount of lactose (0.4-0.8%) is broken down. Thanks to the use of lactic acid bacteria, up to 30% of the initial lactose content is fermented (Minorova *et al*., 2022). When fermentation using starter cultures is used in the production of yoghurt, in addition to lactic acid fermentation, biochemical processes occur, resulting in the accumulation of lactose breakdown products – volatile and organic acids, alcohols, diacetyl, which determine the taste and aroma characteristic of fermented milk products (Li *et al*., 2023). Among lactic acid bacteria, thermophilic lactic acid streptococci show the highest activity in lactose fermentation. The β-galactosidase enzyme of thermophilic streptococcus actively hydrolyses lactose, demonstrating high activity and stability. In addition, according to I. Romanchuk (2020), cations in milk raw materials help stimulate the fermentation process. Research data on the properties of lactic acid bacteria show that during fermentation, they produce folic acid, niacin, vitamins B12, B6, and enzymes that are essential for the human body (Chen *et al*., 2023). Lactic acid bacteria, by fermenting milk raw materials, increase the absorption of proteins and fats and promote the formation of short-chain fatty acids, which are an important source of energy for the body. The fermentation process also improves the bioavailability of calcium and other minerals, which has a positive effect on bone health (Helikh *et al*., 2022).

Thus, analysis of recent studies indicates significant potential for the use of fermentation in improving food quality. Fermented dairy products have a higher content of vitamins, probiotics and other substances, making them beneficial to human health. However, despite numerous studies in this area, there is a need for further research into fermentation processes to determine the conditions that will allow products with maximum beneficial properties to be obtained. The situation on the dairy market is complicated by the fact that, as a result of hostilities in Ukraine, some milk processing enterprises have ceased operations, leading to a decline in milk production and an increase in prices for Ukrainian dairy products (Yudina & Serenko, 2021). One of the main problems remains the attraction and effective use of the food potential of secondary milk raw materials (skimmed milk, milk whey, and buttermilk) formed during the traditional processing of milk into cream, sour milk cheese, and butter (Singh *et al*., 2021).

The theoretical and practical aspects of the production of food products for special dietary purposes, in particular lactose-free and low-lactose dairy products, have been the subject of research by many scientists in different countries. Scientists G. Polishchuk *et al*. (2020), H. Deynychenko *et al*. (2022), V. Gnitsevych & Yu. Honchar (2022) have identified priority areas for the use of secondary milk raw materials in technologies for food products for special dietary purposes. Research by M. Corgneau *et al*. (2017) confirms the need to expand the range of these products for the special nutrition of people suffering from lactose intolerance. Despite the large number of scientific studies, work in this area continues and focuses on the development of new technologies and the expansion of the range of dairy products with reduced lactose content, thanks to the use of new raw materials that are a natural source of essential nutrients and have a wide range of technological properties.

Materials and Methods

Experimental studies were conducted in the laboratories of the Department of Restaurant and Craft Technologies of the State University of Trade and Economics; the Department of

Milk and Children's Products of the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine during 2023-2024. The subject of the research was buttermilk obtained during butter production by whipping cream (Table 1); milk mixtures based on buttermilk, normalised with milk protein concentrate in terms of dry matter content and hydrolysed lactose (Tables 2, 3); YC-X11 direct-added yoghurt starter culture (manufacturer Christian Hansen, Denmark), containing strains of lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Table 4), low-lactose yoghurt based on buttermilk.

***Table 1.** Physicochemical indicators of buttermilk*

Acidity		Mass fraction of ash, %	Mass fraction of dry matter, %	Mass fraction of fat, %	Mass fraction of lactose, %
titrated, °T	active, pH				
16.0 ± 0.3	6.67 ± 0.01	0.64 ± 0.02	8.22 ± 0.03	0.40 ± 0.05	4.5 ± 0.04

Source: developed by the authors based on T. Yudina & A. Serenko (2022)

***Table 2.** Model compositions of milk mixtures normalised by dry matter content*

Raw material	Mixture 1	Mixture 2
Buttermilk, %	93.5	92.0
Dry milk protein concentrate, %	6.5	8.0
Total	100	100

Source: developed by the authors based on T. Yudina & A. Serenko (2022)

***Table 3.** Physical and chemical indicators of milk mixtures based on buttermilk, normalised for dry matter content and hydrolysed lactose*

Sample	Acidity		Mass fraction of ash, %	Mass fraction of dry matter, %	Mass fraction of fat, %	Mass fraction of lactose, %
	titrated, °T	active, pH				
Buttermilk (Control)	16.0 ± 0.3	6.67 ± 0.01	0.64 ± 0.02	8.22 ± 0.03	0.40 ± 0.05	4.5 ± 0.04
Mixture 1	30.0 ± 0.3	6.53 ± 0.01	0.4 ± 0.01	14.1 ± 0.02	0.48 ± 0.04	1.34 ± 0.04
Mixture 2	32.0 ± 0.4	6.52 ± 0.01	0.44 ± 0.02	15.3 ± 0.03	0.50 ± 0.02	1.21 ± 0.04

Source: developed by the authors based on T. Yudina & A. Serenko (2022)

***Table 4.** Characteristics of the YC-X11 fermentation preparation*

Indicator	Meaning/characteristic
Appearance	Lyophilised cultures in granules
Optimal operating temperature	35-45°C
Fermentation duration	4-5 hours
Recommended dosage	3 mg/100 g

Source: developed by the authors based on Y. Song *et al.* (2023)

For the hydrolysis of lactose in raw milk, neutral lactase was used – an enzyme preparation of yeast-derived β-galactosidase GODO-YNL2, manufactured in Japan, the characteristics of which are given in Table 5. A detailed methodology for the hydrolysis of lactose in milk mixtures based on buttermilk, which was used as the raw material in the current study, is provided in the work of T. Yudina & A. Serenko (2022).

Table 5. *Characteristics of the GODO-YNL2 lactase enzyme preparation*	
Indicator	**Meaning/characteristic**
Activity	50,000 ONPGU/g
Appearance	Yellowish liquid
Solubility	Completely soluble in liquid
Specific gravity	1.17
Optimal active acidity	5.5...6.5 pH
Optimal operating temperature	20...45°C
Thermal stability at temperature	up to 55°C
Recommended dosage	0.1%

Source: developed by the authors based on T. Yudina & A. Serenko (2022)

Fermentation of milk mixtures based on buttermilk with dry matter content of 14.1% and 15.3% was carried out in a thermostat at a temperature of 40±2°C. The dosage of the starter culture varied between 2.2 and 3.0 mg/100 g, and the fermentation time was 30 to 300 minutes with samples taken every 30 minutes. The effectiveness of the starter culture was evaluated according to physical and chemical indicators in accordance with DSTU 4343:2004 (2005), taking into account that the standard titrated acidity for yoghurts should be within the range of 80-140°T. The study of the effect of temperature on the activity of the starter culture was carried out with temperature variations within the range of 15...55°C, a starter culture dose of 2.6...2.8 mg, and a process duration of 210 min. Samples were taken every 30 minutes. For each variant of the experiment, five parallel samples of milk mixture with a volume of 200 ml each were prepared.

The carbohydrate composition of the mixtures and low-lactose fermented milk product was determined using a high-performance liquid chromatograph LC-6A with a refractometric detector (RI detector), column HC-75-Ca++ (250×4.7 mm) (manufactured by Shimadzu, Japan). The method was based on the removal of fat and protein by filtration, the determination of carbohydrates in the filtrate of the samples obtained relative to standard samples with a known concentration of added carbohydrates. The efficiency of lactose hydrolysis was determined using formula (1):

$$E = \left(\frac{Ci - Cc}{Cc}\right) * 100\%, \quad (1)$$

where E – lactose hydrolysis efficiency, %; Ci – initial lactose content in mixtures before hydrolysis g/100 g; Cc – lactose content in milk raw material hydrolysate, g/100 g.

The following physicochemical parameters were determined in the test samples: titrated acidity – by titrimetric method according to DSTU ISO 6091:2007 (2009); active acidity – by potentiometric method according to DSTU 8550:2015 (2017); mass fraction of fat – by gravimetric method according to DSTU ISO 11870:2007 (2009); mass fraction of protein – by the Kjeldahl method according to DSTU ISO 8968-1:2005 (2007), vitamin content – by high-performance liquid chromatography according to DSTU EN 14164:2019 (2019). The physicochemical parameters were determined in three parallel samples for each sample.

The rheological parameters of the finished product were determined using a RHEOTEST II (Ukraine) rotational viscometer with a cylinder-cylinder (S/S3) measuring system by recording the deformation (flow) kinetics curves. The measurements were carried out in mode "a", which was set experimentally taking into account the structural and mechanical properties of the test samples. The measuring cylinder (rotor) S2 was selected so that the gradient layer spread throughout the entire thickness of the product layer located in the annular gap of the viscometer measuring device. A new portion of the product was taken for each experiment. The shear stress τ (Pa) was measured for 12 values of the shear rate gradient γ in the range from 0.33 to 145.8 s-1 in the forward and reverse directions. For this purpose, the value of α was recorded at the maximum angle of deflection of the pointer on the instrument scale.

The organoleptic indicators of the developed products were determined by a tasting commission based on a developed sensory evaluation scale for low-lactose fermented milk drinks based on buttermilk. The tasting commission consisted of scientists in the field of food technology, as well as practitioners from food industry enterprises and catering establishments. The organoleptic evaluation was carried out with the participation of 12 qualified tasters. The evaluation was carried out in a specially equipped tasting room at a temperature of 20 ± 2°C, with samples served in identical 50 ml containers. The work of the tasting commission was organised in accordance with the ethical standards of the WMA (2013). All participants were familiarised with the aim of the study and gave their informed consent to participate. The sensory evaluation of yoghurt was carried out on a 5-point scale, taking into account the main characteristics to be evaluated (appearance, colour, taste, smell, consistency) in accordance with DSTU 4343:2004 (2005).

The results of the experimental studies were statistically processed using the least squares method to determine the error of the obtained data. All experiments were repeated five times. Statistical processing of the experimental data was performed using Statistica 10.0 software (StatSoft Inc., USA). For each indicator, the arithmetic mean (M), standard deviation (SD) and standard error of the mean (SEM) were calculated. The reliability of the difference between the groups was assessed using one-way analysis of variance (ANOVA). The difference was considered statistically significant at $p < 0.05$.

Results

The main stage in the production of low-lactose yoghurts is the fermentation of the hydrolysed milk base, which results in the formation of the properties of the sour milk curd, safety indicators and the quality of the finished product. Based on an analysis of the market for fermentation preparations and the work of scientists, the YC-X11 direct-addition yoghurt fermentation preparation (manufactured by Christian Hansen, Denmark) was selected for the production of low-lactose fermented milk drinks. The fermentation parameters recommended by the manufacturer for the use of the direct-addition starter culture YC-X11 are specified for whole milk as the raw material for yoghurt production. Thus, in order to justify the conditions for the action of the YC-X11 starter culture in another dairy system, the effect of its dosage on the quality indicators of low-lactose yoghurt based on buttermilk was investigated (Figs. 2, 3; Table 6).

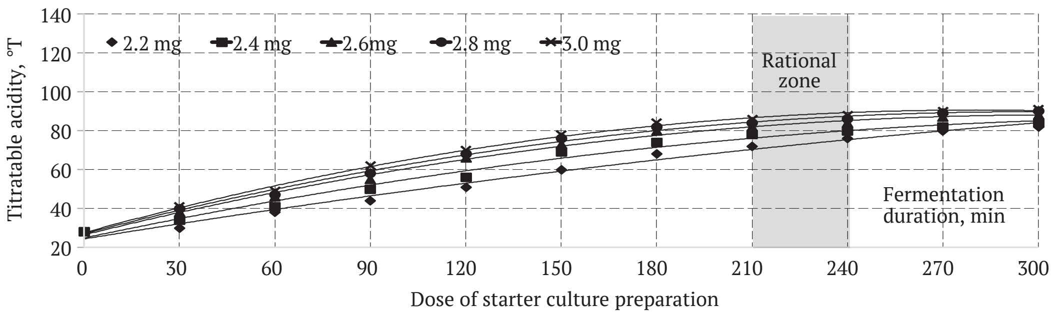

Figure 1. Effect of the dose of starter culture on the duration of fermentation of milk mixture with a dry matter content of 14.1%

Source: developed by the authors

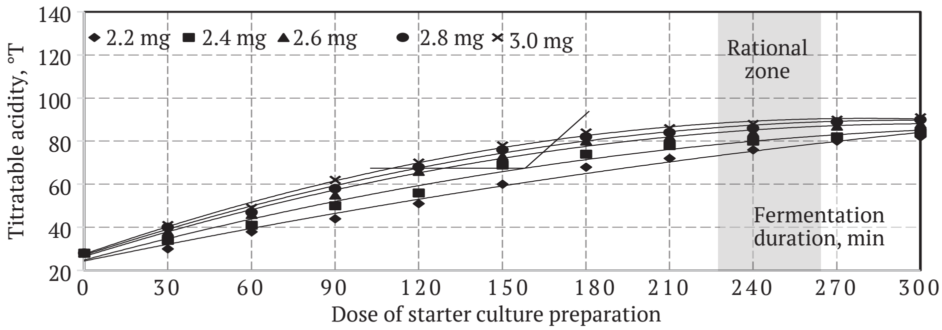

Figure 2. Effect of the dose of starter culture on the duration of fermentation of milk mixture with a dry matter content of 15.3%

Source: developed by the authors

Table 6. *Quality indicators of milk mixtures with hydrolysed lactose after fermentation*

Indicators	Milk mixtures based on buttermilk, normalised with milk protein concentrate									
	Mixture 1					Mixture 2				
Dose of FP, mg	2.2	2.4	2.6	2.8	3.0	2.2	2.4	2.6	2.8	3.0
Titrated acidity, °T	80.0		82.0	83.0	84.0	80.0		81.0	83.0	85.0
Consistency	Heterogeneous, with disturbed curd; slight serum secretion		Homogeneous, tender, with undisturbed curd, moderately dense			Heterogeneous, with disturbed curd; slight serum secretion		Homogeneous, tender, with undisturbed curd, moderately dense		
Colour	Milky white with a yellowish tint									
Smell	Pure, characteristic of fermented milk drinks; extraneous aromas and odours are absent									
Taste	Characteristic of fermented milk products, with a pleasant malty flavour; without extraneous tastes									

Note: FP – fermentation preparation

Source: developed by the authors

The research results indicate that the formation of a fermented milk curd with specified organoleptic properties and regulated titratable acidity within the range of 80-140°T was achieved under the following rational parameters: for a milk mixture with a total solids content of 14.1%, the starter culture dosage was 2.6-2.8 mg/100 g, with a process duration of 210-240 minutes; for a milk mixture with a total solids content of 15.3%, the starter culture dosage was 2.6-2.8 mg/100 g, with a process duration of 180-210 minutes. The use of lower starter culture dosages resulted in a reduction in the intensity of curd formation and an increase in fermentation time, leading to unnecessary energy expenditure, which is undesirable. Applying a starter culture dosage of 3 mg/100 g did not significantly affect the quality indicators of the developed product but did contribute to an increase in production costs.

The strains of lactic acid bacteria in the YC-X11 fermentation preparation are chemoorganotrophic microorganisms (Romanchuk, 2020). This means that they obtain energy by oxidising organic substances, i.e. lactic acid bacteria oxidise lactose, converting it into glucose and galactose. Glucose and galactose serve as substrates for the synthesis of lactic acid. Temperature affects the activity of lactic acid bacteria and their metabolic rate. At higher temperatures, the enzymes involved in the metabolism of lactic acid bacteria work faster, producing more lactic acid per unit of time and, as a result, accelerating the process of sour milk curd formation. Considering the above, in the next series of experiments, the effect of fermentation temperature on the activity of the starter culture in milk mixtures normalised for dry matter content was investigated (Figs. 4, 5).

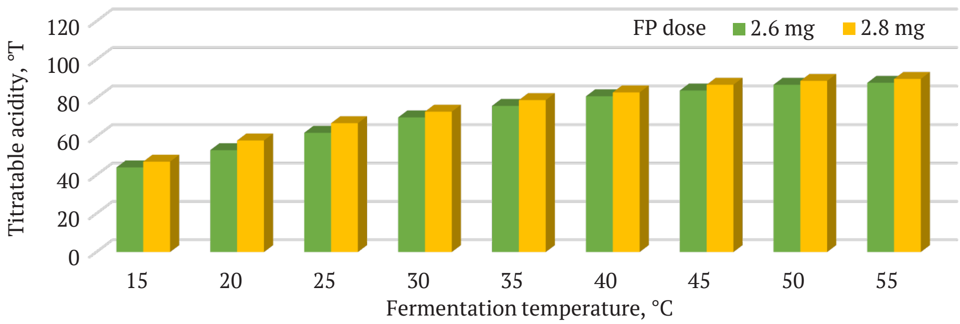

Figure 3. Effect of temperature on the activity of the starter culture in a milk mixture with a dry matter content of 14.1%

Source: developed by the authors

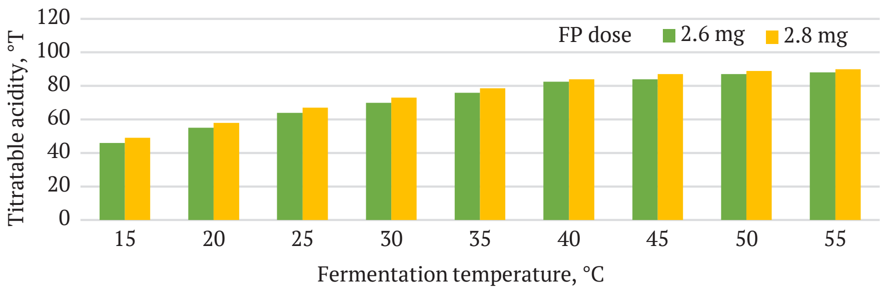

Figure 4. Effect of temperature on the activity of the starter culture in a milk mixture with a dry matter content of 15.3%

Source: developed by the authors

The results showed that the fermentation agent was most effective at temperatures between 40 and 45°C. At lower temperatures, the fermentation process and curd formation were slower, which can be explained by the low activity of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* lactic acid bacteria at these temperatures. When the temperature rose above 45°C, the effectiveness of the starter culture decreased due to the inactivation of lactic acid bacteria at high temperatures. Thus, the most rational parameters for the fermentation process of milk mixtures with a high dry matter content, which ensure the formation of a sour milk curd with the specified organoleptic properties, standardised titrated acidity within the range of 80-140°C and reduce energy consumption, are fermentation temperature – 40-45°C, a starter culture dose of 2.6-2.8 mg/100 g, and a process duration of 180-210 minutes for milk mixtures with a dry matter content of 14.1% and 210-240 minutes for milk mixtures with a dry matter content of 15.3%.

Consistency – one of the most important indicators of the consumer properties of low-lactose fermented milk drinks, including yoghurts. It is formed during the technological process and depends on many factors, including the properties of the milk base, the dose of enzyme and starter cultures, and production parameters. Figures 6 and 7 show the results of a study of the effect of the dose of the starter culture and the duration of fermentation of milk mixtures with different dry matter contents on the rheological properties of low-lactose yoghurt.

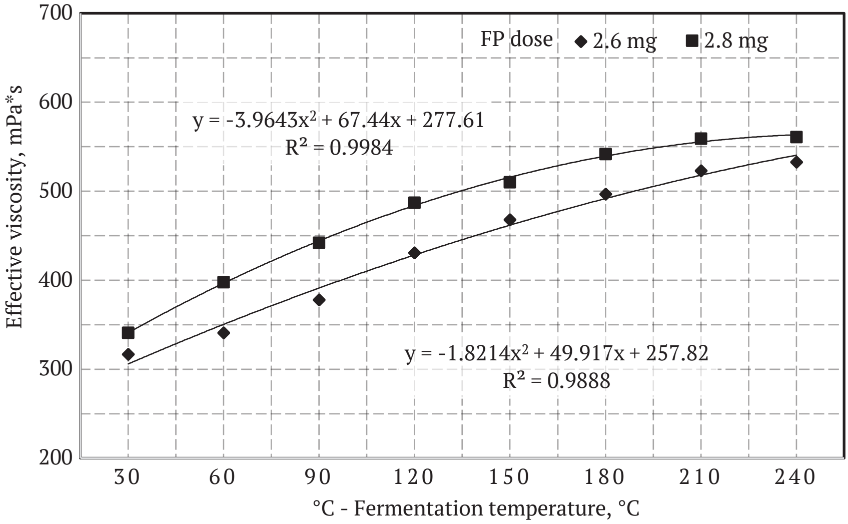

Figure 5. Dynamics of effective viscosity change during fermentation of milk mixture with dry matter content of 14.1%

Source: developed by the authors

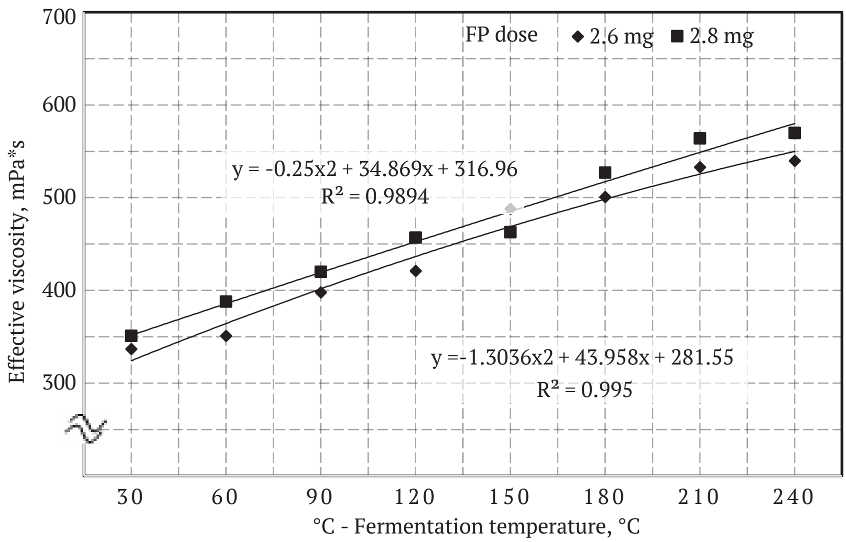

Figure 6. Dynamics of effective viscosity change during fermentation of milk mixture with dry matter content of 15.3%

Source: developed by the authors

The results of the studies show that as the fermentation time increases, the viscosity of the test samples increases. This is because the components of the milk mixture, such as proteins and lactose, are involved in the formation of sour milk curds. The lactic acid bacteria contained in the fermentation preparation convert lactose into glucose and galactose, which serve as substrates for biochemical reactions that result in the formation of lactic acid. Lactic acid causes protein coagulation, followed by the formation of lactic acid curds. Thus, when adding 2.6-2.8 mg/100 g of starter culture and fermenting for 180-210 minutes the viscosity of the finished product was 523-559 mPa*s, and for a milk mixture with a dry matter content of 15.3%, it was 560-564 mPa*s, respectively. The resulting low-lactose yoghurt is characterised by a stable structure and semi-liquid consistency. During the further fermentation process for 210-240 minutes, insignificant changes in viscosity were observed in the test samples, while the consistency of the yoghurt remained homogeneous, semi-liquid, without whey separation. In order to determine the compliance of the products obtained after fermentation with the requirements for low-lactose products set by the European Food Safety Authority (2010), their carbohydrate composition was determined (Fig. 8).

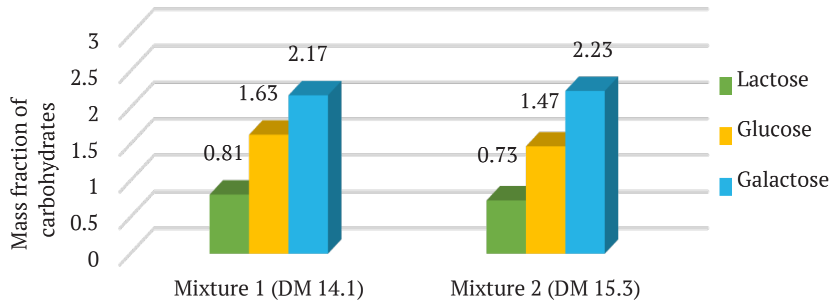

Figure 7. Carbohydrate composition of fermented milk mixtures with hydrolysed lactose
Source: developed by the authors

The results of the studies show that in the obtained milk mixtures with a dry matter content of 14.1% and 15.3%, the lactose content after fermentation is less than 1%, which complies with the recommendations for its content in low-lactose dairy products. For further research and development of low-lactose yoghurt technology, a milk mixture with a dry matter content of 15.3% was selected as the milk base, as it has a lower residual lactose content, and the higher dry matter content in the specified milk base makes it possible to obtain a finished low-lactose fermented milk drink with a stable structure and consistency.

During the fermentation of the milk base in the production of low-lactose yoghurt, biochemical processes occur that affect changes in the chemical composition of the finished product. In particular, under the action of lactic acid bacteria, lactose is converted into glucose and galactose, which, in turn, serve as a substrate for the formation of lactic acid, which participates in the formation of a fermented milk curd (Bolgova *et al.*, 2021). In addition to lactic acid, the lactic acid bacteria that make up the yoghurt starter culture are a source of vitamins, particularly B vitamins. Analysis of the research results (Table 7) shows that the carbohydrate composition changes during the fermentation of the milk base in yoghurt production. Thus, the lactose content in yoghurt after fermentation decreased by 45%, which indicates the high β-galactosidase activity of the lactic acid bacteria included in the starter culture (Wolf *et al.*, 2015).

Table 7. Effect of fermentation on the chemical composition of low-lactose yoghurt based on buttermilk

Indicator	Before fermentation	After fermentation
Carbohydrate content, g/100 g:		
Lactose	1.33	0.73
Glucose	1.69	1.47
Galactose	1.44	2.23
Vitamin content, mg/100 g:		
B_1	0.128	0.131
B_2	0.111	0.122
B_3	0.937	1.0
B_5	0.372	0.386

Source: developed by the authors

Since low-lactose yoghurt based on buttermilk falls into the category of special health foods and is recommended for people with lactase deficiency, the lactose content in the final product is particularly important. The data obtained show that a lactose content of 0.73% per 100 g of product meets the requirements of the European Food Safety Authority (2010). There is also an increase in the vitamin content in the finished yoghurt, which indicates the ability of lactic acid bacteria to produce, in addition to lactic acid, other biologically active substances that increase the nutritional value of the product.

The combination of biochemical transformations in the yoghurt production process affects not only the change in chemical composition, but also the formation of the organoleptic properties of the finished product. During fermentation, in addition to the conversion of lactose to lactic acid, side processes also occur with the formation of organic acids, aldehydes, alcohols, and peptides, which determine the characteristic sour milk taste and aroma of yoghurt. The quality characteristics of the developed low-lactose yoghurt based on buttermilk are shown in Table 8.

Table 8. Quality indicators of low-lactose yoghurt based on buttermilk

Indicators	Characteristics
Titrated acidity, °T	83.0
Active acidity, pH units	4.78
Consistency	Homogeneous, delicate, with intact curds, moderately dense
Colour	Milky white, homogeneous, with a yellowish tint
Smell	Pure sour milk, mild; no foreign odours
Taste	Pronounced sour milk, mild, balanced, with a characteristic sweet taste

Source: developed by the authors

The developed low-lactose yoghurt is characterised by high organoleptic indicators that meet the requirements of DSTU 4343:2004 (2005). Thanks to the accumulation of glucose and galactose, whose sweetness indices are 5-6 times higher than that of lactose, low-lactose yoghurt based on buttermilk has a pleasant sweet taste, which makes it possible not to use sugar in its recipe and recommend the product for low-calorie diets.

Discussion

The results of the study of the fermentation process in low-lactose yoghurt technology based on buttermilk confirm the importance of determining technological parameters to achieve the desired organoleptic and physicochemical properties of the product. Analysis of the results obtained shows significant correspondence with other scientific works devoted to the study of the fermentation process of food products. Thus, the research by S. Sarıtaş *et al*. (2024) confirms that a temperature range of 38-45°C is optimal for the activity of most lactic acid bacteria and ensures the production of fermented milk products of the required quality. The rational fermentation parameters established in the current study ensure the formation of a stable sour milk clot with standardised titrated acidity (80-140°T) and active acidity (pH 4.78), which correlates with the optimal conditions described in the study by A. Asiimwe *et al*. (2021), where a temperature of 40-42°C contributed to better rheological stability of yoghurts with added probiotics. Compared to the study by E. Halbmayr-Jech *et al*. (2020), where the concentration of β-galactosidase from *Lactobacillus paracasei* (0.3%) reduced the lactose content in 100 g of yoghurt to 2.5 g, the approach used in the current study, combining enzymatic hydrolysis of lactose with the action of the YC-X11 starter culture based on lactic acid bacteria, demonstrates a lower final lactose content (0.73%), which makes the product more attractive for people with lactase deficiency.

In the work of F. Tonolo *et al*. (2020), it was determined that the fermentation of dairy products at a temperature of 40-45°C ensures high efficiency of lactic acid bacteria and promotes the formation of a stable sour milk clot. At the same time, according to the results of scientific research, the most effective temperature range for reducing lactose is 42-44°C. Research by Y. Gao *et al*. (2025) shows that temperatures above 45°C can cause the inactivation of lactic acid bacteria, which leads to a decrease in fermentation efficiency. The results obtained in the study prove that fermentation of the milk base at temperatures above 45°C has a negative effect on the activity of starter cultures, confirming the importance of precise temperature control to achieve stable results. Thus, the results of the current study confirm the conclusions of previous studies, according to which the optimal temperature range for reducing lactose content is 42-44°C. The selected fermentation parameters were found to be comparable to scientifically substantiated data.

It is worth noting the study by K. Kondrotiene *et al*. (2024), which examined the effect of fermentation duration on the quality of dairy products. Their study indicated that prolonging fermentation for more than 240 minutes leads to a decrease in the activity of lactic acid bacteria, which negatively affects the organoleptic properties of the product. This coincides with the observations of the authors of the current work: a process duration of more than 210 minutes affects the reduction in the activity of lactic acid bacteria, which indicates the need to control this parameter to achieve high product quality. An increase in the concentration of B vitamins and the accumulation of short-chain fatty acids in the finished yoghurt indicates the biosynthetic activity of lactic acid bacteria during fermentation. This is consistent with the findings of D. Savaiano & R. Hutkins (2021), where fermentation with *Bifidobacterium longum* under similar parameters increased the content of B vitamins and organic acids, improving the bioavailability of minerals, in particular calcium. Research by H. Abbas *et al*. (2024) also demonstrates that bifidobacteria in the fermentation of yoghurt not only enrich the product with biologically active substances, but also increase its antioxidant activity. The data obtained also correlates with the conclusions of B. Namshir *et al*. (2025), where the fermentation of goat's milk with similar strains of lactic acid bacteria led to an increase in the content of B vitamins by 5-15%, increasing the biological value of the product. Scientists W. Li *et al*. (2020) also note that the addition of probiotics to yoghurt promotes vitamin synthesis and improves its stability during storage. Research by L. Li *et al*. (2023) proves that the fermentation of dairy products not only reduces the lactose content but also improves the nutritional value of the product by increasing the concentration of vitamins, in particular B vitamins. This was confirmed by the results of the current study, which note an increase in the content of vitamins, in particular B vitamins, during the fermentation of the milk base of buttermilk yoghurt.

Scientists E. Yamamoto *et al*. (2021) have confirmed that the fermentation of lactose in the production of yoghurt contributes to the accumulation of glucose and galactose, which determines the natural sweetness of the product. According to their data, this process makes it possible to significantly reduce the need to add sugar to achieve the desired taste. The results of the studies also confirm that the process of lactose hydrolysis and monosaccharide accumulation gives yoghurt a natural sweet taste, which makes it less caloric due to the absence of sugar in the recipe. In conclusion, the consistency of the results obtained in the current study with the data from previous studies confirms their scientific validity and emphasises the importance of controlling the fermentation process as a determining factor in the formation of the nutritional value of dairy products.

Conclusions

Based on comprehensive analytical and experimental studies, the role of fermentation in the production of low-lactose yoghurts has been determined. The patterns of influence of technological factors on the fermentation process and the formation of sour milk curd in low-lactose yoghurt have been established. It has been determined that the rational parameters of the fermentation process of milk mixtures with a dry matter content of 15.3%, which ensure the formation of a sour milk curd with the specified organoleptic properties and a standardised titrated acidity within the range of 80-140°T, are a fermentation temperature of 40-45°C, a dose of YC-X11 direct-added yoghurt starter culture of 2.6-2.8 mg/100 g, and a fermentation time of 210 minutes. The effect of fermentation on the nutritional value of the finished product was investigated. It was proven that during the fermentation of the milk base, the lactose content decreased by 45% and did not exceed 1% in the finished yoghurt, which meets the requirements of the European Food Safety Authority for its content in low-lactose dairy products. At the same time, the recorded increase in the concentration of vitamins, in particular group B, indicates an increase in the nutritional value of low-lactose yoghurt. The accumulation of monosaccharides, such as glucose and galactose, in the process of lactose hydrolysis during fermentation determines the natural sweetness of the finished product, which makes it possible to avoid adding sugar to its recipe.

The resulting low-lactose yoghurt based on buttermilk was characterised by favourable consumer properties – a thick consistency, pleasant sour milk taste and natural sweetness, which meet the requirements of DSTU 4343:20024. The technological parameters of the fermentation process determined in the work can be used in the production of yoghurts for people with lactase deficiency, which will contribute to the expansion of the range of low-lactose dairy products of Ukrainian production based on secondary milk raw materials. Further research involves the development of a recipe and technological scheme for the production of low-lactose yoghurt based on buttermilk, as well as research into changes in the quality and safety indicators of the finished product during storage, which will allow determining its shelf life while ensuring the preservation of its beneficial properties.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

References

[1] Abbas, H., Altamim, E., Farahat, E., Mohamed, A., & Zahran, H. (2024). Enhancing *Bifidobacterium* and lactic acid bacteria activity, and improving oxidative stability in functional algal concentrated yoghurt with *Spirulina platensis* powder. *Scientific Horizons*, 27(6), 98-110. doi: 10.48077/scihor6.2024.98.

[2] Asiimwe, A., Kigozi, J., & Muyonga, J. (2021). Physicochemical properties, sensory acceptance and storage stability of yogurt flavored with refractance window dried passion fruit powder. *Asian Food Science Journal*, 20(5), 38-49. doi: 10.9734/afsj/2021/v20i530297.

[3] Bolgova, N., Samilyk, M., Nazarenko, Yu., & Sokolenko, V. (2021). Technology of lactose-free yogurt production in compliance with the principles of the HACCP system. *Tavria Scientific Bulletin. Series: Technical Sciences*, 4, 33-46.

[4] Chen, L., Bagnicka, E., Chen, H., & Shu, G. (2023). Health potential of fermented goat dairy products: Composition comparison with fermented cow milk, probiotics selection, health benefits and mechanisms. *Food & Function*, 14(8), 3423-3436. doi: 10.1039/d3fo00413a.

[5] Corgneau, M., Scher, J., Ritie-Pertusa, L., Le, D.T.L., Petit, J., Nikolova, Y., Banon, S., & Gaiani, C. (2017). Recent advances on lactose intolerance: Tolerance thresholds and currently available answers. *Critical Reviews in Food Science and Nutrition*, 57(15), 3344-3356. doi: 10.1080/10408398.2015.1123671.

[6] Deynychenko, H., Huzenko, V., Dmytrevskyi, D., Zolotukhina, I., & Perekrest, V. (2022). Waste-free technologies implementation for secondary dairy raw materials processing. *Restaurant and Hotel Consulting. Innovations*, 5(1), 82-96. doi: 10.31866/2616-7468.5.1.2022.260878.

[7] DSTU 4343:2004. (2005). *Yoghurts. General technical conditions*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=72933.

[8] DSTU 8550:2015. (2017). *Milk and dairy products. Measurement of pH by potentiometric method*. Retrieved from https://online.budstandart.com/ru/catalog/doc-page?id_doc=71694.

[9] DSTU EN 14164:2019. (2019). *Foodstuffs. Determination of vitamin B by high-performance liquid chromatography*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=84143.

[10] DSTU ISO 11870:2007. (2009). *Milk and dairy products. Determination of mass fraction of fat*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=85098.

[11] DSTU ISO 6091:2007. (2009). *Milk powder. Determination of titrated acidity*. Retrieved from https://zakon.isu.net.ua/sites/default/files/normdocs/dstu_iso_6091_2007.pdf.

[12] DSTU ISO 8968-1:2005 (IDF 20-1:2001). (2007). *Milk. Determination of nitrogen content. Part 1. Kjeldahl method*. Retrieved from https://zakon.isu.net.ua/sites/default/files/normdocs/dstu_iso_8968-1_2005.pdf.

[13] Essa, M.M., Bishir, M., Bhat, A., Chidambaram, S.B., Al-Balushi, B., Hamdan, H., Govindarajan, N., Freidland, R.P., & Qoronfleh, M.W. (2023). Functional foods and their impact on health. *Journal of Food Science and Technology*, 60(3), 820-834. doi: 10.1007/s13197-021-05193-3.

[14] European Food Safety Authority. (2010). Scientific opinion on lactose thresholds in lactose intolerance and galactosaemia. *EFSA Journal*, 8(9), article number 1777. doi: 10.2903/j.efsa.2010.1777.

[15] Faienza, M.F., Giardinelli, S., Annicchiarico, A., Chiarito, M., Barile, B., Corbo, F., & Brunetti, G. (2024). Nutraceuticals and functional foods: A comprehensive review of their role in bone health. *International Journal of Molecular Sciences*, 25(11), article number 5873. doi: 10.3390/ijms25115873.

[16] Gao, Y., Liu, Y., Ma, T., Liang, Q., Sun, J., Wu, X., Song, Y., Nie, H., Huang, J., & Mu, G. (2025). Fermented dairy products as precision modulators of gut microbiota and host health: Mechanistic insights, clinical evidence, and future directions. *Foods*, 14(11), article number 1946. doi: 10.3390/foods14111946.

[17] Gnitsevych, V.A., & Honchar, Yu.M. (2022). Technology and properties of low-lactose semi-finished product based on sweet milk whey. In *Innovative technologies and equipment: Development prospects of the food and restaurant industries* (pp. 118-136). Riga: Baltija Publishing. doi: 10.30525/978-9934-26-205-0-6.

[18] Halbmayr-Jech, E., Kittl, R., Weinmann, P., Schulz, C., Kowalik, A., Sygmund, C., & Brunelle, S. (2020). Determination of lactose in lactose-free and low-lactose milk, milk products, and products containing dairy ingredients by the LactoSens®R amperometry method: First action 2020.01. *Journal of AOAC International*, 103(6), 1534-1546. doi: 10.1093/jaoacint/qsaa080.

[19] Helikh, A., Danylenko, S., Kryzhska, T., & Semernya, O. (2022). Optimization of rheological indicators of yoghurt structure with addition of hemp seed protein isolate. *Food Resources*, 10(18), 51-60. doi: 10.31073/foodresources2022-18-05.

[20] Honchar, Yu., & Gnitsevych, V. (2024). Improving the quality of dairy sauces by using condensed low-lactose milk whey. In O. Priss (Ed.), *Food technology progressive solutions* (pp. 152-168). Tallinn: Scientific Route OÜ. doi: 10.21303/978-9916-9850-4-5.ch6.

[21] Hussein, Z.E.H., Silva, J.M., Alves, E.S., Castro, M.C., Ferreira, C.S.R., Chaves, M.L.C., da Silva Bruni, A.R., & Santos, O.O. (2021). Technological advances in probiotic stability in yogurt: A review. *Research, Society and Development*, 10(12), article number e449101220646. doi: 10.33448/rsd-v10i12.20646.

[22] Ibrahim, S.A., Gyawali, R., Awaisheh, S.S., Ayivi, R.D., Silva, R.C., Subedi, K., Aljaloud, S.O., Siddiqui, S.A., & Krastanov, A. (2021). Fermented foods and probiotics: An approach to lactose intolerance. *Journal of Dairy Research*, 88(3), 357-365. doi: 10.1017/S0022029921000625.

[23] Kondrotiene, K., Zavistanaviciute, P., Aksomaitiene, J., Novoslavskij, A., & Malakauskas, M. (2024). *Lactococcus lactis* in dairy fermentation – health-promoting and probiotic properties. *Fermentation*, 10(1), article number 16. doi: 10.3390/fermentation10010016.

[24] Li, L., Zhou, L., Liu, X., Gong, J., & Xiao, G. (2023). Physicochemical, microbiological, and sensory properties of low-lactose yogurt using *Streptococcus thermophilus* with high β-galactosidase activity. *Journal of Science Food and Agriculture*, 103(15), 7374-7380. doi: 10.1002/jsfa.12840.

[25] Li, W., *et al*. (2020). Fermentation characteristics of *Lactococcus lactis* subsp. *lactis* isolated from naturally fermented dairy products and screening of potential starter isolates. *Frontiers in Microbiology*, 11, article number 1794. doi: 10.3389/fmicb.2020.01794.

[26] Minorova, A.V., Romanchuk, I.O., Danylenko, S.H., Rudakova, T.V., Krushelnytska, N.L., Potemska, O.P., & Narizhnyi, S.A. (2022). Selection and study of the effectiveness of leavening preparations with increased β-galactosidase activity. *Food Resources*, 10(19), 88-98. doi: 10.31073/foodresources2022-19-10.

[27] Namshir, B., Kim, G.-H., Lkhagvasuren, N., Jeong, S.-A., Mijid, N., & Kim, W.-S. (2025). Fermentation and functional properties of plant-derived *Limosilactobacillus fermentum* for dairy applications. *Fermentation*, 11(5), article number 286. doi: 10.3390/fermentation11050286.

[28] Polishchuk, G., Breus, N., Shevchenko, I., Gnitsevych, V., Yudina, T., Nozhechkina-Yeroshenko, G., & Semko, T. (2020). Determining the effect of casein on the quality indicators of cream with different fat content. *Eastern-European Journal of Enterprise Technologies*, 4(11(106)), 24-30. doi: 10.15587/1729-4061.2020.208954.

[29] Romanchuk, I. (2020). *Scientific justification and development of methods for improving resource efficiency in industrial dairy raw material processing*. (Doctoral thesis, National University of Food Technologies, Kyiv, Ukraine).
[30] Ryzhkova, T.M., Danilenko, S.G., Mykhaylov, V.M., & Heyda, I.M. (2024). *Biotechnologies of fermented products from cow and goat milk*. Kharkiv: I.S. Ivanchenko Publishing House.
[31] Sarıtaş, S., *et al*. (2024). The impact of fermentation on the antioxidant activity of food products. *Molecules*, 29(16), article number 3941. doi: 10.3390/molecules29163941.
[32] Savaiano, D.A., & Hutkins, R.W. (2021). Yogurt, cultured fermented milk, and health: A systematic review. *Nutrition Reviews*, 79(5), 599-614. doi: 10.1093/nutrit/nuaa013.
[33] Singh, R., Nikitha, M., Shwetnisha, & Mangalleima, N. (2021). The product and the manufacturing of yoghurt. *International Journal for Modern Trends in Science and Technology*, 7, 48-51. doi: 10.46501/IJMTST0710007.
[34] Song, Y., Li, S., Zhang, R., Tuo, Y., Li, X., Mu, G., & Jiang, S. (2023). Physicochemical properties, antigenicity and allergenicity of yoghurt fermented by *Lactiplantibacillus plantarum* AHQ-14 combined with starter. *International Journal of Food Science and Technology*, 58(5), 2527-2539. doi: 10.1111/ijfs.16396.
[35] Tonolo, F., *et al*. (2020). Identification of new peptides from fermented milk showing antioxidant properties: Mechanism of action. *Antioxidants*, 9(2), article number 117. doi: 10.3390/antiox9020117.
[36] Wolf, I.V., Vénica, C.I., & Perotti, M.C. (2015). Effect of reduction of lactose in yogurts by addition of β-galactosidase enzyme on volatile compound profile and quality parameters. *International Journal of Food Science and Technology*, 50(5), 1076-1082. doi: 10.1111/ijfs.12745.
[37] World Medical Association (WMA). (2013). *Declaration of Helsinki. Ethical principles for medical research involving human subjects*. Retrieved from https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2013-JAMA.pdf.
[38] Yamamoto, E., Watanabe, R., Ichimura, T., Ishida, T., & Kimura, K. (2021). Effect of lactose hydrolysis on the milk-fermenting properties of *Lactobacillus delbrueckii* ssp. *bulgaricus* 2038 and *Streptococcus thermophilus* 1131. *Journal of Dairy Science*, 104(2), 1454-1464. doi: 10.3168/jds.2020-19244.
[39] Yudina, T., & Serenko, A. (2021). Formation of the domestic market for lactose-free and low-lactose dairy products. *Commodities and Markets*, 2, 33-43. doi: 10.31617/tr.knute.2021(38)03.
[40] Yudina, T., & Serenko, A. (2022). Technology of low-lactose milk mixtures for yogurts. *Commodity Science. Technologies. Engineering*, 3(43), 108-116. doi: 10.31617/2.2022(43)09.

Дослідження технологічних параметрів процесу ферментації у технології низьколактозних йогуртів на основі сколотин

Тетяна Юдіна
Доктор технічних наук, професор
Державний торговельно-економічний університет
02156, вул. Кіото, 19, м. Київ, Україна
https://orcid.org/0000-0002-7407-4534

Антон Серенко
Доктор філософії, старший викладач
Державний торговельно-економічний університет
02156, вул. Кіото, 19, м. Київ, Україна
https://orcid.org/0000-0002-0390-369X

Оксана Вітряк
Кандидат технічних наук, доцент
Державний торговельно-економічний університет
02156, вул. Кіото, 19, м. Київ, Україна
https://orcid.org/0000-0002-6614-1928

Любов Ткаченко
Кандидат технічних наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-2731-1178

Альона Альтанова
Кандидат педагогічних наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-2783-4932

Анотація. Розширення асортименту низьколактозних молочних продуктів, зокрема йогуртів, є актуальним напрямом та завданням для харчової промисловості в контексті зростаючої кількості осіб, що страждають на лактазну недостатність. Дослідження технологічних аспектів процесу ферментації є важливим етапом у технології розроблення таких продуктів. Основною метою даної роботи був аналіз технологічних умов ферментаційного процесу при виробництві низьколактозних йогуртів із застосуванням сколотин. У дослідженнях використовувались молочні суміші на основі сколотин, нормалізовані за вмістом сухих речовин та гідролізованою лактозою, заквашувальні препарати, що містять молочнокислі бактерії *Streptococcus thermophilus* та *Lactobacillus delbrueckii* ssp. *bulgaricus*. Досліджено вплив температури сквашування, дози заквашувального препарату та тривалості процесу ферментації на органолептичні та фізико-хімічні показники готового продукту. Встановлено, що раціональними параметрами отримання кисломолочного згустку та зниження вмісту лактози є: температура сквашування 40...45 °С, доза заквашувального препарату – 2,6...2,8 мг/100 г та тривалість процесу ферментації – від 180 до 240 хвилин. Отримані результати показали, що у процесі ферментації вміст лактози в готовому продукті зменшився на 45 %, що дало змогу віднести його до категорії низьколактозних молочних виробів. Водночас зафіксоване зростання концентрації вітамінів, зокрема групи В, свідчить про підвищення харчової цінності продукту. Отриманий низьколактозний йогурт на основі сколотин мав щільний згусток, приємний кисломолочний смак та солодкість завдяки накопиченню моноцукрів – глюкози та галактози, в результаті гідролізу лактози. Визначені в роботі технологічні параметри процесу ферментації можуть бути використані у виробництві низьколактозних йогуртів для осіб із лактазною недостатністю та сприятимуть розширенню асортименту низьколактозних молочних продуктів українського виробництва

Ключові слова: вторинна молочна сировина; лактазна недостатність; заквашувальний препарат; ферментоліз; ферментовані продукти; кисломолочні напої; харчова цінність

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 16(4), 89-104
Received 04.07.2025 Revised 29.10.2025 Accepted 27.11.2025

UDC 635.655:631.56:66.084.8-915.3
DOI: 10.31548/animal.4.2025.89

Improving the quality of soybeans by alkaline microwave treatment

Alla Makarynska
Doctor of Technical Sciences, Associate Professor
Odesa National University of Technology
65039, 112 Kanatna Str., Odesa, Ukraine
https://orcid.org/0000-0003-1879-8455

Olena Kananykhina
PhD in Technical Sciences, Associate Professor
Odesa National University of Technology
65039, 112 Kanatna Str., Odesa, Ukraine
https://orcid.org/0000-0001-6291-7760

Tetiana Turpurova*
PhD in Technical Sciences, Associate Professor
Odesa National University of Technology
65039, 112 Kanatna Str., Odesa, Ukraine
https://orcid.org/0000-0003-3030-7591

Ilya Bozhko
Master
Odesa National University of Technology
65039, 112 Kanatna Str., Odesa, Ukraine
https://orcid.org/0009-0001-6468-964X

Abstract. The article aimed to investigate the effect of combined microwave and alkaline treatment on the nutritional value of soybeans and the degree of inactivation of trypsin inhibitors. Experimental studies were conducted on soybeans of Ukrainian origin with an initial moisture content of 9%. The buffer capacity of soybeans was determined and it was found that to achieve pH 8, it is necessary to add 1% baking soda by weight of the raw material. Three soybean fractions

Suggested Citation:
Makarynska, A., Kananykhina, O., Turpurova, T., & Bozhko, I. (2025). Improving the quality of soybeans by alkaline microwave treatment. *Animal Science and Food Technology*, 16(4), 89-104. doi: 10.31548/animal.4.2025.89.

*Corresponding author

were studied: whole beans, coarse grinding (2-3 mm) and fine grinding (0.5 mm). It was found that the coarsely ground fraction demonstrated the optimal ratio between moisture retention capacity and alkaline solution permeability. Microwave treatment was carried out at a power of 600 W for 30-150 seconds with a step of 30 seconds. To assess the qualitative parameters, an infrared spectrometer was used to determine the activity of trypsin inhibitors and protein solubility in potassium hydroxide. The results showed that the combined microwave-alkali treatment provided more effective inactivation of trypsin inhibitors compared to pure microwave treatment. At a treatment time of 150 seconds, the activity of trypsin inhibitors decreased from 29.8 mg/g in the control sample to 7.1 mg/g in the microwave treatment and to 4.7 mg/g in the combined microwave-alkaline treatment, which is a decrease of 76.2% and 84.2%, respectively. At the same time, the protein solubility remained at a high level – 87.4% for microwave and 88.2% for microwave-alkaline treatment. It has been established that a preliminary increase in pH to 8 contributes to the partial destruction of the buffer properties of protein components and increases the efficiency of subsequent heat treatment. The optimal processing parameters were determined as raw material moistening up to 15%, use of coarsely ground fraction, preliminary alkaline treatment to pH 8, and microwave heating for 120-150 seconds at a power of 600 W. The proposed method allows to significantly increase the nutritional value of soy raw materials while maintaining high protein solubility, which makes it promising for implementation in the food industry and feed production

Keywords: trypsin inhibitors; antinutritional factors; protein denaturation and solubility; alkaline and heat treatment

Introduction

Soybean represents one of the most valuable sources of plant protein in global agriculture and the food industry. However, its widespread application is constrained by the presence of antinutritional factors, amongst which trypsin inhibitors and haemagglutinins occupy a leading position, reducing protein digestibility and the biological value of soy products. Soyabeans account for approximately 25% of global oilseed production and serve as a key component in the food industry and livestock farming (Turpurova & Kurbatov, 2024). The high protein content (35-40%) and balanced amino acid composition render soyabean an indispensable ingredient in the manufacture of food products, compound feeds, and protein concentrates (Kostyna & Bronnikova, 2024). Nevertheless, according to G. Padalkar *et al*. (2023), the full utilisation of soyabean protein is limited by the presence of antinutritional factors, the most significant of which are trypsin inhibitors and haemagglutinins. Trypsin inhibitors account for 5-7% of the total protein content of raw soybeans and are of two main types: Kunitz inhibitor and Bowman-Birk inhibitor. These compounds block the activity of proteolytic enzymes in the digestive tract, reducing protein digestibility and amino acid bioavailability. Long-term consumption of raw soybeans causes pancreatic hypertrophy, reduced growth in animals, and impaired nutrient absorption (Liu, 2024). Therefore, inactivation of anti-nutritional factors is a critically important step in preparing soybean raw materials for use.

As stated by B. Iegorov *et al*. (2023), traditional heat treatment methods, in particular autoclaving and extrusion, although they provide sufficient inactivation of trypsin inhibitors, have significant disadvantages, including high energy consumption, process duration, and the risk of denaturation of a significant number of protein molecules with loss of their functional properties. In this regard, it is urgent to search for alternative processing methods that would combine high efficiency of inactivation of antinutrients with preservation of the nutritional value of proteins. Among modern approaches, the use of microwave heating is of particular interest. This method provides rapid and volumetric heating of the raw material due to dielectric heating, which occurs as a result of the interaction of an electromagnetic field with polar water molecules. Unlike convection heating, where heat is transferred from the surface to the centre, microwave heating provides a more uniform temperature distribution throughout the product, which contributes to more effective inactivation of anti-nutritional factors with lower energy consumption (Xiang *et al.*, 2020).

An additional promising direction is the combination of thermal and chemical treatment by regulating the acid-base balance of the medium. According to Z. Luo *et al.* (2025), alkaline treatment promotes protein denaturation and the breaking of disulphide bonds in the structure of trypsin inhibitors, which increases their sensitivity to thermal inactivation. At the same time, an alkaline environment can improve the solubility of proteins and their functional properties, which is an important factor for further technological use. Despite the existence of separate studies on the effectiveness of microwave processing and alkaline modification of soy protein, a comprehensive approach that would combine both methods, taking into account the optimisation of processing parameters (fractional composition of raw materials, moisture, pH level, duration and power of microwave heating), remains insufficiently studied (Sui *et al.*, 2021; Das *et al.*, 2024). Therefore, the purpose of this study was to determine the optimal parameters of combined microwave-alkali treatment of soybeans for maximum inactivation of trypsin inhibitors while maintaining high protein solubility and nutritional value of the product. To achieve the goal, it was necessary to solve the following tasks: (1) to investigate the effect of moisture and fractional composition of soybeans on the degree of inactivation of trypsin inhibitors during microwave treatment; (2) to establish the buffer capacity of soybeans and calculate the required amount of baking soda to achieve the optimal pH level for preliminary alkaline treatment; (3) to assess the effect of microwave treatment duration on the activity of trypsin inhibitors and protein solubility in samples moistened with distilled water and treated with an alkaline solution.

Literature Review

The problem of inactivation of anti-nutritional factors in legumes has been studied for several decades, and a significant amount of scientific data has been accumulated on the effectiveness of various processing methods. Traditional approaches are based on thermal denaturation of protein inhibitors; however, the intensity and duration of treatment significantly affect not only the inactivation of antinutrients, but also the preservation of the nutritional properties of the final product. The structure of soybean trypsin inhibitors is characterised by the presence of disulphide bonds, which ensure the stability of their three-dimensional conformation. The Kunitz inhibitor contains two disulphide bridges, while the Bowman-Birk inhibitor contains seven such bonds, which makes the latter more heat-resistant (Park *et al.*, 2023). Inactivation of these compounds under the influence of high temperature occurs through the destruction of disulphide bonds and denaturation of the protein structure, which leads to the loss of their inhibitory activity.

Studies by J. Gu *et al.* (2022) showed that complete inactivation of trypsin inhibitors in raw soybeans requires prolonged boiling: 14 minutes provides 80% inactivation, and 30 minutes provides about 90% inactivation. However, such prolonged thermal exposures can negatively affect protein quality, reducing its solubility and bioavailability of amino acids. Therefore, it is urgent to find methods that would ensure effective inactivation at lower temperature-time parameters. Microwave heating demonstrates significant advantages over convection methods due to the dielectric heating mechanism. Studies by I. Munro *et al*. (2003) confirmed that microwave treatment effectively reduces trypsin inhibitor activity and improves protein digestibility: microwaving soy milk at 100°C for 8 minutes increased protein digestibility by 7% compared to traditional heating, which requires 30 minutes to achieve the same effect. A critical factor in the effectiveness of microwave processing is the moisture content of the raw material. Studies of lipoxygenase inactivation in soybeans have shown that at a moisture content of 26.9-56.8%, complete inactivation of the enzyme is achieved after 210 seconds of microwave heating (Cao *et al*., 2023). Moisture acts as a dielectric medium, ensuring uniform distribution of microwave field energy throughout the product volume and contributing to more efficient heating of the internal layers. At the same time, excessive moisture reduction during microwave processing can lead to uneven heating and local overheating. Studies by Z. Luo *et al*. (2025) have shown that microwave heating is more effective in changing the oil distribution in soybean seeds compared to autoclaving, but the degree of protein denaturation remains lower. This highlights the need to optimise raw material moisture to achieve a balance between the efficiency of inactivation of antinutritional factors and the preservation of protein quality.

The particle size of the soybean raw material significantly affects the speed and uniformity of heating. Whole beans are characterised by a slow heating rate due to low thermal conductivity and the presence of a water-impermeable shell. Grinding increases the surface area and improves heat access to the endosperm, but excessively fine fractions can quickly lose moisture during microwave heating, reducing processing efficiency. Studies show that pre-soaking soybeans before microwave processing provides a higher level of trypsin inhibitor inactivation compared to dry raw materials (Munro *et al*., 2003). This is explained by the increase in humidity, which promotes uniform heating, and partial swelling of protein structures, which makes them more accessible for thermal denaturation. However, the duration of the soaking process and subsequent drying may be technologically impractical in industrial conditions.

Adjusting the pH of the medium is a powerful tool for modifying the functional properties of soy protein. Alkaline treatment leads to the destruction of the spatial configuration of protein molecules due to the electrostatic repulsion of negatively charged groups and the destruction of hydrogen bonds. Studies of pH-shifting technology have shown that processing soy protein isolate at pH 12 followed by neutralisation to pH 7 significantly improves solubility, surface hydrophobicity, and emulsifying properties (Tang & Ma, 2009). It is important to note that alkaline treatment (pH 12 → 7) has a more pronounced effect on the structure and functional properties of the protein compared to acid treatment (pH 2 → 7), since the change in protein structure in an alkaline environment is more intense, according to L. Zheng *et al*. (2022). This is confirmed by an increase in the content of free thiol groups by 13-19% and an increase in surface hydrophobicity by 59-61% after heating at pH 12 (Chen *et al*., 2013).

Combined alkaline and heat treatment demonstrates a synergistic effect. Raising the pH to 9 followed by heating provides a greater increase in protein solubility compared to the separate application of alkaline or heat treatment (Wu *et al*., 2009). This occurs due to the partial destruction of the buffering properties of protein components and the facilitation of thermal denaturation of trypsin inhibitors. The Institute of Feed and Agriculture of Podillia of the National Academy of Agricultural Sciences of Ukraine has developed a method for eliminating anti-nutritional factors in soybeans by treating crushed grain in a 2.5% calcium hydroxide solution (Vysochanska & Petrychenko, 2010). The technology involves soaking soybean flour in water (ratio 1:4) with the addition of 25 g/kg of alkali to achieve pH 7. The use of this method allows to reduce the content of antinutrients and improve the digestibility of proteins, but requires a long processing time and subsequent washing of the raw materials.

A generalisation of the literature data shows that the most promising is an integrated approach that combines the advantages of various processing methods. The combination of microwave heating with pre-moistening and pH adjustment can provide a synergistic effect in inactivating anti-nutritional factors at lower energy costs and shorter processing times. However, most studies focus on the separate study of the effect of microwave treatment or alkaline modification, while the comprehensive optimisation of processing parameters (fractional composition, moisture, pH level, microwave heating modes) remains poorly studied. In addition, it is important to determine the optimal balance between the degree of inactivation of trypsin inhibitors and the preservation of the functional properties of the protein, in particular its solubility, which is a critical indicator for the technological use of soy raw materials. Thus, the relevance of this study lies in the development of a scientifically sound approach to combined microwave-alkaline processing of soybeans with the determination of optimal parameters that ensure maximum inactivation of trypsin inhibitors while maintaining high protein solubility and nutritional value of the product.

Materials and Methods

Characteristics of raw materials and reagents. For experimental research, soybeans of the 'Sigaliya' variety from the 2024 harvest in Chernihiv region (Ukraine) were used. The research was conducted in the spring of 2025. The raw materials were stored in paper bags at a temperature of 18-20°C and a relative humidity of 30-40%. The initial moisture content of the beans was 9%, which corresponds to standard storage conditions for oilseeds (DSTU 4964:2008, 2010). The crude protein content in the control samples was 33.7%, and crude fat was 21.5%. Baking soda (sodium bicarbonate, $NaHCO_3$) with a mass fraction of the basic substance of at least 99.9% was used as an alkaline reagent. The choice of soda as an alkaline agent was due to its wide application in the food industry, safety of use, and the ability to effectively regulate the pH level without introducing toxic components or heavy metals. Distilled water with a specific electrical conductivity of no more than 5 μS/cm was used to prepare the solutions.

Determination of the buffering capacity of soybeans. The buffer capacity of soybeans was determined titrimetrically using a standardised 0.1 M baking soda solution. A 5.00 g portion of crushed soybeans (0.5 mm fraction) was placed in a 100 ml beaker and 50 ml of distilled water was added. The suspension was left for 20 minutes at room temperature (22 ± 2°C) for swelling of protein structures and hydration of colloidal components, stirring periodically with a glass rod. After swelling, 0.1 M baking soda solution was gradually added to the suspension in small portions of 0.1-0.2 ml using an automatic pipette, mixing thoroughly after each addition for 30 seconds. One minute after adding the next portion of alkali, the pH of the suspension was measured using universal indicator litmus strips with a pH determination range of 5-9 (determination accuracy ±0.2 pH units) manufactured by Lachema (Czech Republic). The initial pH of the aqueous suspension of soybeans was 6.3 ± 0.1. The volume of soda solution required to increase the pH by one unit was recorded to construct a titration curve and calculate the buffer capacity. The experiment was repeated five times to ensure statistical reliability of the results. The buffer capacity (β) was calculated using the formula (1):

$$\beta = \frac{\Delta C}{\Delta pH}, \quad (1)$$

where ΔC – change in the concentration of added alkali (mol/l); ΔpH – change in the pH of the suspension.

Preparation of samples of different fractions. To determine the optimal particle size of soybean raw materials, three types of samples were prepared: (1) whole beans – soybeans without grinding, previously cleaned of damaged and defective seeds by manual sorting; (2) coarse grinding (2-3 mm) – beans were ground in a laboratory mill OlisLab 2100 (a modernised version of the LZM-1 mill, LLC "Olis", Ukraine), the resulting fraction was sieved through a set of sieves with a mesh size of 2.0 and 3.0 mm to standardise particle size; (3) fine grinding (0.5 mm) – beans were ground using a laboratory mill Retsch ZM-300 (Makrolab LTD, Ukraine) at a rotation speed of 14,000 rpm, using a 0.5 mm ring sieve.

Humidification of raw materials. A control group of samples weighing 100 g each was moistened with distilled water until a humidity of 15% was reached. The volume of water required for moistening was calculated using the formula (2):

$$V = \frac{m \times (W_2 - W_1)}{100 - W_2}, \quad (2)$$

where m – sample mass (g); W_1 – initial humidity (%); W_2 – final moisture content (%). For a 100 g sample with an initial moisture content of 9%, the required volume of distilled water was 7.06 ml. Water was added to the samples evenly using a micro-spray sprayer with constant stirring to ensure uniform moisture distribution. After humidification, the samples were placed in sealed polyethylene bags and kept for 60 minutes at room temperature to evenly redistribute moisture throughout the volume of the raw material. The actual moisture content of the samples after exposure was controlled by the gravimetric method by drying at a temperature of 105 ± 2°C to constant weight.

Alkaline treatment. The experimental group of samples was treated with a solution of baking soda to achieve a pH of 8. Based on the data from the determination of the buffer capacity, it was established that to achieve this pH level, it is necessary to add 1.0% of soda by weight of the raw material (1.0 g per 100 g of soybeans). The soda was dissolved in the calculated volume of distilled water (7.06 ml). The resulting alkaline solution was used to moisten soybean samples similarly to the control group using a spray gun. After moistening, the samples were kept in sealed bags for 60 minutes to evenly distribute the alkali and establish an equilibrium pH value. pH control was performed on aqueous extracts (sample: water ratio = 1:10) 60 minutes after treatment. The actual pH value of the extracts was 7.9 ± 0.1, confirming that the target alkalinity level was achieved.

Microwave processing. Microwave treatment was performed in a household microwave oven Grunhelm 20MX711-B (Ukraine) with a nominal power of 800 W. To ensure the same processing conditions for all samples, the 600 W mode (75% power) was used, which provides more controlled heating conditions and reduces the risk of local overheating. Samples weighing 100 g were placed in plastic containers and distributed in a uniform layer 8-10 mm thick to ensure uniform heating. The containers were placed in the centre of the rotating plate of a microwave oven. The duration of treatment varied from 30 to 150 seconds in 30-second increments, which allowed for assessing the kinetics of trypsin inhibitor inactivation and changes in protein solubility depending on the exposure time. After microwave treatment, the samples were cooled to room temperature in air for 30 minutes, after which the mass loss due to moisture evaporation was determined. The treated samples were ground to a fraction of 0.5 mm and stored in sealed plastic containers at a temperature of

$4 \pm 2°C$ until analytical studies were performed, but not more than 24 hours.

Methods of analysis. To assess the quality indicators of soybean samples, a Bruker TANGO infrared spectrometer (Germany) with Evonik calibration was used, which provides rapid and non-destructive determination of quality indicators. The device operates in the near-infrared spectrum (850-1,050 nm) and uses chemometric models to calculate component concentrations based on spectral characteristics. Determination of trypsin inhibitor activity and protein solubility in potassium hydroxide (KOH) was performed by the spectrometric method using a calibration model developed for soy products. The results were expressed in milligrams of trypsin inhibitors per gram of dry matter (mg/g) and as a percentage of total protein, respectively. The relative error of determination was no more than 1%. Five parallel samples were prepared for each treatment option, which ensured high statistical reliability of the results. Statistical processing of the obtained results was carried out according to V. Matviychuk *et al*. (2021) using variance and correlation analysis ($p = 0.05$).

Results and Discussion

The dependence of the pH change on the amount of added alkali was established by titrating an aqueous suspension of crushed soybeans with a solution of baking soda (Fig. 1). The initial pH value of the suspension was 6.3 ± 0.1, which is consistent with the data of X. Xiong *et al*. (2015) on the weakly acidic reaction of aqueous soybean extracts. Adding 0.2% soda by weight of the raw material increased the pH to 6.8, at 0.5% – to 7.4, and at 1.0% – to 8.0. Further increase in the soda concentration to 1.5% and 2.0% did not cause significant changes in the pH (8.1 and 8.2, respectively), which indicates that a buffer plateau has been reached.

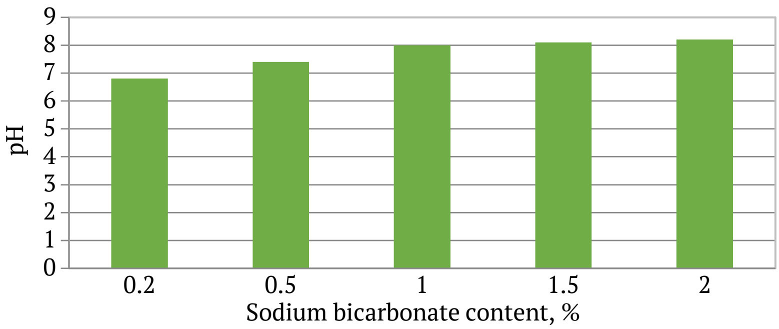

Figure 1. Change in pH depending on the amount of sodium bicarbonate
Source: compiled by the authors

The established buffer curve demonstrated the typical behaviour of protein systems with high buffer capacity in the pH range of 6-8. This was due to the presence of monoaminodicarboxylic amino acids in the composition of soy proteins, in particular glutamic and aspartic acids. The buffer capacity of soybeans in the pH range of 6.3-8.0 was 0.18 ± 0.02 mol/($l \times$ pH unit), which is a relatively high indicator and confirms the ability of soy protein to resist pH changes. For practical use, the optimal concentration of soda was chosen to be 1.0% of the mass of the raw material, which ensured the achievement of pH 8.0 – a level sufficient for partial destruction of the buffer properties of the protein without excessive alkalinity, which can lead to undesirable chemical modifications of amino acids. A similar approach was used in pH-shifting technology, where pH 8-9 was considered optimal for improving the functional properties of soy protein (Wu *et al.*, 2009). Comparative analysis of samples with an initial moisture content of 9% and moistened to a level of 15% revealed the critical role of moisture content in the efficiency of microwave heating. At a microwave power of 600 W, the temperature in the centre of the moistened samples after 30 seconds of treatment was 62 ± 3°C, after 60 seconds – 83 ± 4°C, seconds – 98 ± 5°C, seconds – 112 ± 6°C, and at 150 seconds it reached 125 ± 7°C (Fig. 2). The coefficient of temperature variation at different points of the sample did not exceed 8%, which indicates a fairly uniform heating.

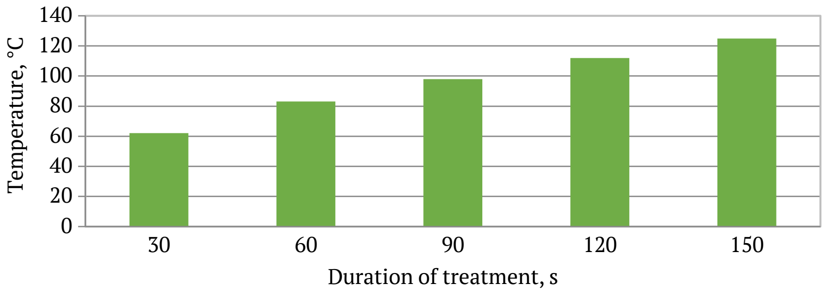

Figure 2. Temperature change in the centre of humidified samples depending on the duration of treatment

Source: compiled by the authors

In contrast to the moistened samples, dry beans (9% moisture) exhibited significant heating non-uniformity with temperature variations ranging from 70°C to 150°C within a single sample. The coefficient of variation of temperature was 25-35%, indicating that heating was localised mainly in the outer layers of the sample. Visual inspection revealed partial burning of the surface layer in the form of dark brown spots, while the internal parts remained insufficiently heated. This is consistent with the data by H. Cao *et al*. (2023) on the critical role of moisture as a dielectric mediator in microwave heating. Water molecules, having a high dipole moment, effectively absorb the energy of the electromagnetic field and convert it into heat. At low humidity, heating occurs less intensively, and local areas with higher moisture content overheat, which leads to uneven processing. Increasing humidity to 15% provides a more uniform distribution of dielectric heating throughout the product volume.

The study of the effect of particle size on the efficiency of combined processing revealed significant differences between the three fractions studied. Whole soybeans were characterised by uniform heating and good moisture retention during microwave processing. The weight loss after 150 seconds of heating was only 3.2 ± 0.4%, indicating effective water retention in the bean structure. However, the activity of trypsin inhibitors after alkaline treatment followed by microwave heating for 150 seconds decreased only to 18.3 ± 1.2 mg/g compared to 29.8 mg/g in the control (a decrease of 38.6%). The low efficiency of alkaline treatment of whole beans is explained by the presence of a waterproof shell, which limits the penetration of the alkaline solution to the endosperm, where the bulk of proteins and trypsin inhibitors are concentrated. The structure of the soybean shell includes a cuticle with a waxy layer and an epidermis with palisade cells, which form a barrier to the diffusion of aqueous solutions (Ma *et al.*, 2004). For effective alkaline treatment of whole beans, a long pre-soaking (8-12 hours) is required, which is technologically impractical.

The fine fraction (0.5 mm) exhibited the opposite problem – excessively rapid moisture loss during microwave heating. The mass loss after 150 seconds was 11.8 ± 1.5%, with intensive evaporation occurring after 60 seconds of treatment. Visual inspection showed the formation of a dry crust on the surface of the sample, which was accompanied by a sharp increase in temperature to 140-160°C in some areas. The activity of trypsin inhibitors during this treatment was almost unchanged. The reason for the low efficiency is the early evaporation of moisture, which occurs before the temperature sufficient to denature the protein inhibitors is reached. Studies by J. Gu *et al*. (2022) show that effective inactivation of trypsin inhibitors requires a temperature of 90-100°C and a sufficient duration of exposure. During rapid drying of the fine fraction, the temperature rises to 140-160°C, but due to the lack of moisture, heat transfer to the internal particles deteriorates, which reduces the efficiency of denaturation.

The coarse grinding fraction (2-3 mm) was found to be optimal, providing a balance between moisture retention capacity and accessibility to alkaline solution. Mass loss after 150 seconds of microwave treatment was 5.8 ± 0.6%, indicating moderate moisture evaporation with sufficient moisture retention for effective heat transfer. It was this fraction that provided the best results in inactivation of trypsin inhibitors during combined treatment. Grinding to a size of 2-3 mm destroys the bean shell and increases the surface area of the endosperm, which facilitates the penetration of the alkaline solution and provides better contact with the protein components. At the same time, particles of this size retain sufficient structural integrity to retain moisture during microwave heating. This is consistent with the data by H. Vagadia *et al*. (2017) on the positive effect of pre-grinding on the efficiency of thermal processing of legumes. The results of determining the activity of trypsin inhibitors in samples after different treatment options are presented in Figure 3. The control sample without treatment was characterised by a trypsin inhibitor activity of 29.8 ± 1.2 mg/g, which is a typical value for raw soybeans and is consistent with the data of M. Friedman & D. Brandon (2001).

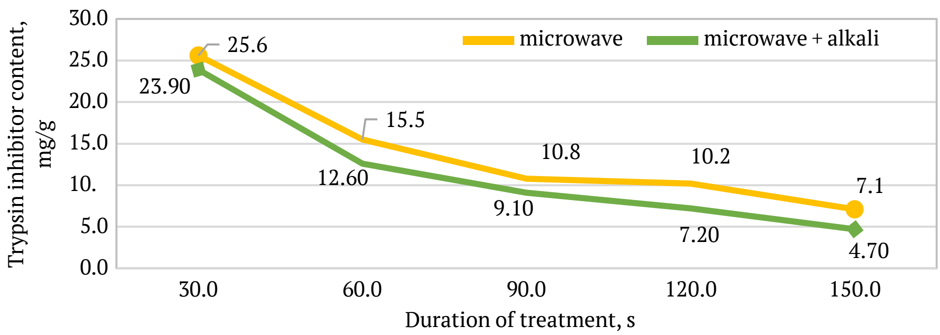

Figure 3. Effect of duration of treatment duration and type on the activity of trypsin inhibitors
Source: compiled by the authors

In microwave-only processes, a gradual decrease in the activity of trypsin inhibitors was observed with increasing heating duration (Fig. 3). The transition between a short interval of 30 seconds provided a decrease in activity to 25.6 mg/g (by 14.1% compared to the control), i.e. to the level of reaching a temperature of 62°C – the level of the beginning of denaturation of the most heat-sensitive protein fractions. The most intense decrease in activity occurs between 30-60 seconds (from 25.6 to 15.5 mg/g), when the sample temperature is 83°C and denaturation of the Kunitz inhibitor occurs, which is less heat-stable compared to the Bowman-Birk inhibitor, according to A. Park *et al*. (2023). Subsequently, with an increase in the treatment duration to 90-120 seconds, the rate of decrease in the activity of trypsin inhibitors decreased (change from 15.5 to 10.2 mg/g), which indicates a higher thermal stability of the Bowman-Birk inhibitor with seven disulphide bonds and requires a longer thermal exposure for complete denaturation (Kumar *et al*., 2006). During treatment for 150 seconds (125°C), the activity decreased to only 7.1 mg/g, corresponding to a 76.2% reduction compared to the control.

Optimal microwave-alkaline treatment demonstrated significantly higher efficiency of inactivation of trypsin inhibitors at all studied time ranges. Already after treatment for 30 seconds, the activity decreased to 23.9 mg/g, which was 6.6% less compared to pure microwave treatment for the same duration. The most pronounced effect was observed in the case of treatment for 120-150 seconds, when the combined treatment provided a reduction in activity to 7.2 and 4.7 mg/g, respectively, which was 29.4% and 33.8% more effective compared to microwave treatment alone. At 150 seconds duration, the combined treatment reduced the activity of trypsin inhibitors by 84.2% to a level of 4.7 mg/g, which corresponds to the limit level considered safe for use in feed purposes (<5 mg/g), according to P. Mittal *et al*. (2021). This confirms the synergistic effect of the combination of alkaline and heat treatment. Increasing the pH to level 8 leads to partial ionisation of carboxyl and amino groups of protein molecules, which leads to electrostatic repulsion and spatial change of the tertiary structure of the protein, as mentioned by C. Tang & C. Ma (2009) and M. Zhong *et al*. (2024). This makes disulphide bonds more accessible for hydrolysis under the influence of high temperature and accelerates the rate of denaturation of trypsin inhibitors. Correlation analysis showed a high negative correlation between the duration of treatment and the activity of trypsin inhibitors both during microwave treatment ($r = -0.986$, $p < 0.001$) and during combined treatment ($r = -0.992$, $p < 0.001$), which confirms the pattern of inactivation of antinutritional factors with increasing thermal exposure time.

The experiments conducted confirm the literature data on the effectiveness of microwave treatment for inactivating trypsin inhibitors. Experimental results obtained by I. Munro *et al*. (2003) confirmed that microwave heating of soy milk at 100°C for 8 minutes reduced the activity of trypsin inhibitors by 70-75%, which is comparable to the observations of the present research with a processing time of 150 seconds. At the same time, the use of combined alkaline-thermal treatment provides the same or greater effect in a shorter duration, which is of practical importance in optimising the energy efficiency of the technological process. It is worth noting that even with the longest processing time (150 seconds), protein solubility remained at a fairly high level – 87.4% for microwave and 88.2% for microwave + alkali. This indicates a relatively mild nature of the thermal effect, which ensures effective inactivation of trypsin inhibitors without excessive denaturation of protein structures, which is important for preserving the technological properties of soy raw materials.

Analysis of the kinetics of inactivation of trypsin inhibitors indicated the most intense decrease in their activity in the range of 30-90 seconds of treatment when the temperature of the samples increased from 60°C to 100°C. This corresponds to the denaturation temperature of the main soy proteins: β-conglycinin (75-80°C) and glycinin (85-95°C). In this temperature range, protein globules unfold and aggregation processes develop, which is accompanied by the destruction of active sites of trypsin inhibitors. With a treatment duration of more than 120 seconds, the rate of inactivation slows down, which may be due to several factors. First, by this point, inactivation of the most thermolabile fraction of inhibitors has already occurred, and the predominantly thermostable Bowman-Birk inhibitor remains. Secondly, at temperatures above 110°C, intensive evaporation of moisture begins, which reduces the efficiency of heat transfer and can lead to local overheating instead of uniform heating. From a practical point of view, the most optimal time for combined microwave-alkaline treatment can be considered to be the interval of 120-150 seconds, when the activity of trypsin inhibitors is 4.7-7.2 mg/g while maintaining protein solubility at 87.8-88.2%. This indicator is close to the results of soybean meal processed by traditional methods (autoclaving, extrusion), where the residual activity of trypsin inhibitors is usually 3-5 mg/g.

Modern soybean processing technologies include autoclaving (121°C, 15-20 minutes), extrusion (130-150°C, 30-60 seconds), and toaster roasting (110-120°C, 20-30 minutes). Autoclaving provides complete inactivation of trypsin inhibitors, but requires a long processing time and significant energy consumption. Extrusion is more energy efficient, but requires special equipment and can lead to excessive protein denaturation with a loss of solubility of up to 70-75%. The proposed method of combined microwave-alkali treatment has several advantages. First, the treatment time is only 120-150 seconds, which is significantly shorter than autoclaving (15-20 minutes) or frying (20-30 minutes). Secondly, the energy consumption is quite low: at a power of 600 W and a duration of 150 seconds, the energy consumption is about 0.025 kWh per 100 g of raw material, which is a competitive indicator. Thirdly, the preservation of high protein solubility (88.2%) makes the processed raw material suitable for a wide range of technological applications. An additional advantage is the possibility of continuous processing in industrial conditions. Conveyor-type microwave plants allow for a continuous process with precise control of processing parameters, which is more difficult for batch autoclave plants. Alkaline pre-wetting can be integrated into the process line as a preparation stage before microwave heating, which does not require additional equipment and does not significantly complicate the process.

Based on the research performed, the following technological recommendations can be formulated for combined microwave-alkali processing of soybean raw materials. Raw material preparation: use soybeans with an initial moisture content of 8-10%, cleaned of impurities and damaged seeds. Grind to a coarse fraction with a particle size of 2-3 mm, which provides an optimal balance between accessibility to alkaline solution and the ability to retain moisture during microwave heating. Alkaline treatment: moisten the crushed raw materials with a solution of baking soda (1% of the mass of the raw materials) until a moisture content of 15% and a pH of 8.0 ± 0.2 are reached. Allow to stand for 60 minutes to evenly distribute the alkali throughout the volume of the raw materials. Monitor the pH of the aqueous extract (ratio 1:10) with indicator strips or a pH meter. Microwave treatment: microwave heating at 600 W for 120-150 seconds. Place the raw materials in an even layer up to 10 mm thick to ensure uniform heating. Monitor the temperature of the samples, which should be 110-125°C at the end of the treatment. Quality control: determine the residual activity of trypsin inhibitors (target value <7 mg/g) and the solubility of protein in KOH (not lower than 85%). If necessary, adjust the processing time depending on the characteristics of the individual batch of raw materials.

Conclusions

The use of combined microwave-alkali treatment of soybeans is an effective treatment method for the effective inactivation of trypsin inhibitors while maintaining high protein solubility. It has been established that the coarsely ground fraction (2-3 mm) provides the best balance between moisture retention capacity and accessibility to the alkaline solution, while whole beans are characterised by insufficient penetration of alkali through the waterproof shell, and the fine fraction (0.5 mm) has excessively rapid moisture loss during microwave heating. It has been established that achieving pH 8 is ensured by adding 1.0% baking soda to the mass of raw materials, which contributes to the partial violation of the buffer properties of protein components and increases the efficiency of subsequent heat treatment. The critical role of raw material moisture for the efficiency of microwave processing has been confirmed. Humidification of soybean raw materials to a level of 15% ensured uniform heating with a temperature variation coefficient of no more than 8%, while processing dry beans with a humidity of 9% was characterised by high uneven heating (variation coefficient of 25-35%) with local overheating of the surface layer and insufficient heating of the internal parts.

It was found that combined microwave-alkaline treatment provides more effective inactivation of trypsin inhibitors compared to pure microwave treatment at all studied time intervals. At a processing time of 150 seconds and a power of 600 W, the activity of trypsin inhibitors decreased from 29.8 mg/g in the control sample to 7.1 mg/g with microwave processing (a 76.2% reduction) and to 4.7 mg/g with combined microwave-alkaline processing (a 84.2% reduction), which is 33.8% more effective compared to pure microwave processing. It has been proven that combined processing provides effective inactivation of anti-nutritional factors while maintaining high protein solubility. Under optimal processing conditions (120-150 seconds), protein solubility remained at 87.8-88.2%, which is sufficient to preserve the technological properties of soy raw materials and its suitability for use in food products and feed. The synergistic effect of combining alkaline and heat treatment is explained by the partial opening of protein structures in an alkaline environment, which makes the disulphide bonds of trypsin inhibitors more accessible for thermal cleavage, while the violation of the buffer properties of protein components reduces the intensity of denaturation under the influence of temperature. A favourable combined processing regime has been formed: moistening the material to 15%, using a coarsely ground fraction (2-3 mm), preliminary alkaline treatment to pH 8.0 ± 0.2 by adding 1% baking soda, microwave heating at a power of 600 W for 120-150 seconds until a temperature of 110-125°C is reached.

The study has certain limitations that must be taken into account when interpreting the results. The experiments were conducted on laboratory equipment with a limited number of samples (100 g). Scaling up to industrial volumes may require correction of processing parameters due to the peculiarities of heat transfer in large volumes of raw materials. Industrial microwave units have a different chamber shape and electromagnetic field distribution system, which may affect the uniformity of heating. Another limitation is that only one pH level (8.0) was investigated during operation. A systematic study of the pH behaviour over a wider range (7.5-9.5) could allow for the establishment of an optimum and the assessment of the sensitivity of the process to changes in this parameter. It is also promising to investigate alternative alkaline agents, such as calcium or potassium hydroxide, which may provide additional technological or nutritional benefits.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

References

[1] Cao, H., Wang, X., Liu, J., Sun, Z., Yu, Z., Battino, M., El-Seedi, H., & Guan, X. (2023). Mechanistic insights into the changes of enzyme activity in food processing under microwave irradiation. *Comprehensive Reviews in Food Science and Food Safety*, 22, 2465-2487. doi: 10.1111/1541-4337.13154.

[2] Chen, N., Zhao, M., Sun, W., Ren, J., & Cui, C. (2013). Effect of oxidation on the emulsifying properties of soy protein isolate. *Food Research International*, 52(1), 26-32. doi: 10.1016/j.foodres.2013.02.028.

[3] Das, D., Panesar, P.S., & Saini, C.S. (2024). Effect of pH shifting on different properties of microwave-extracted soybean meal protein isolate. *Food and Bioprocess Technology*, 17, 640-655. doi: 10.1007/s11947-023-03160-8.

[4] DSTU 4964:2008. (2010). *Soybean. Technical conditions*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=74237.

[5] Friedman, M., & Brandon, D. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry*, 49(3), 1069-1086. doi: 10.1021/jf0009246.

[6] Gu, J., Bk, A., Wu, H., Lu, P., Nawaz, M.A., Barrow, C.J., Dunshea, F.R., & Suleria, H.A.R. (2022). Impact of processing and storage on protein digestibility and bioavailability of legumes. *Food Reviews International*, 39(7), 4697-4724. doi: 10.1080/87559129.2022.2039690.

[7] Iegorov, B., Makarynska, A., Kananykhina, O., & Turpurova, T. (2023). Effect of extrusion on probiotic feed additive. *Grain Products and Mixed Fodder's*, 23(1), 14-19. doi: 10.15673/gpmf.v23i1.2584.

[8] Kostyna, T., & Bronnikova, L. (2024). Formation of productivity indicators of soybean varieties in Vinnitsia region. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 20(2). doi: 10.31548/dopovidi.2(108).2024.006.

[9] Kumar, V., Rani, A., Pandey, V., & Chauhan, G. (2006). Changes in lipoxygenase isozymes and trypsin inhibitor activity in soybean during germination at different temperatures. *Food Chemistry*, 99(3), 563-568. doi: 10.1016/j.foodchem.2005.08.024.

[10] Liu, K. (2024). Enzymatic and algebraic methodology to determine contents of Kunitz and Bowman-Birk inhibitors and their contributions to total trypsin or chymotrypsin inhibition in soybeans. *Journal of Agricultural and Food Chemistry*, 72(20), 11782-11793. doi: 10.1021/acs.jafc.3c06389.

[11] Luo, Z., Zhu, Y., Xiang, H., Wang, Z., Jiang, Z., Zhao, X., Sun, X., & Guo, Z. (2025). Advancements in inactivation of soybean trypsin inhibitors. *Foods*, 14(6), article number 975. doi: 10.3390/foods14060975.

[12] Ma, F. Cholewa, E., Mohamed, T., Peterson, C.A., & Gijzen, M. (2004). Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. *Annals of Botany*, 94(2), 213-228. doi: 10.1093/aob/mch133.

[13] Matviychuk, V.A., Veselovska, N.R., & Shargorodsky, S.A. (2021). *Mathematical modelling of modern technological systems*. Vinnytsia: Vinnitsia National Agrarian University.

[14] Mittal, P., Kumar, V., Rani, A., & Gokhale, S.M. (2021). Bowman-Birk inhibitor in soybean: Genetic variability in relation to total trypsin inhibitor activity and elimination of Kunitz trypsin inhibitor. *Notulae Scientia Biologicae*, 13(1), article number 10836. doi: 10.15835/nsb13110836.

[15] Munro, I.C., Harwood, M., Hlywka, J.J., Stephen, A.M., Doull, J., Flamm, W.G., & Adlercreutz, H. (2003). Soy isoflavones: A safety review. *Nutrition Reviews*, 61(1), 1-33. doi: 10.1301/nr.2003.janr.1-33.

[16] Padalkar, G., *et al*. (2023). Necessity and challenges for exploration of nutritional potential of staple-food grade soybean. *Journal of Food Composition and Analysis*, 117, article number 105093. doi: 10.1016/j.jfca.2022.105093.
[17] Park, A., Kang, S.-H., Kang, B.-H., Chowdhury, S., Shin, S.-Y., Lee, W.-H., Lee, J.-D., Lee, S., Choi, Y.-M., & Ha, B.-K. (2023). Identification of a novel KTi-1 allele associated with reduced trypsin inhibitor activity in soybean accessions. *Agriculture*, 13(11), article number 2070. doi: 10.3390/agriculture13112070.
[18] Sui, X., Zhang, T., & Jiang, L. (2021). Soy protein: Molecular structure revisited and recent advances in processing technologies. *Annual Review of Food Science and Technology*, 12, 119-147. doi: 10.1146/annurev-food-062220-104405.
[19] Tang, C.-H., & Ma, C.-Y. (2009). Effect of high pressure treatment on aggregation and structural properties of soy protein isolate. *LWT – Food Science and Technology*, 42(2), 606-611. doi: 10.1016/j.lwt.2008.07.012.
[20] Turpurova, T., & Kurbatov, S. (2024). Current state and development prospects of the soy market in Ukraine. *Grain Products and Mixed Fodder's*, 24(2), 10-15. doi: 10.15673/gpmf.v24i2.2905.
[21] Vagadia, B.H, Vanga, S.K., & Raghavan, V. (2017). Inactivation methods of soybean trypsin inhibitor – a review. *Trends in Food Science & Technology*, 64, 115-125. doi: 10.1016/j.tifs.2017.02.003.
[22] Vysochanska, M.V., & Petrychenko, V.V. (2010). *Method for producing protein feed additives from soybeans* (Patent of Ukraine No. 52341). Kyiv: Ukrpatent.
[23] Wu, W., Zhang, C., Kong, X., & Hua, Y. (2009). Oxidative modification of soy protein by peroxyl radicals. *Food Chemistry*, 116(1), 295-301. doi: 10.1016/j.foodchem.2009.02.049.
[24] Xiang, S., Zou, H., Liu, Y., & Ruan, R. (2020). Effect of microwave heating on protein structure, digestion properties and Maillard products of gluten. *Journal of Food Science and Technology*, 57, 2139-2149. doi: 10.1007/s13197-020-04249-0.
[25] Xiong, X., Zhao, L., Chen, Y., Ruan, Q., Zhang, C., & Hua, Y. (2015). Effects of alkali treatment and subsequent acidic extraction on the properties of soybean soluble polysaccharides. *Food and Bioproducts Processing*, 94, 239-247. doi: 10.1016/j.fbp.2014.03.001.
[26] Zheng, L., Regenstein, J.M., Zhou, L., & Wang, Z. (2022). Soy protein isolates: A review of their composition, aggregation, and gelation. *Comprehensive Reviews in Food Science and Food Safety*, 21(2), 1940-1957. doi: 10.1111/1541-4337.12925.
[27] Zhong, M., Sun, Y., Qayum, A., Liang, Q., Rehman, A., Gan, R., Ma, H., & Ren, X. (2024). Research progress in soybean lipophilic protein (LP): Extraction, structural, techno-functional properties, and high-performance food applications. *Trends in Food Science & Technology*, 147, article number 104440. doi: 10.1016/j.tifs.2024.104440.

Підвищення якості сої шляхом лужної СВЧ-обробки

Алла Макаринська
Доктор технічних наук, доцент
Одеський національний технологічний університет
65039, вул. Канатна, 112, м. Одеса, Україна
https://orcid.org/0000-0003-1879-8455

Олена Кананихіна
Кандидат технічних наук, доцент
Одеський національний технологічний університет
65039, вул. Канатна, 112, м. Одеса, Україна
https://orcid.org/0000-0001-6291-7760

Тетяна Турпурова
Кандидат технічних наук, доцент
Одеський національний технологічний університет
65039, вул. Канатна, 112, м. Одеса, Україна
https://orcid.org/0000-0003-3030-7591

Ілля Божко
Магістр
Одеський національний технологічний університет
65039, вул. Канатна, 112, м. Одеса, Україна
https://orcid.org/0009-0001-6468-964X

Анотація. Метою статті було дослідити вплив комбінованої мікрохвильової та лужної обробки на поживність сої та ступінь інактивації інгібіторів трипсину. Експериментальні дослідження проводилися на соєвих бобах українського походження з початковою вологістю 9 %. Визначено буферну ємність сої та встановлено, що для досягнення pH 8 необхідно додавання 1 % харчової соди від маси сировини. Досліджено три фракції сої: цілі боби, крупний помел (2-3 мм) та дрібний помел (0,5 мм). Встановлено, що фракція крупного помелу демонструє оптимальне співвідношення між вологоутримувальною здатністю та проникністю лужного розчину. Мікрохвильову обробку проводили при потужності 600 Вт протягом 30-150 секунд з кроком 30 секунд. Для оцінки якісних показників застосовували інфрачервоний спектрометр із визначенням активності інгібіторів трипсину та розчинності білка у гідроксиді калію. Результати показали, що комбінована СВЧ-лужна обробка забезпечує більш ефективну інактивацію інгібіторів трипсину порівняно з чистою СВЧ-обробкою. При тривалості обробки 150 секунд активність інгібіторів трипсину знижувалася з 29,8 мг/г у контрольному зразку до 7,1 мг/г при СВЧ-обробці та до 4,7 мг/г при комбінованій СВЧ-лужній обробці, що становить зниження на 76,2 % та 84,2 % відповідно. При цьому розчинність білка залишалася на високому рівні – 87,4 % для СВЧ та 88,2 % для СВЧ-лужної обробки. Встановлено, що попереднє підвищення pH до 8 сприяє частковому руйнуванню буферних властивостей білкових компонентів та підвищує ефективність подальшої термічної обробки. Оптимальними параметрами обробки визначено зволоження сировини до 15 %, використання фракції крупного помелу, попередню лужну обробку до pH 8 та СВЧ-нагрівання протягом 120-150 секунд при потужності 600 Вт. Запропонований метод дозволяє значно підвищити поживну цінність соєвої сировини зі збереженням високої розчинності білка, що робить його перспективним для впровадження у харчовій промисловості та кормовиробництві

Ключові слова: інгібітори трипсину; антипоживні фактори; денатурація та розчинність білка; лужна та термічна обробка

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Scientific Journal

Volume 16, No. 4. 2025

Founded in 2010. Published four times per year

The original layout of the publication is made in the Department of Scientific and Technical Information of National University of Life and Environmental Sciences of Ukraine

Managing Editor:
N. Shevchenko

Signed for print of November 27, 2025
Format 70*100/16
Conventional printed pages 10.2
Circulation 50 copies

Editors Office Address:
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
E-mail: as@animalscience.com.ua
https://animalscience.com.ua/en

ТВАРИННИЦТВО ТА ТЕХНОЛОГІЇ ХАРЧОВИХ ПРОДУКТІВ

Науковий журнал

Том 16, № 4. 2025

Заснований у 2010 р. Виходить чотири рази на рік

Оригінал-макет видання виготовлено у відділі науково-технічної інформації
Національного університету біоресурсів
і природокористування України

Відповідальний редактор:
Н. Шевченко

Підписано до друку 27 листопада 2025 р.
Формат 70*100/16
Умов. друк. арк. 10,2
Наклад 50 прим.

Адреса видавництва:
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
E-mail: as@animalscience.com.ua
https://animalscience.com.ua/uk