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Effect of breed factor on urea level and blood biochemical parameters in dairy cattle

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Abstract. Dairy cattle breeding has achieved great success due to the targeted improvement of dairy and combined breeds in different climatic and agroecological zones of the world. The level of urea and other biochemical parameters of cows' blood indicates the peculiarities of carbohydrate and protein balance, its impact on reproductive functions, health, feed efficiency, and methane emissions. The aim of the study was to determine the effect of the breed factor on urea levels and

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other biochemical parameters of blood in cows of different breeds. The research was conducted on cows of three contrasting breeds. The first breed is a combined (cross-border) Simmental, the last two are local – Ukrainian Red and White dairy (created by crossbreeding Simmental with Ayrshire, Montbéliard, Holstein) and Ukrainian Black and White dairy (created by crossing local Black and White with Holstein). The experiment was conducted under standard conditions when feeding cows "without restrictions" with the analysis of productive indicators and blood biochemical parameters. The most significant factor influencing the productive and biochemical parameters was the breed factor, which accounted for 14.6% and 51.2%, respectively, in terms of fat content in milk and milk yield in terms of total energy value. A significant influence of the breed was noted in blood biochemical parameters such as total bilirubin 24.7%, urea 33.2%, creatinine 49.8%, alanine aminotransferase 10.4%, aspartate aminotransferase 46.3%, albumin 35.1% and total protein 13.2%. A significant correlation between the concentrations of urea and creatinine, creatinine and aspartate aminotransferase, and albumin in the blood plasma of the studied animal groups was established. A preliminary conclusion was made about the presence of a significant genetic component of influence on the level of urea, blood biochemical parameters and productivity traits. The analysis of the manifestation of such traits in different breeds can be taken into account in selection programmes and the creation of appropriate feeding conditions, taking into account the peculiarities of protein nutrition

Keywords: blood urea level; blood urea nitrogen; dairy breeds of Ukraine; Simmental breed; degree of breed influence

Introduction

In management or herd management programmes, much attention is paid to assessing the genetic predisposition of animals to certain abnormalities or health problems. Urea, as a product of protein metabolism, in combination with other biochemical indicators, is an important indicator of dietary balance for managing nutrition strategy, health status, and controlling nitrogen emissions to the environment (Ruban *et al.*, 2021). Blood urea levels are related to urine and milk nitrogen levels, which makes these indicators, when abnormal, accurate predictors of certain problems (Hossein-Zadeh, 2024). Given the agro-climatic conditions of Ukraine and the breeding of local dairy breeds (Black and White and Red and White) and such cross-border breeds as Simmental, there is a need to test both the level of manifestation and the possibilities of using such biochemical indicators in animal breeding and herd management (Dymchuk & Ponko, 2023). The work is based on the data obtained from the results of biochemical blood tests. According to V. Kondratiuk *et al.* (2024), the value of milk urea (MU) is a useful tool for monitoring the protein nutrition of dairy cows, as it is closely correlated with the level of blood urea (BU). Depending on farms and feeding conditions, this correlation ranged from +0.7543 to +0.9017, with more accurate blood tests being performed when blood samples were taken two hours after feeding. Thus, these indicators serve as a certain indicator of the peculiarities of the manifestation of biochemical processes. In addition, MUN values tend to be higher in the summer months (Webb & de Bruyn, 2021). The University of Pennsylvania's practice guidelines for urea nitrogen values suggest that the observed differences may not be so much related to the breed as to their live weight (Ishler, 2023). For example, large Holsteins tend to have lower MUN and BUN than medium-sized Jerseys.

In the experiments of M. Prahl *et al.* (2022), high-yielding cows had higher plasma levels of urea, uric acid and hypuric acid. These differences were not associated with changes in gene expression and urea cycle or nitrogen metabolism, but were primarily due to hepatic metabolism. According to the authors, feeding a low diet led to an increase in the expression of enzymes that catabolise fatty acids, but the reason for this remains to be determined in future studies. Thus, studies on the influence of genetic and organised (environmental) factors on the characteristics of biochemical processes in cows make it possible to use certain forecasts and select optimal solutions in modern production conditions. The aim of the study was to evaluate and compare the level of urea and basic biochemical parameters of blood of cows of different breeds under similar feeding and housing conditions.

Literature Review

Urea is the end product of protein metabolism, a marker of cow health and an indicator of the balance of cattle rations. The average protein value in milk ranges from 3.0 to 3.9%, with a urea concentration of 8-25 mg/100 ml (Ruban *et al.*, 2021). Any deviations in urea content indicate a carbohydrate-protein imbalance in the diet, which affects reproductive function, hoof problems, reduced feed efficiency, etc. In addition, the level of urea nitrogen in blood (BUN), milk (MUN) or urine (UUN) is used as a control for certain environmental problems associated with methane emissions in ruminants (Zhao *et al.*, 2025). Urea has a low molecular weight, which allows it to rapidly cross the blood-brain barrier into milk, resulting in a strong association between blood urea nitrogen (BUN) and milk urea nitrogen (MUN) concentrations. Although MUN is a minor component of milk, it is an effective tool to identify potential problems in dairy herds (Sosa *et al.*, 2010). Many studies have shown that the intake of plant protein and energy and their synchronised interaction in the rumen are the main factors affecting MUN concentrations. Thus, MUN measurement can serve as a reliable indicator for improving nutrition management.

The urea nitrogen concentration in milk can also be used as an indicator in managing the nutrition strategy on dairy farms to increase the protein utilisation efficiency of dairy cows. Recent studies show that the nitrogen content of milk urea depends mainly on the nitrogen to energy ratio in the diet, but many other factors can influence ureogenesis. According to N. Munyaneza *et al.* (2017), measuring milk urea nitrogen can serve as a control for nitrogen use efficiency in dairy cows and optimise milk nitrogen levels. However, the reference values of milk urea nitrogen for optimising nitrogen use efficiency are different from the required level for milk protein production. Thus, an increase in milk protein production can be expected at milk urea nitrogen levels > 11 mg/dl (Ruban *et al.*, 2021), while protein utilisation efficiency is below this level (< 11 mg/dl). Optimal MUN values range from 10 to 14 mg/dl, but for many countries, the recommended milk urea nitrogen values for cow's milk range from 10 to 16 mg/dl milk. The studies by S. Nozad *et al.* (2012) showed that the highest blood concentrations of blood urea nitrogen (BUN), serum protein (SPtn), creatinine, triglycerides (TG), cholesterol and beta-hydroxybutyric acid (BHB) were found in high-yielding cows. Finally, to ensure a balance between milk protein production and reduced urinary and milk urea nitrogen excretion, recent evidence suggests that dairy cows should be fed a crude protein diet of 16.5%.

M. Štolcová *et al.* (2020) monitored changes in the protein status of the parameters of energy metabolism of the Holstein and Fleckvih breeds by urea and other blood biochemical parameters. At the beginning of lactation, changes in key metabolic parameters were faster and more pronounced in Holstein cows, and the dynamics of these parameters indicated the levels of adaptive performance and processes of body energy

mobilisation in the two breeds as a whole. Complex processes occur in the ruminant rumen with additional formation of microbial nitrogen and nucleic acids, circulation of such a toxic compound as hydrogen nitrite and subsequent neutralisation (conversion) into urea. Urea is hydrolysed into carbon dioxide and ammonia by the urease enzyme. The activity of urease increases the pH of the environment, as ammonia is formed (Ruban & Vasilevsky, 2015). In the 50s of the last century, M. Wall & K. Laidler (1953) studied the hydrolysis of urea catalysed by urease. The work was carried out in trihydroxymethylaminomethane-H_2SO_4 buffer, which was shown to have no activating or inhibitory effect on the reaction. It was found that the reaction passes through a sharp maximum pH of 8.00 at a wide range of substrate concentrations and temperatures, which, in the case of ruminant digestion, leads to certain complications in microbial processes (Ruban & Vasilevsky, 2015).

In the convincing opinion of P. Gregorini *et al.* (2013), the improvement of the functional version of Molly to Molly84 and Molly85, where the biochemical processes of digestion in the cow were described in a comprehensive and interdependent manner, led to a reduction in the proportion of the average relative prediction error and better predictions of methane production (CH_4) in ruminants. Further development of such a mathematical model should focus on potentially improving the prediction of rumen FFA production, as well as the intake pattern and associated changes in rumen nutrient excretion and the model of fermentation itself.

According to A. Bedford *et al*. (2020) investigated the effect of short-term elevated ambient temperature on rumen volatile fatty acid (VFA) dynamics and gene expression (the process where hereditary information from a gene (DNA nucleotide sequences) is converted into a functional product – RNA or protein) of the rumen epithelium associated with VFA transport and metabolism. The results show that under low feed intake and heat stress, there are shifts in the dynamics of rumen VFA and the ability of the rumen epithelium to absorb and transport acids of this series. During protein digestion, ammonia is released in the rumen, and if it is in excess, it is absorbed through the rumen wall into the bloodstream and converted to urea in the liver. Most of the urea is excreted in the cow's urine, although some of it passes into the milk. If the diet lacks nitrogen, the urea is not excreted, but returns to the rumen and is converted back to ammonia (Zhao *et al.*, 2025). G. Cozzi *et al.* (2011) observed that the production season significantly impacts several blood parameters, including total protein, globulin, creatinine, alkaline phosphatase, phosphorus, sodium, and chloride. They propose that these values can be used by dairy farmers to evaluate the metabolic health of lactating cows. Given the kidneys' continuous filtering function, stable blood creatinine levels are expected in healthy individuals, and deviations can indicate kidney problems. X. Du *et al*. (2017) found notably elevated levels of aspartate aminotransferase (AST), a marker of liver damage, in cows experiencing ketosis compared to healthy cows. They suggested that AST levels in blood tests could serve as an indicator of apoptosis, or programmed cell death, in liver cells.

R. Danielsson *et al.* (2017) discovered that despite consistent dietary intake, individual animals exhibited variations in methane (CH4) output. To examine the microbial makeup of the rumen, they performed 16S rRNA gene sequencing on the V4 region of rumen fluid. The study revealed that lower methane levels were associated with a greater abundance of the Methanobrevibacter ruminantium clade, a group of archaea within the Methanobacteriaceae family that produces methane primarily through the reduction of carbon dioxide with hydrogen. Archaea, a specialised group of unicellular organisms found in oxygen-deprived environments such as the rumen, are responsible for methane production (methanogenesis). Within the rumen, these microorganisms

primarily utilise hydrogen (H2) and carbon dioxide (CO2) as fuel for methane synthesis, thus occupying a vital ecological role. Animal waste, including urine and feces, contributes to global nitrogen emissions. Milk urea nitrogen (MUN) levels in dairy cows are directly linked to urinary urea nitrogen (UUN) emissions; both diminish when crude protein intake is reduced. However, even with consistent feeding, MUN levels can vary among individual cows. Furthermore, research by Y. Yao *et al*. (2018) has demonstrated that, in the pursuit of sustainable biogas energy, pre-treating wheat straw with urea allows for substantial methane generation.

C. Müller *et al.* (2021) proposed that variations in internal nitrogen processing lead to differing urinary urea nitrogen (UUN) excretion, with cows exhibiting higher milk urea nitrogen (MUN) levels releasing more UUN than those with lower MUN. Their research examined nitrogen distribution and urea metabolism in dairy cows with varying MUN concentrations, comparing a diet with optimal crude protein (NP) to one with low crude protein (LP). The LP diet resulted in reduced total nitrogen (N) and UUN excretion, as well as lower ammonia emissions from feces. However, methane emissions, urinary nitrogen excretion, and ammonia excretion remained relatively consistent across the groups. Notably, cows with high MUN (HMU) showed lower renal creatinine and uric acid levels and excreted less uric acid (specifically on the LP diet) and creatine in their urine. The authors' initial prediction that cows with high milk urea (HMU) would excrete more urinary urea nitrogen (UUN) than those with low milk urea (LMU) was not supported by their findings. The reduced urinary creatine excretion in HMU cows suggests a potentially smaller environmental nitrogen impact from these animals. J. Spek *et al*. (2012) propose that milk urea nitrogen (MUN) levels in dairy cows can be used on farms to guide dietary adjustments and strategies for minimising environmental nitrogen (N) emissions. While urinary urea nitrogen excretion (UUN) is generally correlated with MUN, this relationship is highly variable. The reliability of MUN as a UUN predictor could be enhanced by considering several influencing factors. These include variations in water consumption, urine production, protein levels, body weight, and the timing and frequency of feeding and milking. Incorporating these factors into the MUN-UUN relationship can significantly improve the accuracy of MUN as a marker for protein utilisation efficiency and UUN.

Therefore, a complicated yet interconnected dynamic exists between milk yield and composition (specifically protein content) and urea concentrations in blood, urine, and milk. Current models that describe nitrogen digestion, absorption, and metabolism, often utilising urea analysis, facilitate the creation of more precise animal diets. However, further investigation is needed to fully optimise not only animal nutrition but also health, reproduction, and methane reduction. These investigations are, in part, influenced by the ecological and genetic features of the research locations.

Materials and Methods

The research was conducted in the conditions of the farm "Grigorovich S.A." Kyiv region in the winter period of 2022-2023 on cows of combined Simmental, dairy Ukrainian Red-White and Black-White breeds. The Ukrainian Red-and-White dairy cow was created by crossbreeding Simmental with Ayrshire, Montbéliard and Holstein, and the Ukrainian Black-and-White dairy cow was created by crossing the local Black-and-White with Holstein. Each of the groups of animals in the experiment consisted of 33 heads. During the experimental studies presented in this paper, all manipulations with the cows involved in the research were carried out taking into account the basic principles of bioethics, in accordance with Article 26 of the Law of Ukraine No. 3447 "On the Protection of Animals from Cruelty" (2006), the European Convention for the Protection

of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the Procedure for Conducting Experiments and Experiments on Animals by Scientific Institutions (2012). Automated milking took place twice a day. The daily mixed diet of the cows consisted of the following feed components: alfalfa haylage – 3.3 kg; corn silage – 21 kg; mixed fodder – 9.7 kg; grass haylage – 5.5 kg; beet pulp – 3 kg; corn – 2.5 kg, straw – 0.5 kg; vitamin and mineral supplements. The level of feed dry matter intake was within 21.5 kg with a content of 15% crude protein, 17% acidic detergent fibre and 26% neutral detergent fibre. The general characteristics of the studied groups are given in Table 1.

Table 1. *General characteristics of the studied groups of cows during standard lactation (305 days) and blood biochemical parameters at the time of the experiment*

Indicator	Breed		
	Simmental (n = 33)	**Ukrainian Red-White (n = 33)**	**Ukrainian Black-and-White (n = 34)**
Hope for 305 lactations	4,359.7 ± 39.15	7,320.9 ± 47.24	8,617.1 ± 85.96
Fat content in milk, %	4.30 ± 0.059	3.82 ± 0.443	3.48 ± 0.043
Protein content in milk, %	3.29 ± 0.019	3.28 ± 0.014	3.25 ± 0.025
ECM[(1)], kg	4,487.8 ± 65.40	7,094.5 ± 296.86	7,969.2 ± 109.11
Total bilirubin, μmol/l	6.12 ± 0.749	3.43 ± 0.160	2.75 ± 0.157
Direct bilirubin, μmol/l	1.67 ± 0.096	1.74 ± 0.101	1.39 ± 0.075
Urea, mmol/l	2.91 ± 0.046	3.41 ± 0.058	3.06 ± 0.047
Creatinine, μmol/l	86.54 ± 1.699	111.16 ± 2.436	86.31 ± 2.016
ALT[2], units/l	37.16 ± 0.625	34.81 ± 1.012	33.44 ± 0.697
AST[3], units/l	34.24 ± 3.344	48.39 ± 1.900	18.43 ± 1.251
Glucose, mmol/l	2.59 ± 0.079	2.83 ± 0.077	2.74 ± 0.096
Albumin, g/l	31.30 ± 0.241	34.45 ± 0.473	31.67 ± 0.222
Total protein, g/l	67.46 ± 0.743	72.02 ± 1.247	72.12 ± 0.917
GGT[4], units/l	13.22 ± 0.424	16.15 ± 1.096	15.04 ± 1.005

Note: ECM[(1)] – milk fat and protein corrected milk yield; ALT[2] – alanine aminotransferase; AST[3] – aspartate aminotransferase; GGT[4] – gamma glutamyl transferase
Source: developed by the authors on the basis of research

Milk production (milk yield, fat and protein content) was monitored according to the requirements of ICAR (n.d.). The value of ECM – Energy-corrected milk or milk yield adjusted for fat and protein content was calculated using the formula (1):

$$ECM = (\text{milk fat, kg} \times 38.3 + \text{milk protein, kg} \times 24.3 + \text{milk yield} \times 0.7832)/3.14. \quad (1)$$

The analysis of serum biochemical parameters was measured on an automatic biochemical analyser HTI BioChem FC-120 (High Technology, Inc., USA) using reagents from this company in the Educational and Scientific Laboratory of Veterinary Diagnostic Research of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine.

The analysis of variance of the effect of breed, month of lactation and month of pregnancy on the studied traits of cows was carried out using a linear model (2):

$$y_{ijk} = a_i + b_j + c_k + e_{(ij)(k)}, \quad (2)$$

where y_{ijk} is the trait under study, a_i is the effect of the i-th breed, b_j is the effect of the j-th month of lactation, c_k is the effect of the k-th month of pregnancy, and $e_{(ij)(k)}$ is the residual.

The degree of influence of factors on the studied traits of beef cattle was calculated by the ratio of variances according to formula (3):

$$\eta 2 = (SSA/SSD)\ 100\%, \quad (3)$$

where SSA is the sum of squared deviations due to the influence of factor A; SSD is the total sum of squared deviations.

Statistical analysis (descriptive statistics, analysis of variance, and correlation analysis) was performed using RStudio software. The following were selected as the main biochemical characteristics:

- total bilirubin – a breakdown product of haemoglobin (red blood cells), myoglobin and cytochromes in the form of direct (bound, conjugated) and indirect (free, unbound or unconjugatedfractions, as an indicator for the diagnosis of liver diseases;
- direct bilirubin as a sensitive marker of liver pathology, which provides a connection of total bilirubin with glucuronic acid (a low-toxic fraction of total bilirubin) in hepatocytes (liver cells);
- urea is the end product of the breakdown of proteins and amino acids, which accounts for more than 50% of residual nitrogen in the body;
- creatinine, a byproduct of protein breakdown, supplies energy to muscles. Blood creatinine levels are directly related to an individual's muscle mass and body weight;
- alanine aminotransferase (ALT) is an enzyme primarily located in liver and kidney tissues, with smaller quantities present in heart and muscle tissues;
- aspartate aminotransferase (AST) is an enzyme distributed throughout the body's cells, with the highest concentration in the liver;
- gamma-glutamyl transferase (GGT) is an enzyme found in liver cells and the bile ducts, where it facilitates specific biochemical processes;
- glucose – blood glucose analysis is considered a useful tool for rapid diagnosis and differentiation of ketosis (Mair *et al.*, 2016);
- albumin – their balance is necessary to prevent fluid loss through blood vessels. Normal levels of total protein, albumin and globulin were 6.70-9.40 g/dl, 2.87-4.29 g/dl, and 3.03-5.73 mg/l, respectively;
- total protein – shows the concentration of proteins in the blood plasma, which consists of three main groups of components: albumin, globulins and fibrinogen.

Blood samples for subsequent analysis were taken two hours after feeding.

Table 2. *Influence of the breed factor on the main productive indicators*

Factor, indicator	Middle square	Fisher's F-test	η^2, %
Fat content	18.132	8.089***	14.6
ECM	95,921,886.182	49.790***	51.2

Note: ECM – total energy content of milk; *** – P > 0.999; ** – P > 0.99; * – P > 0

Source: developed by the authors on the basis of research

Results and Discussion

The general results of the impact of such a variable as breed are presented in Table 2.

The breed factor played a significant role in influencing the traits of fat content in milk and ECM, the values of which were 14.6% and 51.2%, respectively (Table 2). Significant differences in the performance data between the combined Simmental, Ukrainian Red-and-White and Black-and-White dairy breeds were caused by their production orientation, which affected the digestion characteristics in general and especially nitrogen digestion.

To accurately describe the characteristics of nitrogen digestion, absorption and metabolism, urea levels, as well as nitrogen utilisation efficiency and profit maximisation, M. Meng *et al.* (2019) recommend using the "Molly the Cow Model" (Fig. 1).

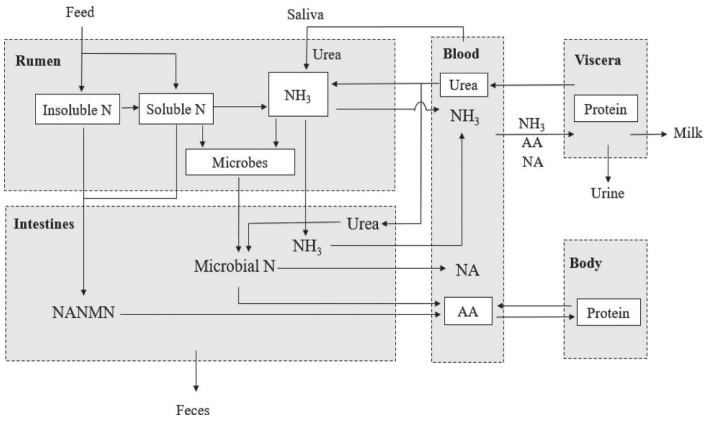

Figure 1. Conceptual diagram of nitrogen metabolism in ruminants – the "Molly model"
Note: NANMN – nonammonia, nonmicrobial nitrogen; NA – nucleic acids; AA – amino acids; N – nitrogen; NH_3 – hydrogen nitrite (ammonia). The darkened areas represent organs and tissues where biochemical processes take place, and arrows indicate the direction of movement of these processes
Source: M. Meng *et al.* (2019)

According to P. Gregorini *et al.* (2013), this model is a deterministic, mechanistic, dynamic model that represents the digestion, metabolism and production of a dairy cow based on mathematical equations. The authors compared the predictions of enteric (when the feed substrate passes through the entire gastrointestinal tract) methane production with the original mathematical version of Molly Origin and two updated versions based on a number of biochemical and genetic parameters that correspond to the design of this experiment. Thus, these versions included the effect of scar tissue, digestive parameters and hormonal parameters (Molly84) with a revised version of digestive and ruminal parameters (Molly85). This model was partly used to explain the influence of genetic (breed) and organisational factors (month of lactation, month of pregnancy), the results of which are shown in Table 3.

Table 3. *Effect of breed, month of lactation and month of pregnancy on the studied blood traits of cows*

Factor, indicator	Sum of squared deviations	Number of degrees of freedom	Mean square of deviations	Fisher's F-test	η^2, %
Total bilirubin, μmol/l					
Breed	212.0	2	106.02	15.566***	24.7
Month of lactation	56.6	7	8.09	1.187	6.6
The month of pregnancy	24.9	6	4.15	0.610	2.9
Balance	565.3	83	6.81		
Direct bilirubin, μmol/l					
Breed	2.261	2	1.1303	3.857*	7.7
Month of lactation	0.507	7	0.0725	0.247	1.7

Table 3. Continued

Factor, indicator	Sum of squared deviations	Number of degrees of freedom	Mean square of deviations	Fisher's F-test	η^2, %
The month of pregnancy	2.086	6	0.3477	1.187	7.1
Balance	24.324	83	0.2931		
Urea, mmol/l					
Breed	4.060	2	2.0300	25.506***	33.2
Month of lactation	0.950	7	0.1357	1.704	7.8
The month of pregnancy	0.625	6	0.1042	1.310	5.1
Balance	6.606	83	0.0796		
Creatinine, µmol/l					
Breed	13,632	2	6,816	57.393***	49.8
Month of lactation	1,719	7	246	2.068	6.3
The month of pregnancy	2,167	6	361	3.041**	7.9
Balance	9,857	83	119		
ALT, U/l					
Breed	236.1	2	118.06	5.569**	10.4
Month of lactation	157.3	7	22.47	1.060	6.9
The month of pregnancy	120.2	6	20.04	0.945	5.3
Balance	1,759.6	83	21.20		
AST, U/l					
Breed	14,964	2	7,482	38.432***	46.3
Month of lactation	578	7	83	0.424	1.8
The month of pregnancy	618	6	103	0.529	1.9
Balance	16,158	83	195		
Glucose, mmol/l					
Breed	0.811	2	0.4054	1.713	0.004
Month of lactation	0.742	7	0.1061	0.448	0.004
The month of pregnancy	2.386	6	0.3977	1.680	0.01
Balance	19.646	83	0.2367		
Albumin, g/l					
Breed	191.34	2	95.67	28.461***	35.1
Month of lactation	36.48	7	5.21	1.550	6.7
The month of pregnancy	37.63	6	6.27	1.866	6.9
Balance	279.00	83	3.36		
Total protein, g/l					
Breed	479.7	2	239.87	7.809***	13.2
Month of lactation	514.8	7	73.54	2.394*	14.2
The month of pregnancy	86.7	6	14.45	0.470	2.4
Balance	2,549.7	83	30.72		
GGT U/l					
Breed	135.8	2	67.90	1.855	3.5
Month of lactation	246.2	7	35.18	0.961	6.3
The month of pregnancy	504.9	6	84.16	2.298*	12.9
Balance	3,039.0	83	36.61		

Note: *** – P > 0.999; ** – P > 0.99; * – P > 0.95

Source: developed by the authors on the basis of research

A significant influence of the breed factor was noted in such blood biochemical parameters as total bilirubin 24.7%, urea 33.2%, creatinine 49.8%, alanine aminotransferase (ALT) 10.4%, aspartate aminotransferase (AST) 46.3%, albumin 35.1% and total protein 13.2% (Table 3). The influence of the breed factor had a significant impact due to the contrasting performance indicators between the compared groups for the entire lactation period (Table 2), which was implemented under the same feeding conditions and carried out on the principle of "no restrictions".

According to DairyNZ (2023), a New Zealand livestock breeding website aimed at farmers, milk urea "indicates" the level of crude protein in a cow's diet, which is produced by the metabolism of amino acids and protein in the body. If a cow's diet is rich in protein, she can produce more urea, which is usually excreted in urine and milk. On the other hand, when the diet lacks nitrogen, the cow's body redirects the urea back into the rumen. Urea levels in milk have been shown to differ in early, mid and late lactation (Table 4).

Table 4. *Recommended nutritional requirements for cows in crude protein (CP) and MUN levels at different stages of lactation*

Indicators	Early lactation	Mid-lactation	Late lactation
Minimum CP*% in the diet	18	16	14
Approximate MUN (mg/dl)	25-40	25-30	20-25

Note: * – crude protein
Source: DairyNZ (2023)

In New Zealand's pasture-based systems, MUN levels are higher than where cows are fed a general mixed diet (i.e. USA, Western Europe, the conditions of this study). This is due to the high amount of crude protein (around 20 and sometimes 30 mg/dl) that cows receive on good quality pasture, travelling long distances. Other factors include water intake, cow condition, lactation stage, season, genetics, milking frequency, rumen health and liver function. Table 5 shows the degree of correlation between biochemical parameters.

A correlation was established between the concentrations of urea, which is the main nitrogenous end product of protein and amino acid catabolism, and creatinine, a product of creatine phosphate breakdown in muscles, including Simmental, Ukrainian Red-White, and Ukrainian Black-White dairy cows (Table 5). The total correlation coefficient for plasma urea and creatinine is 0.552, which shows a positive relationship with the value of $m_r = 0.000$ ($p \leqslant 0.01$), indicating an increase in the level of creatinine in the blood plasma of the studied groups of cows with an increase in the value of urea and vice versa. As in the work of M. Edvardsson *et al.* (2018), a positive correlation was established between creatinine and aspartate aminotransferase, which characterises the functional state of the liver, as well as albumin in the blood plasma of the studied animal groups. The total correlation coefficient is 0.457 and 0.744, respectively, with a value of $m_r = 0.000$ ($p \leqslant 0.01$) and is consistent with changes in the level of these indicators in each of the studied groups of cows. A negative correlation was observed between the concentration of urea and the level of alanine aminotransferase in the blood plasma of cows with a correlation coefficient $r = 0.229$, $m = 0.022$ ($p \leqslant 0.05$). The total correlation coefficient for urea, albumin and total plasma protein is 0.454 with a value of $m_r = 0.000$ ($p \leqslant 0.01$) and 0.0234 with a value of $m_r = 0.019$ ($p \leqslant 0.05$), respectively.

Table 5. *Matrix of correlation coefficients between biochemical parameters of cows' blood (total sample – 100 cows)*

Indicators	Total bilirubin, μmol/l	Direct bilirubin, μmol/l	Urea, mmol/l	Creatinine, μmol/l	ALT, U/l	AST, U/l	Glucose, mmol/l	Albumin, g/l	Total protein, g/l
Bilirubin total	1.00	-	-	-	-	-	-	-	-
Bilirubin direct	0.336 ± 0.001**	1.00	-	-	-	-	-	-	-
Urea	-0.171 ± 0.091	0.111 ± 0.272	1.00	-	-	-	-	-	-
Creatinine	-0.089 ± 0.384	0.103 ± 0.306	0.552 ± 0.000**	1.00	-	-	-	-	-
ALT	0.114 ± 0.263	0.183 ± 0.068	-0.229 ± 0.022*	-0.008 ± 0.937	1.00	-	-	-	-
AST	0.333 ± 0.001**	0.238 ± 0.017*	0.182 ± 0.071	0.457 ± 0.000**	0.144 ± 0.152	1.00	-	-	-
Glucose	-0.021 ± 0.838	-0.301 ± 0.002**	0.098 ± 0.331	0.110 ± 0.276	-0.129 ± 0.200	0.001 ± 0.996	1.00	-	-
Albumin	-0.140 ± 0.168	-0.002 ± 0.986	0.454 ± 0.000**	0.744 ± 0.000**	0.112 ± 0.268	0.368 ± 0.000**	0.211 ± 0.035*	1.00	-
Total protein	-0.152 ± 0.133	-0.126 ± 0.211	0.234 ± 0.019*	0.024 ± 0.812	-0.548 ± 0.000**	-0.016 ± 0.873	-0.005 ± 0.958	0.064 ± 0.527	1.00
GGT	-0.192 ± 0.058	-0.048 ± 0.637	0.125 ± 0.217	-0.053 ± 0.604	-0.108 ± 0.286	-0.184 ± 0.069	-0.149 ± 0.141	-0.086±0.397	0.189 ± 0.061

Note: significance level where *p ⩽ 0.05, **p ⩽ 0.01

Source: developed by the authors on the basis of research

A positive correlation was found between the concentration of direct bilirubin, which is an endogenous antioxidant and the level of glucose and AST in the blood of the studied groups of cows, the correlation coefficient of which is 0.301 with a value of $m_r = 0.002$ ($p \leqslant 0.01$) and 0.238 with a value of $m_r = 0.017$ ($p \leqslant 0.05$). The values of the correlation coefficients are consistent with changes in the level of indicators in each of the studied groups of cows (Table 1).

Thus, the breed factor introduces significant differences in a wide range of biochemical parameters, which can be used for targeted selection programmes and achieving positive results in improving both health and reducing methanogenesis in ruminants. Both excessively high and low values of BUN and MUN are undesirable for dairy cows due to their negative impact on reproductive performance, health and nitrogen use efficiency (Fig. 2).

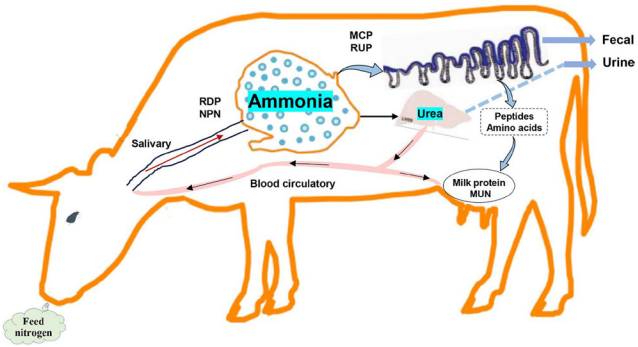

Figure 2. The process of nitrogen metabolism in cattle
Note: RDP – rumen degradable protein; RUP – rumen unprocessable protein; MCP – microbial protein; NPN – non-protein nitrogen; MUN – milk urea nitrogen
Source: X. Zhao *et al*. (2025)

Studies have shown that MUN, like BUN, is a trait with low to moderate heritability and is positively correlated with nitrogen release. According to X. Zhao *et al.* (2025), there are still inconsistencies in the selection of cows with low MUN values, which can effectively reduce nitrogen excretion and affect other economic parameters of dairy cows, as well as reduce nitrogen emissions. In addition, O. Fedota *et al.* (2020; 2022) proved that changes in blood parameters may depend on the body's response to xenobiotics, depending on the genotype, in particular on liver enzyme genes, which was observed both in animals and in medical practice. Thus, the analysis of correlations between biochemical blood parameters in cows of different breeds suggests the existence of breed specificity of metabolic processes, which should be taken into account when developing breeding programmes and animal breeding technologies.

Conclusions

A significant influence of the breed factor (Simmental, Ukrainian Red-and-White dairy and Ukrainian Black-and-White dairy breeds) on the fat content in milk (14.6%), total energy value of milk (51.2%), urea level (33.2%) and blood biochemical parameters was established. Breed or breed factor has a significant impact on blood chemistry parameters such as total bilirubin (24.7%), urea (33.2%), creatinine (49.8%), alanine aminotransferase (ALT) (10.4%), aspartate aminotransferase (AST) (46.3%), albumin (35.1%) and total protein (13.2%). This relationship indicates the possibility of using these indicators in selection programmes aimed at reducing nitrogen emissions and the use of

certain biochemical indicators to monitor animal health and welfare.

The correlation between the concentrations of urea and creatinine in the blood plasma of the studied groups of animals was established. The general correlation coefficient for plasma urea and creatinine indicates an increase in the level of creatinine in the blood plasma of the studied groups of cows with an increase in the value of urea and vice versa. A positive correlation is observed between creatinine and aspartate aminotransferase, as well as albumin in the blood plasma of the studied groups of animals. A negative correlation was observed between the concentration of urea and the level of alanine aminotransferase in the blood plasma of cows. A positive correlation was found between the concentration of direct bilirubin and the level of glucose and AST in the blood of the studied groups of cows, and between the content of urea and albumin, urea and total plasma protein. The values of the correlation coefficients are consistent with changes in the level of indicators for each of the studied groups of cows - Simmental, Ukrainian Red-and-White dairy, Ukrainian Black-and-White dairy breeds.

In order to correctly describe the peculiarities of ruminant digestion, assimilation and metabolism of feed resources, urea levels and a wide range of biochemical parameters of blood and milk, it is necessary to use the "Molly the Cow Model". Building experiments on this principle to assess the effect of a wide range of genetic and organised (non-genetic) factors on nitrogen digestion opens up the possibility of a systematic view of solving environmental, economic and biological problems. Further research should be aimed at a comprehensive assessment of the factors influencing the peculiarities of nitrogen metabolism, which finds its application not only in the agricultural sector but also in medicine.

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Conflict of Interest

The authors declare no conflict of interest.

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Вплив породного фактору на рівень сечовини та біохімічні показники крові в молочному скотарстві

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Анотація. Молочне скотарство досягло великих успіхів завдяки цілеспрямованому удосконаленню молочних та комбінованих порід у різних кліматичних і агроекологічних зонах світу. Рівень сечовини та інших біохімічних показників крові корів свідчить про особливості вуглеводно-білкового балансу, його вплив на відтворювальні функції, стан здоров'я, ефективність використання корму, інтенсивність викидів метану. Мета дослідження полягала у визначенні впливу породного фактору на рівень сечовини та інші біохімічні показники крові корів різних порід. Дослідження проведені на коровах трьох контрастних між собою порід. Перша порода – комбінована (транскордонна) симентальська, дві останні місцеві – українська червоно-ряба молочна (створена шляхом комбінаційного схрещування симентальської породи з айрширською, монбельярдською, голштинською) та українська чорно-ряба молочна (створена на основі схрещування місцевої чорно-рябої з голштинською). Дослід проведено у стандартних умовах при годівлі корів «без обмежень» з аналізом продуктивних показників та біохімічних показників крові. Найбільш суттєвим фактором впливу на продуктивні та біохімічні показники був фактор породи, який склав за ознаками вмісту жиру в молоці та надою в перерахунку на загальну енергетичну цінність відповідно 14,6 % та 51,2 %. Вірогідний вплив породи відзначено за такими біохімічними показниками крові, як рівень загального білірубіну 24,7 %, сечовини 33,2 %, креатиніну 49,8 %, аланінамінотрансферази 10,4 %, аспартатамінотрансферази 46,3 %, альбуміну 35,1 % та загального білка 13,2 %. Встановлена вірогідна кореляційна залежність між концентраціями сечовини та креатиніном, креатиніном та аспартатамінотрансферазою, а також альбуміном у плазмі крові досліджуваних груп тварин. Зроблено попередній висновок про наявність суттєвої

генетичної компоненти впливу на рівень сечовини, біохімічних показників крові та ознак продуктивності. Аналіз прояву таких ознак у різних порід можна враховувати в програмах відбору та створенні відповідних умов годівлі, враховуючи особливості білкового живлення

Ключові слова: рівень сечовини в крові; азот сечовини крові; молочні породи України; симентальська порода; ступінь впливу породи

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Characteristics of beef traits in crossbred bulls with different degrees of its marbling

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Abstract. The purpose of this experimental study was to characterise the quantitative and qualitative traits of beef in the most common domestic animals in Ukraine from Ukrainian Black-and-White dairy and Holstein cattle bulls at different classes of marbling severity of *m. longissimus dorsi*. Twenty-six carcasses from 20-22-month-old bulls of the farm "Zhuravushka" located in Brovary district of Kyiv region, Ukraine, served as material for the study. The marbling of *m. longissimus dorsi* was evaluated according to the JMGA standard (2000) and the morphological composition and quality characteristics of the carcasses were determined. The obtained results showed that in the medium and good (3 to 5 points) class of marbling, the marbling of the *m. longissimus dorsi* in carcasses was higher than in the unsatisfactory and below average grade (1 and 2 points), the content of muscle tissue of the second grade was 6.5 points ($P \leq 0.01$), bone – 2.5 points ($P \leq 0.01$), and the carcass conformation (meatiness) was better developed by 33.3% ($P \leq 0.01$), the development of its cover with adipose tissue – by 31.8% ($P \leq 0.05$) and muscle colour – by 10.2% ($P \leq 0.05$). At the highest (from 3 to 5 points) class of marbling of beef, the content of muscle tissue of the highest (3.9 points) and first (2.6 points) grades was significantly ($P \leq 0.05$) lower, and the size of the "muscle eye" area was 22.5% lower (the difference was not statistically significant). Accounting for the probable difference in slaughter signs depending on the marbling of beef, the data

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obtained can be used to predict the content of certain grades and quality characteristics of muscle tissue in carcasses of crossbred bulls from Ukrainian Black-and-White and Holstein dairy cattle

Keywords: conformation (meatiness) of carcasses; marbling of beef; meat productivity; meat characteristics; quality characteristics of carcasses

Introduction

In an increasingly competitive meat market, beef quality is becoming a crucial factor in consumer choice and economic efficiency of production. The defining feature of beef quality in international classification systems is marbling, as it has close correlations with the sensory characteristics of meat, determining its nutritional quality (Otto *et al*., 2024). In particular, a study by T. Erena *et al*. (2024) confirmed the link between marbling, tenderness and overall taste characteristics of meat, which can be useful for the meat processing industry and product quality control. According to T. O'Quinn *et al*. (2024), beef flavour is a key driver of consumer demand and is influenced by many factors throughout the animal's life cycle and after slaughter – from marbling and genetics to cooking and storage methods.

In accordance with the marbling scales, beef quality is assessed according to the Beef Carcass Grading Standard from the Japan Meat Grading Association (JMGA, 2000) in Japan, United States Standards for Grades of Feeder Cattle (USDA, 2000) in the United States, Korea Institute for Animal Products Quality Evaluation (KAPE, 2019) in South Korea, Meat Standards Australia (MSA, 2015) in Australia. Marbling of *m. longissimus dorsi* as the main trait of beef is included in the Australian Meat Standards Index and is used in the USA to predict the expected difference in the productivity of bulls' progeny according to the Expected Progeny Difference (EPD) system. The standards of the Commission Regulation (EC, 2008) and DSTU 4673:2006 "Cattle for slaughter. Technical specifications" do not take into account the marbling of meat in Europe and Ukraine when assessing cattle carcasses. They focus on live and slaughter weights of animals. As noted by M. Gagaoua *et al*. (2020), these features do not indicate their real value. Marble inclusions in the middle of the muscles were identified by E. Cardenas *et al*. (2024) as the content of adipose tissue. The degree of marbling of beef depends on the sex of the cattle, the level of feeding, age and live weight before slaughter, and the technological process of its rearing. This creates certain economic difficulties for efficient cattle breeding. According to T. Erena *et al*. (2024), bull calves with better carcass quantitative characteristics produce worse meat quality than bullocks and heifers.

In the context of dairy cattle, a modest correlation was observed between the marbling of meat and the content of internal adipose tissue (Martín *et al*., 2022). Furthermore, O. Kruk *et al*. (2024) indicated that an increase in marbling of *m. longissimus dorsi* is associated with a reduction in the muscle tissue of the higher and first grades in beef from bull calves of the Ukrainian Black-and-White dairy breed (UBWD) at the age of 18 to 24 months. The findings of F. Drachmann *et al*. (2024) further corroborated the absence of a relationship between beef marbling and other traits. In recent years, there has been a significant increase in the demand for marbled beef in Ukraine. This is evidenced by the assessment of beef marbling in accordance with the DSTU 4673:2006 standard, which stipulates the requirements for cattle intended for slaughter. The absence of a relationship between marbling and other traits was demonstrated in the experiments of F. Drachmann *et al*. (2024). However, given that

the assessment of marbling of beef in accordance with DSTU 4673:2006 "Cattle for slaughter. Technical specifications" is not foreseen in Ukraine, it is important to establish quantitative and qualitative characteristics of carcasses at different levels of marbling in animal meat. A better understanding of this issue will help to practically prove the need to include the classification of marbling of cattle carcasses in Ukraine in regulatory documents to prove the production of quality beef in accordance with optimal standards.

Therefore, the aim of the research was to investigate the manifestation of quantitative and qualitative characteristics of beef in the carcasses of 20-22-month-old crossbred bull calves from Ukrainian Black-and-White dairy cows and Holstein bulls in accordance with the classes of marbling of muscle tissue of *m. longissimus dorsi*, which would allow to ensure optimal yield of the main components of meat to satisfy consumer demand.

Materials and Methods

The study was carried out in 2014-2016 on bull calves (n = 26) obtained from cattle of Ukrainian Black-and-White dairy (UBWD) and Holstein (H) breeds of the farm "Zhuravushka", located in Brovary district of Kyiv region, Ukraine. After birth, the animals were kept in a group. From the age of 4 months to 20-22 months, they were raised and fattened at the farm's feedlot. The cattle's need for nutrients was satisfied by rough, juicy, green, concentrated fodder and mineral fertilisers from the farm's automated feeders. After the bull calves were slaughtered, their carcasses were weighed. The carcasses were visually assessed for conformation (meatiness) and development of adipose tissue in accordance with the Commission Regulation (EC, 2008). The conformation of the carcasses was classified on a 5-point scale: E (perfect) – all profiles are fairly convex, perfectly developed muscles (5 points); U (excellent) – all profiles are convex, very well developed muscles (4 points); R (good) – profiles are normal, well developed muscles (3 points); O (satisfactory) – profiles are weakly expressed, muscles are moderately developed (2 points); P (unsatisfactory) – all profiles are weakly expressed, muscles are poorly developed (1 point).

The development of the fat cover was classified on a 5-point scale: low – almost no fat coating (1 point); minor – slight fat coating, muscles are visible almost throughout the carcass (2 points); medium – almost the entire carcass is covered with fat, fat accumulation is in the thoracic part (3 points); high – the carcass is covered with fat, fat accumulation in the thoracic and shoulder parts (4 points); very high – the entire carcass is covered with fat without gaps, large accumulation of fat in the thoracic part (5 points).

After sawing the carcasses in half, the half carcasses were cut between the 12^{th} and 13^{th} rib. The length and depth of the "muscle eye" of *m. longissimus dorsi* were measured with a ruler. The area of the "muscle eye" was calculated in accordance with the order of the Ministry of Agrarian Policy of Ukraine No. 290 (2004) using the formula (1):

$$S = L \times D \times 0.8, \quad (1)$$

where S is the area of the "muscle eye", cm 2; L is the length of the "muscle eye", cm; D is the depth of the "muscle eye", cm; 0.8 is the coefficient. The colour of muscle and adipose tissue was determined using a scale from 1 to 7 according to the method (JMGA, 2000). According to JMGA (2000), the degree of marbling of *m. longissimus dorsi* was assessed on a 12-point scale (Table 1). According to the marbling, carcasses (n = 9) with a marbling score of 1 to 2 points were assigned to the first group, and carcasses (n = 17) with a marbling score of 3 to 5 points were assigned to the second group. Using Microsoft Excel 2016, statistical analysis was performed to determine the arithmetic mean, its error, and the reliability criterion.

Table 1. *Classification of beef marbling*

Quality grade	Parameters of marbling degrees – BMS (beef marbling standard)
5 – excellent	from No. 8 to No. 12
4 – good	from No. 5 to No. 7
3 – average	from No. 3 to No. 4
2 – below average	No. 2
1 – unsatisfactory	No. 1

Source: authors' development based on the data of JMGA (2000)

All the manipulations during this study were carried out taking into account the basic principles of bioethics, in accordance with the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006) and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986).

Results and Discussion

The assessed marbling of the beef ranged from unsatisfactory (1 point) to good (5 points). The average grade was 4 points. In carcasses with medium and good (3 to 5 points) classes of marbling of *m. longissimus dorsi*, compared to unsatisfactory and below average (1 to 2 points) marbling, such signs as the relative amount of second-grade muscle tissue by 6.5 points and bone by 2.5 points were significantly ($P \leq 0.01$) higher (Table 2). As indicated in their work E. Peña-Gonzalez *et al*. (2020), marbling (fat in the middle of the muscle) in cattle matures late. It becomes noticeable after the formation of other fat depots. According to S. Sugii *et al*. (2022), fat is first formed around the internal organs in the abdominal cavity, then under the skin, between and in the middle of the muscles (marbling).

Table 2. *Indicators of carcass morphological composition in crossbred bull calves based on different marbling classes of m. longissimus dorsi, M ± m*

Trait	Class of marbling, points	
	from 1 to 2 (n = 9)	from 3 to 5 (n = 17)
Pre-slaughter live weight, kg	400 ± 7.3	410 ± 8.3
Carcass weight (slaughter), kg	184 ± 5.7	183 ± 3.5
Slaughter yield (carcass), %	46.0 ± 0.16	44.6 ± 0.11**
Muscle tissue, kg	129.3 ± 4.35	130.9 ± 2.76
Muscle tissue, %	70.3 ± 0.71	71.5 ± 0.71
Including higher grade, kg	31.7 ± 2.27	27.0 ± 1.01
Including higher grade, %	24.5 ± 0.74	20.6 ± 0.65*
First grade, kg	62.1 ± 2.40	59.4 ± 1.26
First grade, %	48.0 ± 0.54	45.4 ± 0.20*
Second grade, kg	35.5 ± 1.54	44.5 ± 1.46*
Second grade, %	27.5 ± 1.00	34.0 ± 0.77**
Adipose tissue, kg	11.1 ± 1.17	5.2 ± 0.64*
Adipose tissue, %	6.0 ± 0.19	2.9 ± 0.14***
Tendons and ligaments, kg	3.3 ± 0.39	2.1 ± 0.16
Tendons and ligaments, %	1.8 ± 0.13	1.2 ± 0.13*
Bones, kg	40.3 ± 1.16	44.6 ± 0.69
Bones, %	21.9 ± 0.31	24.4 ± 0.27**

Note: * $P \leq 0,05$; ** $P \leq 0,01$

Source: authors' development

The increase in the absolute and relative amount of muscle tissue classified as the second grade with better marbling development can be explained by the fact that it also includes fat located between the muscles, which was released during tenderisation. When the marbling score was 3-5 points, the slaughter yield was significantly ($P \leq 0.01$) lower (by 1.4 points). A decrease in the percentage of carcass yield with an increase in the marbling class of beef was also proven in previous studies (Greenwood, 2021). This is due to the fact that at meat processing plants, when processing beef with better marbling, processors remove excess fat deposited in the body under the skin from animals. Therefore, the increase in adipose tissue under the skin, which has a low market value, is considered waste from beef production (Yamada *et al.*, 2020).

The relative content of muscle tissue of the higher and first grades, as well as tendons and ligaments, was significantly ($P \leq 0.05$) lower by 3.9 points, 2.6 and 0.6 points, respectively, with medium and good (3 to 5 points) marbling degree. The absolute amount of adipose tissue in the carcass was 2.1 times higher ($P \leq 0.05$) with unsatisfactory and below average (1 and 2 points) degrees of marbling of *m. longissimus dorsi*. In dairy cattle, the deterioration of carcass quality traits with an increase in the degree of marbling was noted in the studies by S. Liu *et al.* (2024). Because of this, the authors believe that beef producers should not breed dairy cattle with a high content of fatty tissue in the middle of the muscles, either by genetic selection or by adjusting diets.

The average and good (3 to 5 points) class of marbling of *m. longissimus dorsi* significantly ($P \leq 0.01$) improved the conformation (meatiness) of carcasses by 33.3% compared to the unsatisfactory and below average (2 points) class of marbling (1 point) (Table 3). Evaluating the quality attributes of carcasses according to the EC Standard (2008), similar results were also obtained in the studies of L. Schulz & A. Sundrum (2020). According to their data, the correlation is stronger between beef marbling and carcass conformation than with the development of fatty tissue. The conformation of cattle carcasses is a breed factor and depends on the interaction between the genotype of the animals and their growth characteristics. With an average and good assessment (from 3 to 5 points) of the level of marbling of *m. longissimus dorsi* compared to unsatisfactory and below average (1 and 2 points), the development of fatty tissue on the carcass was significantly ($P \leq 0.05$) 31.8% higher and there was a tendency to better thickness (28.6%) of subcutaneous adipose tissue.

Table 3. *Qualitative traits of bull carcasses according to different marbling classes of m. longissimus dorsi, M ± m*

Assessment of marbling class *m. longissimus dorsi*, points	Traits					
	Carcass conformation, points	Development of adipose coating, points	Thickness of subcutaneous adipose tissue, cm	Colour of muscle tissue, points	Colour of fatty tissue on the carcass, points	Area of the "muscle eye", cm^2
from 1 to 2 (n = 9)	2.4 ± 0.13	2.2 ± 0.11	0.7 ± 0.07	4.9 ± 0.08	4.4 ± 0.18	88.9 ± 5.34
from 3 to 5 (n = 17)	3.2 ± 0.09**	2.9 ± 0.21*	0.9 ± 0.10	5.4 ± 0.12*	4.6 ± 0.13	72.6 ± 4.99

Note: * $P \leq 0,05$; ** $P \leq 0,01$

Source: authors' development

The development of adipose tissue is used to classify, grade and describe the value of carcasses for the meat industry (Brito *et al.*, 2024). According to M. Ju *et al*. (2024), fatty tissue has both negative and positive effects on beef quality. It protects the carcass from moisture loss during evaporation, which leads to increased meat toughness, and is less desirable because it leads to a decrease in slaughter yield, muscle eye area, and water retention capacity of meat (Kruk *et al.*, 2024). Therefore, for high productivity and meat quality, the thickness of fatty tissue under the skin for bulls should be 8.0 mm (Zurbriggen *et al.*, 2024). In addition, the quality of meat from cattle depends not only on the amount and thickness of adipose tissue under the skin, but also on its content in the muscles, the breed of animals, growth rate, and inbreeding.

With medium and good grades (3 to 5 points) of marbling compared to unsatisfactory and below average grades, there is a tendency to reduce the area of the "muscle eye" of *m. longissimus dorsi* by 22.5%. This indicates that with more inclusions of adipose tissue in the *m. longissimus dorsi*, its growth slows down, and therefore the carcass contains less valuable edible parts, including the highest and first grade of muscle tissue. The *m. longissimus dorsi* muscle is located in sections (thoracic and lumbar) that occupy a significant proportion of the carcass.

Carcass cuts with a higher marbling content have better flavour characteristics (O'Quinn *et al.*, 2024). Therefore, due to the deterioration in the yield of valuable cuts, it is not possible to use the values of the marbling class of *m. longissimus dorsi* to predict the production of the highest and first grades of beef in the carcasses of bulls from Ukrainian dairy cows and Holstein bulls at the age of 20-22 months. The highest grade of marbling of muscle tissue was significantly ($P \leqslant 0.05$) better by 10.2% and there was a tendency to improve the colour of subcutaneous fat tissue. The colour of muscle and adipose tissue is an indicator of beef freshness. Yellow adipose tissue is negatively assessed in many countries around the world.

Thus, with the best evaluation of carcasses according to marbling classes, many quantitative and qualitative characteristics of beef, in particular the content of muscle tissue of the highest and first grades, the area of the "muscle eye" *m. longissimus dorsi*, are worse in 20-22-month-old bulls from Ukrainian Black-and-White dairy cows and Holstein bulls. Therefore, improving the quality of beef by regulating the formation of its marbling in cattle remains problematic and expensive for a number of reasons. First of all, there are no breeds in Ukraine that have an optimal intramuscular fat content (marbling). Thus, according to J. Thompson (2004), for good sensory properties, its amount in the muscles should be 15-17%.

According to S. Raza *et al*. (2019), the black Wagyu cattle breed in Japan has the highest amount of adipose tissue (over 30%) in the middle of the muscles. In Korea, the Hanwu breed ranks second in this respect. Among European breeds, Aberdeen Angus cattle have the best concentrations of intramuscular fat and sensory properties (Bureš & Barton, 2018). In Ukraine, beef from Wagyu and Hanwu cattle is not produced because they are not available. The existing Aberdeen Angus breed is not a purebred of Scottish origin, but a hybrid (Aberdeen Angus x Black and White Holstein) bred from embryos imported from the United States. As L. Mueller *et al*. (2019) found, heifers and cows have more adipose tissue in their bodies than bull calves. And scientists E. Kul *et al*. (2019) indicate that castration of bull calves increases the deposition of adipose tissue inside the muscles.

Beef from uncastrated bull calves is of poorer quality and is valued less by consumers (Terevinto *et al.*, 2020; Nechyporenko *et al.*, 2024). In Ukraine, bull calves are rated the best and heifers the worst when delivered to meat processing plants. Since bull calves are not castrated at a young age on private farms, they are hardly ever sold. Not beef, but mainly

veal, which has virtually no intramuscular fat, is sold on the markets. Marbling of beef is better in adult animals and at higher live weight, which makes it more expensive to produce (Hudson *et al.*, 2020). Animals consume 2.25 times more feed nutrients for the formation of adipose tissue than for the growth of carcass muscles. Excess energy supplied to animals in the late fattening period significantly reduces the effectiveness of feed by reducing its digestibility, increasing the amount of fat waste and worsening the efficiency of livestock management.

I. Randhawa *et al.* (2021) found that the deposition of adipose tissue in cattle muscles is promoted by feeding them concentrated feeds with a high energy content. However, when animals are raised on concentrated feed, beef has lower levels of mono- and polyunsaturated fatty acids than those fed on grass pastures. Grazing increases the content of unsaturated fatty acids in the muscles and produces various aldehydes, ketones, alcohols, esters and carboxylic acids that contribute to the flavour and extraordinary juiciness of beef. The processor does not pay the producer for the significant amount of fatty tissue removed from carcasses that are cut off at high fatness. Payment is made only for the slaughter weight (carcass). According to Y. Li *et al.* (2020), during the life of cattle, intramuscular fat reduces lipogenesis and increases the activity of the enzyme cholesterol-25 hydroxylase, which is synthesised from glucose carbon, not acetate, during intensive fattening after the accumulation of "excess" fat between the muscles.

The marbling of beef can be determined in several ways: subjectively by visual inspection, using special equipment, and by chemical analysis. Since the structure of marbling is complex and there are no clear boundaries between each class, it is almost impossible to accurately assess the content of adipose tissue in the middle of the muscles visually. The determined fat content by chemical means does not coincide with the visual assessment of marbling, as it can detect deposits of fatty inclusions that are not visible during visual examination. In live animals, the number of marbling particles in the muscle, including the percentage and their average size, can be determined by computer image analysis (Giaretta *et al.*, 2018). The use of ultrasound for this purpose requires sophisticated equipment, its periodic calibration and adherence to standardised protocols for scanning and interpreting the images.

In this regard, the question arises of further research to solve the problem of combining the quality of beef with the quality characteristics of carcasses of dairy and dairy-meat breeds, which are fed in large quantities for slaughter. In the future, researchers should focus on determining the marbling of beef using ultrasonic devices to establish links between it and carcass quality traits in beef cattle, as this will improve its visual and sensory quality. In Ukraine, research should be conducted to determine the optimal management factors for the cultivation of cattle of common breeds to achieve a compromise between the marbling of beef and its technological, sensory characteristics and chemical composition.

Conclusions

The obtained results of the study indicated a multifaceted influence of marbling of beef on its qualitative and quantitative characteristics in crossbred bull calves. It was established that in 20-22-month-old bull calves of Ukrainian Black-and-White and Holstein dairy breeds, the marbling score ranges from unsatisfactory class (1 point) to good (5 points). At the average and good (from 3 to 5 points) classes of marbling development of *m. longissimus dorsi*, the relative content of muscle tissue of the highest (by 3.9 points) and first (by 2.6 points) grades, tendons and ligaments (by 0.6 points) was significantly ($P \leq 0.05$) lower in the carcass, and there was a tendency (by 22.5%) to deteriorate the area of the "muscle eye". With a better (3 to 5 points) assessment of the marbling

of the *longissimus dorsi* compared to classes from 1 to 2 points, the conformation (meatiness) of carcasses significantly increased by 33.3% ($P \leq 0.01$), the bone content by 2.5 points ($P \leq 0.01$), the development of fatty tissue by 31.8% ($P \leq 0.05$) and the intensity of muscle tissue colour (by 10.2%; $P \leq 0.05$). At an increased level (3-5 points) of marbling compared to its score of 1-2 points, there was a tendency to increase the thickness of the fatty layer by 28.6% and its colour by 4.5%.

Thus, achieving an optimal balance between marbling and other quality characteristics of beef is an important task for breeders and producers. In the future, Ukraine should investigate the factors that determine effective management practices for growing and fattening cattle for the production of beef that combines quantitative and qualitative traits and is attractive to buyers for marbling and justify proposals for the introduction of a national standard for carcass evaluation based on marbling to support the livestock economy.

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Характеристика ознак яловичини у помісних бугайців за різної вираженості її мармуровості

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Анотація. Метою даного експериментального дослідження було охарактеризувати кількісні та якісні ознаки яловичини у найбільш розповсюджених в Україні помісних тварин від української чорно-рябої молочної та голштинської худоби за різного класу вираженості мармуровості *m. longissimus dorsi*. Матеріалом для проведення дослідження послужили 26 туш від 20-22-місячних бугайців фермерського господарства «Журавушка», що розташоване у Броварському районі Київської області, Україна. Оцінили мармуровість *m. longissimus dorsi* відповідно до стандарту JMGA (2000) та визначили морфологічний склад і якісні ознаки туш. Отримані результати засвідчили, що за середнього та доброго (від 3 до 5 балів) класу мармуровості продовгуватого м'яза у тушах були більшими, ніж за незадовільного та нижче середнього оцінювання (у 1 та 2 бали), вміст м'язової тканини другого сорту – на 6,5 пункта ($P \leq 0,01$), кісток – на 2,5 пункта ($P \leq 0,01$), та краще розвиненою конформацією туш (м'ясистість) на 33,3 % ($P \leq 0,01$), розвиток її покриву жировою тканиною – на 31,8 % ($P \leq 0,05$) і колір м'язів – на 10,2 % ($P \leq 0,05$). За вищого (від 3 до 5 балів) класу мармуровості яловичини у ній вірогідно ($P \leq 0,05$) меншими були вміст м'язової тканини вищого (на 3,9 пункта) і першого (на 2,6 пункта) сортів, та розмір площі «м'язового вічка» – на 22,5 % (різниця статистично не вірогідна). За урахування вірогідної різниці в ознаках забою залежно від мармуровості яловичини отримані дані можливо використовувати для прогнозування в тушах вмісту певних сортів і якісних ознак м'язової тканини у помісних бугайців від худоби молочних порід української чорно-рябої та голштинської

Ключові слова: конформація (м'ясистість) туш; мармуровість яловичини; м'ясна продуктивність; характеристики м'яса; якісні ознаки туш

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Use of the probiotic preparation "SVITECO-PWC" in the cultivation of broiler chickens

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Abstract. The aim of the study was to identify changes in the intestinal immune system of broiler chickens under the influence of a probiotic preparation based on *Bacillus subtilis* as a tool for improving the technological results of rearing. Two experimental groups were formed: a poultry house where the drugs were given according to the usual preventive programme (control) and a poultry house where the probiotic preparation based on *Bacillus subtilis* "SVITECO-PWC" was

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given. Methods of the study were: pathomorphological examination (autopsy), microbiological examination of broiler chickens, quantitative microbiological examination of the microflora of the cecum, polymerase chain reaction in intestinal tissue and litter, biochemical examination of blood serum parameters of broiler chickens, determination of interferon-α content in cells of the 12th cecum and cecum, determination of E-cadherin content in samples of the 12th cecum. Statistical analysis of production indicators: live weight, feed conversion, broiler liveability. The necropsy results showed that the main disease of the poultry in both groups was erosive-desquamative gastroenterocolitis, but the poultry in the experimental group showed a tendency to less erosive damage to the small and large intestine; the number of *E. coli* bacteria in the cecum of the experimental group was on average lower than in the control group, and the homogeneity of the indicator was also higher in the experimental group; the use of probiotic mixture in poultry contributed to a significant reduction in the number of *Eimeria aservulina* in the intestines of broilers and enhanced the colonisation of the intestines by *Clostridium perfringens*, which led to an increase in the number of the latter in the litter, improved protein metabolism and the amount of interferon in the experimental group. The conclusions of the study were that the addition of the probiotic to the preventive programme improved the intestinal immunity of broilers and production performance

Keywords: broiler; *Bacillus subtilis*; intestinal immune system; microbiome

Introduction

The relevance of the study is driven by the need to improve product quality amid growing demand for safe and environmentally friendly poultry meat, the main source of protein in the human diet. The use of antibiotics in poultry farming, while contributing to increased technological efficiency, has serious negative consequences, including the development of antibiotic resistance and residues in meat (Sirenko *et al*., 2024). In this context, probiotic preparations are a promising alternative that can improve the intestinal immune system of broiler chickens, increase resistance to infections and ensure optimal rearing results without compromising consumer health. The use of antibiotics, according to R. Kulkarni *et al*. (2022), causes resistance in both poultry bacteria and resistance of bacteria that cause infectious diseases in humans who eat poultry meat that has been given antibiotics during the growing process is a negative phenomenon in veterinary medicine. The way out of this situation is to find alternatives to antibiotics in poultry farming. As noted by M. Abd El-Hack *et al*. (2020), acidifiers, vegetable oils and probiotics are an alternative to antibiotics.

According to E. Baéza *et al*. (2022), S. Biswas *et al*. (2023), almost half of all animal protein in food will be poultry meat in 2030. And these data do not include the most affordable animal protein – eggs. Infectious diseases that are zoonotic anthroponoses require constant veterinary control and necessitate the search for means to contain constantly changing pathogens. Research has led to the fact that since 2006, European countries have been searching for drugs that will replace antibiotics (Kramarenko *et al*., 2023). This method of control is natural and cannot cause resistance to the main pathogens of poultry production: *Salmonella*, *E. coli*, campylobacteriosis, and others. R. Jha *et al.* (2020) mention several probiotic cultures used in poultry production. Their effectiveness depends on the concentration of probiotics *Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus, Bacillus, Pediococcus*,

as well as the frequency of their use to colonise the intestines, respiratory tract and even the premises where poultry are kept. According to E. Oviedo-Rondón (2019), J. Plaza-Diaz *et al.* (2019), and D. Horyanto *et al.* (2024), the use of probiotics has an effect that takes time but has long-lasting indirect results. This is the normalisation of the intestinal microflora – the main factor in the formation of intestinal immunity, the competition of safe probiotic cultures that form natural and effective symbiotic interactions with the villi of the poultry intestinal epithelium. Objective data of W. Kim & H. Lillehoj (2019), J. Wang *et al.* (2022), and S. Iqbal *et al.* (2023) even indicate modelling of the poultry immune system, improving the number and effectiveness of cellular immunity carried out through the organs of cellular immunity: the bursa and thymus. The way out of the situation when it is necessary to look for an alternative to drugs that inhibit the ability of pathogens to multiply is microorganisms that displace them and do not harm the host.

Inflammatory processes are triggered in response to any type of health threat or microbial infection or abnormalities in the functioning of cells and tissues (Kirimbayeva *et al.*, 2023). The physical embodiment for detecting such reactions in laboratory studies were the levels of chemokines, interleukins, enzymes, antimicrobial peptides and other bioactive substances. *Lactobacillus acidophilus*, *Lactobacillus reuteri,* and *Lactobacillus salivarius* are the probiotic bacteria species that, when introduced into the poultry intestine, promoted the expression of cytokine genes in the intestinal lymphatic tissues (Bogatko & Utechenko, 2024).

These processes, which occurred and developed in the intestine, are directly related to the level of immunity and the potential for broiler growth and feed conversion. The gut can be seen as a place of constant struggle for the resources of active commensal or pathogenic microorganisms. The immune system of the poultry gut constantly responds to this struggle by supporting commensal microorganisms and fighting off microbes. Intestinal-associated lymphoid tissue includes lymphoid cells, Meckel's diverticulum, Peer's spots, and cecal glands. The immune response to pathogens consists of a standard chain of receptor recognition of the pathogen and the production of cytokines. Probiotics entering the poultry intestine give certain reactions: an increase in cytokines, the number of T cells, the production of antibodies, and the level of intestinal immune response.

The immune system of a poultry differs from other immune systems by the presence of tonsils and the glandular pouch or bursa (organs associated with the ontogeny of T and B cells), which are located directly next to the cloaca, the last part of the intestine (Bogatko *et al.*, 2024). The presence of aggressive pathogens in the poultry intestine leads to an increase in the number of leukocytes. Conversely, the presence of symbiotic microflora in the poultry intestine, such as probiotic cultures, will allow the poultry body not to waste energy resources on the production of leukocytes, which was not necessary.

The *Lactobacillus reuteri* species has been used since 1997 as an organism capable of producing bacteriocins that prevent the development of pathogens in the intestines of broilers. The aim of the study was to identify patterns in the changes in the broiler gut under the influence of a probiotic preparation.

Materials and Methods

The research was conducted on the basis of two poultry houses of the Mykolaiv National Agrarian University Educational and Research Centre during the standard poultry rearing cycle of 45 days in January-March 2024. For the study, two groups of Ross 208 broiler chickens were formed: a control group (poultry house No. 1) and an experimental group (poultry house No. 2). The poultry house has standard lighting with an intensity of 25 lux. The lighting programme provides for the absence of light for a period of 7 to 30 days for 4 hours per day.

The watering and feeding system are standard Roxell equipment. The feed programme in both poultry houses is the same, the company provides poultry with feed of its own production, the composition of which is a trade secret. The temperature, humidity, and ventilation system are automatic. The bedding in both houses was the same: sunflower processing waste (husk). Laboratory studies were conducted at the Mykolaiv National Agrarian University.

The control group received drugs during the growing period according to a preventive programme drawn up by veterinarians and approved by the university management. These medicines include Lovit Va + Se vitamin, Novion acidifier, standard vaccines against infectious bronchitis of chickens and Newcastle disease. The experimental group received a course of Lovit Va + Se vitamin, standard vaccines against infectious bronchitis in chickens and Newcastle disease, and a probiotic preparation "SVITECO-PWC" (manufacturer – SIRION Limited Liability Company) containing *Bacillus subtilis*. Pathological examinations were performed to identify intestinal lesions in both groups. The autopsy was performed according to the following scheme. Once a week, starting from day 14, five dead broilers taken from the control house and five dead broilers from the experimental house were necropsied. The study lasted for 44 days, which is the time required to raise Ross-208 broilers. Pathological changes were recorded.

Microbiological studies were carried out in both research groups, these were quantitative microbiological studies of the intestinal microflora. To do this, at the end of the study, namely at 44 days, five live chickens from the experimental and five live chickens from the control poultry house were randomly selected, then killed in a humane manner and samples of the intestinal microbiota were taken. The procedure involved transporting the gut samples to the laboratory and storing them at -80 °C until DNA isolation. DNA was extracted using a Qiagen Powerlyzer kit (USA). The purpose of such studies was to identify the number of *E. coli* bacteria in the blind intestines, and to examine the homogeneity and number of enterococci. A microbiological examination of faeces and litter was also carried out. A polymerase chain reaction (PCR) test was conducted to identify the genetic material of infectious agents. Biochemical studies of blood serum parameters of broiler chickens in the control and experimental poultry houses were carried out. The purpose of such studies is to identify trends or lack thereof towards an increase in immunoglobulins in the poultry of the experimental group. Immunoblotting studies were performed to determine the content of interferon-α in the cells of the 12 duodenum and cecum.

All experimental studies were carried out in accordance with modern methodological approaches and in compliance with relevant requirements and standards, in particular, they meet the requirements of ISO/IEC 17025:2017 (2017). The conditions of detention and all manipulations were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986).

The experimental component of the "SVITECO-PWC" drug administration was the method of administration and dose. The drug was administered as follows: in the system of watering lines using a mediator: concentration of the working solution was 0.01% (1 litre of drug per 10,000 litres of water). The stock solution was prepared in a clean container, the water for dissolving the drug had a temperature of 25-40°C, but not higher than 50°C. The number of *E. coli* bacteria in the ileum of broilers was determined in colony forming units (CFU) by Nitzsch. This method allowed indirectly determining the number of viable cells by counting colonies in the culture medium or on its surface. In general, this technique did not work in the study by P. Cronin *et al.* (2021), when the authors tested this indicator in the ileum, and

not in the cecum, where more pronounced biochemical processes are constantly taking place. Therefore, it was the cecum that was studied. Feed intake and control weighing was carried out at the end of each feeding phase. Poultry mortality was recorded daily. At the end of broiler rearing, production results were summarised for the main biotechnological indicators in both groups: conversion, preservation, live weight. Feed conversion was calculated by dividing the total amount of feed by the total weight gain.

Results

The most affordable method of continuous monitoring of changes in poultry condition is necropsy of dead poultry from the poultry houses under study. Necropsy data are presented in Table 1.

Table 1. *Pathomorphological study data of broilers*

Growing days		14	21	28	35	44
Experimental group	1	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis. Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis	Lymphohistiocytic infiltration of the liver portal tracts	Erosive-desquamative gastroenterocolitis
	2	Trauma	Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis	Probable colibacillosis. Dystrophy, Necrosis of the femoral head	Femoral head necrosis
	3	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis	Erosive-desquamative gastroenterocolitis	Trauma	Femoral head necrosis
	4	Femoral head necrosis	Chronic atrophic-hyperplastic tracheitis	Femoral head necrosis	Lymphohistiocytic infiltration of portal tracts	Femoral head necrosis
	5	Femoral head necrosis	Erosive-desquamative gastroenterocolitis	Trauma	Lymphohistiocytic infiltration of portal tracts	Erosive-desquamative gastroenterocolitis
Control group	1	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis. Erosive-desquamative gastroenterocolitis. Probable colibacillosis	Erosive-desquamative gastroenterocolitis probable colibacillosis	Probable colibacillosis	Splenitis – probable colibacillosis
	2	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis. Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis probable colibacillosis	Probable colibacillosis	Splenitis erosive-desquamative gastroenterocolitis
	3	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis. Erosive-desquamative gastroenterocolitis. Probable colibacillosis	Erosive-desquamative gastroenterocolitis probable colibacillosis	Probable colibacillosis	Splenitis erosive-desquamative gastroenterocolitis
	4	Vitelline peritonitis	Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis	Aerosacculitis, a "starry sky" pattern in the liver	Erosive-desquamative gastroenterocolitis
	5	Inflammation of the umbilical ring	Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis	A picture of a "starry sky" in the liver	Erosive-desquamative gastroenterocolitis

Source: created by the authors

The main disease of the studied poultry of both groups was erosive-desquamative gastroenterocolitis. The combined pathology was chronic atrophic-hyperplastic tracheitis, endobronchitis with polyps. Complications were probable colibacillosis, a picture of "starry sky" in the liver and lymphohistiocytic infiltration of the portal tracts. Uneven hyperplasia of the lymphoid tissue of the spleen and bursa against the background of atrophic processes can be considered as background processes. The described signs of digestive tract lesions can also occur against the background of mycotoxicosis. Thus, the pathological and histological changes in the internal organs of poultry of both groups were generally similar, but in the experimental group there was a tendency for less erosive damage to the small and large intestine, and signs of uneven epithelialisation of erosions (the presence of multiple rows of epithelium and its numerous mitoses) were noted. Based on the results of studies of broiler chickens' cadavers, it was found that:

- pathogenic *E. coli* was isolated in all 5 cadavers in experimental poultry house 2;
- *E. faecalis* in 4 cases and *E. coli* in 3 cases out of 5 in the control house 1.

Thus, the etiological structure of bacterial infections in the poultry houses differed (two types of microorganisms were detected in the control house), with *E. faecalis* was insensitive to all groups of antibiotics studied. The presence of *E. coli* bacteria was also investigated (Fig. 1).

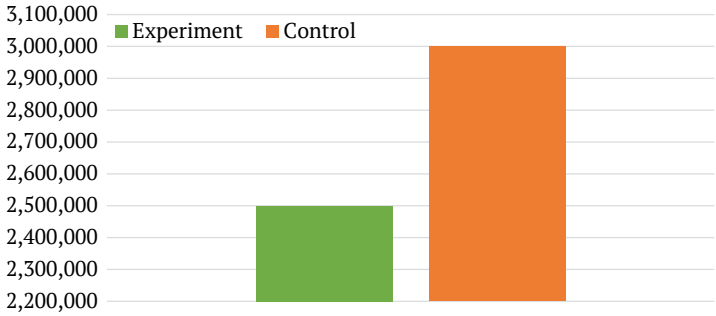

Figure 1. Number of bacteria of the *E. coli* group in the blind intestines, CFU/g, cecum
Source: created by the authors

As a result of quantitative microbiological studies of the intestinal microflora, the following data were found: the number of bacteria of the *E. coli* group in the blind intestines of the experimental group is on average lower than in the control group, and the homogeneity of the indicator is also higher in the experimental group. The number of enterococci in the blind cats was investigated. The number of enterococci in the chickens of the experimental group was on average higher and homogeneity lower than in the control group (Fig. 2).

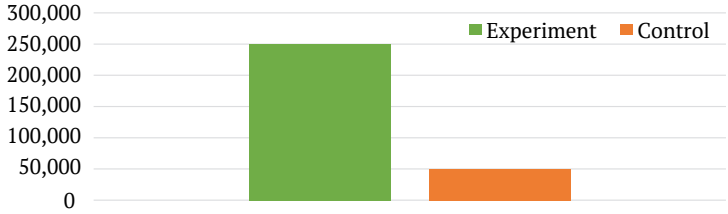

Figure 2. The number of enterococci in the blind intestines, CFU/g, cecum
Source: created by the authors

According to the results of quantitative microbiological studies of faeces and litter, the number of *E. coli* bacteria was higher in the experimental poultry houses than in the control house, but the sample was not representative (1 sample from each house). Thus, the main differences between the poultry houses are characterised by coinfection with *E. coli* and *E. faecalis* in the control house, in contrast to the experimental house, where mono-infection with *E. coli* was detected, as well as a decrease in the number of *E. coli* bacteria and, conversely, an increase in enterococci in the experimental house. The results of the PCR study reflect the presence of genetic material of *Clostridium perfringens* and the eimeria pathogen in the tissues and contents of the intestines and litter of broilers (Table 2).

Table 2. *PCR results of the study of the genetic material of microorganisms in tissues and intestinal contents of broilers*

Microorganism Number of positive samples, %		**Poultry house No. 1 (control)**		**Poultry house No. 2 (experiment)**	
		Genome equivalents	Number of positive samples, %	Genome equivalents	
Poultry					
Clostridium perfringens		33.33	$2.97 \times 103 \pm 0$	100	$7.09 \times 103 \pm 2.46 \times 103$
Eimeria	*Acervulina*	100	$4.03 \times 108 \pm 9.54 \times 107$	100	$6.15 \times 107 \pm 3.49 \times 106$
	Praecox	0	-	0	-
	Mitis	0	-	0	-
	Maxima	0	-	0	-
	Necatrix	0	-	0	-
	Brunetti	0	-	0	-
	Tenella	0	-	0	-

Note: ($M \pm m$, $n = 3$) (the difference between the experimental and control groups is significant at $p \leqslant 0.05$; "-" – no genetic material of target microorganisms was detected)
Source: created by the authors

It was found that 1 out of 3 animals of the control group contained *Clostridium perfringens* genetic material in the tissues and intestinal contents, while all animals of the experimental group contained *Clostridium perfringens* DNA. Against this background, the amount of genetic material in the animals of the control and experimental groups did not differ significantly and ranged from 103 genome equivalents. The results of the study of the causative agent of eimeria indicate the circulation of only one species of Eimeria – *Eimeria aservulina* – among poultry. It should be noted that the genetic material of this microorganism was identified in all animals of the experimental and control groups. At the same time, the amount of *Eimeria aservulina* DNA extracted from the intestines of the experimental poultry was significantly lower by more than 6.5 times ($p \leqslant 0.05$) compared to the values of the control group. The results of the study of broiler litter indicate the absence of genetic material of *Clostridium perfringens* and the pathogen of eimeria (Table 3).

Table 3. *Results of PCR study of the genetic material of microorganisms in broiler litter (n = 1)*

Microorganism		P 1 (control)	P 2 (experiment)
Clostridium perfringens, genome equivalents		-	1.25 × 104
Eimeria	*Acervulina*	-	-
	Praecox	-	-
	Mitis	-	-
	Maxima	-	-
	Necatrix	-	-
	Brunetti	-	-
	Tenella	-	-

Source: created by the authors

The genetic material of *Clostridium perfringens* was detected in the litter of the poultry of the experimental group, and the DNA of microorganisms from the genus *Eimeria* was not detected. Thus, the use of probiotic mixture in poultry contributes to a significant reduction in the number of *Eimeria aservulina* in the intestines of broilers and enhances the colonisation of the intestines by *Clostridium perfringens*, which leads to an increase in the number of the latter in the litter. Blood samples were taken from pre-slaughter broiler poultry, i.e. on the 44th day of rearing. A total of 100 blood serum samples were taken in each group of 50. The average data of biochemical studies are presented in Table 4.

Table 4. *Biochemical parameters of blood serum of broiler chickens in control and experimental poultry houses*

Indicators	Control (M±m, n=10)	Experimental (M±m, n=10)
Total protein, g/l	34.9±1.75	41.1±2.47
Albumin, g/l	14.6±0.40	15.8±0.53
Globulins, g/l	20.3±1.38	25.3±2.08
A/G ratio, units	0.76±0.03	0.65±0.05
Uric acid, µmol /l	297.7±31.14	326.3±33.80
Creatinine, µmol /l	36.1±2.74	33.2±3.35
AST, U/l	284.8±13.28	300.6±20.39
ALT, U/L	13.5±1.1	10.7±1.35
Ritis index, units	22.79±2.4	32.21±3.95
Alkaline phosphatase, U/L	4,117.7±393.4	3,895.6±576.5
Glucose, mmol/l	13.3±0.6	12.79±0.47
Total calcium, mmol/l	2.41±0.12	2.56±0.08
Inorganic phosphorus, mmol/l	3.89±0.41	3.65±0.23
Ca/P ratio	0.68±0.07	0.75±0.06
Vitamin A, mcg /g	74.99±12.74	88.08±9.1

Note: P ≤ 0.1 – there is a trend towards probable changes
Source: created by the authors

There is a tendency to increase the content of total protein, mainly due to globulin protein fractions. Such changes are characteristic of an increased antigenic load on the body of animals with a higher production of immunoglobulins. Despite a slight increase in globulin content, this resulted in a decrease in the protein coefficient. Higher levels of albuminous protein fractions may indicate better digestion of feed proteins and/or improved

liver function. No significant changes in other biochemical parameters were found.

The content of vitamin A in the liver of broiler chickens of both groups did not differ significantly with a slight tendency to increase in the experimental poultry. In general, both groups showed increased alkaline phosphatase activity, which is more typical of control poultry. Such changes may be a consequence of impaired calcium-phosphorus metabolism and indicate increased osteolysis processes. Other biochemical parameters in poultry of both groups are within the reference values. The results of determining the content of interferon-α in the cells of the 12 duodenum and cecum showed significant differences between the control group of broiler chickens and the experimental group of poultry (Fig. 3).

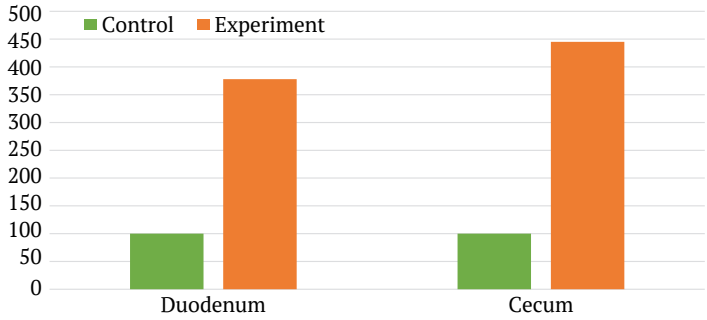

Figure 3. Results of immunoblotting and relative content of α-interferon in the duodenum and cecum of broiler chickens of research groups

Note: (M ± m, n = 3, P < 0.001) significance of changes compared to control
Source: created by the authors

A relatively moderate increase in the production of interferon-α by small intestinal cells indicates the stimulation of innate immunity mechanisms in the integumentary system of broilers kept under conditions of probiotic exposure. It should be noted that the production of interferon-α was 3.78 and 4.45 times higher in the 12 duodenum and in the cecum, respectively. Thus, probiotic exposure may contribute to the effectiveness of innate immunity by initiating the expression of cellular response genes that provide immune resistance and protection against infectious agents.

The results of determining the content of E-cadherin in the samples of the 12 duodenum showed a moderate increase of 1.22 times in the experimental group compared to the control group. No differences in the content of E-cadherin were found in the cecum samples. Taking into account the fact that E-cadherin provides intercellular adhesion of enterocytes and is a marker of epithelial cell density specific to epithelial cells, the results obtained indicate an increase in the integrative properties of the duodenum and the effectiveness of the integumentary barrier. It is the duodenum that provides the first and most critical protection against the invasion of enterogenic microorganisms and viruses, due to its forward localisation after the stomach. Thus, enhancing the efficiency of the barrier function through E-cadherin adhesion may help protect broiler chickens from enteropathogenic infections.

Considering all the above results, it can be concluded that the detected changes in markers of innate immunity and barrier function of the intestinal system under the conditions of probiotic action indicate a multidirectional protective effect that can contribute to the improvement of intestinal health in broiler chickens. The final stage of the research was the obtaining of statistical data on the main production indicators, which are presented in Table 5.

Table 5. *Production indicators of broiler farming*

Indicators	Live weight, g			Feed conversion y/y			Livestock conservation
	10 days	20 days	35 days	10 days	20 days	35 days	
Control group	238	820	2,086	1.094	1.344b	1.533b	96.5%
Experimental group	239	831	2,114	1.082	1.316a	1.508a	97.5%
Standard error of the mean	1.803	7.036	18.91	0.011	0.07	0.08	-
P-value	0.628	0.303	0.312	0.425	0.003	0.037	

Source: created by the authors

An increase in the preservation of broiler livestock in the experimental group was noted, which indicates the effectiveness of the probiotic preparation in preventing stress and infectious diseases and a positive effect on the immune system. The addition of investigational probiotic product improved the intestinal microbial balance and increased broiler productivity. Mortality during the day did not exceed 0.1% and the statistical data on broiler preservation were satisfactory. For feed conversion, statistically significant differences were observed in the period of 0-20 days and in the whole experiment. In particular, the positive effect of the investigational drug on the gastrointestinal tract and intestinal health was observed in the first 20 days of poultry rearing, when broilers experience a high intestinal load. The results of feed conversion obtained at the end of the experiment correspond to the recommended value for Ross-208 broilers (1.537 g/g).

When nutrient requirements are fully met and poultry are not exposed to physiological stress due to suboptimal rearing conditions and pathogens, the positive effect of probiotics may be less pronounced. This fact is the reason why there were no significant differences in poultry live weight between the experimental groups. However, regardless of the conditions of poultry rearing, the addition of the investigational drug to the feed significantly improved the biotechnology of poultry rearing.

Discussion

The issue of gut health and its resolution is a multifactorial research area. Studies on laboratory animals by A. Slawinska *et al*. (2021), K. Yu *et al.* (2021), and M. Paradowska *et al.* (2022) have proven that gut microorganisms directly influence the immunity of the respiratory tract and other immune system components. Inflammatory processes, as noted by H. Al-Khalaifa *et al.* (2019), S. Barbut & E. Leishman (2022), and K. Yue *et al*. (2024) represent the simplest defence mechanisms in poultry. Both theoretical analyses and experimental research have demonstrated that the impact of probiotics on the intestine involves several aspects, including histological changes (villus condition and density), microbiota (taxonomy, representative count, qualitative composition, presence, or absence of pathogenic microorganisms), intestinal feed content and its digestibility, and several other parameters. According to I. Ogbuewu *et al*. (2022) and N. Burkhardt *et al.* (2022), the development of immune defence systems in poultry intestines through symbiotic interaction with probiotics is a preventive measure against pathogens. Indirect evidence of the effect of probiotics on broiler health includes blood serum analysis, changes in biochemical indicators, immunoblotting, cytokine levels, leukocyte count, and more. A key argument for using probiotics in poultry farming is the feed conversion ratio (FCR), as improved intestinal function enhances nutrient absorption and reduces energy expenditure in fighting pathogens. Other production indicators, such as live weight and meat yield per square meter of poultry house area, may not be as conclusive. The safety of broiler flocks is directly related to resistance to infectious diseases and, therefore,

to gut health and the positive impact of probiotic cultures (Uazhanova *et al*., 2024). Data showed that broiler survival increased by 1% in the experimental group compared to the control group, confirming the probiotic efficacy of *Bacillus subtilis* (hay bacillus). Additional production indicators demonstrated the effectiveness of the probiotic supplement, and the use of "SVITECO-PWC" was accompanied by a significant improvement in feed conversion efficiency.

The study established a direct relationship between immunity and gut microbiota in broiler chickens. As described in the work of P. Cronin *et al.* (2021), gut-associated lymphoid tissue recognises pathogenic microorganisms without harming commensal microbes. According to F. Larsberg *et al.* (2023), immune functions also include the formation of B- and T-lymphocyte populations, immune response, and immune memory. Probiotics based on *Bacillus* promote the production of antimicrobial substances that inhibit the growth of pathogenic microorganisms. Moreover, the use of probiotics does not affect the withdrawal period for meat products. Other methods of preventing infectious diseases without antibiotics, such as acidifiers and plant extracts, as reported by B. Panea & G. Ripoll (2020) and J. Kowalczyk *et al*. (2020), do influence poultry meat quality and have a specific withdrawal period. In Ukraine, the transition from antibiotics to probiotics in livestock and poultry farming, according to A. Kolechko *et al.* (2023), is still ongoing, and the practical aspects of probiotic use require further refinement in agricultural production. The substances produced by bacteria of this genus include lichenysin (an antimicrobial peptide), bacteriocins, or bacteriocin-like compounds such as subtilin and coagulin. Bacteriocins are cationic (positively charged) peptides exhibiting hydrophobic or amphiphilic properties. The authors stated that a combination of bacterial strains from this genus effectively inhibits the most common poultry pathogens responsible for secondary infections following primary and viral infections, including aggressive pathogens such as Salmonella in chickens. The taxonomy of microorganisms (bacteria and coccidia) found in the intestines of agricultural poultry is directly linked to the continuous immune burden on broilers (Berezin *et al*., 2008). Pathogenic microorganisms, even in healthy poultry, force their bodies to constantly expend energy and structural resources to counter these pathogens. The balance and quantity of different gut microbiota representatives also play a significant role. Escherichia coli naturally supports broiler immunity but can become pathogenic if the domestic fowl's health weakens (Szeląg-Sikora *et al*., 2024). *Bacillus subtilis*, also known as hay bacillus, is not a natural microorganism in the broiler intestine and thus cannot become a pathogen. To utilise its unique properties, it was necessary to continuously administer supplements containing *B. subtilis* to maintain a high concentration in the gut, ensuring its ability to suppress other pathogenic microorganisms. Studies by X. Tang *et al.* (2021) demonstrate that the effectiveness of probiotics becomes evident only after prolonged use. Immediate improvements in production metrics such as flock survival rate, productivity index, feed conversion, average daily weight gain, and live weight per square meter of poultry house area do not occur. However, after two cycles of transitioning to probiotic supplements while discontinuing other treatments, improvements in these indicators were observed. The authors noted an increase in broiler weight during the first three weeks of growth. In later stages, the dynamic system of gut microbiota reached equilibrium. Thus, the population of *B. subtilis* reached a certain level and ceased to grow further. This resulted in a stable microbiome resistant to coccidia and *E. coli*, promoting nutrient absorption. The reduction of intestinal inflammation in broilers was indirectly confirmed through post-mortem examinations, where intestinal lesions were nearly absent in the experimental group.

In-depth studies by I. Ogbuewu *et al*. (2022), the authors noted a significant downregulation of genes associated with immune processes and inflammatory reactions in the gut – CCL4, IL-1β, IL-8, and LITAF. The author also observed a decrease in cytokine levels in broilers treated with probiotic supplements. At the same time, increased interferon production in the broiler intestine indicated an enhanced protective function, as observed in studies evaluating the effects of *B. subtilis*-containing supplements on the experimental group of broilers.

The ability of the small intestine to absorb feed substances is a separate component of the broiler's protection against stress and diseases of infectious and non-infectious origin (Montayeva *et al*., 2023). In addition to the prescribed feed ingredients, recipes include several other substances that play a therapeutic and prophylactic role in poultry metabolism. These are coccidiostatics that fight single-celled intestinal parasites, special vitamin and mineral supplements that are anti-stress agents and sorbents that adsorb toxins. Improving the functional properties of the intestine under the influence of probiotics allows all these drugs to be fully absorbed, which will certainly improve the immunity and health of poultry. Evidence of improved functional intestinal absorption of feed substances is provided by a biochemical study of broiler blood serum, which showed an improvement in protein metabolism.

An analysis of the mechanisms of interaction between Escherichia coli and the poultry intestinal microbiota showed that the therapeutic and prophylactic effect of probiotic preparations is selective. An increase in beneficial bacteria in the cecum and inhibition of the development and reproduction of pathogenic and opportunistic microbiota were observed. Adaptive protection of the intestinal microbiota partially relieves the burden on the poultry immune system and allows it to respond more easily to vaccination and form more stable immunity to vaccine pathogens (Daskalova *et al*., 2023). Indirect evidence of this is provided by the arguments of a pathological autopsy, which showed more frequent hyperplasia of lymphoid tissue, inflammation of the spleen and bursa in broilers of the control group. A direct proof of the effectiveness of the positive impact on the poultry immune system is the change in leukocyte activity, namely an increased percentage of CD4 + CD25T helper cells and other positive changes.

The investigational probiotic reduces the level of pathogenic microflora and the risk of disease in drinking water. Drinking water in poultry farming is a source of pathogens that develop in drinking systems. Veterinary drug residues and sugars contained in these residues are an ideal breeding ground for bacteria and fungi. The conglomeration of microorganisms creates a thin layer on the inside of the drinker line, which is called a biofilm. The development of the biofilm microorganisms is inhibited by the development of hay bacillus, which absorbs the nutrients contained in the biofilm and prevents pathogenic bacteria and fungi from obtaining these nutrients. The same process occurs when hay bacillus, together with poultry faeces, gets into the bedding. This allowed us to reduce the number of pathogens in it, namely the number of coccidia – single-celled intestinal parasites that suppress the immunity of poultry.

Conclusions

The established changes in morphological, cellular and molecular parameters comprehensively reflect the beneficial effect of the probiotic on the state and function of the small intestine of broiler chickens. Involvement of *E. faecalis* in the control poultry house is due to a more pronounced erosive and desquamative lesion of the intestine, while in the experimental group, signs of uneven epithelialisation of erosions were noted. The better condition of the intestinal wall may be associated with less pressure from *Eimeria aservulina* in experimental

chickens. The absence of *E. faecalis* in the experimental poultry, against the background of an increase in the population of enterococci in the blind intestines, indicates a better intestinal resistance compared to the control group.

The obtained results of determining molecular markers of innate immunity, density and intercellular connections of the intestinal epithelium indicate the presence of a protective effect of the probiotic aimed at improving the efficiency of barrier function and protection against enteropathogens. Thus, the complex of preventive treatments of broilers during rearing with the investigational probiotic preparations helps to increase the barrier and immune function of the intestine, as indicated by the results of immunoblotting (α-interferon), histological (uneven epithelialisation of erosive intestinal lesions) and biochemical (increased total protein content due to globulin fractions) studies. Such changes contribute to better absorption of feed proteins and vitamins, as indicated by an increase in blood albumin and vitamin A levels in the liver, and restructuring of the intestinal microbiome, which was reflected in a significant decrease in the number of *Eimeria aservulina* and *E. coli* bacteria against the background of increased colonisation by enterococci and *Clostridium perfringens*.

However, the study of the immune effect of probiotic preparations on broilers should include a more thorough study of CD4 + T helper cell proliferation, CD28 + αβ T cell activation and other studies of leukocyte activity, which has not been carried out. In addition, SIRION has products for washing poultry houses, aerosol treatment of poultry, and products for the sanitation of poultry drinking systems, the effect of which has not been studied.

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Conflict of Interest

None.

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Використання пробіотичного препарату «SVITECO-PWC» при вирощуванні курчат-бройлерів

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Анотація. Метою дослідження було виявлення змін у імунній системі кишечника курчат-бройлерів під впливом пробіотичного препарату на основі *Bacillus subtilis* як інструменту поліпшення технологічних результатів вирощування. Сформовано дві дослідницькі групи: пташник, де давали препарати згідно зі звичайною превентивною програмою (контроль), і пташник, де давали пробіотичний препарат на основі *Bacillus subtilis* «SVITECO-PWC». Методи дослідження включали: патоморфологічне дослідження (розтин), мікробіологічні дослідження тушок курчат-бройлерів, кількісні мікробіологічні дослідження мікрофлори сліпих кишок, полімеразну ланцюгову реакцію у тканинах кишечника та у підстилці, біохімічне дослідження показників сироватки крові курчат-бройлерів, визначення вмісту інтерферону-α у клітинах 12-палої та сліпої кишки, визначення вмісту Е-кадгерина у зразках 12-палої кишки. Проведено статистичний аналіз виробничих показників: живої маси, конверсії корму, збереження поголів'я бройлерів. За результатами розтину виявлено, що основним захворюванням дослідженої птиці обох груп був ерозивно-десквамативний гастроентероколіт, але у птахів дослідної групи спостерігалася тенденція до меншого

ерозивного ураження тонкого і товстого кишечнику; кількість бактерій *E. coli* в сліпих кишках дослідної групи в середньому була нижча, ніж в контрольній, гомогенність показника також була вища в дослідній групі. Застосування пробіотичної суміші сприяло достовірному зменшенню в кишечнику бройлерів кількості *Eimeria acervulina* та посилювало колонізацію кишок *Clostridium perfringens*, що обумовило збільшення кількості останньої у підстилці, покращення білкового обміну і кількості інтерферону в дослідній групі. Висновками роботи було те, що додавання досліджуваного пробіотичного препарату в превентивну програму підвищувало кишковий імунітет бройлерів та виробничі показники

Ключові слова: бройлер; *Bacillus subtilis*; імунна система кишечнику; мікробіом

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Influence of the type of livestock by-products on biogas yield and composition

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Abstract. The study of the effects of various types of animal waste on the quantity and composition of biogas is significant and relevant for optimising anaerobic fermentation processes, increasing the efficiency of biogas production and adapting technologies to farm conditions. The purpose of this study was to evaluate the effects of livestock by-products,

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specifically cattle manure, pig manure, and chicken manure, on the quantity and quality of biogas produced. The methods employed in the study included statistical analysis, gas analysis, and fermentation. The study analysed the physicochemical properties of several types of raw materials for biogas production. The study found that chicken manure had the highest potential for biogas production due to its high content of volatile solids (25-30%) and the optimum ratio of methane in the biogas composition (65%). Cattle manure was characterised by a stable average biogas yield (0.15-0.18 m^3/kg volatile solids in feedstock (VT, %)), while pig manure had the lowest yield (0.12-0.14 m^3/kg volatile solids in feedstock). According to the study results, the addition of carbonaceous materials (e.g., chopped straw) improved the carbon to nitrogen ratio to optimise the fermentation process. The analysis of the organic matter content before and after fermentation revealed a significant decrease for chicken manure (51%), which indicated the effectiveness of biodegradation. The study included an assessment of the composition of biogas, including methane (50-65%), carbon dioxide (30-40%), and hydrogen sulphide (1-3%). The change in pH in all types of raw materials after fermentation indicated that the environment in the bioreactors had stabilised, providing favourable conditions for microorganisms. The findings of this study can be used in practice by ecologists, agronomists, livestock technologists, and biogas producers to create energy-independent farms through the integration of biogas plants into farms

Keywords: waste; manure; chicken manure; bioreactor; methane; carbon dioxide; fermentation

Introduction

Regular research into methods of optimising the processing of organic livestock waste is significant for ensuring energy efficiency and the long-term development of the agricultural sector of Ukraine's economy. As one of the principal problems of modern agriculture, the problem of utilisation of livestock by-products, such as chicken manure, requires research with maximum economic and environmental benefits in focus. The problem of the study is that the lack of sufficient understanding of the effects of the type of livestock by-products on the yield and composition of biogas makes complicates the optimisation of anaerobic digestion processes. According to V. Shmatenko (2024), distinct types of feedstocks significantly affect the efficiency of fermentation, which determined the quantity and quality of biogas produced. The existence of the problem is confirmed by the fact that when using feedstocks with a high content of organic matter, such as chicken manure, there was a need to adjust the carbon:nitrogen (C: N) ratio to achieve the best conditions for the biogas fermentation process.

A prominent aspect is the problem of reducing nitrogen and phosphorus emissions into the environment for pig production, as it has reduced the environmental impact of the industry and improved the efficiency of nutrient use in animal production. S. Zinoviev & M. Pushkina (2023) investigated this issue. Their study proved that the use of multiphase feeding systems and optimisation of the amino acid composition of diets can improve the efficiency of nutrient absorption by pigs. A significant issue is the need for environmentally safe disposal of organic waste (Muminova *et al.*, 2023). In this regard, Y. Palamarenko & I. Chikov (2023) investigated the effects of various methods of organic waste processing on the environment. According to their findings, the use of biogas plants for the disposal of organic waste has reduced greenhouse gas emissions. The development of biogas technologies is a topical issue for modern environmentalists. S. Tkachenko *et al.* (2020) studied the efficiency

of biogas plants and their effects on the environmental situation. The researchers proposed a technology for using agricultural by-products for biogas production. Their study showed that this technological innovation has enabled the reduction of greenhouse gas emissions and contributed to the development of energy production by renewable energy facilities.

The replacement of natural gas consumption with alternative energy sources has become particularly significant due to the threat of an energy crisis caused by the military conflict and the possible termination of Russian gas supplies (Moshenskyi *et al.*, 2024; Strokal *et al.*, 2024). According to G. Geletukha *et al*. (2022), biomethane is a promising alternative to natural gas that has a wide range of applications, including energy production and raw materials for the chemical industry. Aspects of the environmental and economic assessment of the introduction of bioenergy technologies in the context of reducing anthropogenic and military risks and improving energy security of Ukraine were investigated by V. Dudin *et al*. (2024). As a result, they analysed current trends in bioenergy, modelled technological and economic parameters of biogas plants.

The problem of intensification of biogas production processes stays urgent in the context of growing demand for renewable energy sources and the need for efficient waste processing, as proved by V. Chubur *et al*. (2022). According to their findings, the combination of physical and chemical pretreatment methods, such as cavitation and electrolysis, greatly increased the efficiency of anaerobic digestion. One of the key environmental issues is the rational use of organic and mineral fertilisers, as well as the disposal of livestock waste (Kravchenko & Bykova, 2023). R. Lohosha *et al.* (2023) investigated the effects of various fertilisation systems on the yield of maize and red beet. The researchers found that the use of artificial fertilisers in combination with high doses of bio-organic fertilisers, specifically digestate, provided a considerable increase in yields. The issues related to optimising the anaerobic co-digestion of pig manure and maize stalks to increase the efficiency of biogas and methane production are still significant in the context of growing demand for renewable energy sources, the necessity of recycling organic waste and reducing greenhouse gas emissions, as proven by H. Wang *et al*. (2020). The researchers found that the addition of maize stalks to pig manure during anaerobic co-digestion increased microbial diversity.

The study of unexplored aspects of increasing the efficiency of anaerobic digestion, improving biogas purification technologies and optimising the use of various types of raw materials requires a comprehensive approach in modern conditions. K. Obileke *et al.* (2024), T. Manushkina *et al.* (2024) recommended the development of technologies aimed at reducing greenhouse gas emissions, such as the introduction of renewable energy sources and technologies for the extraction of carbon dioxide (CO_2) from industrial sources.

Studies have not paid sufficient attention to the aspects of using alternative energy sources in combination with innovative organic waste treatment technologies. The purpose of this study was to investigate the effects of types of livestock by-products on the yield and composition of biogas, considering the varying physical and chemical properties of the raw materials used. The objectives were to determine the effects of distinct types of organic livestock waste on the amount and composition of biogas; to assess the effectiveness of adding carbonaceous materials to optimise the C: N ratio in the feedstock; to analyse the effects of the physical and chemical properties of waste on the performance of the anaerobic fermentation process.

Materials and Methods

The sampling of raw materials for biogas production took place on farms specialising in cattle (Dairy Alliance Company in Kyiv region),

pigs (Podilsky Bacon Farm in Khmelnytsky region) and poultry (Vinnytsia Poultry Farm in Vinnytsia region). The study was conducted in 2023-2024 in accredited laboratories of the State Service of Ukraine for Food Safety and Consumer Protection and the State Agency on Energy Efficiency and Energy Saving of Ukraine, which have the relevant permits for biogas analysis.

During the preparation of the raw materials, the livestock by-products were sieved to remove large solid inclusions. Subsequently, water was added to obtain the same dry residue (10% dry weight). After the selection of raw materials for each type of livestock by-product, the key physicochemical parameters were analysed in the laboratory: moisture content (%) was determined according to the method of drying to constant weight; volatile solids content (VT, %) was determined as a proportion of organic matter; C:N ratios were measured using a CHNS analyser. For each portion of carbonaceous materials, the weight of the additive was determined as follows (1):

$$m_{straw} = \frac{N_{initial} \times C{:}N_{desired}}{C_{straw}} - C_{initial}, \quad (1)$$

where $N_{initial}$ is the initial nitrogen content in the substrate, g/kg; $C{:}N_{desired}$ is the desired carbon to nitrogen ratio in the substrate; C_{straw} is the carbon content in straw, g/kg; $C_{initial}$ is the initial carbon content in the substrate, g/kg.

When calculating the biogas yield, the theoretical biogas yield for each type of feedstock was estimated based on the organic matter content (2):

$$V_{theoretical} = VT \times K_{degradation} \times 0.35\ m^3/kg, \quad (2)$$

where $V_{theoretical}$ is the theoretical volume of biogas, m^3; VT is the mass of volatile solids in the feedstock, kg; $K_{degradation}$ is the degradation coefficient of organic matter (0.4-0.6 for solid organic matter), 0.35 m^3/kg is the coefficient responsible for the volume of biogas produced by the decomposition of 1 kg of volatile matter.

The organic degradation factor was defined as the percentage of volatile solids decomposed (3):

$$K_{degradation} = \frac{VT_{initial} - VT_{final}}{VTinitial} \times 100\%, \quad (3)$$

where $K_{degradation}$ is the percentage of volatile solids decomposition; $VT_{initial}$ is the initial volatile solids content in the raw material, %; VT_{final} is the final volatile solids content after fermentation, %.

Preliminary alkaline treatment of the feedstock with sodium alkali (NaOH) solution and the use of a hydraulic shredder press to mechanically grind the materials under high pressure before fermentation can improve the efficiency of the biogas production process. A gas analyser was used to determine the biogas composition on a daily basis. The methane (CH_4) concentration in the biogas was estimated by chromatography, carbon dioxide (CO_2) – by infrared spectroscopy, and hydrogen sulphide (H_2S) – by spectrophotometry

The following equipment and facilities were used to determine the fermentation performance and evaluate the efficiency of using different types of raw materials: BioFlo laboratory anaerobic bioreactor with controlled temperature (37 °C to optimise methanogenesis) (USA), biogas collection system equipped with a gas meter and gas analyser EnviTec Biogas GmbH (Germany), Shimadzu UV-1800 spectrophotometer for biogas composition measurements (Japan), Agilent 7890A gas chromatograph (USA) for accurate analysis of CH_4, CO_2, and H_2S concentrations.

Research methods were employed to investigate the effects of the type of livestock by-products on the yield and composition of biogas, as established by legislative acts: State Standard of Ukraine (DSTU) ISO No. 11722:2004 “Solid mineral fuels. Hard coal. Determination of moisture in a sample for general analysis by the nitrogen drying method” (2005), DSTU ISO No. 5725-4:2005 “Accuracy (correctness and precision) of measurement methods and results” (2005).

The study employed the method of statistical analysis of data (analysis of variance (ANOVA)) obtained during the evaluation of the physical and chemical properties of distinct types of raw materials to compare the biogas yield and methane composition for each type of by-product. Two-way ANOVA was used in the study. To determine the statistical significance of the findings, the p-value was used, set at 0.05, meaning that $p < 0.05$ is considered statistically significant, i.e., the difference between the groups is significant.

A gas analysis and fermentation method for determining biogas yield and assessing its composition. The effect of fermentation time on gas yield was analysed by regression analysis. The average fermentation time for raw materials (organic waste, food residues, manure) was 30-45 days. For anaerobic fermentation, the optimum temperature ranged within 35-40 °C. The optimum pH for anaerobic fermentation was 6.5-7.5. Stirring was carried out 1-2 times a day or as needed to avoid sedimentation of the material. Each experiment with distinct types of raw materials was repeated 3-5 times. To ensure an optimum start of fermentation, a starter containing special anaerobic bacteria was used. The starter was obtained from previous experiments or from industrial biogas plants. 10-20% of the starter was added to 1 litre of substrate.

Results

The study described the characteristics of various types of feedstocks for biogas production, as well as the calculation of the expected biogas yield. Evaluation of the chemical and physical properties of the feedstock helped to plan the fermentation process efficiently and provide optimised conditions for the biogas plant. Carbon additives, specifically chopped straw, were used to improve the C:N ratio in the feedstock, which contributed to the optimisation of the fermentation process and increase the biogas yield (Havrysh *et al.*, 2020). Table 1 provides data on the main physical and chemical parameters of the feedstock before loading into the biogas plant for further calculations of biogas yield, as well as determining the necessary corrective measures to optimise the fermentation process.

Table 1. *Characteristics of raw materials before loading*

Indicator	Cattle manure	Pig manure	Chicken manure
Humidity (%)	75-80	70-75	60-65
Volatile solids (VT, %)	20-25	18-22	25-30
Carbon: nitrogen (C:N)	25:1	18:1	10:1

Source: compiled by the authors

The analysis of the feedstock showed that each type of waste had specific physicochemical characteristics that could affect the fermentation process: the highest moisture content (75-80%) was found in cattle manure, which required additional measures to regulate the consistency; the highest volatile solids content was observed in chicken manure (25-30%), which indicated its high potential in the biogas process; for chicken manure (10:1), an imbalance of C:N ratio was found, which negatively affected the fermentation, so carbonaceous materials such as chopped straw were added to improve this indicator (Formula 1). The desired C:N ratio is considered to be 20:1 to 30:1. For cattle manure, calculations were made using formula (1).

The initial nitrogen content ($N_{initial}$) was 1 g/kg (based on standard data for chicken manure). The desired C:N ratio ($C{:}N_{desired}$) was 20:1 (selected for optimum fermentation). The carbon content of the straw (C_{straw}) was 450 g/kg (approximate value). The initial carbon content of cattle manure ($C_{initial}$) was 250 g/kg (standard

value for cattle manure). The values of formula (1) were substituted into the equation:

$$m_{straw} = \frac{1 \times 20}{450 - 250} = \frac{20}{200} = 0.1.$$

Therefore, to achieve the desired C:N ratio of 20:1, 0.1 kg of straw should be added for each kilogram of cattle manure. For pig manure and chicken manure, the same formula (1) was used to calculate the ratio.

The initial nitrogen content ($N_{initial}$) was 1.5 g/kg (standard value for pig manure). The desired C:N ratio ($C{:}N_{desired}$) was 20:1 (selected for optimum fermentation). The carbon content of the straw (C_{straw}) was 450 g/kg (approximate value). The initial carbon content of pig manure ($C_{initial}$) was 300 g/kg (standard value for pig manure). The values of formula (1) were substituted into the equation:

$$m_{straw} = \frac{1.5 \times 20}{450 - 300} = \frac{30}{150} = 0.2.$$

The calculation shows that 0.2 kg of straw should be added for every kilogram of pig manure. The initial nitrogen content ($N_{initial}$) was 1 g/kg (based on standard data for chicken manure). The desired C:N ratio ($C{:}N_{desired}$) was 20:1 (chosen for optimum fermentation). The carbon content of the straw (C_{straw}) was 450 g/kg (approximate value). The initial carbon content of the chicken manure ($C_{initial}$) was 300 g/kg (standard value for chicken). The values of formula (1) were substituted into the equation:

$$m_{straw} = \frac{1 \times 20}{450 - 300} = \frac{20}{150} = 0.133.$$

Thus, to achieve the desired C:N ratio of 20:1, 0.133 kg of straw should be added for each kilogram of chicken manure. Based on the analysis of the physicochemical properties of distinct types of biogas feedstocks, the expected biogas yield was calculated for each of them. These data can be used to assess the potential of different substrates in the biogas process and determine the best conditions for their use. The calculations results presented in Table 2 show the values of biogas yield depending on the type of raw material.

Table 2. *Expected biogas yields*

Raw material	Expected biogas yield (m^3/kg VT)
Cattle manure	0.15-0.18
Pig manure	0.12-0.14
Chicken manure	0.2-0.25

Note: biogas yield was calculated using the formula (2)
Source: compiled by the authors

The calculation of the expected biogas yield demonstrated the following key aspects: chicken manure provided the highest biogas yield (0.2-0.25 m^3/kg VT), and therefore this component became a promising feedstock for biogas plants. Cattle manure had an average biogas yield (0.15-0.18 m^3/kg VT), which required optimisation of fermentation conditions. Pig manure was characterised by the lowest biogas yield (0.12-0.14 m^3/kg VT), but it can be effectively used in mixed substrates. The study confirmed the expediency of factoring in the physicochemical properties of the feedstock to improve the efficiency of the biogas process.

For optimum biogas production, chicken manure should be used in combination with carbon additives (e.g., straw). To minimise the impact of H_2S, excessive use of pig manure should be avoided or adsorbents (e.g., iron oxide) should be used. For efficiency, large farms can combine several types of feedstocks for

a stable C:N ratio (Havrysh *et al.*, 2019). The data on the volume of biogas produced from each type of organic feedstock after 30 days of fermentation were presented. After 30 days of fermentation in each bioreactor, the parameters listed in Tables 3, 4, 5, and 6 were evaluated.

Table 3. *Biogas yields from different types of raw materials*

Raw material	Biogas volume (m^3/kg VT)
Cattle manure	0.16
Pig manure	0.13
Chicken manure	0.23

Source: compiled by the authors

After 30 days of fermentation, chicken manure showed the highest biogas yield (0.23 m^3/kg VT), indicating its higher efficiency as a feedstock for biogas production compared to cattle manure (0.16 m^3/kg VT) and pig manure (0.13 m^3/kg VT). Table 4 shows the content of the key components of biogas collected from each type of feedstock. The results are presented in percentage terms for methane (CH_4), carbon dioxide (CO_2) and hydrogen sulphide (H_2S).

Table 4. *Composition of biogas by components*

Component	Cattle manure (%)	Pig manure (%)	Chicken manure (%)
Methane	60	55	65
Carbon dioxide	38	40	33
Hydrogen sulphide	1	3	2

Source: compiled by the authors

According to the findings of the study, chicken manure had the highest methane content (65%) and, accordingly, the feedstock demonstrated an advantage in terms of biogas energy value. Cattle manure provided an average biogas yield with the lowest H_2S content (1%), which reduced the need for additional treatment. Pig manure contained the highest level of H_2S (3%), which required additional measures to clean the biogas from impurities. Table 5 shows the dynamics of biogas yield over three 10-day fermentation periods. The data shows how the volume of biogas changed over time for each type of feedstock.

Table 5. *Dynamics of biogas yield in different fermentation periods*

Period (days)	Cattle manure (m^3/day)	Pig manure (m^3/day)	Chicken manure (m^3/day)
1-10	0.04	0.03	0.05
10-20	0.07	0.05	0.09
20-30	0.05	0.04	0.07

Source: compiled by the authors

The highest average daily biogas yield was observed for chicken manure during all days of fermentation, with a maximum in the second period (0.09 m^3/day). Cattle manure showed an average level of dynamics, while pig manure had the lowest biogas yields, especially in the

first period (0.03 m^3/day). Table 6 presents the volatile solids before and after fermentation, as well as the calculated organic matter degradation factor for each type of feedstock.

Table 6. *Degradation factor of organic matter (volatile solids, VT)*

Raw material	VT before fermentation (%)	VT after fermentation (%)	Degradation rate (%)
Cattle manure	25	11	55
Pig manure	22	11	50
Chicken manure	30	12	60

Note: organic degradation factor was determined by the established formula (3)
Source: compiled by the authors

Chicken manure showed the highest organic matter degradation rate (60%), indicating its high biodegradability. Cattle manure had an average degradation factor (55%), while pig manure had the lowest (50%), which could affect the efficiency of processing. Chicken manure showed the highest biogas yield and methane content due to its high organic matter content, optimum C:N ratio and high organic matter degradation factor. Pig manure had the lowest yield due to its high sulphur content, which contributed to the formation of the H_2S component. Cattle manure provided a stable average gas yield but required balancing to improve the methane ratio. Removal of H_2S is necessary to protect equipment and reduce emissions. Chicken manure could cause ammonia accumulation, which required adjustment of the substrate concentration. Biogas production from chicken manure is the most cost-effective due to the high methane yield. Cattle manure has proved to be a suitable feedstock for biogas production and economically viable for farms with large livestock.

Table 7. *Distribution of organic matter before and after fermentation*

Raw material	Organic matter before fermentation (%)	Organic matter after fermentation (%)	Difference (%)
Cattle manure	80	36	44
Pig manure	78	39	39
Chicken manure	85	34	51

Source: compiled by the authors

Prior to the biofermentation process, it is vital to assess the organic matter content of distinct types of feedstocks, as this is a key indicator for the efficiency of the biogas process. After fermentation, the organic matter is partially decomposed and some of it is converted into biogas. Table 7 shows the changes in the organic matter content of the feedstock types under study before and after fermentation.

Changes in organic matter content after fermentation are significant for all types of feedstocks. The largest decrease in organic matter was observed in chicken manure (51%), suggesting a high level of decomposition of organic compounds during fermentation. For effective fermentation, it is vital to understand changes in the chemical composition of the substrate, specifically pH and C:N ratio. These parameters affect the activity of microorganisms that decompose organic matter, as well as the final quality and quantity of biogas. Table 8 shows the changes in pH and C:N ratio before and after fermentation for each type of feedstock.

Table 8. *Chemical characteristics of the substrate before and after fermentation for three types of raw materials*

Raw material	pH before fermentation	pH after fermentation	C:N before fermentation	C:N after fermentation
Cattle manure	7.2	7.8	25:1	15:1
Pig manure	7	7.5	18:1	12:1
Chicken manure	6.8	7.4	10:1	7:1

Source: compiled by the authors

The increase in pH after fermentation indicated the stabilisation of the substrate, which reduces the risk of developing an acidic environment in the biogas plant. Changes in the C:N ratio showed an improvement in the conditions for microorganisms responsible for the decomposition of organic matter, which may contribute to a better activity of the microbiological process. The energy efficiency of biogas depended on the amount of methane produced during fermentation and its calorific value. As methane is the principal energy component of biogas, its amount directly affected the energy yield. Table 9 shows the energy efficiency of biogas from distinct feedstock types based on the amount of methane and its calorific value.

Table 9. *Energy efficiency of biogas*

Raw material	Volume of methane (CH_4) m^3/kg VT	Calorific value (kWh/m^3 CH_4)	Energy output (kWh/kg VT)
Cattle manure	0.1	9.94	0.994
Pig manure	0.07	9.94	0.696
Chicken manure	0.15	9.94	1.491

Source: compiled by the authors

Chicken manure demonstrated the highest energy yield (1.491 kWh/kg VT), and therefore it should be considered as the most efficient feedstock for biogas plants among the types under study. Components in biogas, such as H_2S and ammonia (NH_3), can be harmful to health and to biogas plant equipment. It is therefore crucial to monitor their content during the fermentation process and in the final biogas. Table 10 shows the level of harmful components in biogas produced from distinct types of raw material.

Table 10. *Environmental indicators (content of harmful components in biogas)*

Component	Cattle manure (%)	Pig manure (%)	Chicken manure (%)	Permissible level (%)
H_2S	1	3	2	≤1
NH_3	0.8	1.5	2.1	≤1

Source: compiled by the authors

The level of harmful components in biogas varied depending on the type of feedstock. Pig manure had the highest level of H_2S – 3%, which required additional biogas treatment to reduce its negative effects, while cattle manure and chicken manure had lower levels of harmful components in biogas. Temperature is one of the most significant factors affecting the speed of biological fermentation processes. Since temperature conditions change the activity of

microorganisms, this can substantially affect the amount and composition of biogas. Table 11 shows the dependence of biogas yield on fermentation temperature for each type of feedstock.

Table 11. *Biogas yield depending on the fermentation temperature*

Temperature (°C)	Cattle manure (m^3/kg VT)	Pig manure (m^3/kg VT)	Chicken manure (m^3/kg VT)
25	0.14	0.11	0.2
30	0.16	0.13	0.23
35	0.18	0.14	0.25
40	0.17	0.13	0.24

Source: compiled by the authors

The temperature of 35 °C is optimal for fermentation of all types of feedstocks, providing maximum biogas yield. Increasing the temperature to 40 °C resulted in a decrease in biogas volumes, probably due to the inhibition of microbial activity. The biogas yield from chicken manure was the highest among the studied feedstock types at all mentioned temperatures. The CH_4 and CO_2 content in biogas is a significant indicator, since methane is the principal energy component, while carbon dioxide is a product of decomposition of organic matter. The ratio of these gases can give an indication of the efficiency of the methanogenesis process. Table 12 shows the ratio of methane:carbon dioxide (CH_4:CO_2) in biogas produced from distinct types of raw material.

Table 12. *CH_4:CO_2 ratio in biogas*

Raw material	CH_4:CO_2 ratio
Cattle manure	1:0.6
Pig manure	1:0.7
Chicken manure	1:0.5
Optimal ratio	1:0.6 or higher

Source: compiled by the authors

Chicken manure showed the highest methane content compared to other feedstocks, making it the most efficient source of biogas. The high CO_2 content of biogas from pig manure may require additional treatment to improve energy efficiency. The time to reach the maximum biogas yield is another significant factor that determines the efficiency of the fermentation process. The time required to reach the highest level of biogas yield can vary depending on the type of feedstock under study. Table 13 shows the time to reach the maximum biogas yield depending on the type of feedstock used.

Table 13. *Time to reach the maximum biogas yield*

Raw material	Time to maximum biogas yield (days)
Cattle manure	22
Pig manure	18
Chicken manure	20

Source: compiled by the authors

Pig manure provided the fastest achievement of the maximum biogas yield in 18 days, which positively affected the efficiency of fast biogas processes. According to the study

results, cattle manure and chicken manure showed a longer fermentation period, which may affect the overall efficiency of biogas production. The effect of mechanical shredding or chemical treatment on biogas yield is vital for process optimisation. Table 14 shows the effects of raw material pretreatment on biogas yield.

Table 14. *Effects of raw material pretreatment on biogas yield*

Raw material	No treatment (m^3/kg VT)	After mechanical shredding with a hydraulic shredder press device (m^3/kg VT)	After chemical treatment with NaOH solution (m^3/kg VT)
Cattle manure	0.16	0.18	0.19
Pig manure	0.13	0.14	0.15
Chicken manure	0.23	0.26	0.28

Source: compiled by the authors

Pretreatment with NaOH solution significantly increased biogas yields for all feedstock types. Chicken manure showed the largest increase in biogas yield after mechanical grinding (13% increase) and chemical treatment (22% increase). These data confirmed the significance of pretreatment to increase the efficiency of the fermentation process. The fermentation temperature directly affected the biogas yield. The highest yield was observed at 35 °C, after which the fermentation efficiency began to decrease. For all feedstock types, the CH_4:CO_2 ratio varied, with the highest methane content in the biogas produced from chicken manure. Pig manure reached the maximum biogas yield in 18 days, indicating an acceleration of the fermentation process compared to other feedstocks. Mechanical and chemical treatment of the feedstock significantly increased the biogas yield, which is essential for increasing the efficiency of biogas plants.

Discussion

According to the findings of this study, chicken manure is the most promising raw material for biogas production due to its high methane yield, optimum carbon to nitrogen (C:N) ratio (ensured by the addition of carbonaceous materials), and high degradation coefficient of organic matter. One of the most pressing problems is the formation of undesirable impurities such as H_2S. Adjustment of the substrate composition, maintenance of the optimum pH level and control of the C:N ratio are possible with the combined use of such raw materials as chicken manure with the addition of straw or cattle manure. It was found that pig manure had high levels of hydrogen sulphide, which required additional measures aimed at treating the biogas to reduce its harmful effects. K. Akamati *et al*. (2022) raised an analogous issue. The researchers found that rational changes in feed composition reduced the H_2S content of biogas, and effective management of manure treatment systems, including aeration or the use of chemical inhibitors, significantly reduced the level of hydrogen sulphide and greenhouse gas emissions, contributing to a reduction in impact. This statement can be agreed with, as the concentration of H_2S in biogas can actually depend on the composition of feed and animal housing conditions. Changes in the diet can genuinely affect the metabolic processes in the animal body, altering the chemical composition of the manure and the level of H_2S in the biogas (Golub *et al.*, 2020).

The present study noted that each type of biogas feedstock had unique physical and chemical characteristics that significantly affected the biogas yield and composition. Specifically, chicken manure demonstrated the highest biogas yield and optimum methane content

due to the high level of decomposed organic material. A. Uwizeye *et al*. (2019) investigated an analogous issue. The researchers found that the overall level of methane emissions in major pig meat-producing countries fluctuated depending on changes in livestock production methods and management, with an increase in methane emissions from enteric fermentation in Spain. The findings obtained in the present study differed from the conclusions presented by A. Uwizeye *et al*. (2019), since the present study focused on the physicochemical characteristics of the feedstock that determine biogas yield, which was distinct from the approaches proposed by researchers in analysing methane emissions in the context of specific livestock production methods. The authors' study focused on factors related to livestock management and agricultural practices.

Chicken manure proved to be the most promising feedstock for biogas production due to its high methane yield and organic matter degradation factor, making it an economically viable and energy-efficient source for biogas plants. S. Singh *et al*. (2024) covered this subject in their study, showing that methanogenic bacteria in anaerobic mull, cattle rumen and manure played a significant role in the decomposition of organic matter and methane production. The present study reached analogous conclusions, as the findings revealed that methanogenic bacteria are essential for the breakdown of organic matter, which confirmed the feasibility of using anaerobic methanogenesis for the treatment of organic waste.

According to the findings of the present study, it is known that chicken manure has become the best feedstock for biogas production due to its high content of volatile solids and high methane yield, which ensured the highest biogas production. S. Chozhavendhan *et al*. (2020) also studied this problem. The researchers found that biogas production technology proved to be effective in converting renewable energy sources such as agricultural, livestock, industrial, and municipal waste into a clean form of energy. Their study confirmed that biogas technology can reduce greenhouse gas emissions and promote development by efficiently utilising renewable resources and reducing dependence on fossil fuels.

It was noted that the physicochemical properties of distinct types of raw material, specifically moisture content, volatile solids content, and C:N ratio, positively affected the biogas fermentation process. C. Mutate *et al*. (2023) found that feedstocks with an optimum C:N ratio, as well as high levels of organic matter, provided the best fermentation results and biogas yields. This statement can be agreed with, as a high content of organic matter provides sufficient energy for microorganisms, which stimulates anaerobic fermentation and leads to a high biogas yield (Kucher *et al*., 2022).

It was found that temperature fluctuations and the type of feedstock positively affect the methane production, which was significant for the efficiency of biogas technologies. N. Lovanh *et al*. (2023) pointed to the efficiency of using livestock waste, specifically wastewater from poultry and dairy manure processing, for biogas production, including methane. N. Lovanh *et al*. (2023) focused on the efficiency of agricultural waste processing for biogas production, while the present study covered three principal types of biogas feedstocks and compared their efficiency in terms of physical and chemical characteristics.

Cattle manure showed an average level of biogas yield, but it was necessary to correct its moisture content and C:N ratio to increase fermentation efficiency. L. Dong *et al*. (2019) found that the plunger reactor provided stable production of high-quality biogas under conditions of waste disposal with hydraulic retention for 25 days, temperature conditions of 37-40°C, and a 7-10% concentration of solids in the substrate. The statement of L. Dong *et al*. (2019) can be agreed with, as the researchers confirmed the effectiveness of large-scale

bioreactors for the treatment of organic waste, specifically cattle manure, which led to an increase in biogas and methane production under optimum conditions of hydraulic retention and temperature.

After mechanical grinding and chemical treatment of chicken manure, an increase in biogas yield was found (by 13% and 22%, respectively). I. Mahmoud *et al*. (2022) found that the co-digestion of sludge and raw chicken manure increased the total biogas production and improved the sludge treatment process. The findings of the present study showed an increase in biogas yield after pretreatment and grinding of the feedstock, while I. Mahmoud *et al*. (2022) employed another technology and accordingly indicated that the increase in biogas yield was achieved through co-digestion of sludge and chicken manure.

It is recommended to use chicken manure in combination with carbon additives (straw), which is necessary to obtain maximum biogas yield. E. Orhorhoro & O. Oghoghorie (2024) concluded that the highest level of biogas yield was observed when chicken manure was co-digested with seaweed. The present study obtained partially analogous findings to those of E. Orhorhoro & O. Oghoghorie (2024), which showed that the use of an organic additive in the form of seaweed was effective in increasing biogas productivity. However, in the present study, the greatest effect was observed when chicken manure was combined with various additives, including carbon-rich straw, which created favourable conditions for microbial activity and optimisation of the carbon to nitrogen ratio.

This study found that the use of chicken manure produced the highest proportion of methane compared to other types of raw materials, making it the most efficient source of biogas. J. Di Mario *et al*. (2024) found that the use of untreated olive mill wastewater for biogas production led to an increase in biogas yield. The statement of J. Di Mario *et al.* (2024) can be agreed with, because according to scientific data, wastewater contains organic compounds that can be broken down by microorganisms during anaerobic fermentation, which leads to an increase in the content of methane and other biogas components.

Chicken manure has the highest proportion of methane compared to other types of raw materials. M. Ajao *et al.* (2024) showed that the addition of silica nanosupplementation increased the amount of methane in cow and sheep manure biogas. The statement of M. Ajao *et al.* (2024) can be agreed with, because according to scientific data, the added nanosupplement can have a catalytic effect, stimulating biochemical reactions that increase the amount of methane yield in biogas systems.

It was found that chicken manure showed the highest biogas yield after 30 days of fermentation, which indicated its greater efficiency as a feedstock for biogas production compared to cattle manure and pig manure. O. Ojo (2022) presented the results, according to which poultry manure was the most effective feedstock for biogas production compared to cow and pig manure, as it provided the highest volume of biogas production and high level of gas production. The findings of O. Ojo's (2022) work can be agreed upon, as analogous studies confirm that poultry manure has a high potential for biogas fermentation due to its high content of easily digestible organic compounds such as proteins and fats. This is why better gas production rates were achieved compared to the other two types of manure studied, which contain a high amount of cellulose and are more difficult to break down during fermentation.

The study of the content of harmful components in biogas revealed that cattle manure and chicken manure had a lower content of harmful components in biogas compared to pig manure contaminated with hydrogen sulphide, which contributed to the reduction of anthropogenic emissions. Z. Akyürek (2023) found that the use of livestock waste for biogas production helped reduce greenhouse gas

emissions. The researchers' conclusions should be accepted, as the use of livestock waste for biogas production does have benefits for reducing harmful emissions, including greenhouse gases such as methane and hydrogen sulphide.

The highest pH level was found in cattle manure. S. Ejiko *et al*. (2024) noted that a high pH was recorded in pig waste, which affected the speed and quality of anaerobic digestion. It is possible to agree with the opinion of S. Ejiko *et al*. (2024), since high pH in pig manure does reduce the efficiency of the process, since an acidic environment is optimal for anaerobic bacteria.

Compared to other types of raw materials, pig manure showed the highest level of carbon dioxide (CO_2), which contributed to the decomposition of organic compounds. B. Žalys *et al*. (2023) showed that pretreatment of cow, pig, and chicken manure with CO_2 gas led to an increase in biomethane yield compared to untreated manure. The findings of B. Žalys *et al*. (2023) should be agreed with, as pretreatment with CO_2 gas does indeed increase biomethane yields and reduces the amounts of harmful gases, specifically hydrogen sulphide, during the biogasification process.

According to the obtained indicators, the average biogas yield with the lowest hydrogen sulphide content was obtained from cattle manure compared to other types of raw materials, which reduced the need for additional stages of biogas purification before its use. A. Ogunkeyede *et al*. (2024) found that cow belching and manure sludge produced high levels of biogas, which helped to reduce the amount of organic waste while contributing to energy production. The conclusions of A. Ogunkeyede *et al*. (2024) should be agreed with, since the use of the types of raw materials under study actually enables not only the reduction of organic waste, but also their use for energy production.

It was found that an elevated level of biogas yield from cattle manure was achieved at pH 7.8 after fermentation. M. Mohammed *et al*. (2022) found that the highest biogas yield was achieved at pH 7, while the use of an isolated digester greatly increased the volume of biogas produced compared to a transparent digester. This statement can be agreed with, as the optimum pH level for the activity of methanogenic microorganisms is within the range of neutral or slightly alkaline environment.

The study found that the use of the technology of preliminary chemical treatment of biomass (solutions of sodium alkali or potassium hydroxide were used for alkaline treatment), which consisted of chicken manure, improved the biogas yield. K. Venslauskas *et al*. (2024) found that biogas production was improved in untreated straw, specifically by applying a biological pretreatment method using microorganisms, namely *Trichoderma* species. The conclusions of K. Venslauskas *et al*. (2024) can be agreed with, as the scientific data confirms that biomass pretreatment using a biological method can contribute to the efficient decomposition of structural carbohydrates, thus increasing the availability of fermentable sugars for microorganisms, and improving biogas yields.

Conclusions

The study revealed that the greatest biogas yield was recorded when using chicken manure (0.23 m^3/kg VT), due to the high content of organic matter and the optimum C:N ratio. Pig manure provided the lowest biogas yield (0.13 m^3/kg VT) due to the high H_2S content, and therefore additional measures for biogas treatment were required. Cattle manure demonstrated an average biogas yield (0.16 m^3/kg VT) but required optimisation of fermentation conditions to improve performance. Chicken manure showed the highest biogas yield during all fermentation periods, reaching a maximum (0.09 m^3/day) in the second period, and therefore the use of this substrate is effective for biogas plants.

According to the observations, chicken manure had the highest organic degradation

rate (60%), indicating the high efficiency of its biological decomposition, while cattle manure and pig manure had degradation rates of 55% and 50%, respectively. Chicken manure, with its high organic matter degradation factor and high methane content of 65%, proved to be the most energetically valuable feedstock for biogas production. Considering the energy efficiency of this substrate, its use is beneficial for biogas plants. Reduction of H_2S emissions from pig manure is possible with limited use of pig manure, addition of adsorbents, or combination of various types of feedstocks, which will achieve a stable C:N ratio and reduce the effects of the harmful component. The findings of the study of the physical and chemical properties of the feedstock revealed that the highest moisture content (75-80%) was observed in cattle manure, which required additional consistency adjustment, while the highest content of volatile solids (25-30%) was found in chicken manure, which emphasised its high energy quality. The C:N ratio of the chicken manure (10:1) was too low, and therefore carbonaceous materials, such as straw, were added to improve the optimum biogas yield.

Areas for further research may include the introduction of a comprehensive approach to analysing the effects of seasonal changes in temperature and humidity on fermentation processes and biogas yields to assess the optimum conditions for the operation of biogas plants using animal by-products and their effects on biogas production.

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Conflict of Interest

None.

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Вплив типу побічних продуктів тваринництва на вихід і склад біогазу

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Анотація. Дослідження впливу різних видів відходів тваринного походження на кількість та склад біогазу є важливим і актуальним для оптимізації процесів анаеробного бродіння, підвищення ефективності виробництва біогазу та адаптації технологій до умов господарств. Метою роботи була оцінка впливу побічних продуктів тваринництва, зокрема гною великої рогатої худоби, свинячого гною та курячого посліду на кількість і якість отриманого біогазу. В ході дослідження застосовувалися методи: статистичний аналіз, метод газоаналізу і ферментації. Під час проведення дослідження проаналізовані фізико-хімічні властивості різних видів сировини для виробництва біогазу. Встановлено, що курячий послід мав найвищий потенціал для утворення біогазу через високий вміст летких твердих речовин (25-30 %) і оптимальне співвідношення метану в складі біогазу (65 %). Також виявлено, що гній великої рогатої худоби характеризувався стабільним середнім рівнем виходу біогазу (0.15-0.18 м3/кг маса летких твердих речовин у сировині (VT, %)), тоді як свинячий гній мав найнижчий вихід (0.12-0.14 м3/кг маса летких твердих речовин у сировині). Згідно з результатами дослідження продемонстровано, що додавання вуглецевих матеріалів (наприклад, подрібненої соломи) сприяло покращенню співвідношення вуглецю до азоту для оптимізації ферментаційного процесу. Проведений аналіз вмісту органічної речовини до та після ферментації показав значне її зниження для курячого посліду (51 %), що свідчило про ефективність біологічного розкладу. Дослідження включало оцінку складу біогазу, зокрема метану (50-65 %), вуглекислого газу (30-40 %) і сірководню (1-3 %). Зміна показників pH у всіх типах сировини після ферментації вказувала на стабілізацію середовища в біореакторах, що забезпечило сприятливі умови для мікроорганізмів. Результати дослідження можуть бути використані на практиці екологами, агрономами, технологами тваринництва і виробниками біогазу з метою створення енергетично незалежних господарств через інтеграцію біогазових установок у фермерські господарства

Ключові слова: відходи; гній; курячий послід; біореактор; метан; вуглекислий газ; ферментація

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Methods of microbiological analysis: Monitoring and ensuring food safety

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Abstract. Modern methods for detecting indicator microorganisms in food production facilitate the early identification of microbiological risks, thereby preventing the contamination of equipment and food products. These methods also enable the timely implementation of sanitary measures, ensuring the microbiological quality of food products. This study focused on the practical application of microbiological techniques for rapid sanitary and microbiological

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monitoring of raw materials, food products, and contact surfaces of technological equipment. Particular emphasis was placed on evaluating highly efficient and cost-effective approaches to ensuring food safety. During production control, methods that simplify microbiological testing are employed, such as using ready-to-use nutrient media, which significantly reduces the time and resources required for microbiological examinations. The study analysed the primary modern nutrient media used for transporting, cultivating, isolating, identifying, and differentiating microorganisms in food products. Modern microbiological methods have been thoroughly analysed, with an evaluation of the quality of nutrient media and the stability of their properties, which directly impact the validity and reliability of microbiological testing of food products. The study identified and evaluated various test systems and the methodologies according to which they have been validated. The use of Petrifilm test plates – ready-to-use nutrient media designed for the quantitative assessment of different groups of microorganisms – has been analysed. These included quantitative testing for yeasts and moulds, *Escherichia coli*, mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), coliform and Enterobacteriaceae during the production of food products, including semi-finished poultry products. The study also highlighted the current validation certificates for these methods and their compliance with both national and international standards, such as ISO 9001, in the field of development and production. Growing attention to food safety and increased quality requirements for microbiological laboratories promote the adoption of advanced methods for analysing raw materials, food products, and surfaces of technological equipment. These methods significantly accelerate the acquisition of microbiological testing results while optimising resource usage. The study of cost-effective and highly efficient tools and rapid sanitary-microbiological monitoring methods enhances the competitiveness of food products

Keywords: safety; pathogen identification; test systems; Petrifilm

Introduction

The state regulatory framework addressing food safety requires reform, particularly regarding compliance with safety and quality standards by food producers. In Ukraine, efforts are ongoing within the EU4SaferFood 2 project (State Service of Ukraine on Food Safety and Consumer Protection – Development Strategy of the State Service of Ukraine on Food Safety and Consumer Protection, 2024), which aims to harmonise Ukrainian food safety legislation with that of the EU. The project facilitates the development and implementation of key reforms based on EU law in the domain of food safety, including improving Ukraine's state control system to meet EU standards. One of the anticipated outcomes of this collaboration is the provision of enhanced services in the area of food safety, alongside the reform of the laboratory infrastructure (NGO Agrarian Union of Ukraine. Agropolitical Report, 2022). This reform is expected to highlight the benefits of monitoring food products produced by enterprises adhering to the European farm-to-fork strategy.

Quality control of food products is a critical aspect of production management, safeguarding businesses from the supply of substandard materials and raw ingredients. The selection of control methods and instruments ensures the desired level of food quality, as the testing process demands the highest levels of precision, reliability, and data accuracy (Varenina *et al.*, 2022). Modern, hightech methods for analysing food quality offer significant advantages over traditional techniques,

reducing the number of research stages, costs associated with the production and validation of culture media, and labour requirements. M. Castro *et al.* (2024) highlight that comprehensive approaches to food safety monitoring, combining theoretical and practical aspects, are essential tools. Moreover, these methods play a crucial role in assessing the quality of finished products and providing timely results for implementing sanitary measures.

The implementation of high-tech, cost-effective tools and rapid methods for routine sanitary and microbiological monitoring is a crucial step in enhancing the quality and safety of food products both in Ukraine and internationally. Traditional methods for the qualitative and quantitative determination of indicator microorganisms suffer from several drawbacks, including being labour-intensive, low-throughput, and time-consuming. These methods are often unsuitable for rapid monitoring, especially in medium-sized and small food processing enterprises that lack accredited bacteriological laboratories. In light of these limitations, V. Chiozzi *et al.* (2022) highlight the need for simpler, more objective, and high-throughput tools and methods for the sanitary and microbiological monitoring of raw materials, semi-finished products, and food products.

Given Ukraine's challenging energy situation and the global trend towards resource reduction, minimising water and electricity consumption has become a pressing issue. Since the early 1990s, global practices have increasingly incorporated methods that simplify microbiological testing of food products, alongside traditional methodologies, particularly in production control. The use of ready-to-use nutrient media reduces the time and resources required for microbiological testing (Hartantyo *et al.*, 2023).

The ability to rapidly obtain results from microbiological analysis allows for the prompt release of food products to the market, providing manufacturers with a competitive advantage. This paper aimed to investigate the practical application of rapid sanitary and microbiological monitoring of raw materials, food products, and equipment surfaces using modern microbiological methods, and to evaluate highly efficient and cost-effective tools. The objective of this study was to systematise microbiological control methods and assess methods for detecting indicator microorganisms in food production, enabling timely implementation of sanitary measures and ensuring the microbiological quality of food products.

Literature Review

As highlighted by G. Birgand *et al.* (2022), the history of culture media began in 1861 with the work of French microbiologist L. Pasteur, who used beer wort, wine, grape juice, and meat broth as culture media. The aim was to create a fermentation medium. The primary nitrogen sources were ammonium salts; sugar was used as a carbon source for microbial growth, while ash provided essential micronutrients. Pasteur observed that specific chemical properties and individual nutrients within a medium could either promote or inhibit the growth of particular microorganisms. Given the competitive nature of microorganisms for nutrients, certain cultures can exhibit excessive growth. Pasteur's research led to the modern understanding of preventive measures and proactive monitoring of food products, enabling the implementation, scaling, and utilisation of culture media. As noted by T. Giles-Vernick *et al.* (2022), this distinguished scientist developed microbiological culture media from initial work as a sideline to meat production to the onset of mass production, and to the ongoing development required to meet the demands of modern microbiology laboratories.

As highlighted by H. Craven *et al.* (2021), German bacteriologist R. Koch demonstrated a new technique for using culture media at the London International Medical Congress. At the time, the challenges of using broth-based

culture media were well recognised. Scientists were actively seeking and researching reliable alternatives to replace existing media. Coagulated egg white, starch paste, and aseptically sliced potato were proposed as alternative culture media. In 1881, R. Koch used meat extract with added gelatin to isolate pure cultures of microorganisms. The resulting "nutrient gelatin" was poured into glass plates, inoculated, and placed under a bell jar. This new method of using solid media allowed for both the isolation of pure bacterial cultures and their subculture on plates or gelatin surfaces in tubes sealed with cotton plugs. Although a significant advancement, gelatin had two major drawbacks as a gelling agent. It underwent a gel-to-liquid phase transition at relatively high temperatures, specifically around 25°C, which hindered the incubation of plates at these temperatures. Additionally, gelatin could be liquefied by gelatinase, an enzyme produced by proteolytic microorganisms.

In 1882, Frau Hesse introduced a revolutionary idea: using agar as a replacement for gelatin in the preparation of solid culture media. It is worth noting that this hydrocolloid was already used in the production of fruit jellies at the time. The polysaccharide mixture of agarose and agaropectin possesses unique physical properties: it remains solid up to 85°C but does not solidify into a gel until 34-42°C upon cooling. Agar's transparency aids in observing colonies, and unlike gelatin, it is not degraded by most microorganisms (Mainardi & Bidoia, 2024).

Meat extract is a valuable source of various growth factors for bacteria, but it has several drawbacks: it does not contain sufficient amino acids to support the optimal growth of many microorganisms; its imprecise chemical composition leads to variable experimental results; and it is susceptible to evaporation. As noted in their book by R. Owusu-Apenten & E. Vieira (2022), F. Loeffler modified the medium by adding peptone and salt to Koch's original meat extract composition. Peptone was a meat enzymatic digest, used in the 19th century as a pharmaceutical product for digestive disorders. The addition of meat peptone expanded the amino acid composition (as a nitrogen source); salt enhanced the viability of microorganisms, which was crucial for their selective cultivation.

A new type of culture dish for media was introduced in 1887. J. Petri modified a flat glass plate to prevent physical and biological contamination. By the 1890s, nutrient media had been developed that formed the basis for microbiological practice and are still used in modified forms in laboratories today.

Selective media are special nutrient media designed to cultivate specific types of microorganisms. The first selective medium was developed by M. Beijerinck in 1888. Bacteriologist A. McConkey developed the first chromogenic medium in 1905. It contained selective components (neutral red and bile salts) that inhibited the growth of Gram-positive bacteria and a specific substrate – lactose. In their study, R. Singh *et al.* (2020) determined that intestinal bacteria ferment lactose, leading to a decrease in pH and a change in the indicator's colour. As a result, colonies of *Salmonella*, *Shigella*, and coliform bacteria appear red and are surrounded by a turbid zone of bile salt precipitation. In 1945, A. Fleming, H. Florey, and E. Chain were awarded the Nobel Prize for the development of penicillin, which made it possible to use antibiotics as a practical tool. However, in the 1960s, antibiotics began to be used as selective agents for growing microorganisms, allowing for the avoidance of contamination and creating conditions for the selective growth of specific types of bacteria and fungi.

Chromogenic media contain chromogens, molecules designed to mimic colourless metabolic substrates. These molecules are not cleaved by the target enzyme but accumulate within the cell. Once cleaved, the chromogens become insoluble and coloured, allowing for easy differentiation and identification. The development of selective basal media with chromogenic substrates has facilitated the

creation of advanced nutrient media. As noted by K. Anith *et al.* (2021), this, in turn, has enabled the separation and identification of groups of microorganisms.

In a collaborative study, A. Simpson *et al.* (2022) determined that the use of film-based identification media requires significantly less sample preparation, generates less waste, and occupies less incubator space compared to traditional agar-based methods. Studies have shown that nutrient-coated film plates provide a good correlation for microbial counts with agar-based nutrient media. An additional advantage of film media is the ability to process large sample volumes. This makes them suitable for counting microorganisms in environments with very low biomass, such as extraterrestrial environments. Numerous chromogenic media are now used for various organisms, including *Candida, E. coli* and other coliforms, *Listeria monocytogenes, Salmonella*, and *Clostridium perfringens*. It is worth noting that much of 19th-century microbiology had a clinical focus, but today the food and processing industries face different challenges. There is a need for new approaches in the development and use of specialised methods capable of detecting minimal quantities of pathogenic microorganisms in 25 g of food products.

Materials and Methods

The methodology of the study was based on contemporary theoretical and experimental methods of microbiological analysis. The main experimental part of the research was conducted in the laboratory of the Department of Standardization and Certification of Agricultural Products, National University of Life and Environmental Sciences of Ukraine, utilising novel methods and tools for the sanitary and microbiological control of raw materials and food products. Alongside classical culture media for the isolation and differentiation of microorganisms from various taxonomic groups, chromogenic culture media (CCMs) from various manufacturers (Oxoid, Merck, bio Merieux, HiMedia, Sigma, Fluka) were employed. This method is based on the identification of microorganisms based on specific enzymes using special chromogenic substrates.

The study involved the determination of microbiological contamination in two poultry semifinished products. Samples (Fig. 1) included: chicken thigh (broiler), chilled; chicken wing (broiler), chilled.

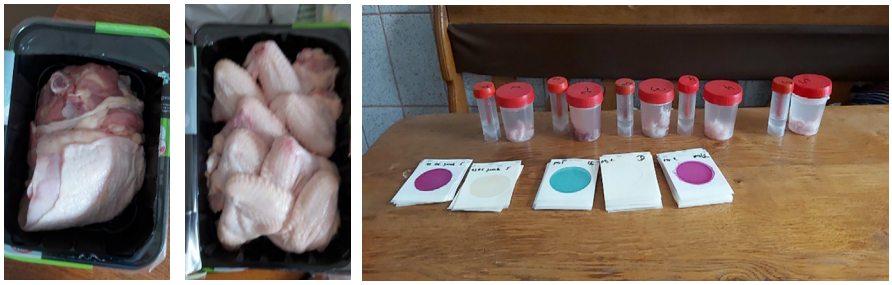


Figure 1. Samples for analysis to determine microbiological indicatorsy
Source: authors' photo

Sample preparation, detection, and enumeration of Enterobacteriaceae in the test samples were conducted according to the ISO 21528 standard on classical culture media. The initial suspension and decimal dilutions of the test sample were prepared according to ISO 6887. For this, a 10 g sample was aseptically transferred from the combined sample into a sterile filter bag, 90 cm^3 of sterile peptone saline was added, and the mixture was

homogenised to obtain the initial suspension. Subsequently, a series of decimal dilutions were prepared from the initial suspension by transferring 1 cm^3 of inoculum into a tube containing 9 cm^3 of sterile peptone saline.

To isolate Enterobacteriaceae, 1 cm^3 of the initial suspension and its decimal dilutions were inoculated into sterile Petri dishes. The inoculum was overlaid with Violet Red Bile Glucose Agar (VRBGA) cooled to 45°C and gently mixed by rotating. After the medium solidified, a second layer of the same medium was poured on top. The plates were incubated at 37°C for 24 hours. Plates containing fewer than 150 characteristic colonies were selected, and these colonies were counted. Five characteristic colonies were then selected for further identification.

The characteristic colonies were transferred to non-selective media and incubated for 24 hours at 37°C. Identification was then carried out by determining oxidase activity and the ability to ferment glucose. Oxidase-negative colonies were inoculated into a glucose agar slant. Inoculated tubes were incubated at 37°C for 24 hours. A positive reaction was indicated by a colour change of the agar to yellow. The number of Enterobacteriaceae per cm^3 or 1 g of the test sample was calculated based on the number of confirmed typical colonies on the plate (in each plate).

Certain species of Enterobacteriaceae may form colourless colonies or cause decolourisation of the medium. Therefore, if no characteristic colonies were observed, whitish colonies were selected for confirmation. The enumeration of Enterobacteriaceae, *Escherichia coli* (SEC), and coliform bacteria was performed using Petrifilm (EC) test plates (Fig. 2).

Petrifilm Yeast and Mold Count Plates (Fig. 3) were used to enumerate yeast and mould colonies and to determine lactic acid bacteria. The initial suspension and decimal dilutions of the test sample were prepared according to ISO 6887.

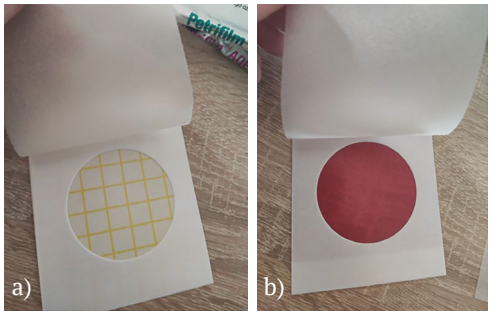


Figure 2. Petrifilm test plates for colony counting
Note: a – *Escherichia coli* (SEC); b – coliform bacteria and *Escherichia coli*
Source: authors' photo

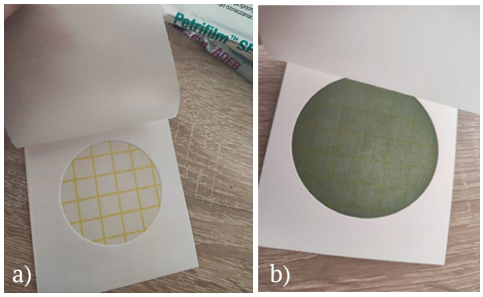
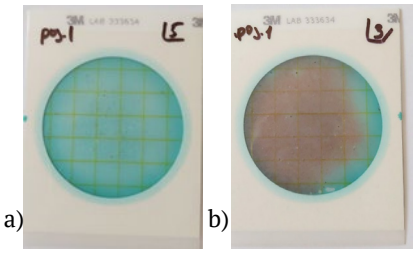


Figure 3. Petrifilm test plates
Note: a – Yeast and Mold Count Plate for counting yeast and mould colonies; b – determination of lactic acid bacteria
Source: authors' photo

The Petrifilm EB plate was placed on a flat surface. The top film was lifted, and 1 cm^3 of the suspension or its dilution was applied to the centre of the bottom film. The film was gently lowered onto the sample, avoiding the entrapment of air. The inoculum was then spread over the surface of the Petrifilm EB using a spreader. The Petrifilm plates were left for one minute to allow the gel to form. Petrifilm EB plates were incubated in a horizontal position with the clear side up at 37°C for 24 hours. The Petrifilm plate for the determination of lactic acid bacteria contains a compound that absorbs oxygen and creates anaerobic conditions. Heterofermentative lactic acid bacteria are identified

as red colonies with gas bubbles. Red colonies without gas are identified as homofermentative lactic acid bacteria (Fig. 4).



a) b)

Figure 4. Petrifilm test plates
Note: Petrifilm test plates for the determination of lactic acid bacteria
Source: authors' photo

Petrifilm plates for the detection of lactic acid bacteria exhibited an excessive number of colonies, characterised by numerous small colonies, abundant gas bubbles, and a colour change of the gel from blue to pinkish-violet. The high concentration of colonies led to the entire plate becoming dark blue-violet with a pink halo around the outer edge. To obtain a more accurate count, further dilution of the sample was necessary.

Following incubation, results were recorded. In cases where more than 100 colonies were observed on Petrifilm EB plates, the number of colonies in two or more characteristic square areas was counted, and the mean value for each area was determined. To estimate the total number of colonies on each plate, the average number of colonies per square area was multiplied by 20, as the area of the circular inoculation zone is approximately 20 cm^2. The total number of yeasts and moulds serves as an indicator of the overall cleanliness of food products throughout their lifecycle, from production and processing to distribution and storage. It is important to note that after use, the test systems contain viable cultures of yeasts and/or moulds and should therefore be autoclaved at 120°C for 15 minutes.

Results and Discussion

The quality of culture media and the stability of their cultural properties directly influence the reliability of microbiological testing of raw materials, semi-finished products, and culinary products. Research conducted by scientists has evaluated the applicability of methods and test systems, particularly Petrifilm indicator plates, for counting microbial groups in food products and on processing surfaces. Researchers M. Bilan *et al.* (2024) conducted a study using traditional microbiological methods and indicator test plates to assess the safety of goat's milk. The results of the study showed that the level of bacterial contamination of goat's milk with mesophilic aerobic and facultatively anaerobic microorganisms was within acceptable limits. No significant differences were found in the results when using alternative methods. The study demonstrated the potential of using test plates to ensure the quality and safety indicators during the production of goat cheese and other dairy products made from it.

The aim of the research by M. Morin *et al.* (2021) was to evaluate the accuracy of the Petrifilm system for detecting excessive bacterial contamination in colostrum. Using Petrifilm for bacterial contamination analysis, the results demonstrated that the test systems were a reliable alternative to standard laboratory methods, with high sensitivity and specificity for detecting aerobic and coliform contamination. The results presented by authors A. Ríos-Castillo *et al.* (2021) indicate that production surfaces and commercial equipment are a source of food contamination. As products can become recontaminated from these surfaces, it is necessary to maintain hygiene standards in retail settings. Given the large number of employees, food products, sales volumes, and customer flow, it is crucial to maintain strict hygiene standards in retail settings to prevent foodborne outbreaks. Procedures must be effective in ensuring food safety.

In collaborative research on frozen food products, M. Silbernagel *et al.* (2003) conducted

comparative studies using the 3M Petrifilm Staph Express Count Plate and the official AOAC 975.55 method for enumerating *Staphylococcus aureus*. Frozen semi-finished products such as lasagna, custard, vegetable mixes, mushrooms, and breaded mushrooms were selected as samples, and the presence of *S. aureus* was determined. The obtained values for contamination of readytoeat food products using the 3M Petrifilm Staph Express Count Plate method were similar to the official standard method, guaranteeing the reliability of the results.

L. Nero *et al.* (2020) aimed to assess the applicability of Petrifilm plates for enumerating lactic acid bacteria in bacon. Bacon samples were analysed using four protocols, including conventional control protocols and Petrifilm test plates. Based on the reliable results obtained, researchers determined the ease and speed of using Petrifilm. They verified the adequacy, selectivity, and similarity of results to official protocols, establishing the applicability of alternative indicator plates for microbiological testing of bacon.

M. Sharan *et al.* (2022) emphasised the importance of developing standard procedures for disinfection and hygienic treatment of equipment and utensils to prevent and control microbiological contamination and highlighted the priority of continuous sanitary monitoring. To prevent biofilm formation, it is essential to systematically identify production areas and food processing locations and monitor the microbial load in these zones. They stressed the importance of understanding the mechanisms of microbiological contamination and developing new, costeffective control methods in the food industry.

Scientists at the US Food Safety Center, S. Jones *et al.* (2020), identified the advantages and disadvantages of bacterial enumeration methods depending on the type of production surface. However, researchers noted the inconsistency of sampling methods in real-world processing plant conditions, which are reliably ensured in laboratory settings. There is a demand for rapid and reliable methods for assessing bacterial contamination of surfaces to ensure comprehensive food safety and thus guarantee the effectiveness of sanitation procedures.

In their research, M. Gaare & S. Mishra (2021) determined that to prevent food contamination resulting from contact with various production surfaces, a microbiological monitoring program should be developed. The program involves several steps, including a proper procedure for sanitising production equipment, disinfecting production equipment and work surfaces. The next step is to identify potential microbiological hazards during food production or processing, to ensure that production and product distribution comply with international standards. The goal of the research by A. Schumacher *et al.* (2022) was to compare the identification of microorganisms for different 3M Petrifilm test systems. The authors recognised the use of YM as a reliable method for counting viable yeasts and moulds in dried cannabis flowers. They established that 3M Petrifilm YM test systems provide convenience for conducting research and save space in the incubator.

In recent times, test systems in the form of plates (cards) have gained widespread use. The most common among them are Petrifilm, Rida Count, and Compact Dry test systems. Depending on their purpose (detection of specific types of microorganisms), the composition of the nutrient medium is formulated during the production of the test plate. During manufacturing, the nutrient medium is applied to the surface of a mesh polymer base. The nutrient medium contains a special substance that, when a liquid phase (dilutions) of the product is added at room temperature, transforms into a gel. In addition, depending on the type of microorganism, an indicator substance is added to the nutrient medium during the production of the test plates, which colours the colonies a specific colour.

Following the testing methodology, inhibitory substances were added to the nutrient

medium, such as antibiotics (novobiocin), 2,3,5-triphenyl tetrazolium chloride, as well as sodium deoxycholate, bile, and other agents that suppress the growth of accompanying microflora. Chromogenic plates contain substrates that change the colour of the nutrient medium under the action of enzymes produced by specific types of microorganisms. For example, lysine, which is decarboxylated to cadaverine, is added to the medium for the isolation of *Salmonella*. This raises the pH and produces hydrogen sulphide, which, when combined with iron, forms iron sulphide, colouring the colonies black. Chromogenic substrates that are broken down by the enzyme β-galactosidase to form blue colonies are included in the nutrient medium of plates for the isolation of coliform bacteria. COMPACT DRY medium for the detection of *E. coli* contains a substance that, when cleaved by the enzyme glucuronidase present in *E. coli* cells, causes the colonies to turn blue.

RIDA COUNT. The monitoring method utilises ready-to-use sterile dry test cards. These systems consist of essential components: nutrient media and chromogenic substrates, specific to particular determinations. The cards are hermetically sealed with an impermeable membrane that is removed before testing. After applying the sample to the card, it is resealed with the same film, ensuring sterility, and then incubated horizontally at the specified temperature. Following incubation, the coloured colonies are counted, taking into account the dilutions and initial volume of the medium. The range of test systems is quite diverse and constantly expanding, including RIDA COUNT TOTAL, RIDA COUNT COLIFORM, RIDA COUNT *E. COLI*, RIDA COUNT *E. COLI* / COLIFORM, and RIDA COUNT *STAPH. AUREUS*. These test systems are designed to determine the quality and assess the microbiological indicators of food raw materials, finished products, animal feed, and swabs from various objects. Currently, test systems are actively used to research the possibility of determining the level of microbiological cleanliness of various production surfaces. The RIDA COUNT plate for the simultaneous detection of coliforms and *E. coli* contains an enzyme that, when interacting with their metabolic products, colours the former blue and the latter purple, as shown in Figure 5.

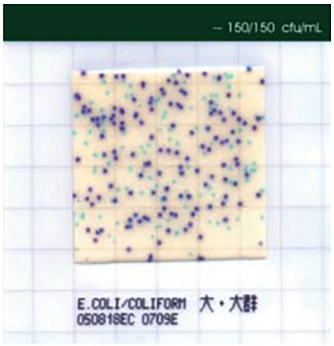


Figure 5. RIDA COUNT plate, *E. coli*/coliform, colony count after 24 hours
Source: RIDA COUNT

To detect *S. aureus*, mannitol salt agar is added to the RIDA COUNT plate. This medium contains sodium chloride and antibiotics that inhibit the growth of many microorganisms except staphylococci. The fermentation of mannitol is linked to the acid phosphatase activity of staphylococci and leads to a decrease in pH, which in turn causes the colonies of staphylococci to turn a greenish-blue or black with a green tint, as shown in Figure 6.

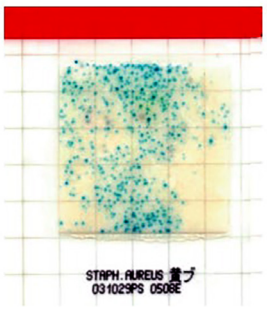


Figure 6. RIDA COUNT plate, *Staphylococcus aureus*, colony count after 24 hours
Source: RIDA COUNT

Petrifilm test plates have certificates of certification from AOAC INTERNATIONAL Official Method of Analysis, AOAC Performance Tested MethodSM, NF Validation by AFNOR Certification, as well as Ukrainian State Standards DSTU 7089-2009 and DSTU 7140-2009 for the dairy industry. Neogen Petrifilm test plates are designed for the quantitative determination of Enterobacteriaceae; coliforms; *E. coli*; lactic acid bacteria; yeasts and moulds; mesophilic aerobic and facultatively anaerobic microorganisms (MAFAnM); *Staphylococcus aureus*. Table 1 lists the different types of test systems and the methods according to which they are validated.

Table 1. *Validation Certificates for Neogen Petrifilm*

Neogen® Petrifilm®	**International standards compliance**	**DSTU/ ISO Compliance**
Neogen Petrifilm for Rapid Coliform Count (RCC)	ISO 4832[1], ISO 4832[2] NF Validation by AFNOR Certification	DSTU ISO 4832:2015 Microbiology of food and animal feed. Horizontal method for coliform enumeration. Colony count technique (ISO 4832:2006, IDT).
Neogen Petrifilm Test Method for Coliform Count (CC)		
Neogen Petrifilm Rapid Yeast and Mould Count (RYM)	ISO 21527 (1-2) NF Validation by AFNOR Certification	ISO 21527-1:2013 Microbiology of food and animal feed. Enumeration of yeasts and moulds. Part 1: Products with water activity greater than 0.95. ISO 21527-2:2008 Microbiology of food and animal feed. Horizontal method for the enumeration of yeasts and moulds. Part 2. Colony count technique in products with water activity less than or equal to 0.95.
Neogen Petrifilm Yeast and Mould Count (YM)		
Neogen Petrifilm for Rapid Aerobic Count (RAC)	ISO 4833 NF Validation by AFNOR Certification	DSTU ISO 4833:2006 Microbiology of food and animal feed. Horizontal method for microbial enumeration. Colony count at 30°C.
Neogen Petrifilm Aerobic Count (AC)		
Neogen Petrifilm Lactic Acid Bacteria Count (LAB)	ISO 15214:2007 NF Validation by AFNOR Certification	DSTU ISO 15214:2007 Microbiology of food and animal feed. Horizontal method for enumeration of mesophilic lactic acid bacteria at 30°C.
Neogen Petrifilm Enterobacteriaceae Count (EB)	ISO 21528-2 NF Validation by AFNOR Certification	DSTU ISO 21528-2:2014 Microbiology of food and animal feed. Horizontal method for detection and enumeration of Enterobacteriaceae. Part 2: Colony count method (ISO 21528-2:2004, IDT).
Neogen Petrifilm Staph Express Count (STX) + Staph Express Disk for Coagulase Activity Confirmation	ISO 6888-1NF Validation by AFNOR Certification	DSTU ISO 6888-1:2003 Microbiology of food and animal feed. Horizontal method for coagulase-positive *Staphylococci* (*Staphylococcus aureus* and others). Part 1: Using Baird-Parker agar medium (ISO 6888-1:1999, IDT).
Neogen Petrifilm *E. coli* Count (SEC)	ISO 16140-2 NF Validation by AFNOR Certification	ISO 16649-2 Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli.*

Note: standard names are presented as specified by the responsible Technical Committees
Source: developed by the authors

The use of such systems provides a significant (1.5-2 times) reduction in direct costs for conducting analyses and allows for a shorter study duration. Petrifilm plates are designed for the quantitative determination of various groups of microorganisms. They consist of a nutrient medium on a substrate. Petrifilm has a multilayer structure and comprises a substrate with a nutrient medium; and a transparent film covering the plate to maintain sterility. The media contain necessary components: chromogenic substrates and indicators, which

give the microbial colonies a characteristic colour. Modified substrates are also added to the chromogenic nutrient medium of rapid tests, allowing for the detection of specific biochemical properties of microorganisms. The use of Petrifilm plates begins directly with sample preparation for microbiological analysis. Stages such as preparing glassware and nutrient media, and conducting quality control of the media in independent laboratories for activity and selectivity are eliminated.

Petrifilm test plates are available in a variety of types, each designed for specific applications. Unopened packages of Petrifilm should be stored in a refrigerator or freezer at a temperature not exceeding 8 °C (46 °F). Before opening a package of Petrifilm, allow it to reach room temperature (20-25 °C, RH < 60%). To avoid moisture exposure, resealed packages should be stored in a cool, dry place for no more than one month. If the laboratory temperature exceeds 25 °C (77 °F) with a relative humidity above 50% (except for air-conditioned rooms), resealed packages of Petrifilm should be stored in a freezer.

Opened packages of Petrifilm should be stored in a tightly sealed container in the freezer. It is not recommended to use Petrifilm plates after the expiration date. Specific requirements for laboratory freezing equipment are recommended. The freezer used to store opened packages should operate without an automatic defrost cycle to avoid damaging the plates through repeated contact with moisture. Petrifilm plates that have changed colour should not be used.

Petrifilm RAC is a ready-to-use nutrient medium designed for the rapid enumeration of aerobic bacteria. It contains nutrients, a cold-water-soluble gelling agent, and a dual-perception indicator to facilitate colony counting. Petrifilm RAC plates are intended for the enumeration of aerobic bacteria in raw materials, finished products, environmental samples (air, swabs, imprints), and beverages. Petrifilm RAC plates are registered by Neogen for use solely in the food and beverage industry. For example, Petrifilm RAC plates are not registered by Neogen for the analysis of water, pharmaceuticals, or cosmetics. Petrifilm RAC plates are not intended for the diagnosis of human or animal diseases.

Petrifilm RAC plates cannot differentiate between various strains of microorganisms. Petrifilm AC is an Aerobic Count Plate used for enumerating aerobic bacteria in food and beverages. Petrifilm YM is a Yeast and Mold Count Plate designed for quantifying yeasts and moulds. Petrifilm YM is a ready-to-use culture medium containing nutrients, antibiotics, a cold-water-soluble gelling agent, and an indicator to simplify the counting of yeasts and moulds. Petrifilm YM plates are designed for the enumeration of yeasts and moulds in food and beverage production (Fig. 7).

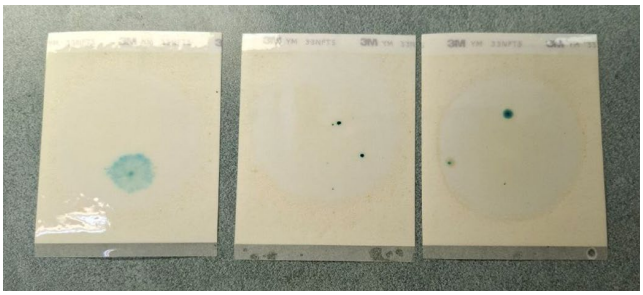


Figure 7. Typical colonies of moulds and yeasts

Note: typical mould colonies are large with a dark centre and diffuse edges, flat, blue-green or with colour variations; typical yeast colonies are small with smooth edges, convex, pinkishbrown, or blue-green

Source: authors' photo

Petrifilm Aerobic Count Plates (AC) are designed to enumerate mesophilic aerobic and facultatively anaerobic microorganisms (MAFAnM) in food production and on contact surfaces (Fig. 8). They can also be used for environmental monitoring. Using Petrifilm AC reduces labour costs and allows for more frequent monitoring at critical control points in production, ensuring effective food safety and quality control.

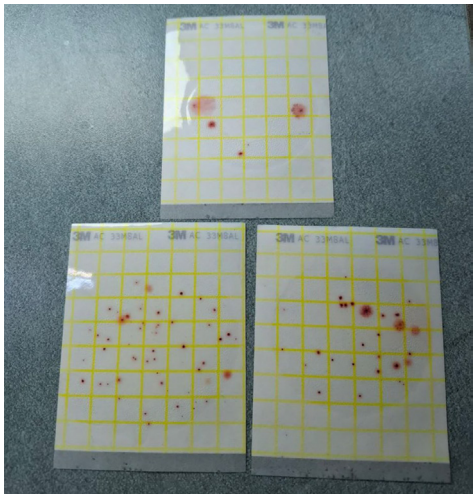


Figure 8. Petrifilm Aerobic Count Plate (AC) for counting MAFAnM
Note: top – 5 CFU, left – 50 CFU, right – 28 CFU
Source: authors' photo

Petrifilm *E. coli* Count Plates (SEC) are ready-to-use nutrient media containing selective agents, nutrients, a cold-water-soluble gelling agent, and a glucuronidase indicator BCIG (5bromo4-chloro-3-indolyl-D-glucuronide) to facilitate colony counting. Petrifilm SEC plates are designed for the enumeration of *Escherichia coli* (*E. coli*) in food and beverage production. Most strains of *E. coli* are thermotolerant and produce beta-glucuronidase, an enzyme that reacts with the BCIG indicator in 3M Petrifilm SEC plates, forming a dark green or blue-green substance that colours the colonies.

Each batch of Petrifilm test plates is accompanied by a quality certificate from the independent laboratory AFNOR. Petrifilm test plates are validated according to standard methodologies. The results obtained from cultures on Petrifilm plates are consistent, reliable, and 100% reproducible. When conducting microbiological tests using Petrifilm plates, compared to traditional methods, there is a 76% reduction in electricity consumption and a 79% reduction in water usage. Studies conducted by the manufacturer show a significant reduction of up to 75% in greenhouse gas emissions and up to 66% in waste. Additionally, the use of Petrifilm plates allows for a 45% reduction in average labour costs and an 80% increase in the efficiency of technical personnel. The accuracy, reliability, and speed of determining food quality allow for increased product competitiveness and rapid release for sale.

Switching from agar to Petrifilm count plates reduces labour costs by an average of 45%. A study of 292 food processing plants showed an average increase in the efficiency of quality control personnel of 80.5% using Petrifilm – or 3.7 hours of saved time for technical personnel per day. By obtaining results within 24 hours or less, Petrifilm can provide data approximately twice as fast as traditional agar plate methods. Stable in storage and as thin as paper, Petrifilm test plates take up 85% less space when incubated. Petrifilm plates use less glassware and equipment and optimise the workspace and incubator space compared to traditional methods. Studies have shown that when conducting quality analysis of raw materials, individual production stages, and the disposal of packaging materials, the use of Petrifilm plates is an energy-saving, labour-saving, and environmentally friendly method.

Conclusions

Food testing and quality control demand highly sophisticated technology. This article analyses contemporary methods for the microbiological examination of food products; it explores highly efficient and cost-effective tools and

methods for the rapid sanitary-microbiological monitoring of raw materials, food products, and the surfaces of processing equipment. It presents current validation certificates, demonstrating their compliance with the International Organisation for Standardisation (ISO) 9001 standards in research and development. The application of modern validated methods for microbiological monitoring and ensuring food safety optimises turnaround times, enhances the accuracy and reliability of results and reduces energy and labour costs for such studies.

Research has demonstrated that using Petrifilm test plates for microbiological analysis significantly reduces the consumption of electricity, water, and greenhouse gases compared to traditional methods. This leads to a 45% reduction in labour time and an 80.5% increase in staff efficiency. Due to the rapid result delivery within 24 hours, Petrifilm is twice as fast as traditional methods. Test plates also save incubator space and optimise the use of laboratory equipment. Overall, Petrifilm is an energy-efficient, labour-saving, and environmentally friendly method for food quality analysis. The advantages of Petrifilm test plates allow for savings in the time spent preparing media, simplify the testing process through quality identification, and reduce disposal costs. The use of simple, technological tools and objective methods for the sanitary-microbiological control of raw materials, food products, and the surfaces of processing equipment guarantees food safety.

Future research plans include refining processes for the detection and control of microbiological hazards in the food industry using advanced analytical methods. Continued research is planned to evaluate and select highly effective yet cost-effective sanitary control tools, such as Petrifilm test plates. In particular, further investigation into specific culture media for the quantitative analysis of microorganisms and their compliance with ISO standards will enhance the competitiveness of food products by ensuring their microbiological safety.

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Conflict of Interest

None.

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Методи мікробіологічного аналізу: контроль та гарантування безпеки харчових продуктів

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Анотація. Сучасні методи виявлення індикаторних мікроорганізмів на виробництві харчових продуктів дозволяють завчасно виявити мікробіологічні ризики і попередити контамінацію обладнання та харчових продуктів мікроорганізмами та своєчасно організувати санітарні заходи, тим самим забезпечуючи якість харчової продукції за мікробіологічними показниками. Стаття була спрямована на дослідження практичного застосування мікробіологічних методів для проведення оперативного санітарно-мікробіологічного моніторингу сировинних матеріалів, харчових продуктів та контактних поверхонь технологічного обладнання, з акцентом на оцінку високоефективних та економічно рентабельних засобів для забезпечення безпечності харчових продуктів. Під час виробничого контролю застосовують методи, що спрощують мікробіологічні випробування харчових продуктів, зокрема використання готових поживних середовищ дозволяє скоротити витрати часу і ресурсів на проведення мікробіологічних випробувань. Проведено аналіз основних сучасних поживних середовищ, що використовуються для транспортування, культивування, виділення, ідентифікації та диференціації мікроорганізмів харчових продуктів. Детально проаналізовано сучасні мікробіологічні методи; здійснено оцінку якості поживних (живильних) середовищ і стабільність їхніх властивостей, що позначається на обґрунтованості й адекватності мікробіологічних випробувань харчових продуктів. У статті визначено та оцінено різновиди тест-систем та методи, згідно з якими вони валідовані. Проведено аналіз застосування тест-пластин Petrifilm – готових поживних середовищ, які призначені для кількісного визначення різних груп мікроорганізмів, зокрема для кількісного аналізу дріжджів та цвілевих грибів, *Esherichia coli*, мезофільних аеробних і

факультативно-анаеробних мікроорганізмів (МАФАнМ), ентеробактерій та коліформних бактерій, під час виробництва харчових продуктів (напівфабрикатів з сільськогосподарської птиці). Наведено сучасні сертифікати їх валідації, їх відповідність в державній та міжнародній організації зі стандартизації (ISO) 9001 у сфері розробок та виробництва. Підвищення уваги до безпеки харчових продуктів та вимог до якості мікробіологічних лабораторій сприяє застосуванню сучасних методів аналізу сировини, харчових продуктів і поверхонь технологічного устаткування. Використання методів дозволяє значно пришвидшити процес отримання результатів мікробіологічних досліджень, а також оптимізувати використання ресурсів. Вивчення засобів, що вирізняються високою ефективністю і невисокою ціною, та методів оперативного санітарно-мікробіологічного моніторингу забезпечить конкурентоздатність харчової продукції

Ключові слова: безпечність; ідентифікація збудників інфекцій; тест-системи; петрифільми

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Innovative technologies for the creation of low-calorie dairy products using plant components

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Abstract. The development of low-calorie dairy products using plant-based ingredients is becoming increasingly relevant due to the growing demand for healthy eating and alternatives to traditional dairy products. The study background was based on the need to develop functional products with a reduced calorie load that combine high nutritional value with attractive organoleptic

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properties. The study aimed to develop low-calorie products based on plant-based raw materials using innovative technologies such as protein extraction, fermentation and ultrafiltration, ensuring a reduction in calories without losing texture, taste and shelf life. High-quality plant-based ingredients (soya, almonds, oats) and probiotic cultures were used, and the methodology included the isolation of protein isolates through extraction, further fermentation to improve flavour and enrich beneficial microorganisms, and ultrafiltration to help concentrate proteins, remove sugars and ensure product stability. The results showed that the developed products, namely fermented soya yoghurt (55 kcal/100 g), almond milk (30 kcal/100 ml) and oatmeal cream dessert (80 kcal/100 g), demonstrated a significant reduction in calories by 20-50% compared to traditional dairy analogues. All samples maintained a stable texture, homogeneity and high organoleptic characteristics for 7 days at +4°C, with an average score of 4.8 out of 5 according to the expert panel. Additionally, the study determined that the use of ultrafiltration ensured long-term product stability, while fermentation contributed to improved flavour characteristics and increased biological value. The study results confirmed the effectiveness of innovative technologies in creating competitive low-calorie products from plant components. The practical significance of the study is determined by possibility of implementing the results obtained in industrial production to meet the needs of the modern market focused on healthy eating

Keywords: food industry; healthy food; fermentation; ultrafiltration; protein extraction; probiotic cultures

Introduction

Interest in healthy eating and informed food choices is growing globally. One of the key trends is to reduce the consumption of high-calorie foods, which reduces the risks of obesity, cardiovascular disease and diabetes. In this context, low-calorie dairy products with plant-based ingredients are gaining more and more attention from producers, scientists and consumers. Dairy products are an important source of nutrients such as protein, calcium and vitamins (Ruban & Danshin, 2023). However, the growing demand for lower-calorie alternatives is driving the use of plant-based ingredients such as soya, almonds, oats or coconut. They can be used to create products that retain high nutritional value and organoleptic properties while remaining low in calories. The research relevance is determined by several factors: growing interest in veganism, increased demand for products with improved nutritional properties, and the need to optimise production technologies. Despite the advances in the dairy industry, many questions remain unanswered about the creation of high-quality low-calorie dairy products with plant-based ingredients (Moroz, 2023).

Scientific research in the dairy industry mainly aims to improve production technologies and preserve the health benefits of products. For instance, A. Minorova *et al.* (2022) studied the technology of low-calorie milk ice cream production, focusing on innovative approaches to improving production efficiency. Emphasis was devoted to functional ingredients that provide the required organoleptic characteristics. Another example is the monograph by R. Nikiforov *et al.* (2022), which discussed the introduction of innovative technologies in the production of dessert products based on protein-carbohydrate semi-finished products. The authors presented the results of research on improving the composition, structure and properties of such desserts, considering the requirements for reducing calorie content. The

study by L. Peshuk *et al.* (2023) addressed the development of innovative algae products that are gaining popularity as an alternative to traditional products. The review describes in detail the technological features of such products and their positive impact on health.

M. Arshad *et al.* (2024) analysed innovative technologies that extend the shelf life of plant-based products. This is especially relevant for alternatives to dairy products, where it is important to preserve functional properties. S. El-Aidie & G. Khalifa (2024) examined new ways of using whey protein in the dairy industry. The emphasis is on the environmental and technological prospects of this ingredient for creating sustainable innovative products. A study of the impact of lipoxygenase inactivation on flaxseed protein quality confirms that controlling oxidative processes improves the sensory characteristics and stability of plant components in food systems (Belinska *et al.*, 2024). An analysis of new ingredients and ultrasonic processing methods was presented by C. Rudke *et al.* (2023). These approaches improve dairy production by promoting innovation in food technology. Advances in reducing sugar content in food were described by G. Zhang *et al.* (2022). The use of innovative technologies and ingredients allows to preserve the taste characteristics while reducing their calorie content. Lastly, modern approaches to the development of low-calorie foods enriched with resistant starch were discussed by S. Karunarathna *et al.* (2024). The authors analysed its beneficial properties and potential impact on consumer health. The use of whey in the creation of innovative food products and its importance for the circular economy was studied by M. Tita *et al.* (2024). The study addressed ultrafiltration as a method of concentrating proteins and their further use in products. The functional properties of cereal beverages as an alternative to dairy products were reviewed by K. Kouamé *et al.* (2023). Scientists have investigated the potential of protein extraction and fermentation to improve the nutritional value, texture, and organoleptic characteristics of these products.

Existing technologies do not always produce a product that not only meets the requirements of reduced calories but also retains high nutritional value and organoleptic properties, which are important for consumers (Dymchuk & Ponko, 2023). Accordingly, the need for new approaches to the production of low-calorie dairy products using plant-based ingredients is becoming even more apparent. This includes the development of new technologies that allow for achieving optimal product parameters, such as texture, taste and aroma, which are important for the consumer experience. The study aimed to substantiate and apply innovative technologies to create low-calorie dairy products based on plant components that would provide an optimal balance between reduced calorie content, nutritional value and organoleptic characteristics. The study solved three key tasks: evaluated the effectiveness of innovative technologies, such as protein extraction, fermentation and ultrafiltration, in the production of low-calorie dairy products; studied the impact of various plant ingredients, including soy, almonds and oats, on the organoleptic properties, stability and nutritional value of finished products; developed an optimised production technology that ensures reduced calorie content, balanced composition, long-term stability and high consumer appeal of products.

Literature Review

Protein extraction is one of the key technologies used in the production of low-calorie dairy products from plant-based ingredients. This process allows for the extraction of highly concentrated protein fractions from plant materials such as soya, peas, almonds and oats. Protein isolates obtained by extraction act as substitutes for part of the milk protein, which can significantly reduce the calorie content of the final product while maintaining its nutritional value (Hewage *et al.*, 2024). Protein

extraction technology involves the separation of protein components from other macronutrients, including fats and carbohydrates, which helps to reduce the energy value of the product by 10-15%. For instance, soy protein isolates provide a structure and texture similar to traditional dairy products such as yoghurt or cheese (Ortega *et al.*, 2024). Similarly, pea proteins are used due to their high stability and ability to form a creamy consistency. Almonds and oats add a mild flavour to products and provide an increased concentration of dietary fibre, which has a positive impact on the functional properties of dairy substitutes. Protein extraction also has a positive effect on the texture of the final product, making it thicker and more stable (Alimardanova *et al.*, 2021). In addition, this technology helps to improve the organoleptic properties, creating products that taste and texture similar to traditional dairy products.

Fermentation is another innovative technology used in the production of low-calorie dairy products from plant-based ingredients (Oleksy-Gębczyk *et al.*, 2024). The fermentation process involves the use of probiotic cultures that break down complex carbohydrates in plant materials, reducing their calorie content and improving the nutritional value of the products. The use of probiotic cultures in fermentation not only reduces the content of energy-saturated substances but also contributes to the enrichment of products with beneficial microorganisms (Chen *et al.*, 2024). This increases their biological value and improves their functional properties, such as maintaining the health of the intestinal microflora. For instance, the fermentation of soya or oat milk can create products similar to traditional fermented milk products such as yoghurt or kefir (Tu *et al.*, 2025). Fermentation also plays a key role in improving the organoleptic characteristics of products. This process reduces the bitterness associated with some plant-based ingredients and results in a richer, more pleasant, fermented milk flavour. Additionally, fermentation results in a stable product texture, which is an important factor for consumers (Prasath *et al.*, 2024).

Ultrafiltration is a modern technology that removes low molecular weight compounds such as sugars and concentrates proteins, vitamins and minerals (Hashmi *et al.*, 2024). This process can be used to create products with a high nutrient content while reducing the calorie content of the final product. In the production of plant-based milk products, such as oat or almond, ultrafiltration is used to achieve the creamy consistency of traditional dairy products. This process can reduce the calorie content of the product by 10-15% without losing important nutritional properties (Akshit *et al.*, 2023). For example, ultrafiltrated almond milk retains the natural flavour of almonds but has improved texture and stability. In addition, ultrafiltration prevents the stratification of herbal beverages and allows for the creation of products with a long shelf life, which is important for industrial production (Sağcan *et al.*, 2024).

The combined use of protein extraction, fermentation and ultrafiltration creates a synergy that significantly improves the quality of low-calorie dairy products made from plant-based ingredients. The combination of these technologies not only reduces calorie content but also ensures optimal texture, rich flavour and high nutritional value. For instance, the use of fermentation after protein extraction from soybeans can be used to create thick, creamy yoghurts with a low-calorie content and enriched with beneficial microorganisms (Ismayilov *et al.*, 2023). In turn, the use of ultrafiltration to purify and concentrate protein components ensures the stability and long shelf life of such products. Comparison with traditional dairy products shows that plant-based substitutes created using innovative technologies can provide similar organoleptic properties while maintaining health and environmental benefits. The integrated approach allows producers to meet the growing demands

of consumers seeking a healthy diet without compromising on taste and texture.

Materials and Methods

The study was conducted over a period of 6 months (from July 2024 to January 2025) in the laboratory of food technology and biotechnology of the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine (Kyiv, Ukraine). The study complied with the requirements of current national and international standards governing the quality of raw materials, methods of analysis, technological processes and final characteristics of the products. The quality control of the selected raw materials was conducted per DSTU 4964:2008 (2010), DSTU 4963:2008 Oats (2010) and UNECE DDF-06:2007 Almond kernels (2008). Sampling for analysis was done following DSTU 4601:2006 Oilseeds (2007). The physicochemical parameters of the raw materials, such as protein, fat and moisture content, were determined as per DSTU ISO 20483:2016 Cereals and legumes (2017), DSTU ISO 659:2007 Oilseeds (2009) and DSTU ISO 712:2015 Cereals and products made from them (2016). The main technological processes, including protein extraction, fermentation and ultrafiltration, were conducted based on international standards. The extraction of protein components is done following the requirements of ISO 1871:2009 (2009). The fermentation process was regulated by ISO 7889:2003 (2003). The final characteristics of the products were assessed following DSTU 2212:2003 Dairy Industry (2004), which sets out the requirements for the organoleptic and physicochemical properties of fermented milk products, and DSTU 8552:2015 Milk and Dairy Products (2017), which regulates the determination of moisture and dry matter content in milk and dairy products.

Three main types of plant material were used in the study: soya beans (*Glycine max*), almonds (*Prunus dulcis*) and oats (*Avena sativa*). All raw materials were purchased from certified suppliers and met the requirements of the DSTU for food safety. The soya beans were of the "Annushka" variety, a high-protein variety recommended for food purposes. The producer was Agro-Soyuz Limited Liability Company (Ukraine). Before the experiment, the soybeans were mechanically cleaned of impurities, soaked in distilled water in a ratio of 1:3 at a temperature of +20°C for 12 hours. After soaking, they were blanched in water at +80°C for 2 minutes to reduce the content of anti-nutritional compounds such as trypsin inhibitors and lectins. The almonds were of the "Nonpareil" variety, one of the most widely used varieties for food purposes. The manufacturer was Blue Diamond Growers (USA). Before use, the almonds were immersed in hot water (+90°C) for 30 seconds to facilitate peeling. After that, the skin was removed by hand, the kernels were dried to 5% moisture content at +50°C and ground to a particle size of <0.5 mm using a laboratory mill. The oats belonged to a naked-grain variety recommended for food. The manufacturer was Grain Company LLC (Ukraine). The oats were mechanically dehulled, washed with distilled water to remove residual impurities, dried at +50°C to a moisture content of 10%, and ground to particles <0.8 mm in size.

Before the experiment, the main quality indicators were monitored. The protein content was determined by the Kjeldahl method (36-38% for soybeans, 21-23% for almonds, and 10-12% for oats), the fat content by the Soxhlet method (18-20% for soybeans, 48-52% for almonds, and 6-8% for oats), and the moisture content did not exceed 8% for soybeans, 5% for almonds, and 12% for oats. Protein extraction was conducted for soya and oat raw materials using the alkaline method. A 0.1 M NaOH solution was used to maintain the pH of the medium at 8.5. The ratio of raw material to solution was 1:10 (g:ml), and the process temperature was maintained at +50°C. The extraction time was 60 minutes under constant stirring with a

magnetic stirrer at 250 rpm. After extraction, the proteins were precipitated when the isoelectric point (pH 4.6) was reached, which was set by adding 1 M HCl. The precipitate was separated by centrifugation at 4,000 rpm for 10 minutes, after which the protein isolates were washed with distilled water and used in further technological steps.

Fermentation was conducted for soy protein isolate and oat cream using probiotic cultures *Lactobacillus acidophilus* (strain LA-5) and *Bifidobacterium bifidum* (strain BB-12). Both strains were provided by Chr. Hansen (Denmark). The lyophilised powder was stored at -18°C in an airtight container. Before use, the cultures were activated in a sterile milk medium at +37°C for 2 hours. The inoculum concentration was 10^7 CFU/ml. The process was carried out at +37°C for 8 hours in a thermostatically controlled incubation chamber Binder BD 56. The pH was monitored using a Hanna HI 2211 pH meter, with the final pH value reduced to 4.4-4.6, which ensured the development of the fermented milk characteristics of the products and contributed to the improvement of organoleptic properties.

Ultrafiltration was used to reduce calories and stabilise the products. The process was carried out on a Sartorius Vivaflow 200 with membranes made of polyethersulfone with a pore size of 10 nm. This separated low molecular weight compounds such as residual sugars and increased concentration of protein components. The process was conducted at a pressure of 1.5 bar with a flow rate of 50 ml/min. The total volume of the final concentrate was 30% of the initial volume. The process temperature was maintained at +10°C to prevent protein denaturation, and the total ultrafiltration time did not exceed 2 hours. The calorific value was assessed by bomb calorimetry using an IKA C200 device. Measurements were made in triplicate, and the results were expressed in kcal/100 g of product. The measurement error did not exceed ±2%. The texture characteristics were analysed using a TA.XTplus Texture Analyzer. The test parameters included a compression test with a probe penetration rate of 1 mm/s and a penetration depth of 5 mm. Characteristics such as elasticity, cohesion, and viscosity were determined, which were used to assess the structure homogeneity and texture stability of the resulting products.

The organoleptic evaluation was conducted by an expert panel of 10 tasters. The tasters evaluated the samples according to four main criteria: taste (balance, fermented milk and nutty notes), texture (uniformity, creaminess), aroma (proneness, absence of foreign odours) and overall attractiveness. The evaluation was conducted on a 5-point scale, and the results were analysed statistically. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) v.26 software. The reliability of the results was determined by the method of one-factor analysis of variance (ANOVA), and the significance level was $p < 0.05$.

The quality of the products was compared with five similar commercial products purchased in retail chains. These included plant-based yoghurts, almond milk and oatmeal cream desserts from three manufacturers. In addition, to evaluate the effectiveness of calorie reduction and organoleptic characteristics, a comparison was also made with traditional dairy products: cow's milk yoghurt, cow's milk and milk cream dessert. The analysis was based on the following parameters: caloric content (determined by bomb calorimetry), texture characteristics (measured with a textrometer) and organoleptic evaluation (average score of the expert panel).

The first stage involved a thorough selection and evaluation of raw materials. Selected samples of soybeans, almonds and oats were preliminarily tested for compliance with such indicators as protein, fat and carbohydrate content, as well as their organoleptic properties. Only raw materials with a high protein and fibre content were used for the experiment (Table 1).

Table 1. *Characteristics of the selected raw materials for the experiment*

Type of raw material	Protein content (%)	Fat content (%)	Carbohydrate content (%)	Fibre content (%)	Amount of raw materials sampled (kg)	Exclusion criteria
Soybeans	36.5	18.3	30.5	4.8	50	Protein content <34%, fat content >20%, impurities >2%.
Almonds	21.8	49.2	21.6	12.5	40	Fat content <45%, damaged kernels >5%, bitter taste
Oats	11.2	6.5	55.4	10.3	60	Protein content <10%, moisture content >12%, impurities >3%

Source: compiled by the authors

Protein extraction was conducted using alkaline hydrolysis, which dissolved protein molecules in an environment with an elevated pH. After that, the proteins were precipitated at the isoelectric point (pH 4.5-4.8) and subjected to filtration to obtain pure isolates. The resulting protein concentrates were used as the basis for the creation of low-calorie dairy products. Fermentation was conducted by adding probiotic cultures to the prepared protein mixtures. This process lasted 8 hours at 37°C, which created optimal conditions for the growth of microorganisms and the breakdown of complex carbohydrates. In the last stage, the fermented mixtures were subjected to ultrafiltration to remove residual sugars and increase the concentration of proteins. This stage also ensured the long-term stability of the product's texture during storage.

Results

The pilot study resulted in the development of three types of low-calorie dairy products made using plant-based raw materials. Fermented soya yoghurt is produced by using the probiotic cultures *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. It was produced using 1,000 g of soya protein isolate obtained from 2,500 g of "Annushka" soya beans. The final product yield was 1,800 g after fermentation and stabilisation. The concentration of probiotic cultures in the final product was 10^7 CFU/ml. As determined, the developed sample is characterised by a creamy texture, typical for fermented milk products, and a balanced fermented milk taste. The almond milk, enriched with protein fractions, was produced by ultrafiltration. It was produced using 1,500 g of peeled "Nonpareil" almonds, which were extracted in 5,000 ml of water after grinding. The resulting emulsion was subjected to ultrafiltration, resulting in a final milk volume of 4,000 ml. As determined, this drink has a homogeneous texture, a pronounced nutty flavour and reduced calorie content compared to similar products on the market. The oatmeal cream dessert was developed using ultrafiltration to concentrate proteins and remove low molecular weight compounds. The production process used 1,200 g of oat flour obtained by grinding 2,000 g of hulled oats. The final product yield after ultrafiltration and stabilisation was 2,200 g. The prepared suspension in a ratio of 1:5 was subjected to fermentation using *Lactobacillus acidophilus*, followed by ultrafiltration. It was found that the resulting product has a thick, stable consistency without signs of stratification or precipitation. As determined, it is characterised by a rich taste with a natural sweetness inherent in oats and a reduced calorie content.

All samples had a stable, creamy texture with no signs of stratification or sedimentation throughout the shelf life. This was made

possible by a combination of protein extraction and ultrafiltration, which ensured the homogeneity of the products. The organoleptic evaluation conducted by the expert panel demonstrated the high consumer appeal of the samples. The average score of all the samples was 4.8 out of 5, based on criteria such as aroma, texture and balance of taste. The products also showed stability during storage. For 7 days at +4°C, the samples retained their organoleptic and physicochemical properties. The texture remained stable, the taste did not change, and there were no signs of delamination or spoilage (Table 2). This highlights the potential of the developed products for commercial production and long-term storage.

Table 2. *Comparative characteristics of the products obtained*

Product	Calorie content (kcal/100 g or ml)	Calorie reduction compared to traditional dairy analogues (%)	Texture	Taste (score out of 5)	Stability (days)
Fermented soya yoghurt	55	21%	Creamy, stable	4.8	7
Almond milk	30	50%	Light, homogeneous	4.8	7
Oatmeal cream dessert	80	33%	Thick, stable	4.9	7

Source: compiled by the authors

The results of the study demonstrated the significant potential of the developed products as an alternative to traditional dairy products. Fermented soy yoghurt was lower in calories compared to traditional yoghurt based on cow's milk (70 kcal/100 g). Almond milk had a lower calorie content compared to cow's milk (60 kcal/100 ml). Oatmeal cream dessert had a lower calorie content than traditional dairy cream desserts (120 kcal/100 g). Fermented soya yoghurt, almond milk and oatmeal cream desserts were characterised by low-calorie content, stable texture and high organoleptic properties. A 21-50% reduction in calories compared to traditional dairy products and a 10-25% reduction compared to commercial plant-based analogues confirms the effectiveness of the technologies used, such as protein extraction, fermentation and ultrafiltration.

Protein extraction, fermentation and ultrafiltration provided a comprehensive approach to achieving high-quality products with optimal organoleptic characteristics and reduced energy value. Each technology has a defined functional role at individual stages of production. The separation of protein isolates contributed to the formation of a stable texture and reduced fat content in the products, which is important for ensuring low-calorie content. The fermentation process using probiotic cultures not only improved the taste properties but also increased the biological value of the products. Ultrafiltration made it possible to remove up to 40-60% of residual sugars, increase the concentration of protein components by 15-25% and ensure product stability for 7 days of storage at +4°C. In particular, in the process of almond milk production, the content of mono- and disaccharides decreased from 4.2 g/100 ml to 1.7 g/100 ml (a 59.5% decrease), and the concentration of proteins increased from 1.2 g/100 ml to 1.5 g/100 ml (a 25% increase). Fermented soya yoghurt showed a decrease in sugars from 5.5 g/100 g to 3.3 g/100 g (40%), while the protein component increased from 3.8 g/100 g to 4.6 g/100 g (21%). The oatmeal cream dessert after ultrafiltration had a sugar content of 2.9 g/100 g (against the initial 6.8 g/100 g, i.e., a 57% decrease), and the protein component increased from 2.5 g/100 g to 3.1 g/100 g (by 24%) (Table 3).

Table 3. *Impact of the technologies used on the quality of the products developed*

Technology	Effect on product quality	Application examples
Protein extraction	Provided a thick, stable texture and reduced fat content. Create a homogeneous consistency that is not inferior to traditional dairy products.	The extraction of protein isolates from soya, almonds and oats to form the basis of reduced-calorie products.
Fermentation	Improved the organoleptic properties of the products, providing a characteristic fermented milk aroma and taste. The breakdown of complex carbohydrates reduces calories and increases the biological value.	The use of probiotic cultures (*Lactobacillus acidophilus, Bifidobacterium bifidum*) to produce soya yoghurts.
Ultrafiltration	Concentrate protein fractions, reduce calories and remove residual sugars. Ensured product stability and long-term texture uniformity during storage.	Processing of almond milk and oatmeal cream dessert to ensure stability and long shelf life.

Source: compiled by the authors

The developed samples showed significant advantages over popular commercial analogues. One of the key advantages is texture stability. While some commercial products based on plant-based ingredients often experience stratification or sedimentation during storage, the developed samples retained their homogeneity and thick consistency. This is the result of the optimal use of ultrafiltration and protein extraction technologies. The developed samples had 21-50% lower calorie content compared to traditional dairy products (fermented soy yoghurt 55 kcal/100 g vs. 70 kcal/100 g, almond milk 30 kcal/100 ml vs. 60 kcal/100 ml, oatmeal cream dessert 80 kcal/100 g vs. 120 kcal/100 g). The organoleptic characteristics of the developed samples received high marks from the expert committee, which included the aroma, balance of taste and texture. The taste and aroma of the samples were found to be richer and more natural compared to commercial analogues (Table 4).

Table 4. *Comparison of the characteristics of the developed products with commercial analogues*

Characteristic	Developed samples	Commercial products
Calorie contents (kcal/100 g)	55 (yoghurt), 30 (milk), 80 (dessert)	70 (yoghurt), 60 (milk), 120 (dessert)
Texture	Thick, homogeneous, stable	Often delamination or sedimentation
Taste	Rich, natural fermented milk or nutty flavour	Sometimes overly sweetened or artificial
Stability	Retains properties for 7 days at +4°C	May lose uniformity during storage

Source: compiled by the authors

The obtained results demonstrated that the developed products have a stable texture, pronounced natural flavour characteristics and reduced calorie content compared to commercial analogues. Thanks to the use of protein extraction, fermentation and ultrafiltration technologies, an optimal balance between nutritional value and organoleptic properties was achieved. In addition, the stability of the products during storage is ensured, which is an important criterion for their further use in production.

Discussion

The results of the study demonstrated the effectiveness of innovative technologies of protein extraction, fermentation and ultrafiltration in the creation of low-calorie dairy products from plant components. The obtained indicators of caloric content, texture, stability and organoleptic characteristics indicate the feasibility of using these technologies in industrial production. A comparison of the results with previous scientific studies concluded the innovativeness

and practical significance of the study. The results of the study were compared with the data of other scientists, which made it possible to assess their compliance with current trends in the creation of low-calorie products. H. Ijaz & S. Sun (2025) discussed structured lipids as fat substitutes that help reduce the calorie content of foods. These conclusions are consistent with the effectiveness of the applied ultrafiltration, which reduced the fat content, ensuring texture stability and preservation of organoleptic characteristics. I. Monroy-Rodríguez *et al.* (2024) noted that protein microparticles can be used as an effective fat substitute in low-calorie foods. A similar effect was observed in the developed samples, where the use of protein isolates from soy, almond and oat provided a thick consistency and high stability, which correlates with the trends described in this study. The study by Q. Xu *et al.* (2024) addressed the functional properties of emulsion gels used to replace fats in dairy products. The conclusions drawn by these authors confirm the effectiveness of technologies aimed at improving texture and reducing calorie content, which was achieved in the developed samples through protein extraction and ultrafiltration. In addition, O. Kovalova *et al*. (2024) demonstrated that the use of cold plasma makes it possible to intensify the process of lentil germination, increasing its biological value by increasing the content of essential amino acids (by 3.6%) and B vitamins (B1, B2, B5, B9), PP and C. Also, the authors note that cold plasma-derived ribbon malt has improved nutritional characteristics and can be used in the production of functional foods, including reduced-calorie dairy products.

R. Munteanu-Ichim *et al.* (2024) highlight the benefits of using plant ingredients and probiotics to create functional non-dairy yoghurts. The high organoleptic and functional characteristics obtained during the study of fermented soy yoghurt are consistent with the results described in the above work, which confirms the effectiveness of fermentation with probiotic cultures. The results of the study were compared with modern scientific developments in the field of low-calorie products from plant components. M. Tosif *et al.* (2025) investigated the properties of mucilage substances that act as functional fat substitutes in vegan products. The authors' conclusions confirm the effectiveness of the use of innovative fat substitutes, which is consistent with the results obtained, where ultrafiltration was used to reduce calorie content without losing textural characteristics.

The safety and quality of dairy products based on cow's and mare's milk with the addition of plant components was studied in the study by M. Iztileuov *et al.* (2024). The identified positive changes in organoleptic properties due to plant ingredients correlate with the results of the study, where the use of protein isolates from soy, oats and almonds ensured high stability and attractiveness of the developed samples. I. Nyambayo *et al.* (2024) studied consumer acceptance of plant-based milk and dairy products. The authors emphasise the growing interest in products with reduced calorie content and functional properties, which confirms the importance of the low-calorie products developed in this study. The high evaluation of the organoleptic characteristics confirms that they meet the consumer expectations described in the study. The study by T. Zhang *et al.* (2023) analysed the use of plant-based fat substitutes with functional and health-promoting properties. The technologies used in this study, such as protein extraction and ultrafiltration, confirm the effectiveness of the proposed approach to reducing the fat content in products without losing their texture and taste.

M. Alam *et al.* (2025) analysed the integration of sensor technologies into the food industry, which contributes to improving product quality and meeting modern consumer requirements. The introduction of modern methods of quality control and improvement of production processes in this study also corresponds to these trends. The results of

the study were compared with modern scientific developments related to the creation of low-calorie products with plant components. A. Gupta *et al.* (2025) examined the physicochemical and sensory characteristics of plant milk for coffee drinks. The authors noted the importance of a stable texture and flavour, which coincides with the findings of a study in which stability and organoleptic characteristics were achieved using ultrafiltration. S. Shaheen *et al.* (2023) highlighted the functional and metabolic properties of fat substitutes. Their analysis confirms the possibility of reducing the calorie content of products without losing functional properties, which is consistent with the results of the study, where protein extraction contributed to a reduction in fat content and preservation of texture.

D. McClements *et al.* (2021) examined the introduction of innovative technologies in the food industry to create a sustainable and healthy food system. The results obtained in this study, such as the use of protein extraction, fermentation and ultrafiltration, confirm the promise of innovative technologies to meet modern consumer requirements. S. Chen *et al.* (2023) studied colloidal strategies for inhibiting lipid degradation in the development of low-calorie foods. Their findings on the importance of controlling lipid components are consistent with the results of a study where ultrafiltration and fermentation reduced fat content and ensured product stability. The findings confirm the effectiveness of innovative technologies for creating low-calorie products, demonstrating their compliance with current scientific trends.

Conclusions

As a result of the study, the main goal was achieved: innovative technologies for the creation of low-calorie dairy products based on plant components that combine reduced calories with high organoleptic characteristics and nutritional value were substantiated and developed. The use of protein extraction, fermentation and ultrafiltration technologies has made it possible to produce products that meet modern consumer requirements for healthy eating and quality. The study confirmed the effectiveness of using plant ingredients such as soya, almonds and oats in the production of low-calorie dairy products. The resulting protein isolates from plant-based raw materials provide a stable texture that is not inferior to traditional dairy products. Fermentation reduces calorie content by breaking down complex carbohydrates and further improves the taste due to the fermented milk flavour. Ultrafiltration ensured the concentration of protein components, reduced calorie content and long-term texture stability of the products.

The results of the study showed that the developed products, such as fermented soya yoghurt (55 kcal/100 g), almond milk (30 kcal/100 ml) and oatmeal cream dessert (80 kcal/100 g), had 20-50% lower calorie content compared to their traditional counterparts. The organoleptic evaluation showed high consumer appeal, with an average score of 4.8 out of 5 for taste and texture. The products also demonstrated stability for 7 days of storage at +4°C. The practical significance of the results includes the possibility of introducing these technologies into commercial production. The developed products can meet the demand in the functional and dietary food market, providing consumers with a healthy alternative to dairy products. Further research should optimise technologies for production costs reduction, expanding the product range by using new plant-based ingredients (such as algae proteins and chia seeds), and studying the long-term stability of products under different storage and transportation conditions, which in general creates the basis for further development of the low-calorie dairy products segment with plant-based ingredients.

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Conflict of Interest

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Інноваційні технології створення низькокалорійних молочних продуктів із використанням рослинних компонентів

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Анотація. Створення низькокалорійних молочних продуктів із використанням рослинних компонентів набуває дедалі більшого значення через зростання попиту на здорове харчування та альтернативи традиційним молочним виробам. Бекграунд дослідження базувався на необхідності розробки функціональних продуктів зі зниженим калорійним навантаженням, які поєднують високу поживну цінність і привабливі органолептичні властивості. Метою дослідження було розробити низькокалорійні продукти на основі рослинної сировини із застосуванням інноваційних технологій, таких як екстракція білків, ферментація та ультрафільтрація, забезпечуючи зниження калорійності без втрати текстури, смаку та стабільності під час зберігання. У роботі використовувалися високоякісні рослинні інгредієнти (соя, мигдаль, овес) і пробіотичні культури, а методологія включала виділення білкових ізолятів шляхом екстракції, подальшу ферментацію для покращення смаку та збагачення корисними мікроорганізмами, а також ультрафільтрацію, що сприяла концентрації білків, видаленню цукрів і забезпеченню стабільності продуктів. Результати показали, що розроблені продукти – ферментований соєвий йогурт (55 ккал/100 г), мигдальне молоко (30 ккал/100 мл) та вівсяний крем-десерт (80 ккал/100 г) – продемонстрували суттєве зниження калорійності на 20-50 % у порівнянні з традиційними молочними аналогами. Усі зразки зберігали стабільну текстуру, однорідність і високі органолептичні характеристики протягом 7 днів при температурі +4°C, отримавши середній бал 4,8 із 5 за оцінкою експертної комісії. Додатково було встановлено, що використання ультрафільтрації забезпечило тривалу стабільність продуктів, а ферментація сприяла покращенню смакових характеристик

і підвищенню біологічної цінності. Висновки дослідження підтвердили ефективність інноваційних технологій у створенні конкурентоспроможних низькокалорійних продуктів із рослинних компонентів. Практична значимість полягає у можливості впровадження отриманих результатів у промислове виробництво для задоволення потреб сучасного ринку, орієнтованого на здорове харчування

Ключові слова: харчова промисловість; здорове харчування; ферментація; ультрафільтрація; екстракція білків; пробіотичні культури

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Biotechnological solutions for improving the quality and environmental friendliness of sugar in the food industry

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Abstract. Sugar production has a significant impact on the environment, which makes it necessary to introduce more efficient and environmentally friendly technologies. One such alternative is the use of biotechnological approaches to improve the quality of sugar syrups and the production of organic sugar without the use of chemicals. The purpose of this study was to evaluate the efficiency of enzymatic methods of syrup purification using pectinesis and amyloglucosidase and determine their impact on the quality of the final product, as well as to investigate the possibilities of processing sugar by-products into useful substrates for other food industries. Enzymatic purification and ultrafiltration methods were used to achieve this goal. During the experiments, a syrup with a residual pectin content of 0.02% ± 0.005 and starch content of no more than 0.01% was obtained, which indicates a high level of purification. The combined use of enzymatic and membrane methods allowed improving the stability of the process and increasing productivity to 120 litres of syrup per hour, which also helped to reduce energy costs by 8% and reduce the duration

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of technological stages by 10%. The results showed that enzymatic purification of the syrup reduced the level of impurities in molasses and contributed to an increase in the yield of bioethanol to 88% ± 2, which is 9% higher than in the control group. It was found that the feed obtained from pulp after biotechnological treatment had high quality indicators: a decrease in moisture content to 10% ± 0.5, a decrease in microbial activity to 5 × 103 CFU/g and increase the mechanical strength of pellets to 92% ± 4. The results showed significant benefits of enzymatic technologies, in particular, reducing the cost of chemical reagents and waste disposal. The practical significance of the study lies in the fact that the use of biotechnological methods can significantly increase the efficiency of organic sugar and feed production, helping to reduce the environmental burden

Keywords: chemical impurities; enzymatic purification; membrane purification; molasses; by-products

Introduction

Biotechnologies are increasingly influencing food production, especially in the sugar industry, opening up new opportunities to improve the quality of the final product and reduce the negative environmental impact. Sugar production faces many challenges, such as the need to reduce impurities in the final product as well as to process significant amounts of by-products. In this context, research on the impact of innovative biotechnologies is critical. Biotechnological methods allow producing pure organic sugar without the use of chemicals, while contributing to sustainable development through ecological waste processing. For example, the study by G. Kovalenko (2019) showed that the introduction of biotechnological solutions can significantly optimise waste processing costs and increase the environmental compliance of production processes. Similar ideas were reflected in the paper by A. Bezpala *et al*. (2024), who focused on the rational use of sugar by-products, such as pulp, for the production of feed or substrates for other industries.

Improving the quality of the final product in biotechnological production is made possible by the use of advanced microbial cultures and enzymes (Nabiyev *et al*., 2024). For example, V. Nikulshyn *et al*. (2022) considered energy-saving options to optimise production processes that simultaneously reduce the negative impact on the environment. M. Steinwand & P. Ronald (2020) noted that the use of genetically modified sugar beet crops can not only reduce the number of pests and chemical treatments, but also provide a product with improved organoleptic qualities. Processing of sugar by-products opens up significant opportunities for ensuring sustainable industrial development (Panasiuk & Myskovets, 2023). M. Meghana & Y. Shastri (2020) explored ways to use waste efficiently, such as converting it into biofuels or biopolymers. This increases the value of waste and helps to reduce environmental pollution. Such innovations correspond to global trends in sustainable development and allow creating circular economic models that integrate several branches of the food industry. The relevance of this problem was emphasised by M. Rajaeifar *et al*. (2019), who analysed the transition to a waste-to-energy conversion strategy as an efficient way to reduce the negative impact on the environment, which promotes more sustainable use of resources and reduces the environmental footprint. P. Sukphun *et al*. (2023) considered the double stages of processing sugar industry waste to produce biogas. This approach allows not only reducing the amount of waste, but also obtaining renewable energy sources, which can affect the sustainable development of the industry as a whole.

In the context of the development of biotechnology in the food industry, it is also important to consider the economic and political factors that influence the introduction of innovations (Kolisnik & Polishchuk, 2023). K. Alekseieva *et al*. (2023) highlighted the importance of government support for businesses, especially in crisis situations, to ensure economic sustainability and food security. These researchers analysed state support programmes in Ukraine, in particular, in the agro-industrial sector, which directly affects the production of sugar and other food products. They emphasised the need to create a favourable environment for the functioning and reproduction of businesses in the new economic environment, which is especially relevant for innovative biotechnological enterprises in the food industry. As noted by K. Alekseieva *et al*. (2023), the effectiveness of government support programmes and clear criteria for selecting viable enterprises are key factors for stimulating the development of promising technologies, including biotechnological innovations in the sugar industry.

The studies by B. Biyashev *et al*. (2023), E. Shahini *et al*. (2024), and D. Breus *et al*. (2024) highlighted a critical issue of the environmental impacts of war, which can be long-term and irreversible for ecosystems and human health. The researchers emphasised the importance of understanding the extent of the impact of such events on natural resources and developing effective measures to restore them. These aspects are also directly related to the sugar industry, since a violation of the ecological balance can significantly affect the quality of raw materials and production conditions. As noted by E. Shahini *et al*. (2024), planning for the recovery of affected areas, including ecosystem restoration, is an important component of ensuring the sustainable development of various food industries, including sugar production, which highlights the need to integrate environmental considerations into the development of biotechnological innovations for this sector.

M. Nawaz *et al*. (2021) considered the concept of zero waste through bio-wastewater treatment, which allows the conversion of contaminated water typical of the sugar industry into useful resources and contributes to the production of valuable products. This is another example of a strategy for converting industrial waste into useful resources, contributing not only to the sustainable development of the industry, but also to the preservation of the natural environment. It is also worth noting the study by K. Bueno-Zabala *et al*. (2020), which focused on the optimised production of glucose syrup using membrane reactors, which simultaneously allows the production of valuable by-products. This approach increased the efficiency of raw material use and reduced production costs, which is important for improving environmental sustainability and economic benefits.

Special attention should also be paid to the latest technologies for processing sugar beet, the development of which was considered by B. Muir (2022), highlighting innovative approaches designed to improve the efficiency of final product production and reduce the impact on the environment. These technologies are essential for optimising production processes and conserving resources, ensuring increased environmental sustainability of the sugar industry. Thus, the main areas of scientific research aimed at innovation in the sugar industry are waste processing, reducing the ecological footprint of production and improving the efficiency of resource use, which ensures the sustainable development of the industry and contributes to the preservation of ecological balance. The purpose of this study was to identify key biotechnologies that can improve the quality of both the final product and by-products of production, namely, molasses and pulp, and to minimise the environmental impact by recycling waste.

Materials and Methods

Samples of sugar beet (*Beta vulgaris*) were provided by LLC Agrofirma im. Dovzhenka

(Astarta-Kyiv Group, Poltava Oblast). The inclusion criteria were conditions under which the samples met maturity standards (beet sugar content ⩾ 16%, was declared by suppliers), had no defects or visible signs of disease. The sample did not include specimens with damage, infested with pests or fungal diseases, such objects were excluded after visual inspection and microbiological analysis. Microorganisms used at the stage of disposal of by-products (bacteria *Zymomonas mobilis*, strain ATCC 31821, providing a high level of ethanol fermentation) were provided by D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The study was conducted from March to September 2024 at the Institute of Agricultural Microbiology and Agro-Industrial Production of the National Academy of Sciences of Ukraine (Chernihiv, Ukraine). The experimental part included working with control and experimental samples, where the former characterised the usual chemical approach to syrup processing, and the latter – testing the proposed biotechnological methods. The study focused on evaluating enzymatic methods for syrup purification to reduce the impurity content without using chemical reagents.

Sugar beet samples of the control and experimental groups were cleaned of dirt and ground to a uniform consistency (particle size – 2-3 mm). To obtain the syrup, the raw material was extracted with hot water (temperature 80°C, mass/volume ratio 1:2), and thorough mixing was ensured during extraction to maximise the release of sugars from the cell mass. Control samples were prepared according to conventional technology, which involves the use of chemical reagents such as limestone milk and sulphur anhydride to purify the syrup. In the test samples, an enzyme complex was added to the resulting syrup, including: pectinase for the breakdown of pectin compounds (dosage 20 U/mL, activity 100 PE); amyloglucosidase for the conversion of starch residues to glucose (dosage 15 U/mL, activity 120 AO). Fermentation was carried out in a Biostat B bioreactor (Sartorius, Germany) at a temperature of 50°C and a pH of 5.5 for 6 hours. This temperature was chosen because used enzymes have optimal activity in the range of 45-55°C, which ensured maximum efficiency in the breakdown of pectins and starch residues and increased the purity of the final product. The temperature of 50°C was also favourable for enzymatic activity, but too high for the development of unwanted bacteria. The pH value of 5.5 maintained the stability of enzymes, microbial activity, and prevented the development of contaminants.

In the control group, impurities were measured after chemical treatment, and in the experimental group – after enzymatic purification. Analysis of the pectin content was performed by gravimetry: the selected syrup sample was subjected to pectin precipitation using alcohol (96% ethanol) in a ratio of 1:2. The resulting precipitate was centrifuged in an Eppendorf 5804 R centrifuge (Germany) (10 minutes at 5,000 rpm) to remove liquid residues, dried in a Memmert UF 260 drying cabinet (Germany) at a temperature of 105°C to a constant mass. The residual pectin content was calculated as a percentage of the total syrup mass. Analysis of the residual starch concentration by the enzymatic method was based on hydrolysis of the residual starch to glucose, followed by quantitative determination of glucose. The syrup sample was incubated with alpha-amylase with an activity of 50 U/mL at 37°C for 30 minutes. The resulting glucose was determined using the glucose oxidase method, which included colorimetric measurement with a BIO-TEK instruments Synergy H1 colorimeter (USA) absorption at 540 Nm. The concentration of residual starch was calculated considering the conversion factor. In the control and experimental groups, these processes were performed in the same way. After purification, the samples of the control and experimental groups were further purified by ultrafiltration. This process involved passing the syrup through a Millipore Pellicon

2.0.2 µm membrane (USA) with a pore size of 0.2 microns, which helped to remove macromolecules, cell mass residues, and impurities.

To ensure the suitability of the syrup for further use, it was concentrated by vacuum distillation. The pressure was reduced to 50-100 mm Hg in the LabTech LT 3000 vacuum unit (China), which allowed water to evaporate at a temperature of 50-60°C. This low-temperature process prevented thermal degradation of sugars, preserving their natural composition and organoleptic properties. The syrup concentration reached 65-70% of dry substances, which provided optimal viscosity for further production stages. Determination of the colour characteristics of the syrup corresponding to its quality was carried out on the ICUMSA scale with a Lovibond PFX 8800 meter (Germany). The presence of undesirable impurities resulting from caramelisation was assessed by optical microscopy using an Olympus BX41 microscope (Japan). The experimental and experimental groups used the same technological parameters, but considered the effect of the absence of chemical reagents on the quality of the final product. In both groups, the stability of the syrup's organoleptic characteristics, such as smell, colour, and taste, was assessed by sensory analysis, and the indicators were evaluated on a 10-point scale.

Processing of by-products. The process of fermentation of molasses was carried out using microorganisms *Zymomonas mobilis*. The prepared molasses solution was inoculated at pH 4.5-5 in a Biostat B bioreactor (Sartorius, Germany). The fermentation temperature was 30°C and the duration was 24-48 hours, during which the ethanol yield was monitored by analysis on an Agilent 8890 gas chromatograph (USA). The resulting ethanol was distilled in a LabTech LT 3000 vacuum distillation unit (Italy), which made it possible to effectively separate ethanol while preserving its properties.

A Flottweg Z Series dewatering press (Germany) was used to process the pulp. Further drying was carried out in an Alvan Blanch DR 800 drum drying unit (Great Britain) at 60-90°C to achieve a residual moisture content of 10%. The dried material was ground in a Buhler MultimpactMax hammer mill (Bühler Group, Switzerland) to a particle size of 2-3 mm. Granulation was carried out on a CPM Europe Century Series granulator (CPM Europe, Netherlands), which provided the formation of feed pellets with a diameter of 8-12 mm with high mechanical strength. The quality of granular feed was evaluated by several indicators. The moisture content was determined by drying in a Binder FD115 thermostat (Germany) at 105°C, and microbiological purity was assessed by seeding on TSA agar for aerobic bacteria and SDA agar for fungi with the number of colony-forming units (CFU/g). The mechanical strength of the pellets was measured on the PDT 1100 Pellet Durability Tester (Luxembourg), while fractional analysis of particle size uniformity was performed on the RETSCH AS 200 laboratory sieve analyser (Germany).

Results

The enzymatic approach to syrup purification showed significantly better results compared to conventional chemical methods. The study showed that the content of residual pectin in control samples treated with chemical reagents was 0.08% ± 0.01, while in samples, purified with pectinase and amyloglucosidase, this indicator decreased to 0.02% ± 0.005 (Fig. 1). In parallel, the residual starch in the syrup after enzymatic purification was significantly lower – ⩽ 0.01% compared to 0.04% ± 0.007 in the control samples. These results clearly demonstrate a high degree of impurity removal due to enzymatic purification. The mechanism of pectinase was to break down pectin chains, which greatly facilitated the removal of residual macromolecules. Amyloglucosidase, in turn, effectively broke down the residual starch, ensuring its minimum concentrations in the final product.

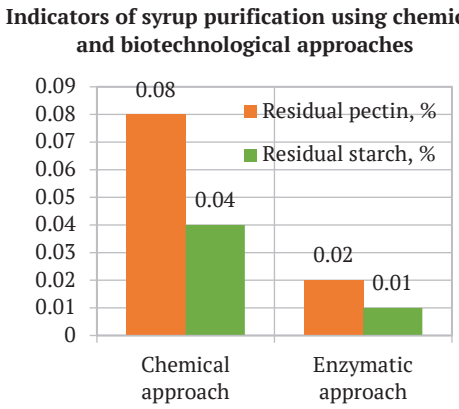

Figure 1. Indicators of syrup purification using chemical and biotechnological approaches
Source: compiled by the authors

One of the most important advantages of biotechnological purification is the absence of chemical by-products in the final product. Chemical purification methods such as lime and sulphur compounds, despite their efficiency, lead to the development of undesirable precipitates, residues of chemical agents in the syrup, and harmful by-products such as calcium salts (Muir, 2022). In comparison, the enzymatic approach provided not only a high degree of purification, but also the preservation of the natural composition of the syrup, which is critical for organic production. The study also revealed the economic benefits of biotechnological purification. The use of enzymes has reduced the duration of the purification process to 6 hours, while the chemical approach often requires additional stages of precipitation, filtration, and regeneration of reagents, which extends the technological process to 10-12 hours and increases costs.

To test the reliability of the method, 10 repeated experiments with enzymatic purification were performed, and in all cases the values of residual pectin and starch corresponded to the established values (0.02% and ⩽ 0.01%, respectively). All experiments were performed under the same conditions – temperature 50°C, pH 5.5, enzymatic treatment lasting 6 hours. Fluctuations in the values did not exceed 5%, which indicates a high reproducibility of the method (Table 1).

Table 1. *Results of repeated experiments on enzymatic purification of sugar syrup*

No.	Pectin (%)	Pectin standard (%)	Starch (%)	Starch standard (%)
1	0.02	0.02	0.009	0.01
2	0.019	0.02	0.008	0.01
3	0.021	0.02	0.01	0.01
4	0.018	0.02	0.009	0.01
5	0.02	0.02	0.009	0.01
6	0.019	0.02	0.008	0.01
7	0.02	0.02	0.009	0.01
8	0.021	0.02	0.01	0.01
9	0.018	0.02	0.008	0.01
10	0.019	0.02	0.009	0.01

Source: compiled by the authors

Another advantage of the enzymatic approach is the reduce in the environmental impact of production. Chemical treatment leads to the development of sediments that require special disposal, and to excessive water consumption during the regeneration of chemical agents (Nawaz *et al.*, 2021). The enzymatic method significantly reduces the need for additional resources, such as chemical reagents and energy, as it reduces the use of harsh chemicals for cleaning and stabilising products by 30-40%. In addition, the reduction of energy requirements for cleaning and stabilisation processes is up to 25%, which helps to reduce costs and minimise

the amount of harmful residues. This makes the method the most successful for use in environmentally oriented production.

Membrane ultrafiltration technology has shown high efficiency in removing macromolecules and other impurities in syrups of both the control and experimental groups. However, the purification results were significantly better in the case of samples from the experimental group that underwent the enzymatic purification stage before membrane treatment. The formation of precipitation on the membranes was an important parameter that demonstrated the advantage of the combined approach. In the control samples, the level of deposits on the membrane increased after 20 minutes of system operation, which led to a gradual decrease in throughput by up to 60% from the initial level. Instead, in the experimental samples, deposits on the membranes were reduced by 45% compared to the control group, and stable operation of the plant was maintained for up to 30 minutes, which allowed a larger volume of purified syrup to be obtained in one filtration cycle.

The results of the analysis of residual pectin substances and starch in syrups after ultrafiltration demonstrate a higher purity of the final product in the experimental group. In the control samples, the level of residual pectin was ⩽ 0.02%, and starch – ⩽ 0.01%. In the samples of the experimental group, these indicators were lower than the detection limit of the analytical equipment used (< 0.01%). This indicates a deeper purification of the syrup due to the synergistic effect of pre-enzymatic treatment, which significantly reduced the content of impurities before the membrane stage. The more efficient operation of the membranes in the experimental group is explained by the reduced load on the filter elements. Enzymatic treatment reduced the level of macromolecules and the viscosity of the syrup, which facilitated the passage of syrup through the membranes and reduced the risk of contamination.

In addition to the cleaning quality, the performance of the ultrafiltration unit was also better for the experimental group. On average, the volume of purified syrup per hour was 120 litres for experimental samples compared to 90 litres for control samples, which provided an additional productivity of 30%. The absence of the need for frequent membrane washing during the processing of experimental samples also significantly increased the cost-effectiveness of the process. The combination of enzymatic and membrane purification not only improved the quality of the final product, but also demonstrated environmental benefits. The use of enzymes in the initial stages allowed reducing the volume of precipitation formed by 22-25% and reducing water consumption for cleaning the system by 25-35%, which is usually necessary for the regeneration of chemical membranes in the control group. This approach also reduced the use of chemical reagents by 40%, which will help to improve the environmental friendliness of the entire technological process. Consequently, the results of membrane syrup purification confirm the effectiveness of the proposed combined approach. Based on enzymatic pretreatment, it was possible to significantly reduce the level of precipitation on the membranes, improve the quality of cleaning, and the stability of the ultrafiltration system. This indicates the prospects of using this method to ensure high quality of organic sugar and reduce the environmental impact of production.

Syrup concentration by vacuum distillation. Vacuum distillation provided a stable syrup concentration process for both the control and experimental groups. However, the results of the final product analysis clearly demonstrated the advantages of the combined enzymatic and membrane purification approach applied to the samples of the experimental group. One of the key evaluation parameters was the determination of the colour characteristics of syrup on the ICUMSA scale, which is used to determine the degree

of purity and clarification of sugar products. As a result of the combined approach to syrup purification, the samples of the experimental group showed lower values in the range of 190-210 units, which indicates a much cleaner product with a natural amber colour. For the control group, this indicator was noticeably higher and ranged from 240-270 units, which indicates the presence of residual impurities and the absence of maximum transparency.

The preservation of aromatic compounds is another important parameter of the quality of the final product, since these components provide the smell and natural taste profile of the syrup. In the process of vacuum concentration under low pressure conditions (50-100 mm Hg) and the temperature range of 50-60°C, it was possible to minimise the loss of volatile substances. The samples of the experimental group showed a 15% higher level of preservation of volatile aromatic compounds compared to the control samples, which provided improved organoleptic properties of the product. Losses in the control group are explained by residues of chemical impurities, which could cause additional evaporation or degradation of aromatic components when heated.

The concentration of syrup to a sugar density of 76-78% occurred without noticeable degradation of natural sugars or the development of undesirable products (caramelisation) in both groups. However, the experimental samples had a more uniform texture without microscopic inclusions, which were sometimes recorded in the control group during microscopic analysis. This may be caused by the higher purity of the syrup before the vacuum concentration, which prevented the development of foreign crystal formations or microparticles that could remain in the control samples. Due to the high initial purity of the syrup of the experimental group, the concentration duration was reduced by 10% compared to the control samples. Reducing the level of impurities that need to be removed during distillation has reduced energy costs by 8%. This has had a positive impact on the overall performance and profitability of the production process, which is important for industrial implementation.

Tasting evaluation of concentrated syrups in both groups showed that the samples of the experimental group had a more natural taste with a pronounced beet aroma, while in the control group there was a slight bitterness, which probably arose due to the presence of residual chemicals. The average score of the organoleptic test for samples from the experimental group was 9.2 ± 0.3 on a 10-point scale, while in the control group this indicator was 7.8 ± 0.4 ($p < 0.05$). Table 2 illustrates the key differences in the concentration process parameters between the two groups, confirming the overall advantage of the technological approach used for experimental samples.

Table 2. *Comparison of sugar syrup concentration process parameters*

Parameter	Control group	Experimental group
Colour indicator (ICUMSA)	240-270	190-210
Level of preservation of aromatic substances (%)	85 ± 3	100
Duration of concentration (min)	60 ± 2	54 ± 1
Energy consumption (kWh)	25 ± 1	23 ± 1

Source: compiled by the authors

The use of vacuum distillation in the experimental group, which used syrup purified by enzymatic and membrane methods, made it possible to achieve significantly better quality of the final product, including colour, aroma, and texture indicators. The high efficiency of the process, its energy saving and environmental friendliness make this

technology promising for large-scale implementation in the production of organic sugar (Bulgakov *et al.*, 2017).

Results of by-product disposal. The fermentation process of molasses, one of the main by-products of sugar production, showed significant differences in bioethanol yield between the control and experimental groups. Molasses obtained after enzymatic purification of the syrup showed a significantly higher degree of processing of sugars into ethanol. In the control group, where molasses was obtained after conventional chemical purification of syrup, the bioethanol yield was 79% ± 3 of the theoretical maximum (Table 3). This is caused by the high content of residual chemical impurities, which suppressed the activity of microorganisms and reduced the efficiency of fermentation. Instead, in molasses purified by biotechnological method (experimental group), impurities were minimised, which allowed achieving a bioethanol yield of 88% ± 2 ($p < 0.01$).

Table 3. *Results of molasses fermentation in two groups*

Parameter	Control group	Experimental group	Difference (p-value)
Bioethanol yield	79% ± 3% of the theoretical maximum	88%±2% of the theoretical maximum	$p < 0.01$
Time to reach maximum ethanol concentration (hours)	36 hours	30 hours	$p < 0.01$
Organic acid concentration (g/l)	0.5 ± 0.08 g/l	0.2 ± 0.05 g/l	$p < 0.01$
Y_ethanol/sugar (g ethanol/g sugar)	0.42 ± 0.03 g/g	0.48 ± 0.02 g/g	$p < 0.05$

Source: compiled by the authors

Comparison of the dynamics of the fermentation process also showed a significant difference between the groups. In the control group, the maximum ethanol concentration was reached in 36 hours, while in the experimental group, this process was reduced to 30 hours. The reduction in the duration of fermentation can be explained by a favourable environment for *Zymomonas mobilis* in molasses purified without chemical reagents. Additional quality analysis of the obtained bioethanol showed that ethanol obtained from molasses of the experimental group had a higher degree of purity, with the content of undesirable side components 20% lower than in the control samples. In the control group, traces of organic acids (for example, acetic acid) were often found in bioethanol, which were formed due to chemical stress on microbial cells caused by residual impurities in molasses. In the bioethanol samples of the experimental group, the concentration of organic acids was 0.2 ± 0.05 g/l, while in the control group – 0.5 ± 0.08 g/l ($p < 0.01$), which confirms the increased efficiency of the process in the absence of chemical contamination in the raw material. Reducing the level of impurities in molasses, as a result of the use of enzymatic purification of syrup, improved the viability and metabolic activity of microorganisms. The experimental group demonstrated a stable value of the Y_ethanol/sugar parameter (efficiency of converting sugars to ethanol), which was 0.48 ± 0.02 g/g, which is very close to the theoretical maximum (0.51 g/g). In the control group, this parameter reached 0.42 ± 0.03 g/g, which is 12% less ($p < 0.05$), which leads to a lower ethanol yield.

Sugar beet pulp, which is formed as a by-product after extraction of sugar substances, was further processed in this study to produce granular feed. Evaluation of the final product showed significant advantages of the feed obtained in the experimental group, according to the following key indicators. The experimental pellets achieved a moisture content of 10% ± 0.5 compared to 12% ± 0.8 in the control samples.

The reduced moisture content provided an increased duration of feed storage without loss of quality or mould formation. Granulated feed of the experimental group contained microorganisms in the amount of 5×10^3 CFU/g, which is significantly less than in the control group (2×10^4 CFU/g). Such low microbial activity was ensured by the absence of residual chemical impurities in the pulp and careful heat treatment. The pellets obtained in the experimental group showed mechanical strength at the level of 92% ± 4, which is significantly higher than in the control group (80% ± 5). This is important to ensure the stability of the product during transportation and storage, and to prevent dust formation (Table 4).

Table 4. *Comparison of the characteristics of granulated feed from pulp between groups*

Parameter	Control group	Experimental group
Moisture content, %.	12 ± 0.8	10 ± 0.5
Microbiological purity	2×10^4 CFU/g	5×10^3 CFU/g
Mechanical strength of pellets	80 ± 5%	92 ± 4%

Source: compiled by the authors

The absence of synthetic preservatives, the high quality of pellets, and their stability allows avoiding the risk of accumulation of undesirable substances in the feed. The use of the enzymatic approach at the initial stages helped to reduce the need for chemical reagents to stabilise the product, and reduce waste disposal costs due to increased drying efficiency. The long shelf life of experimental pellets without additional chemical treatment also reduced the manufacturer's costs and reduces the environmental burden. Obtained results demonstrate the superiority of biotechnological methods in all aspects of the study: syrup purification, concentration, preservation of organoleptic properties, and disposal of by-products. Comparison of control and experimental samples confirmed that the enzymatic approach allows obtaining a better final product with minimal environmental impact and better efficiency indicators.

Discussion

The results obtained in the course of the study show a certain similarity with the results of previous research, including individual differences, which can be explained by different approaches to processing raw materials and the variability of experimental conditions. In this regard, it is important to compare the results with studies by other researchers that focus on the environmental and technological aspects of sugar beet processing and sugar production. This study on optimising the use of sugar industry by-products has shown that beet processing and syrup production processes have significant potential to reduce waste and increase efficiency. These results correlate with the study by B. Muir & A. Anderson (2022), who noted the significance of developing sugar beet as an important area in Europe to increase yields and reduce production costs. In particular, improving the efficiency of syrup production and processing by-products can significantly improve the environmental and economic component of sugar production. However, the results obtained demonstrate that the integration of the latest technologies for preserving syrup in limited seasonality conditions can significantly increase the production period.

Such approaches are confirmed by the findings of A. Adbhai *et al*. (2022), who focused on using patent molasses left over from the sugar extraction process to create secondary products. The researchers also noted the high potential for energy production from residual molasses. This study, on the contrary, focused more on the efficiency of their processing in the context of reducing the amount of waste when using raw materials for the production

of not only sugar, but also bioenergy resources. The results of S. Singh *et al*. (2021) regarding the processing of sugar cane waste into commercial products, in particular, through the optimisation of production methods and solving environmental problems that arise in the process, are consistent with this study, since in both cases it is about the use of biotechnologies for processing sugar by-products to improve the quality of the final product and reduce environmental impact, in particular, through fermentation and reducing the need for chemical reagents, which also indicates opportunities for improving the environmental efficiency of the agricultural sector.

Similarly, the results of the studies by A. Hernández-Pérez *et al*. (2020) and A. Babu & S. Adeyeye (2024) related to sugar extraction and sweetener production through biotechnological processes, support observations on the potential of using environmentally friendly methods to reduce waste and improve the efficiency of production processes. Although studies conducted by other researchers focus more on aspects of technological development within the one-sided use of sugar beet, the results highlight the need for multifunctional solutions to solve the problem of waste in production. The results of the development of biotechnological processes for the production of fructose-rich syrups coincide and in some aspects complement the conclusions from previous works of international researchers, in particular, the study by M. Garcia-Aguirre *et al*. (2009) identified significant potential for using enzymatic hydrolysis of agave fructose-oligosaccharides to produce fructose-rich syrups. This study highlighted the importance of optimising technological processes, which is directly compatible with the results obtained, which shows that modern technologies of bioconversion of raw materials can lead to an increase in the quality and efficiency of syrup production.

The study by R. Singh *et al.* (2017) is also important. It described enzymatic methods for the synthesis of high fructose syrups. The researchers noted the importance of using specific enzymes to improve the process of converting monosaccharides to higher fructose-rich compounds. These results support these conclusions, as it was found that the choice of specific enzymes can actually increase the yield of the product without losing quality. Similarly, research by H. Atiyeh & Z. Duvnjak (2005) on the use of ultrafiltration membranes and activated carbon for the purification of fructose syrups produced from cane molasses indicates the importance of such technologies in the syrup purification process. The results obtained indicate the effectiveness of these methods, in particular for achieving the desired purity of syrups while preserving their useful properties.

The study by J. Hubbuch & M. Kula (2007), with a focus on the isolation and purification of biotechnological products, confirms the effectiveness of the approach used in providing high-quality fructose-rich syrups. The methods recommended by these researchers can be adapted for cleaning products at the production stage, which will allow achieving the maximum level of purification with minimal energy costs. Other studies, for example by D. Lima *et al*. (2011), which highlighted the biotechnological potential of fructose syrup, are consistent with the results in terms of improving the use of fructose as a biologically active product. These results support the possibility of optimising the use of fructose-rich syrups for industrial purposes, especially given their wide range of applications in the food industry. The fundamental research by E. Vandamme *et al*. (1987) examined microbial sucrosophosphoratases in the context of fermentation and biotechnological applications that improve the process of converting sugar to fructose syrups. The results of this sucrose bioconversion study also confirmed the ability of enzymatic technologies to significantly increase production efficiency when using microbial agents,

similar to what was found in the current study. The studies by J. Bicas *et al*. (2010) on the biotechnological production of bioflavours and functional sugars support conclusions about the broad potential for the production of not only basic bio-products, but also additives with high functional properties. This proves that fructose syrups can serve as a basis for the development of products with additional functional properties, which increases their value in the food and pharmaceutical industries.

The results of this study confirm the possibility of obtaining syrups from organic raw materials under a similar concept, but with a greater emphasis on maximising resource recovery and reducing costs. T. Kwan *et al*. (2019) focused on the possibility of using food and beverage waste for the production of syrups using biorectification technologies: a technical and economic assessment showed the high efficiency of this approach. The study by H. Olsen (1995), describing the enzymatic production of glucose syrups, was useful for understanding the mechanisms of starch and sugar hydrolysis in the manufacturing process. In particular, theoretical approaches to the use of enzymes such as amylases and glucosidases confirm their own results regarding the use of microbial enzymes for the efficient production of glucose syrups. The use of enzymatic technologies significantly improves the yield and purification of syrups, which coincides with the data on high results in optimising enzymatic hydrolase (Bekbayev *et al*., 2024; Osokina *et al*., 2024).

Conclusions on the technology of raw material preparation considering biotechnological processing also confirm the findings of A. Bušić *et al*. (2018) regarding the production of bioethanol from renewable raw materials, a similar approach to the efficient use of organic waste is shown. Although this study focuses more on syrup production, these results confirm the correctness of the chosen waste reuse strategy. Studies by P. Singh & S. Kumar (2019) on microbial enzymes in food biotechnology also support conclusions about the role of enzymes in the processes of sugar breakdown and syrup production. The researchers focused on the improvement of processes using various types of microbial enzymes. The results of the study, in particular, demonstrate significant advantages as syrups due to the use of specific microbial enzymes that activate bioconversions in the early stages. In addition, the study by E. Martínez *et al*. (2015), which discussed xylitol purification and crystallisation strategies, provides key guidance on such processes in the context of ongoing research. Although the work was done with fructose syrups, some of the purification principles proposed in their study may be useful for improving the characteristics of syrups. D. Peters (2006) revealed the importance of carbohydrates for fermentation, which confirmed the chosen approach to fermentation of syrups based on simple and complex sugars. These results confirm the ability of carbohydrates in syrups to optimise processes in the production of high-calorie products for the food industry, as indicated by D. Peters (2006).

The findings coincide with the conclusions of S. McKelvey & R. Murphy (2017) regarding the use of fungal enzymes in biotechnology. Their study demonstrated the effectiveness of using such enzymes to break down complex organic compounds, which was confirmed in the production of sugar syrups. However, in contrast to their approach, this study focused more on optimising conditions for more efficient fermentation, which allowed for improved yield and quality of finished products. Thus, it can be argued that the results of this study are consistent with a number of important international trends in the field of technological progress and environmental efficiency of sugar beet production. Moreover, the presence of some differences related to the conditions and methods of

research suggests additional opportunities for improving the processing processes and use of raw materials at different stages of production.

Conclusions

The enzymatic method of syrup purification using pectinase and amyloglucosidase showed significantly better results than conventional chemical approaches. The obtained indicators of the content of residual pectin (0.02% ± 0.005) and starch (⩽ 0.01%) indicate a high level of purification of impurities, which confirms the feasibility of a biotechnological approach in the sugar industry. Repeated experiments showed the reliability of the enzymatic purification method, where fluctuations in the results did not exceed 5%. The stability of the results allows considering this approach as a standard method for ensuring product quality. The use of membrane ultrafiltration technology after enzymatic treatment significantly improved the quality of purification. Reducing the level of impurities before membrane filtration reduced the amount of precipitation on the membranes, improved the stability of the plant and provided an increase in productivity of up to 120 litres of syrup per hour. This approach provided a synergistic effect, significantly improving the characteristics of the final product.

Samples of syrup purified by combined methods are characterised by a lower colour index (190-210 ICUMSA units), better preservation of aromatic compounds (+ 15%) and the absence of micro-inclusions, which indicates an improved texture and organoleptic characteristics. This provided an increased consumer score (9.2 ± 0.3 vs. 7.8 ± 0.4 in control samples), while also reducing energy costs by 8% and reducing the duration of technological stages by 10% indicates the economic advantage of the combined method of syrup purification. In addition, the enzymatic approach significantly reduces the environmental impact of production, reducing the development of harmful waste and water consumption. Vacuum distillation of the syrup after combined purification allowed achieving a stable sugar density (76-78%), minimising the loss of volatile aromatic compounds, and also ensured the preservation of the natural taste and aroma of the syrup. The duration of the concentration process was reduced due to the higher initial purity of the syrup, which reduced production costs. Molasses obtained after enzymatic purification allowed achieving a high bioethanol yield (88% ± 2), which significantly exceeds the indicators of the control group (79% ± 3). Additional processing of pulp using enzymatic membrane technology helped to reduce residual impurities, improving its quality as a raw material for feed or bioenergetics use. This approach ensured more efficient use of resources and reduced the environmental burden.

One of the main limitations of the study was the dependence on specific enzymatic preparations that are used to purify the syrup. This limits the ability to scale the technology to large production volumes due to the high cost of certain enzymes and their availability. In addition, some aspects of the process, such as optimising fermentation time and membrane purification, require additional research to achieve even better results in terms of productivity and cost-effectiveness. Further research should consider integrating this technology with other biotechnological processes to provide a more integrated approach to the purification and processing of sugar syrups.

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Conflict of Interest

None.

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Біотехнологічні рішення для покращення якості та екологічності цукру в харчовій промисловості

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Анотація. Виробництво цукру має значний вплив на навколишнє середовище, що зумовлює необхідність впровадження більш ефективних та екологічно чистих технологій. Однією з таких альтернатив є використання біотехнологічних підходів для покращення якості цукрових сиропів та виробництва органічного цукру без застосування хімічних речовин. Метою даної роботи було оцінити ефективність ферментативних методів очищення сиропів з використанням пектинезу та амілоглюкозидази і визначити їх вплив на якість кінцевого продукту, а також дослідити можливості переробки побічних продуктів цукрового виробництва в корисні субстрати для інших галузей харчової промисловості. Для досягнення поставленої мети використовували методи ферментативного очищення та ультрафільтрації. В ході експериментів було отримано сироп із залишковим вмістом пектину 0,02 % ± 0,005 та вмістом крохмалю не більше 0,01 %, що свідчило про високий рівень очищення. Комбіноване використання ферментативного та мембранного методів дозволило підвищити стабільність процесу та збільшити продуктивність до 120 л сиропу на годину, що також сприяло зниженню енерговитрат на 8 % та скороченню тривалості технологічних стадій на 10 %. Результати показали, що ферментативне очищення сиропу знизило рівень домішок у мелясі та сприяло підвищенню виходу біоетанолу до 88 % ± 2, що на 9 % вище, ніж у контрольній групі. Встановлено, що корм, отриманий з жому після біотехнологічної обробки, має високі показники якості: зниження вологості до 10 % ± 0,5, зменшення мікробної активності до $5 \times 10_3$ КУО/г та підвищення механічної міцності гранул до 92 % ± 4. Отримані результати продемонстрували значні переваги ферментативних технологій, зокрема зниження витрат на хімічні реагенти та утилізацію відходів. Практичне значення роботи полягає в тому, що використання біотехнологічних методів дозволяє значно підвищити ефективність виробництва органічного цукру та кормів, сприяючи зменшенню навантаження на навколишнє середовище

Ключові слова: хімічні домішки; ферментативне очищення; мембранне очищення; меласа; побічні продукти

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Optimisation of parameters for obtaining milk-plant concentrates

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Abstract. A priority direction in the dairy processing industry is the development of innovative resource-saving technologies that enable raw material conservation, increased yield, and expanded product range. In particular, there is a growing demand for plant-based milk analogs in mixtures for producing milk-plant concentrates using accelerated technologies. The study aimed to determine the optimal parameters of the thermoacid coagulation process for milk-plant protein mixtures to obtain milk-plant concentrates and analyse their impact on the yield and qualitative characteristics of the final product. Milk-plant concentrates were obtained by thermoacid precipitation of proteins from a milk-plant mixture. The Box-Wilson central composite design method was used to optimise the parameters of concentrate production. It was established that thermoacid precipitation of proteins in a milk-plant mixture containing 35% whey-plant suspension with a water-to-solid ratio of 1:3 and a self-pressing duration of 30 min ensured a 36% transition rate of both milk and plant proteins into the concentrate. These parameters were 8-12% higher than those for protein precipitation from a mixture of skim milk and whey-plant suspension (20%) with water-to-solid ratios of 1:5 and 1:7. The addition of a 20% whey-plant suspension with a water-to-solid ratio of 1:7 and a curd self-pressing duration of 10 min resulted in the least protein destabilisation and the highest moisture content (78%) in the milk-plant concentrates. Analysis of the active acidity (pH) of the concentrates revealed a general trend: as the proportion of whey-plant suspension in the mixture increased from 20% to 40% and its water-to-solid ratio changed from 1:7 to 1:3, the pH decreased linearly. The lowest impact on water-holding capacity (72%) was observed under the following conditions: 20% whey-plant suspension, 1:7 water-to-solid ratio, and 10 min self-pressing duration. The obtained results allow for the regulation of qualitative indicators of milk-plant concentrates depending on their intended use, enabling the diversification of protein products with controlled moisture content, water-holding capacity, and active acidity

Keywords: milk; *Arachis hypogaea*; thermoacid coagulation; mathematical modeling; quality indicators

Introduction

The growing interest in a healthy lifestyle has contributed to the increasing popularity of plant-based diets. The food industry is actively responding to this demand by developing new product categories. Among these are alternative versions of traditional foods for vegans and vegetarians, including meat substitutes, plant-based eggs, mayonnaise, ready-made meals, sauces, and plant-based milk. Notably, plant-based dairy products have gained significant consumer popularity (Tachie *et al.*, 2023). According to Y. Motuzka & A. Koshelnyk (2019), the modern dairy industry in Ukraine exhibits a trend toward increasing the production of low-fat products, as well as incorporating alternative ingredients and various additives to enhance taste, aroma, and texture. These changes align with global trends; however, in the Ukrainian context, they are primarily driven by the shortage of natural dairy raw materials. One potential solution to this issue is the incorporation of additional plant-based sources of protein and fat in dairy processing, as well as the more efficient utilisation of secondary dairy raw materials.

In recent years, the food market has shown a growing trend in expanding the range of plant-based protein products. This is driven by the search for new and renewable raw material sources (Kushnir & Nikolaienko, 2023). Particular attention has been given to the development of plant-based milk analogs (Sharma, 2023). According to F. Reyes-Jurado *et*

al. (2021), the increasing popularity of plant-based milk can be attributed to certain consumer groups preferring it over cow's milk due to its taste characteristics and rich composition, which includes natural plant-derived vitamins, minerals, and dietary fiber. Scientific research is actively exploring the feasibility of producing dairy products using plant-based ingredients, involving the partial or complete replacement of dairy components. Researchers utilise proteins from various grains and legumes, such as soy, chickpeas, peas, rice, and flaxseed, aiming to identify alternative ingredients that reduce dairy protein consumption while maintaining balanced nutritional value (Ayodeji *et al*., 2020; Golchin *et al*., 2023; Grasso *et al*., 2023).

Legumes such as soy, peanut (*Arachis hypogaea*), and lupin milk serve as valuable ingredients for food enrichment (Goldstein & Reifen, 2022). Investigating their potential applications in dairy product manufacturing is a promising field, considering their unique chemical composition and properties (Gharibzahedi & Smith, 2021). Legumes are known for their high protein content with significant biological value, as well as carbohydrates, minerals (especially calcium and iron), and vitamins (thiamine and niacin). Furthermore, as noted by M. Carbonaro and A. Nucara (2022), legumes have a low-fat content and a low glycemic index (GI 31) due to their high fiber, oligosaccharide, and slowly digestible starch content. Additionally, research by M. Pina-Pérez & M. Ferrús Pérez (2018) indicates that legumes possess antioxidant, antimicrobial, and anti-inflammatory properties.

The analysis of both Ukrainian and international scientific studies suggests that the development of milk-plant products should prioritise raw materials that are either traditional or widely available in a specific region. In Ukraine, peanut cultivation (*Arachis hypogaea*) is gaining popularity due to its high nutritional value, particularly its easily digestible protein and fat content. The protein composition of peanuts includes albumins, globulins, and glutelins. The high biological value of peanut protein is attributed to the presence of eight essential amino acids, which the human body cannot synthesise, along with ten non-essential amino acids, making its composition comparable to animal protein (Zahran & Tawfeuk, 2019).

One approach to enhancing the nutritional value of plant proteins is the development of combined products that employ the principle of mutual protein enrichment. It is well established that plant proteins are better assimilated when combined with animal proteins. Therefore, the development of new products where animal proteins are partially or completely replaced with plant proteins, as well as the expansion of dairy product assortments incorporating plant-based ingredients with enhanced nutritional value and improved taste characteristics, is a crucial task for the dairy industry. The objective of this study was to optimise the parameters for obtaining milk-plant concentrates (MPCs) through thermos-acid coagulation of proteins in milk-plant mixtures using mathematical modeling methods and to investigate the impact of these parameters on the yield and quality characteristics of the resulting concentrates.

Materials and Methods

Method for obtaining milk-plant concentrates

The research was conducted in 2024 at the laboratories of the Department of Milk and Dairy Product Technology at the National University of Food Technologies of Ukraine. The experiment utilised skimmed milk obtained through the separation of whole milk, characterised by a total solids content of 11.5%, protein content of 3.6%, and active acidity of pH 6.5. Milk-plant mixtures (MPMs) were prepared by blending skimmed milk with a whey-plant suspension (WPS). The WPS had an active acidity of 6.7 ± 0.1 pH and a dispersed phase particle size ranging from 10 to 60 μm. To prepare the WPS, peanuts (*Arachis hypogaea*) were pre-soaked for

8-10 hours to allow for swelling, followed by washing 2-3 times. Afterward, the peanuts were mixed with rennet whey in a 1:5 ratio. The resulting mixture was homogenised for 5-7 minutes using a disperser operating at 1,000 s^{-1} until a uniform consistency was achieved. The prepared WPS was filtered and added to the pre-treated skimmed milk.

The milk-plant concentrate (MPC) was obtained through thermos-acid coagulation of proteins from the milk-plant mixture. The acidity and amount of coagulant were adjusted to achieve a pH range of 5.3-5.8, corresponding to the isoelectric point of casein and the primary fractions of plant proteins. The coagulation process was carried out at a temperature of 92 ± 2°C by mixing the milk-plant mixture with whey, which had an acidity of 150 ± 10°T, in ratios ranging from 1:10 to 5:10, followed by holding for 4 ± 1 minutes. Under these conditions, the reaction medium reached the isoelectric point of the proteins, disrupting their salt bonds and forming a concentrate, which was then subjected to self-pressing. The control sample consisted of a milk-protein concentrate obtained without the addition of the whey-plant suspension.

Mathematical modeling of milk-plant concentrate quality indicators

To achieve the research objective, a full-factorial experiment (FFE) was applied. A parametric scheme for the process of obtaining milk-plant concentrates using the thermos-acid coagulation method was developed (Fig. 1), with defined input (controlling) and output (controlled) factors, as well as selected and justified optimisation criteria.

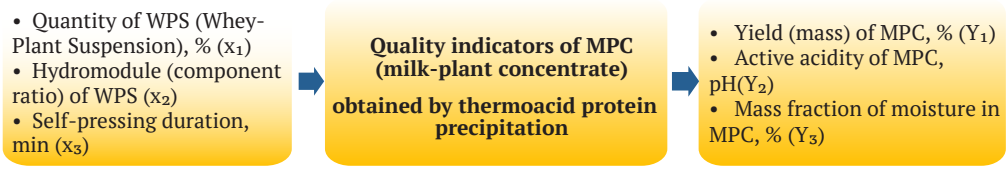

Figure 1. Parametric scheme of the process of obtaining MPC by thermoacid coagulation of milk-plant mixture proteins

Source: developed by the authors

For parameter optimisation, we determined the zero level of factors based on a priori information and the interval of their variation beyond the measurement error. The zero level corresponded to the following requirements: at these points, the optimisation parameter values were the best among all known values; the zero-level coordinates were located within the definition area. As control factors with significant impact on the qualitative indicators of milk-plant concentrates (MPC), the following were selected: x_1 – whey-plant suspension quantity, %, x_2 – hydro-module of whey-plant suspension (component ratio), x_3 – self-pressing duration, min. The output-controlled indicators that most significantly characterise thermoacidic coagulation and influence the formation of milk-plant concentrate quality indicators are: Y_1 – yield (mass), %, Y_2 – active acidity, pH units, Y_3 – moisture mass fraction, %, Y_4 – water-retaining capacity, %. The milk-plant mixture quantity was varied from 20% to 40% with a variation step of 10%, the hydro-module of the whey-plant suspension was changed to 1:3, 1:5, 1:7, and the self-pressing duration was from 10 min to 30 min with an interval of 10 min.

During the optimisation of the MPC obtaining process by thermoacidic protein precipitation of the milk-plant mixture, the Box-Wilson

cube mathematical modeling method was used. Functional dependencies were determined by the least squares method. The method is based on determining the regression equation coefficients that provide the minimum sum of squared deviations of experimental data (Y_e) from the values calculated by the regression equation, the general form of which is presented by formula (1), where coefficients are calculated using formulas (2) and (3).

$$Y = b_0 + b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + b_{12} \cdot \cdot x_1 \cdot x_2 + b_{23} \cdot x_2 \cdot x_3 + + b_{13} \cdot x_1 \cdot x_3 + b_{123} \cdot x_1 \cdot x_2 \cdot x_3, \quad (1)$$

$$b_i = \frac{\sum_{i=1}^{N} x_{iu} \cdot y_u}{N}, \quad (2)$$

$$b_{ij} = \frac{\sum_{i=1}^{N} x_{iu} \cdot x_{ju} \cdot y_u}{N}, \quad (3)$$

where x_{ij} – variable value in the corresponding column of the experiment plan; y_u – result of the u-th experiment; N – total number of experiments ($N = 8$); u – experiment variant number; i – factor number; j – factor number different from i.

To analyse the significance of regression equation coefficients, the adequacy of equations was determined using the Fisher criterion (F_p) according to standard methods through applied software. The selected criterion was compared with the tabular value. The equation is considered adequate under the condition of $F_p < F_m$, where F_p, F_m are the calculated and theoretical values of the Fisher criterion, respectively. Experimental conditions: F_m = 3.050; confidence probability – 4; number of free members $S_{\Delta HA}(f_1)$ = 8; number of experiments $S_{exp}(f_2)$ = 5. Experimental data were processed using the mathematical statistics method STATISTICA (StatSoft). To determine the functional dependence that most accurately reproduces the change in indicators, the approximation reliability coefficient (R^2) for each function was found. The accuracy of the obtained results is ensured by three to five-fold repetition of experiments. The results were visualised in the form of graphs, which allowed a visual demonstration of the changes in the studied parameters.

Methods of research on the quality indicators of milk-plant concentrates

The yield (mass) was determined by the gravimetric method – the sample was weighed after self-pressing the concentrate, obtained from 1 dm³ of milk-plant mixture. Active acidity was determined potentiometrically using a Sartorius PB-20 universal pH meter (Germany). The mass fraction of moisture was measured by an accelerated method on the KVARC-21M-33 moisture meter (Ukraine), by drying the sample to a constant mass, and thermogravimetrically on laboratory electronic balance-moisture meters of the ADGS 50 series (Poland). The water-retaining capacity (WRC) of milk-vegetable concentrates was determined using the Grau-Hamm method, with A. Alekseev's modification, based on measuring the amount of water released from the product under light pressure and absorbed by filter paper.

Results and Discussion

To determine the optimal parameters for obtaining milk-plant concentrates through thermocoagulation of proteins from the milk-plant mixture, a full-factorial experiment was designed and conducted. The mathematical processing of the results allowed the formulation of analytical dependencies in the form of regression equations for calculating the impact of the amount of whey-plant suspension on the mass of skimmed milk, the hydro module (ratio of components) of the WPC, the duration of self-pressing on the yield (1), active acidity (2), mass fraction of moisture (3), and water-retaining capacity (4) of the milk-plant concentrates. For the given equations, the condition $F_P < F_T$ was satisfied, where F_P and F_T are the calculated and theoretical Fisher's criterion values, respectively, allowing us to conclude the adequacy of the obtained equations to the actual state of the process. These equations describe

the influence of three main factors: the hydromodule (x_1), the amount of whey-plant suspension (WPS) (x_2), and the duration of self-pressing (x_3) on the yield of MRC, considering their interactions and quadratic dependencies.

$$Y_{1(yield)} = 13.416 - 3.1675 \cdot x_1 + 4.6236 \cdot x_2 + 2.2040 \cdot \cdot x_3 - 1.5775 \cdot x_1 \cdot x_2 - 1.0086 \cdot x_2 \cdot x_3 - 3.0879 \cdot x_1 \cdot \cdot x_3 + 5.9429 \cdot x_1^2 + 14.6335 \cdot x_2^2 + 11.7776 \cdot x_3^2,$$

$B_{kp\,i} = 0.380$; $B_{kp\,ii} = 0.978$; $F_r = 0.213$.

The free term 13.416 represents the base yield level that can be expected in the absence of the influence of independent variables. The negative coefficient for x_1 (–3.1675) indicates that as the hydromodule increases, the yield decreases, since the dilution of the mixture reduces the concentration of proteins and fats, which complicates the precipitation process. The positive coefficient for x_2 (4.6236) demonstrates that an increase in the amount of CMC significantly increases the yield, as a higher amount of dry substances contributes to a higher amount of precipitate. The coefficient for x_3 (2.2040) indicates the positive effect of the duration of self-pressing, as a longer process promotes better moisture removal and higher yield. Interaction effects also play an important role. The negative coefficient for $x_1 \cdot x_2$ (–1.5775) shows that the interaction between the hydromodule and the amount of CMC reduces the efficiency of precipitation. Similarly, the negative coefficient for $x_2 \cdot x_3$ (–1.0086) suggests that an increase in the amount of CMC and the duration of self-pressing may lead to oversaturation of the system, reducing the yield. The interaction between x_1 and x_3 also has a negative impact (–3.0879), indicating the need for an optimal selection of the hydromodule and the duration of self-pressing to improve the yield. The quadratic terms reflect nonlinear dependencies. The positive coefficient for x_1^2 (5.9429) indicates that there is an optimal hydromodule value that maximises the yield. The significant positive impact of x_2^2 (14.6335) suggests the importance of an optimal CMC level for increasing the yield. The positive coefficient for x_3^2 (11.7776) confirms the effectiveness of increasing the self-pressing duration, although within the optimal value. Therefore, the equation shows that to achieve maximum yield, it is necessary to balance the hydromodule, the amount of CMC, and the duration of self-pressing, taking into account both direct and interaction effects.

$$Y_2(pH) = 5.3902 - 1.0482 \cdot x_1 + 0.4454 \cdot x_2 + + 0.7078 \cdot x_3 + 0.0725 \cdot x_1 \cdot x_2 - 0.4104 \cdot x_2 \cdot x_3 - - 0.2050 \cdot x_1 \cdot x_3 + 1.2706 \cdot x_1^2 + 2.8033 \cdot \cdot x_2^2 + 1.9539 \cdot x_3^2,$$

$B_{kp\,i} = 0.161$; $B_{kp\,ii} = 0.036$; $B_{kp\,ii} = 0.087$; $F_r = 1.107$

The constant 5.3902 reflects the initial pH level observed in the absence of factor influences. The negative coefficient for x_1 (–1.0482) indicates that an increase in the hydromodule leads to a decrease in pH, which may be due to the lower amount of dry substances in the mixture. The positive coefficient for x_2 (0.4454) shows that as the amount of CMC increases, the active acidity rises, as plant proteins contain amino acids that affect pH. The coefficient for x_3 (0.7078) indicates a positive effect of the duration of self-pressing on acidity, which may be related to changes in protein structure and the release of substances with higher acid content. The interaction effects indicate more complex dependencies. The positive coefficient for $x_1 \cdot x_2$ (0.0725) suggests a weak positive influence of the interaction between the hydromodule and the amount of CMC. In contrast, the negative coefficients for $x_2 \cdot x_3$ (–0.4104) and $x_1 \cdot x_3$ (–0.2050) suggest that an excessive combination of CMC with the self-pressing duration or the hydromodule with the duration can lead to a decrease in acidity. The quadratic terms in the equation show nonlinear effects. The positive coefficient for x_1^2 (1.2706) indicates that there is an optimal hydromodule value to achieve higher active acidity. The significant positive

impact of x_2^2 (2.8033) confirms that the amount of CMC is an important factor affecting pH. The quadratic term x_3^2 (1.9539) also indicates that the self-pressing duration positively affects acidity within the optimal value. Overall, the equation demonstrates that active acidity is largely dependent on the amount of CMC and the duration of self-pressing, while the hydromodule has a negative impact. Interactions between factors can have both positive and negative effects, depending on their combinations. To achieve an optimal pH level, both direct and interaction effects must be taken into account.

$$Y_{3\,(W)} = 82.2035 - 9.2774 \cdot x_1 - 1.9996 \cdot x_2 - 0.1329 \cdot \cdot x_3 - 6.8050 \cdot x_1 \cdot x_2 - 2.1104 \cdot x_2 \cdot x_3 - 2.9817 \cdot x_1 \cdot \cdot x_3 + 13.8341 \cdot x_1^2 + 33.3881 \cdot x_2^2 + 30.1849 \cdot x_3^2,$$
$$B_{kp\,i} = 0.17;\ B_{kp\,ii} = 0.202;\ F_r = 0.053$$

The free term 82.2035 represents the initial value of the moisture content, observed in the absence of the influence of independent variables. The negative coefficient for x_1 (–9.2774) indicates that as the hydromodule increases, the moisture content decreases. This can be explained by the fact that with an increase in the hydromodule, proteins and fats precipitate less effectively, which reduces the ability to retain water. The coefficient for x_2 (–1.9996) is also negative but smaller in value, suggesting a decrease in moisture content as the amount of CMC increases. The negative coefficient for x_3 (–0.1329) demonstrates that an increase in the duration of self-pressing slightly reduces moisture content. Interaction effects show more complex relationships. The negative coefficient for $x_1 \cdot x_2$ (–6.8050) indicates that the interaction between the hydromodule and the amount of CMC reduces moisture content, likely due to a decrease in the ability of proteins to retain water. The negative coefficient for $x_2 \cdot x_3$ (–2.1104) suggests that a combination of a higher amount of CMC and longer self-pressing reduces moisture content. A similar negative effect is observed with the interaction of $x_1 \cdot x_3$ (–2.9817). The quadratic terms indicate the existence of optimal values for each factor. The positive coefficient for x_1^2 (13.8341) suggests that there is an optimal hydromodule value that maximises moisture retention. The significant positive coefficients for x_2^2 (33.3881) and x_3^2 (30.1849) confirm that both the amount of CMC and the duration of self-pressing are important factors for the concentrate's ability to retain moisture. Overall, the equation shows that the moisture content decreases with an increase in the hydromodule, the amount of CMC, and the duration of self-pressing. However, to achieve optimal moisture content, it is necessary to account for the interactions between factors and their quadratic effects.

$$Y_{4\,(WHC)} = 84.1000 - 15.0714 \cdot x_1 + 8.1691 \cdot x_2 + + 8.9998 \cdot x_3 + 3.0975 \cdot x_1 \cdot x_2 - 5.8641 \cdot x_2 \cdot x_3 - - 4.2857 \cdot x_1 \cdot x_3 + 18.4696 \cdot x_1^2 + 40.4773 \cdot x_2^2 + + 28.0679 \cdot x_3^2.$$
$$B_{kp\,i} = 0.095;\ B_{kp\,ii} = 0.191;\ F_r = 0.001$$

The constant 84.1000 reflects the initial moisture-holding capacity observed in the absence of the influence of independent variables. The negative coefficient for x_1 (–15.0714) indicates that as the hydromodule increases, the moisture-holding capacity significantly decreases. This may be due to the fact that a larger hydromodule reduces the concentration of proteins and fats, which contribute to water binding. The positive coefficient for x_2 (8.1691) suggests that an increase in the amount of CMC contributes to an increase in moisture-holding capacity, as plant-based proteins have high hydrophilicity. The coefficient for x_3 (8.9998) is also positive, indicating a positive effect of the duration of self-pressing on the concentrate's ability to retain moisture, as longer pressing promotes the removal of free water while retaining bound moisture. Interaction effects reflect more complex relationships. The positive coefficient for $x_1 \cdot x_2$ (3.0975) suggests that the interaction between the hydromodule and the amount of CMC partially compensates

for the negative impact of the hydromodule. In contrast, the negative coefficients for $x_2 \cdot x_3$ (−5.8641) and $x_1 \cdot x_3$ (−4.2857) indicate that a combination of a higher amount of CMC or hydromodule with the duration of self-pressing can reduce moisture holding capacity. The quadratic terms highlight the existence of optimal values for the factors. The positive coefficient for x_1^2 (18.4696) suggests that there is an optimal hydromodule level that ensures maximum moisture-holding capacity. An even greater positive impact from x_2^2 (40.4773) confirms that the amount of CMC is a key factor influencing the concentrate's ability to retain water. The positive coefficient for x_3^2 (28.0679) indicates that the duration of self-pressing also has a significant impact within optimal values. Overall, the equation shows that to achieve a high moisture-holding capacity, it is necessary to ensure an optimal balance between the hydromodule, the amount of CMC, and the duration of self-pressing, taking into account both direct and interaction effects.

The complex changes and response surfaces of the physicochemical properties of milk-plant concentrates obtained through thermocoagulation of milk-plant protein mixtures are presented in Figures 2 a, b, c, d as 3D graphs, reflecting the dependence of controlled parameters on controlling factors.

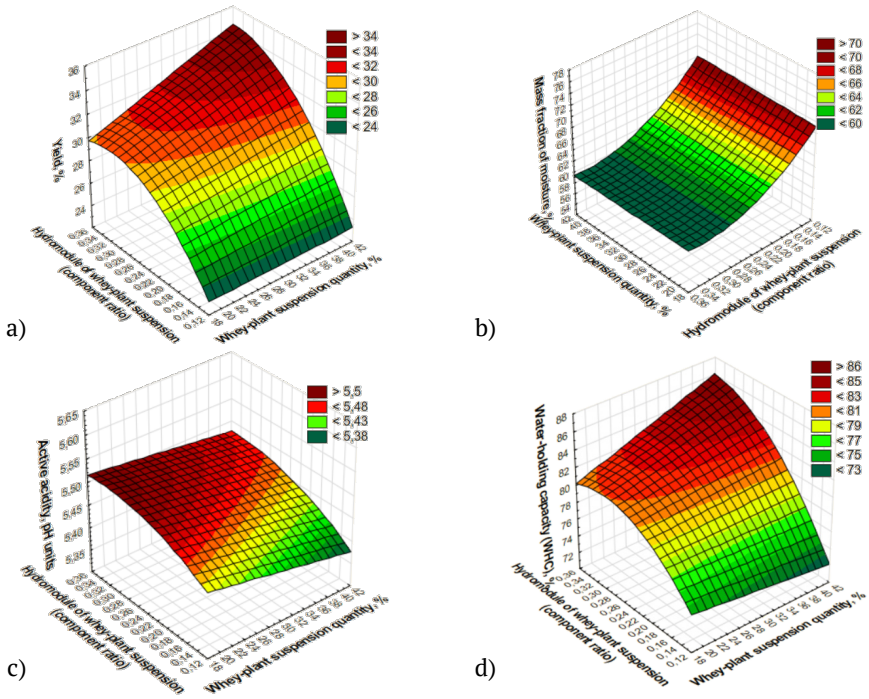

Figure 2. Response surfaces showing changes in yield (a), mass fraction of moisture (b), active acidity (c), and moisture-holding capacity (d) of milk-plant concentrates depending on the parameters of processing the milk-plant mixture

Source: developed by the authors

In the production of milk-plant concentrates (MPC), significant roles are played by the processes of structure formation and syneresis, involving protein substances. The obtained MPC are polydisperse colloidal systems, where the dispersion medium is whey, and the dispersed phase consists of proteins and fats of both plant and animal origin. The stability of protein

globules is determined by the conformation of the particles, their charge, and the presence of a hydration shell (Lamichhane *et al.*, 2018). The concentration change of individual components of milk, as well as the type and conditions of protein precipitation, leads to a change in the nature of the bonds betweenprotein parts.

To isolate milk and plant proteins, it is necessary to disrupt the equilibrium of at least two stability factors, which occurs during thermal denaturation. This process is accompanied by changes in the configuration, hydration, and aggregate state of the particles, which become less stable. Additionally, the degree of thermal denaturation of protein globules in milk-plant mixtures depends on temperature and heating duration (Li *et al.*, 2023). Using the proposed thermal and time regimes allows for the simultaneous coagulation of both milk proteins and plant proteins. Furthermore, as noted by authors Y. Nazarenko *et al.* (2023), the initial processing of plant proteins improves the coagulation of the milk-plant mixture, contributing to the formation of cheese curd. During the hydrolysis of soy proteins, peptides and amino acids are also formed, which create better complexes with casein micelles.

Studies have shown that during the thermocoagulation of proteins from the milk-plant mixture, increasing the amount of whey-plant suspension added to defatted milk, as well as changing its hydromodule (1:3) and increasing the duration of self-pressing, leads to an increase in the yield of MPC (Fig. 2a). This is generally characterised by an increase in the transition of both milk proteins – caseins, albumins, globulins, – and plant proteins – glutelins. The majority of fat in *Arachis hypogaea* fruits concentrates with proteins during precipitation, while the rest goes into the whey. As a result of heating the milk-plant mixtures to a temperature of 92 ± 2 °C in the presence of a coagulant (acid whey), proteins co-precipitate in a single protein complex, which also includes the fat component. The transition coefficient for the above components during the coagulation of MPC proteins, which contains 35% whey-plant suspension with a hydromodule (component ratio) of 1:3, was about 36%. These parameters are 10-12% higher than the precipitation of proteins from the mixture of defatted milk and whey-plant suspension (20%) with hydromodules of 1:5 and 1:7.

In their studies, O. Tsisaryk *et al.* (2023) also noted an increase in the yield of the cheese product, which is related to the properties of plant proteins that facilitate the formation of structure. This ensures the formation of a dense curd and more efficient use of raw materials. The increase in cheese yield is explained by the fact that plant proteins have the ability to hold water, converting it into a bound state. This leads to their swelling, increasing mass and volume, which, in turn, increases the yield of the final product by 7-9%.

Analysing the regression equation and graphical interpretation of the change in the mass fraction of moisture in MPC (Fig. 2b), it can be concluded that increasing the amount of whey-plant suspension in the mixture from 20% to 40%, decreasing the hydromodule from 1:7 to 1:3, and the duration of self-pressing from 10 to 30 minutes, resulted in a decrease in the mass fraction of moisture in the concentrates. During the production of MPC, the hydromodule of the whey-plant suspension had almost the same effect on the final result. The maximum value of this parameter in MPC (78%) was observed when processing the milk-plant mixture containing 20% whey-plant suspension with a hydromodule of 1:7 and a self-pressing time of 10 minutes. Under the condition of processing the mixture (35% whey-plant suspension, hydromodule 1:3), the moisture in the MPC decreased by 22%. The obtained values of the mass fraction of moisture in the concentrates varied from 28.28% to 41.36% and were within the range obtained by A. Arise *et al.* (2020) in the production of a protein product from a mixture of soy and

almond milk. A. Ajayi *et al*. (2023) emphasised that the moisture content is crucial in forming the consistency and textural properties of protein products. M. Balogun *et al*. (2019) reported that a higher moisture content can promote the growth and spread of microorganisms, thus shortening the shelf life of cheese. High moisture values in cheese samples may be related to the hydrophilic nature of plant proteins.

The analysis of the effect of the variable factor space on the active acidity of MPC indicates that this parameter primarily depends on the pH of the milk-plant mixture and the coagulant: the pH decreased with a change in the hydromodule from 1:7 to 1:3 and an increase in the amount of MPC from 20% to 40%. Based on the analysis of the active acidity, a general trend was established – when increasing the amount of whey-plant suspension from 20% to 40% and changing its hydromodule from 1:7 to 1:3, the pH decreased linearly (Fig. 2c). It was found that the addition of 35% whey-plant suspension with a component ratio of 1:3 and self-pressing of the obtained concentrates for 30 minutes ensured the lowest value of active acidity of MPC at a level of 5.35. Comparing the obtained values of active acidity with the pH results of A. Ajayi *et al*. (2023), this aforementioned trend of acidity reduction with an increase in the proportion of plant milk is confirmed, and the specified results are consistently close. O. Tsisaryk *et al*. (2023) recorded that with an increase in the proportion of plant additives, a decrease in the active acidity of the product was observed: from 5.22 in the control sample to 5.11-4.96 pH units in the experimental samples. This phenomenon can be explained by the presence of a significant number of acidic amino acids in chickpea proteins.

The mathematical model describing the change in the moisture-holding capacity of MPC (Fig. 2d) shows a direct dependence of this parameter on the amount of whey-plant suspension in the milk-plant mixture and its hydromodule. The maximum value of the moisture-holding capacity at 88% was observed in the MPC samples obtained under the highest experimental conditions – thermocoagulation of the milk-plant mixture containing 35% whey-plant suspension with a hydromodule of 1:3 and subsequent self-pressing of the curd for 30 minutes. The change in the ability of proteins to absorb ions depends on the acidity of the medium. At the point where the protein's electric charge is close to zero and the dissociation of molecules is minimal, the proteins are least able to hold water. When the pH shifts in either direction from this point, dissociation of the acidic or basic groups of the protein increases, which enhances the charge on protein molecules and, accordingly, their ability to hydrate. There is interaction between κ-casein and plant proteins (glutelins) due to the residues of N-groups and other hydrophobic bonds (Shaghaghian *et al.*, 2022).

The changes in the mass fraction of moisture and moisture-holding capacity in concentrates obtained through the thermocoagulation of proteins from the milk-plant mixture do not match the results of these parameters in the scientific development by D. Borges *et al*. (2024) and can be considered as a result of the continuous interaction between proteins, due to the reduction of the hydrated water content around casein micelles. With an increase in the amount of whey-plant suspension and changes in its hydromodule, the strength of the bonds between protein particles increases, and glutelins combine with each other and with other proteins through disulfide bonds, forming a strong three-dimensional protein network, which results in the easy release of whey contained in its interstices.

The lowest yield (22%) was observed when precipitating proteins from the milk-plant mixture containing 20% whey-plant suspension with a hydromodule (component ratio) of 1:7 and a self-pressing duration of 10 minutes. This process occurred due to the initial denaturation of plant proteins, which, when precipitating onto the surface of casein micelles,

increase their hydrophilicity, thereby reducing the degree of precipitation. The lowest value of active acidity in MPC (5.35) was obtained when processing the milk-plant mixture with 35% whey-plant suspension and a hydromodule of 1:3. The least impact on the moisture-holding capacity of MPC (72%) was observed under the following conditions: 20% whey-plant suspension with a hydromodule (1:7) and a self-pressing time of 10 minutes. Comparing the data obtained during the study with the results published by these scientists shows that the results for yield and physicochemical properties of milk-plant products, as well as the described characteristics of the technological processes used, are not contradictory and are mainly explained by differences in the plant raw materials used in the compared studies.

Conclusions

The proposed method for producing dairy-plant concentrates ensures high consumer properties and a balanced chemical composition, achieved through the optimal combination of protein components of animal and plant origin. The use of a mixture of whey and plant components in the dairy-plant mixture allows for the control of the quality of dairy-plant concentrates. This, in turn, makes it possible to expand the range of protein products by regulating moisture content, its retention, and acidity, depending on the intended use.

The conducted research showed that the most effective mixture contained 35% whey-plant suspension with a hydromodule of 1:3 and a pressing time of 30 minutes. Under these conditions, the maximum degree of protein fraction transfer into the concentrate was achieved – both dairy proteins (caseins, albumins, globulins) and plant proteins (arachin, conarachin, glutenin). The moisture retention capacity of such a concentrate reached 76%, indicating its high functional suitability for further use in various protein products. Reducing the content of whey-plant suspension to 20% and using a hydromodule of 1:7, with a pressing time of 10 minutes, helped minimise the destabilisation of protein structures, which positively affected moisture retention in the concentrate. Under these conditions, the mass fraction of moisture reached 78%, which may be important for producing products with increased moisture, such as paste-like dairy protein desserts or alternative dairy products.

In addition to the main quality indicators, the research also confirmed the relationship between the pH level of the dairy-plant mixture and the protein coagulation processes. It was found that as the content of the whey-plant suspension increases and the hydromodule decreases, the active acidity of the concentrates gradually decreases, which can affect their organoleptic and textural characteristics. The results obtained allow for targeted regulation of the composition of dairy-plant concentrates to produce products with specified functional and technological characteristics. A promising direction for future research is the analysis of changes in quality indicators of concentrates during storage, as well as studying the effect of different temperature regimes on the stability of the protein structure and microbiological resistance.

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Conflict of Interest

None.

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Оптимізація параметрів отримання молочно-рослинних концентратів

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Анотація. Пріоритетним напрямком молокопереробної галузі є розвиток інноваційних ресурсозберігаючих технологій, які дозволяють економити сировину, збільшувати вихід та розширювати асортимент продукції. Зокрема, спостерігається тенденція до зростання попиту на рослинні аналоги молока у складі сумішей для отримання молочно-рослинних концентратів за прискореними технологіями. Метою дослідження було визначення оптимальних параметрів процесу термокислотної коагуляції білків молочно-рослинної суміші для отримання молочно-рослинних концентратів та аналіз їх впливу на вихід і якісні характеристики кінцевого продукту. Молочно-рослинні концентрати отримували термокислотним осадженням білків молочно-рослинної суміші. Для оптимізації параметрів отримання молочно-рослинних концентратів використано метод математичного моделювання – Бокса-Уільсона на кубі. Встановлено, що термокислотне осадження білків молочно-рослинної суміші, що містить 35 % сироватково-рослинної суспензії з гідромодулем 1:3 та тривалістю самопресування 30 хв., забезпечує ступінь переходу в концентрат як молочних білків, так і рослинних – на рівні 36 %. Такі параметри на 8-12 % вище, ніж осадження білків суміші зі знежиреного молока та сироватко-рослинної суспензії в кількості 20 % з гідромодулем 1:5 та 1:7. Додавання сироватко-рослинної суспензії у кількості 20 % з гідромодулем 1:7 та тривалістю самопресування згустку 10 хв.

характеризувалося найменшими процесами дестабілізації білків та максимальним значенням масової частки вологи молочно-рослинних концентратів – 78 %. В результаті аналізу активної кислотності молочно-рослинних концентратів була встановлена загальна тенденція – при збільшенні кількості сироватко-рослинної суспензії в суміші для осадження від 20 % до 40 % та зміни її гідромодуля від 1:7 до 1:3, pH лінійно знижувався. Найменший вплив на вологоутримуючу здатність концентратів (72 %) відзначено за таких умов отримання концентратів: 20 % сироватково-рослинної суспензії з гідромодулем 1:7 та тривалістю самопресування 10 хв. Отримані результати дають можливість регулювати якісні показники молочно-рослинних концентратів у залежності від подальшого їх використання для розширення асортименту білкових продуктів з регульованою масовою часткою вологи, вологоутримуючою здатністю та активною кислотністю

Ключові слова: молоко; *Arachis hypogaea*; термокислотна коагуляція; математичне моделювання; показники якості

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Quality and safety of chicken eggs after washing and disinfection with a chlorine-containing agent

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Abstract. Contamination of food-grade chicken eggs with pathogenic and conditionally pathogenic microorganisms in the process of their production, storage, and sale causes a high risk of toxicoinfections in humans. One of the most effective ways to reduce microbial load is to wash and disinfect the surface of eggshells with active chlorine-based products. The purpose of the study was to determine the quality of washing and disinfection of food-grade chicken eggs during storage in a chilled form. In the experiment, the quality and microbiological indicators of food-grade chicken eggs were determined after washing and disinfection on the 1st, 60th, and 80th days of storage at a temperature of 4°C. The microbiological parameters of eggs were studied using MALDI TOF technology, and the quality was studied using digital ovoscopy. Washing and disinfection with a preparation based on active chlorine at a concentration of 0.5% freed eggshells from colonies of MAFAnM and mould fungi. Storage of washed and disinfected eggs in a chilled form until the 60th and 80th days contributed to an increase in the QMAFAnM by 2.45 lg CFU/cm2 and at 5.68 lg CFU/cm2 accordingly. The quantity of mould fungi on the shell surface of washed and disinfected eggs on the 60th day of storage reached 4.37 lg CFU/cm2, and on the 80th day, their number was equal to the QMAFAnM. The quantity of MAFAnM and mould fungi in the yolks of food-grade eggs had a strong direct dependence on their quantity on the shell and on their shelf life in chilled form. Washing and disinfection of food-grade chicken eggs with a chlorine-containing preparation did not affect the loss of weight, albumen index, yolk colour, strength and thickness of shell, but reduced the HU index to 78.5-79.5 units, which, combined with microbiological indicators, allowed them to be stored in a chilled form up to 80 days. The results obtained should be factored in when choosing the product, washing and disinfection mode, and shelf life of food-grade eggs, considering the species composition of microorganisms characteristic of a particular poultry farm

Keywords: shell; yolk; QMAFAnM; mould fungi; ovoscopy; microbial contamination

Introduction

During the production of food-grade eggs, there is a risk of contamination of the shell with droppings, dust, and microorganisms that pose a danger to human health. Chicken eggs are a source of dietary protein, lipids, polyunsaturated fatty acids, vitamins, minerals and carotenoids, which are necessary for the human body (Shevchenko *et al.*, 2020; 2021). As noted by S. Khan *et al.* (2024), the suitability of eggs for consumption is determined by the level of safety, which is affected by both the conditions of keeping and feeding an industrial herd of chickens, and the sanitary and hygienic conditions of egg production, collection, processing, and storage. Although eggshells are a natural barrier to many species of opportunistic and pathogenic microorganisms, they may not only accumulate on the surface, but also enter the egg (Mahmoud *et al.*, 2023).

According to J. An & H. Lee (2023), contamination of eggshells and food contents is an important public health issue. Eggshells can be contaminated both vertically and horizontally, including infectious diseases of chickens and contamination that can enter eggs from the environment (Borysevich & Lisova, 2020; Khatun *et al.*, 2022). Thus, microbial contamination of eggs depends on the conditions of keeping chickens, storing, distributing, processing, or cooking food. According to K. Petrovič *et al*. (2024), the spread of toxicoinfections through eggs and egg products occupy the first place in the list of human toxicoinfections. Micro-organisms can enter eggs during laying, harvesting, packaging, storage, and transportation. *Salmonella* is one of the most dangerous toxiconifections of food origin, which is often

found in food-grade eggs and affects the human digestive system in EU countries (Chan *et al*., 2023). Contact of eggs with chicken droppings can also cause infection, which can get inside the egg and cause it to spoil. Spoilage of eggs is one of the main reasons that determine their shelf life, so in order to reduce the contamination of the surface of eggshells with microorganisms and mitigate the risk of human infection with dangerous toxicoinfections, eggs are subjected to certain treatments, since infected eggshells cause more than 75% of the 1.2 million cases of salmonellosis and infectious diseases that occur in different segments of the population every year.

Washing and disinfecting eggs can reduce the number of viable *Salmonella* bacteria on the shell. In the United States, eggs are usually washed using chemical disinfectants such as quaternary ammonium salts or chlorine compounds. Recently, a new non-thermal disinfection technique has been developed for washing eggshells using cold plasma-activated water, which allows inactivating *Klebsiella michiganensis* by reducing the number of colonies >5.28 log CFU/egg. It is known that chemicals designed to wash eggs can remain on the shell and damage the cuticle, which increases the risk of penetration and contamination of the eggs with *Salmonella* and other bacteria (Medina-Gudiño *et al*., 2020). Washing food-grade eggs is prohibited in some countries due to the risk of pathogenic microorganisms entering through the shell and cross-contamination. An alternative approach to the inactivation of *Salmonella* in eggs is pasteurisation, which is widely used in the United States. Pasteurisation of eggshells is carried out using infrared radiation, hot water, or hot air. However, such heat treatment causes protein denaturation and the formation of a gel mesh of protein around the inner surface of the shell, which obviously affects the rheological properties of yolk and albumen proteins, leads to a deterioration in texture properties and, as a result, a decrease in attractiveness for consumers. Several non-thermal technologies, such as various types of packaging, the use of ultraviolet irradiation, ozonation, and chilled storage, have been used to extend the shelf life of eggs (Sokołowicz *et al*., 2023). UV radiation is widely used for disinfection of surfaces, but it has disadvantages, the main of which is the low ability to penetrate through dirt on the surface of the shell, under which there may be microorganisms.

Washing improves the appearance of eggs, but does not benefit their storage, as it leads to the opening of pores in the shell. Therefore, the washing strategy is usually used before breaking eggs in the food industry. As noted by S. Sokovnin (2021), toxic waste is generated when washing eggs, in particular, wastewater with residues of disinfectants and detergents, such as formaldehyde or phenolic compounds. Given the large list of methods and means of washing and disinfecting eggs used in the food industry, it can be concluded that there are no universal ones that would meet the requirements of hygiene, ecology, quality, and safety. A reliable and promising method of processing food-grade chicken eggs is the use of complex products that simultaneously have washing and disinfecting properties. They have a milder effect on the structure of the internal components of eggs compared to pasteurisation. Disinfection is applied to soft treatments in which eggshells are disinfected using certain chemicals (such as chlorine) or new technologies. Therefore, the purpose of these studies was to determine the microbiological parameters and quality of chicken eggs after washing and disinfection with a chlorine-containing preparation for storage in a chilled form.

Materials and Methods

For the study, food-grade chicken eggs obtained from chickens of the industrial flock of the High-Line W36 cross at Ovostar LLC, Kyiv Oblast, were used. The study was conducted during September-December 2024. Chicken eggs were selected on the 1st day of laying and

divided into two batches: Batch 1 was subjected to ovoscopy and microbiological studies of the shell and yolks were performed. Batch 2 was washed and disinfected and placed in egg pads for storage in a refrigerator at a temperature of 4°C (Table 1).

Table 1. *Scheme of the experiment to study the quality of washing and disinfection of food-grade chicken eggs*

Characteristics of eggs	Shelf life, days	Storage conditions
Eggs before washing and disinfecting	1	temperature 4°C, relative humidity 80-85%
Eggs after washing and disinfection	1	
	60	
	80	

Source: developed by the authors

Washing and disinfection of chicken eggs was carried out using a 0.5% solution of detergent with disinfectant effect Blanidas-C Gen Deo (Blanidas LLC, Kyiv, Ukraine), which contained sodium hypochlorite and sodium hydroxide, with a mass fraction of active chlorine of 5.6%. Eggs were washed using a Unifortes B.V. line (Unifortes, Netherlands), which is designed to wash 36,000 eggs per hour. The washing line was equipped with a UNI-EW350.6 + drying module, which provided egg drying. Egg washing consisted of the following steps: washing with a detergent and disinfectant solution, rinsing eggs from the remains of detergents and disinfectants, and venting the eggs to remove moisture residues from the shell surface. After drying, the process of packing eggs in egg pads and storing them in the egg warehouse was carried out.

Ovoscopy and microbiological studies were performed on the first day before and after washing and disinfection, and on the 60th and 80th days after washing and disinfection of eggs. For ovoscopy, 15 eggs were used for each study, and 5 samples were prepared for microbiological analysis. Each sample for microbiological studies was formed from 5 eggs. The experiment determined the weight of eggs, albumen index, yolk colour (16-point YolkFan ™ colour scheme), HU units, shell strength and thickness using the DET 6000 digital egg tester (NABEL Co., Ltd., Japan). Among the main indicators that help to determine their quality are the HU unit, yolk colour, shell thickness and strength, albumen index, and egg weight. HU is a common indicator used in poultry farming to measure the quality of egg white. It measures the index of thick albumen surrounding the yolk.

Microbiological studies of eggs were carried out at the Expert Centre "Biolights" LLC, Ternopil (accredited according to ISO/IEC17025). To determine the QMAFAnM (quantity of mesophilic aerobic and facultative anaerobic microorganisms) and mould fungi, egg yolks were used and eggshell washes were prepared. To do this, consecutive tenfold dilutions were made in sterile saline solution. The number of microorganisms on the shell surface and in egg yolks was determined in colony-forming units (CFU), the results were expressed in lg CFU/cm^2 and lg CFU/g, respectively. The QMAFAnM was determined using the Plate count agar M091 medium (HiMedia, India), mould fungi – Sabouraud Agar (HiMedia, India).

To determine the species composition of microorganisms, the samples were inoculated on differential diagnostic media and plated by the method of sectoral cultivation (Baird-Parker Agar (HiMedia, India), XLD Endo Agar (HiMedia, India), Pseudomonas Agar (HiMedia, India), Enterococcus Agar (HiMedia, India), Bacillus Cereus Agar (HiMedia, India)) and grown for 24 ± 1 or 48 ± 1 h at 37 ± 1 or 30 ± 1°C (according to the incubation requirements of the culture

medium). After incubation, the grown microbial colonies were identified using the MALDI TOF method. This method was based on the detector's determination of the flight time of ionised ribosomal proteins of various microorganisms, followed by the conversion of this information into a molecular mass spectrum, which was compared with spectra from a unique database (Singhal *et al.*, 2015). For microbiological studies, a Bruker Daltonics mass spectrometer, Maldi Tof microflex (Bruker Daltonics, Germany) was used. Identification of isolated cultures of microorganisms from eggs was performed using MBT Compass MALDI Biotyper 3.1 Compass 1.4 for FLEX – Volume 1, 2 Software and Manuals (Bruker Daltonik, Bremen, Germany).

The results obtained were processed statistically using univariate analysis of variance using Microsoft Excel 2016. The dynamics of the number of microbial colonies in food-grade eggs was determined by regression and correlation analysis, the data in the tables were presented as x ± SD (mean ± standard deviation). The difference between the values of washed and unwashed chicken eggs during the storage period was calculated using the Tukey test, the probability was considered $P < 0.05$ (taking into account the Bonferroni correction).

Results and Discussion

The results of the study showed that there was a small quantity of MAFAnM on the surface of the shell of freshly laid unwashed eggs, and after washing on the first day of storage, no colonies of viable microorganisms were detected (Table 2).

Table 2. *Microbial contamination of the surface of the shell of chicken eggs after washing and disinfection during storage in a chilled form, x ± SD, n = 5, lg CFU/cm2*

Characteristics of eggs	Shelf life, days	MAFAnM	Mould fungi
Eggs before washing and disinfecting	1	0.43 ± 0.14^{a}	$< 1^{a}$
Eggs after washing and disinfection	1	$< 1^{b}$	$< 1^{a}$
	60	2.45 ± 0.17^{c}	4.37 ± 0.21^{b}
	80	5.68 ± 0.42^{d}	5.61 ± 0.38^{c}

Note: different letters of the upper indexes indicate values that significantly differed in the same column of the table ($p < 0.05$) based on the results of comparison using the Tukey test
Source: developed by the authors

Storage of washed and disinfected eggs up to 60 days in a chilled form contributed to an increase in the QMAFAnM by 2.02 lg CFU/cm^2 compared to unwashed eggs and at 2.45 lg CFU/cm^2 compared to washed and disinfected ones on the 1st day of storage. The increase in the shelf life of washed and disinfected eggs to 80 days contributed to an increase in the QMAFAnM colonies on the shell surface by 5.25 lg CFU/cm^2 compared to unwashed eggs on the 1st day of storage, by 5.68 lg CFU/cm^2 compared to washed and disinfected eggs on the 1st day of storage, and by 3.23 lg CFU/cm^2 – compared to washed and disinfected eggs on the 60th day of storage.

Colonies of mould fungi on the shell surface of unwashed and washed and disinfected eggs were not found on the 1st day of storage. On the 60th day of storage of washed and disinfected eggs using chlorine-containing agent Blanidas-C Gen Deo, the quantity of mould fungi on the shell surface reached 4.37 lg CFU/cm^2, and on the 80th day of storage, the number increased to 5.61 lg CFU/cm^2. As noted by B. Abdoli *et al.* (2024), this is associated with the specificity of the structure of the eggshell cuticle and the presence of soluble matrix proteins in it, which at a concentration of 0.1 mg/mL can inhibit the growth of certain microorganisms, in

particular, *Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus*, for more than 8 hours, and *S. enteritidis* and *E. coli* for 4 hours.

Storage of washed and disinfected food-grade eggs up to 60 days in a chilled form contributed to the growth and reproduction of microorganisms and mould fungi on the surface of the shell, but their total number did not exceed the permissible standard value, which allowed considering such eggs suitable for consumption. On the 80th day of storage on the surface of the shell of washed and disinfected eggs, the quantity of MAFAnM and mould fungi was almost equal, which indicates their spread and intensive reproduction. The shells of unwashed food-grade chicken eggs were contaminated by representatives of genera *Bacillus* and *Staphylococcus*. During the entire storage period, no pathogenic microorganisms such as *Salmonella* spp., *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* were detected. As emphasised by L. Lin *et al*. (2020) and D. Bermudez-Aguirre & B. Niemira (2023), this is particularly important because most of the rules of good hygiene practices in egg poultry farming apply to preventing egg contamination with *Salmonella* spp., *E. coli* and other pathogenic and conditionally pathogenic microorganisms. In research by A. Aygun (2017), various bacteria were isolated and identified from the surface of eggshells and egg contents, including *Salmonella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Campylobacter* spp., *E. coli*, *Clostridium perfringens*, *Listeria monocytogenes*, *Citrobacter freundii*, *Bacillus cereus*. These microorganisms pose a potential risk to human health and can cause food toxicoinfections (Lin *et al.*, 2021; Chousalkar *et al.*, 2021).

A strong direct correlation was found between the QMAFAnM on the shell surface of washed and disinfected chicken eggs and their shelf life in chilled form ($r = 0.937 \pm 0.122$, $P < 0.001$). An increase in the QMAFAnM on the shell surface of washed and disinfected food-grade eggs was characterised by a linear dependence on the shelf life of their chilled form (Fig. 1).

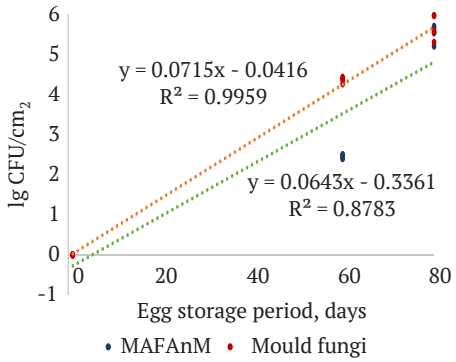

Figure 1. Dependence of the quantity of MAFAnM and mould fungi on the shell surface of washed and disinfected chicken eggs on the shelf life in chilled form, n = 15
Source: developed by the authors

Increasing the number of microorganisms on the surface of the eggshell increases the likelihood of their penetration into the internal environment of the egg (Oliveira *et al.*, 2022; 2024). In the yolks of unwashed, as well as washed and disinfected chicken eggs on the 1st day of storage of colonies, MAFAnM were not observed. On the 60th day of storage of washed and disinfected eggs, an increase in the QMAFAnM to 2.82 lg CFU/g was established, and on the 80th day – to 4.57 lg CFU/g, which almost reached the MPL (maximum permissible level) according to DSTU 5028:2008 (2010) (Table 3).

Table 3. *Microbial contamination of chicken egg yolks after washing and disinfection during chilled storage, x ± SD, n = 5, lg CFU/g*

Characteristics of eggs	Shelf life, days	MAFAnM	Mould fungi
Eggs before washing and disinfecting	1	< 1^{a}	< 1^{a}
Eggs after washing and disinfection	1	< 1^{a}	< 1^{a}

Table 3. Continued

Characteristics of eggs	Shelf life, days	MAFAnM	Mould fungi
	60	2.82 ± 0.19^{b}	1.43 ± 0.12^{b}
	80	4.57 ± 0.34^{c}	2.54 ± 0.17^{c}

Note: different letters of the upper indexes indicate values that significantly differed in the same column of the table ($p < 0.05$) based on the results of comparison using the Tukey test
Source: developed by the authors

Mould fungi in the yolks of unwashed and washed and disinfected food-grade chicken eggs were not isolated on the 1st day of storage. On the 60th day of storage, a small quantity of mould fungi appeared in the yolks of washed and disinfected chicken eggs, the number of which increased by 1.11 lg CFU/g on the 80th day of storage compared to the previous period. Correlation analysis showed a strong direct dependence of the QMAFAnM in the yolks of washed and disinfected chicken eggs on their shelf life in chilled form ($r = 0.984 \pm 0.035$, $P < 0.001$). The increase in the quantity of MAFAnM colonies in the yolks of washed and disinfected chicken eggs during the shelf life was characterised by a linear relationship (Fig. 2).

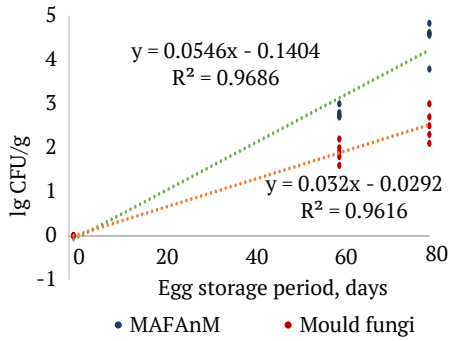

Figure 2. Dependence of the quantity of MAFAnM and mould fungi in the yolks of washed and disinfected food-grade chicken eggs on the shelf life in chilled form, n = 15
Source: developed by the authors

The quantity of mould fungi in the yolks of washed and disinfected chicken eggs had a strong direct dependence on their shelf life ($r = 0.981 \pm 0.038$, $P < 0.01$). The regression line showed that there is a direct linear relationship between the number of mould colonies in the yolk of washed and disinfected chicken eggs and their shelf life in chilled form (Fig. 2). Determination of the dependence of the QMAFAnM in yolks on the QMAFAnM on the shell surface of washed and disinfected chicken eggs showed a strong direct correlation ($r = 0.973 \pm 0.053$, $P < 0.001$). Regression analysis confirmed a linear relationship between microbial contamination of the shell surface and egg yolk (Fig. 3).

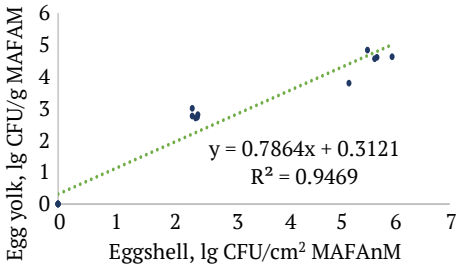

Figure 3. Dependence of the QMAFAnM in yolks on the QMAFAnM on the shell surface of washed and disinfected chicken eggs for storage in chilled form, n = 15
Source: developed by the authors

A strong direct relationship was also found between the level of contamination of the yolk and shell of washed and disinfected eggs by mould fungi ($r = 0.976 \pm 0.048$, $P < 0.001$) during their chilled storage. The regression line confirmed the existence of a direct linear relationship between the number of mould colonies on the shell surface and in the yolks of washed and disinfected food-grade chicken eggs when stored chilled (Fig. 4).

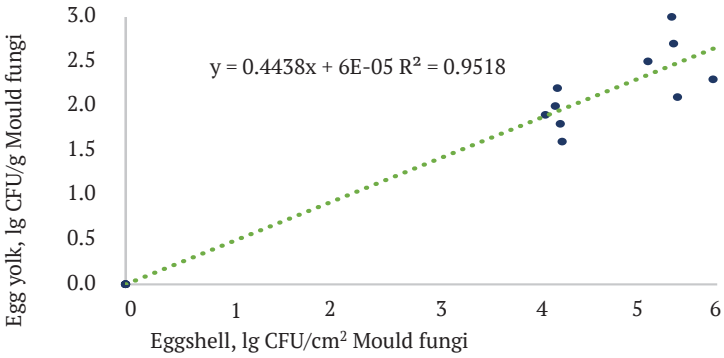

Figure 4. Dependence of the quantity of mould fungi in yolks on the number of quantity fungi on the shell surface of washed and disinfected food-grade chicken eggs for storage in chilled form, n = 15

Source: developed by the authors

Analysis of the species composition of microorganisms in the shells of unwashed chicken eggs showed that the main representatives of microorganisms on the 1st day of storage were the genus *Bacillus* (family Bacillaceae) which includes *B. mycoides* and *B. pumilus* and gender *Staphylococcus* (family Staphylococcaceae), which includes two species: *S. epidermidis* and *S. xylosus* (Table 4).

Table 4. *Microbiological screening with MALDI-TOF identification of chicken eggshells after washing and disinfection for storage in chilled form*

Characteristics of eggs	Shelf life, days	Types of dominant microorganisms
Eggs before washing and disinfecting	1	*Bacillus mycoides, Bacillus pumilus, Staphylococcus epidermidis*, Staphylococcus xylosus,*
Eggs after washing and disinfection	1	*Staphylococcus epidermidis*, Staphylococcus saprophyticus, Lactococcus garvieae*
	60	*Staphylococcus equorum***
	80	*Staphylococcus equorum***

Note: *, ** – identical types of bacteria on the surface of eggshells

Source: developed by the authors

Bacillus mycoides and *B. pumilus* come from the environment, in particular, belong to soil microorganisms that promote plant growth (Di Franco *et al.*, 2002). Thus, these types of bacteria could get into the composition of chicken feed, and from the feed to the surface of the eggshell. After washing and disinfecting food-grade eggs on the 1st day of storage in a chilled form, representatives of microorganisms of two genera were identified on the surface of the shell, in particular the genus *Staphylococcus* which includes *S. epidermidis* and *S. saprophyticus*, and the genus *Lactococcus* (family Streptococcaceae) – *L. garvieae*. On the 60th and 80th days of storage of washed and disinfected eggs, only one representative of the genus Staphylococcus was isolated from the shell surface – *S. equorum*. It is believed that staphylococci make up an important part of the microbiome of the shell and the contents of the egg (albumen and yolk). The results of this study are consistent with the data obtained by D. Stepień-Pyśniak *et al.* (2009), which indicate a relatively high level of contamination of food-grade eggs with staphylococci.

Of the 1,125 bacteriological studies of egg whites, yolks, and shells, staphylococci were

detected in 514 cases in this study. The largest percentage of staphylococci was isolated from the surface of eggshells – 302 strains. 12 types of staphylococci were isolated from the eggs under study, including coagulase-positive strains such as *S. hyicus and S. aureus,* and coagulase-negative, in particular, *S. warneri, S. lentus, S. xylosus* and *S. epidermidis*. Results of the study by S. Hsu *et al*. (2023) also indicate that in unwashed chicken eggs, 197 samples were positive for *Staphylococcus* spp. In addition to staphylococci, there are a number of other organisms that can contaminate the shell of chicken eggs, in particular, *Alcaligenes, Salmonella, Pseudomonas, Arthrobacter, Proteus, Escherichia, Serratia, Bacillus, Hafnia, Aeromonas, Citrobacter* and *Micrococcus* (Legros *et al.*, 2021; Kukhtyn *et al.*, 2024). Mould fungi and yeast were isolated in smaller amounts from eggshells when eggs were stored in high humidity conditions and were represented by five genera *Aspergillus, Penicillium, Cladosporium, Mucor, Rhizopus,* and yeast by one genus – *Rhodotorula*.

Washing and disinfecting chicken eggs with Blanidas-C Gen Deo did not ensure the release of the shell surface from staphylococci and streptococci, which dominated other microorganisms. Moreover, the species composition of staphylococci changed, and on the 60th and 80th day of storage of washed and disinfected food-grade eggs, the shell surface contained only *S. equorum*. The results obtained are consistent with the data of A. Wilson *et al*. (2021), who identified *S. equorum* in poultry houses, in particular, it was most common in bedding (33.7%), nests (25.1%) and egg conveyor belts (35.0%) when using a free-range chicken system. Analysis of the species composition of microorganisms in the yolks of chicken eggs showed their significant difference from the shells of both unwashed and washed and disinfected eggs (Table 4, 5).

Table 5. *Microbiological screening with identification of MALDI-TOF egg yolks of food-grade eggs after washing and disinfection for storage in chilled form*

Characteristics of eggs	Shelf life, days	Types of dominant microorganisms
Eggs before washing and disinfecting	1	*Pantoea agglomerans, Pseudomonas koreensis*
Eggs after washing and disinfection	1	*Staphylococcus epidermidis**
	60	*Pseudomonas alcaliphila, Pseudomonas oleovorans*
	80	*Bacillus subtilis, Staphylococcus epidermidis**

Note: * – identical types of bacteria in egg yolks
Source: developed by the authors

In the yolks of unwashed eggs on the 1st day of storage, two genera were distinguished, which belong to two different families, which included one type of microorganisms each. Genus *Pantoea* (family Erwiniaceae) in the yolks of unwashed washed eggs is represented by *P. agglomerans,* and genus *Pseudomonas* (family Pseudomonadaceae) – *P. koreensis. P. agglomerans* is widespread both in animals, soil and water, and in humans and currently, there are a significant number of contradictory statements about its pathogenicity for humans (Haralampidou *et al.*, 2022; Casale *et al.*, 2023). However, as noted by P. Ezeh *et al*. (2024), it is quite resistant to a number of antibiotics, which requires further research. *P. koreensis* belongs to newly isolated bacteria and is also a natural component of the soil microbiome (Kho *et al.*, 2023).

After washing food-grade eggs on the 1st day of storage, one type of microorganisms was

isolated in the yolks – *Staphylococcus epidermidis*. On the 60th day of storage of washed and disinfected food-grade eggs, microorganisms belonging to the genus *Pseudomonas*, namely *P. alcaliphila* and *P. oleovorans*, were found in the yolks, which differ in species composition from the yolks of unwashed eggs (Table 5). On the 80th day of storage of washed and disinfected chicken eggs, two types of microorganisms belonging to different families were identified: *Bacillus subtilis* and *Staphylococcus epidermidis*. The species composition of microorganisms in the shells and yolks of unwashed and washed chicken eggs depended on the shelf life of chilled form and differed from each other, with the exception of *S. epidermidis,* which was found both in flushes from the shell and egg yolks.

According to A. McWhorter & K. Chousalkar (2020), bacteria, viruses, and protozoa that get on the surface of the shell can enter the shell and survive in the eggs. If the eggs are not washed, they can become infected, and internal pathogens can multiply and grow during harvesting, sorting, transportation, and storage. Washing eggs did not release the yolks from *S. epidermidis* on the 1st day of storage, which also indicated a possible vertical path of contamination. Increasing the shelf life of washed and disinfected food eggs to 60 days indicates that bacteria of the genus *Pseudomonas* penetrate and multiply into the yolks, in particular *P. alcaliphila* and *P. oleovorans*. There is little information about the origin of these bacteria in the literature, but they are also part of the plant and soil microbiome (Chan *et al.*, 2021; Zeng *et al.*, 2023). In this study, it was found that increasing the shelf life of washed and disinfected eggs to 80 days contributed to reproduction *Bacillus subtilis* and *Staphylococcus epidermidis*. Given that the compound feed of industrial herd chickens included a probiotic, it can be assumed that *Bacillus subtilis* had an alimentary origin and ensured the dominance of beneficial microorganisms in the digestive system of chickens (Tsai *et al.*, 2023; Tajudeen *et al.*, 2024). Similar data was obtained by S. Hsu *et al.* (2023) when analysing internal fractions of unwashed and washed chicken eggs. The prevalence of *Staphylococcus* spp., *Enterobacteriaceae* and *E. coli* was 1.9%, 1.9%, and 0.5%, respectively, which allowed the researchers to conclude that washed eggs have better microbial safety and quality.

It is possible to get a guaranteed effect from washing food eggs only considering many factors that relate to their quality. During egg storage, certain physical and chemical changes in albumen and yolk occur that affect their quality. Eggshells have a porous structure that provides a constant gas exchange between the internal contents of the egg and the environment. The porous eggshell also ensures the penetration of microorganisms, in particular, bacteria and mould fungi from the external environment. All of these processes contribute to chemical reactions in eggs, which ultimately leads to undesirable changes in albumen and yolk that degrade egg quality (Caner & Yüceer, 2015; Obianwuna *et al.*, 2022).

In fresh eggs, the thick albumen makes up about 60% of the egg's weight, and its pH is 5.6-7.5 and depends on the exchange of gases and moisture with the environment. The duration of storage and temperature are the main factors that determine the rate of spoilage of eggs. As the shelf life of eggs increases, the thick albumen becomes thinner due to the enzymatic activity of ovomucin-lysozyme, disulfide bonds or reactions between α- and β-ovomucins, and an overall increase in the pH of the contents. K. Fikiin *et al.* (2020) suggest that when storing eggs at a temperature of 10°C, the HU index almost did not change for 20 days, but at 30°C this indicator decreased from 85 to 35, which indicates a reduction in their shelf life. Washing and disinfecting chicken eggs with a chlorine-containing agent in this experiment did not affect egg weight, albumen index, yolk colour, shell strength and thickness during 80 days of storage (Table 6).

Table 6. *Quality of chicken food-grade eggs after washing and disinfection when stored in a chilled form, x ± SD, n = 15*

Indicator	Characteristics of eggs			
	Eggs before washing and disinfecting	Eggs after washing and disinfection		
	Shelf life, days			
	1	1	60	80
Egg weight, g	58.43 ± 1.88[a]	57.66 ± 2.07[a]	57.11 ± 1.54[a]	56.41 ± 1.87[a]
Albumen index, mm	7.67 ± 1.16[a]	7.17 ± 0.79[a]	6.14 ± 0.88[a]	6.21 ± 0.43[a]
Egg yolk colour, points	10.13 ± 0.92[a]	10.20 ± 0.86[a]	11.00 ± 0.93[a]	11.14 ± 1.57[a]
HU units	88.79 ± 5.61[a]	85.78 ± 4.29[ab]	78.48 ± 6.17[b]	79.53 ± 2.59[b]
Shell strength, kgf	4.96 ± 1.06[a]	4.53 ± 1.12[a]	4.52 ± 1.40[a]	3.89 ± 0.67[a]
Shell thickness, mm	0.38 ± 0.03[a]	0.38 ± 0.04[a]	0.39 ± 0.02[a]	0.37 ± 0.04[a]

Note: different letters of the upper indexes indicate values that significantly differed in the same column of the table ($p < 0.05$) based on the results of comparison using Tukey test

Source: developed by the authors

It was found that storing washed and disinfected eggs for 60 days contributed to a decrease in the HU index by 10.31 units, and for 80 days – by 9.26 units compared to unwashed eggs. Moreover, the HU index of washed and disinfected eggs, even before the 80th day of storage, did not reach the critically low value characteristic of spoiled eggs. These data are consistent with the results obtained by K. Drabik *et al*. (2021), who showed that decontamination of eggshells with an aqueous solution of citric acid at concentrations of 10% and 15% for storing eggs at 14°C and 70% humidity after 7, 14, 21, and 28 days reduces weight loss, provides a smaller air chamber, better albumen structure, less intense water diffusion from albumen to yolk.

The quality indicators of washed and disinfected eggs obtained in the study using a chlorine-containing agent also do not contradict the data of G. Akarca *et al*. (2021), where after washing, the eggshells were coated with almond, apricot, or cherry tree resins and stored at 4°C and 22°C for 60 days. In the present study, it was found that air chamber height, weight loss, albumen and yolk pH values increased in all samples during storage, while HU units, albumen and yolk index, on the contrary, decreased. The lowest weight loss (0.54 g) and air chamber height (2.89 mm), the highest HU index (73.9), albumen index (8.8%) and yolk index (40.4%) were found in cherry resin-coated eggs when stored at 4°C. A similar pattern regarding changes in the quality of washed and disinfected eggs using pulsed light technology was obtained by B. Wang *et al*. (2022), who proved that with increasing storage time, the total value of the HU index and yolk index decreases, and albumen pH and weight loss increase. Thus, washing and disinfecting chicken eggs with a chlorine-containing agent ensures their proper quality and safety and allows them to be stored in a chilled form for up to 80 days.

Conclusions

The surface of the shell of unwashed chicken eggs on the 1st day of storage in chilled form did not contain colonies of mould fungi and was contaminated with a small quantity of MAFAnM microorganisms. The use of a chlorine-containing detergent and disinfection of eggs on the 1st day of storage inactivated MAFAnM colonies on their surface. With the increase in the shelf life of washed and disinfected eggs to 60 and 80 days, there was an increase in the quantity of MAFAnM and mould fungi on the surface of the shell and in the yolks, which was linear. The

following representatives of the family Bacillaceae were isolated in flushes from the shells of unwashed chicken eggs on the 1st day of storage in chilled form: *Bacillus mycoides* and *B. pumilus*; the family Staphylococcaceae: *Staphylococcus epidermidis* and *S. xylosus*. On the surface of the shell of washed and disinfected eggs on the 1st day of storage in chilled form, the basis was made up of microorganisms of the family Staphylococcaceae: *S. epidermidis* and *S. saprophyticus*, as well as family Streptococcaceae – *Lactococcus garvieae*. With an increase in the shelf life of washed and disinfected eggs to 60 and 80 days, the shell was dominated by *Staphylococcus equorum*. The surface of the shell of chicken eggs differs in the species composition of microorganisms from the yolks. The contamination of unwashed food egg yolks on the first day of storage with microorganisms of the family Erwiniaceae was revealed: *Pantoea agglomerans*, and Pseudomonadaceae: *Pseudomonas koreensis*. In the yolks of washed and disinfected food eggs, *Staphylococcus epidermidis* dominated on the 1st day of storage; on the 60th day – *Pseudomonas alcaliphila* and *P. oleovorans*; on the 80th day – *Bacillus subtilis* and *Staphylococcus epidermidis*. The main type of microorganisms in the shells and yolks of washed and unwashed eggs for storage in a chilled form was *Staphylococcus epidermidis*. Washing and disinfecting food-grade eggs does not affect their quality, with the exception of the HU index, which decreased on the 60th and 80th days of storage, but was within the limits characteristic of high-quality eggs. The results obtained can be the basis for choosing a means of washing and disinfecting food-grade chicken eggs to reduce their contamination with pathogenic and conditionally pathogenic microorganisms, considering the epidemiological situation in a particular poultry farm. Future research may focus on the quality and safety of egg products made from processed chicken eggs.

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Conflict of Interest

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Якість та безпечність курячих яєць після миття і дезінфекції хлорвмісним засобом

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Анотація. Контамінація курячих харчових яєць патогенними та умовно патогенними мікроорганізмами в процесі їх виробництва, зберігання та реалізації спричиняє високий ризик виникнення токсикоінфекцій у людей. Одними з ефективних засобів зниження мікробного навантаження є миття і дезінфекція поверхні шкаралупи яєць препаратами на основі активного хлору. Метою дослідження було визначити якість миття та дезінфекції харчових курячих яєць під час зберігання в охолодженому вигляді. В досліді визначали якість та мікробіологічні показники харчових курячих яєць після миття та дезінфекції на першу, 60-ту та 80-ту добу зберігання за температури 4°С. Мікробіологічні показники яєць досліджували з використанням технології MALDI TOF, якість – за допомогою цифрової овоскопії. Миття та дезінфекція препаратом на основі активного хлору в концентрації 0,5 % звільняла шкаралупу яєць від колоній МАФАМ і плісеневих грибів. Зберігання митих і дезінфікованих яєць в охолодженому вигляді до 60-ї та 80-ї доби сприяло збільшенню кількості МАФАМ на 2,45 lg КУО/cm2 і на 5,68 lg КУО/cm2 відповідно. Чисельність плісеневих грибів на поверхні шкаралупи митих і дезінфікованих яєць на 60-ту добу зберігання досягала 4,37 lg КУО/cm2, а на 80-ту добу їх кількість зрівнялася з чисельністю МАФАМ. Чисельність МАФАМ і плісеневих грибів в жовтках харчових яєць мала сильну пряму залежність від

їх кількості на шкаралупі і від терміну їх зберігання в охолодженому вигляді. Миття і дезінфекція харчових курячих яєць хлорвмісним препаратом не впливала на втрату маси, висоту білка, колір жовтка, міцність і товщину шкаралупи, але знижувала індекс HU до 78,5-79,5 одиниці, що в поєднанні з мікробіологічними показниками дозволило зберігати їх до 80-ї доби в охолодженому вигляді. Отримані результати необхідно враховувати при виборі засобу, режиму миття і дезінфекції та терміну зберігання харчових яєць з урахуванням видового складу мікроорганізмів, характерного для конкретної птахофабрики

Ключові слова: шкаралупа; жовток; МАФАМ; плісеневі гриби; овоскопія; мікробна контамінація

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