ANIMAL SCIENCE AND FOOD TECHNOLOGY

Founder:
National University of Life and Environmental Sciences of Ukraine

Year of foundation: 2010

Recommended for printing and distribution
via the Internet by the Academic Council
of National University of Life and Environmental Sciences of Ukraine
(Minutes No. 9 of February 26, 2026)

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

The journal is included in the list
of Professional Scientific Publications of Ukraine
Category "B". Branch of sciences: Agricultural. Specialty: 0721 – Food processing,
0811 – Crop and livestock production, 0831 – Fisheries.
(order of the Ministry of Education and Science of Ukraine
of December 23, 2025, No. 1693)

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

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

ТВАРИННИЦТВО ТА ТЕХНОЛОГІЇ ХАРЧОВИХ ПРОДУКТІВ

Засновник:
Національний університет біоресурсів і природокористування України

Рік заснування: 2010

Рекомендовано до друку та поширення
через мережу Інтернет Вченою радою
Національного університету біоресурсів і природокористування України
(протокол № 9 від 26 лютого 2026 р.)

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

Журнал входить до переліку
наукових фахових видань України

Категорія «Б». Галузь знань: Сільськогосподарські науки,
спеціальності – 181 «Харчові технології»,
204 «Технологія виробництва і переробки продукції тваринництва»,
207 «Водні біоресурси та аквакультура»
(Наказ Міністерства освіти і науки України від 23 грудня 2025 р. № 1693)

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

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

Editorial Board

Редакційна колегія

Міжнародні члени редколегії:

Мирослава Качаньова	Професор, інженер (біотехнології) Словацький сільськогосподарський університет в Нітрі, Жешувський університет, Словакія
Джованні Сілія	Кандидат ветеринарних наук, науковий співробітник, Рада сільськогосподарських досліджень та економіки Науково-дослідний центр сільського господарства та навколишнього середовища, Італія
Міхайела Корнеа-Сіпчіган	Кандидат наук у галузі харчових технологій, Університет сільськогосподарських наук та ветеринарної медицини Клуж-Напока, Румунія
Родіка Маргаоан	Кандидат наук у галузі харчових технологій, науковий співробітник, Університет сільськогосподарських наук та ветеринарної медицини Клуж-Напока, Румунія
Марія Клопчіч	Кандидат наук у галузі тваринництва, доцент, Люблянський університет, Словенія
Лукаш Зіта	Кандидат наук у галузі тваринництва, доцент, Чеський університет природничих наук, Чехія
Ян Бріндза	Кандидат наук, доцент, Словацький сільськогосподарський університет в Нітрі, Словакія
Галя Замарацкая	Кандидат наук у галузі харчових технологій, доцент, Шведський університет сільськогосподарських наук, Швеція
Мохаммадабаді Мохаммадреза	Кандидат наук у галузі порівняльної генетики, професор, Керманський університет Шахіда Бахонар, Іран
Бранко Гламузіна	Доктор наук у галузі аквакультури, професор, Дубровницький університет, Хорватія
Олександр Маліновський	Кандидат наук у галузі аквакультури, науковий співробітник, Південночеський університет у Ческі-Будейовиці, Чехія

CONTENTS

Food Technology

M. Madani, A. Makarynska, N. Vorona
Complex processing of brewery waste for obtaining feed additives..... 9

M. Gill, V. Posukhin, D. Chobotar
Gas and thin-layer chromatography in the control of residual levels
of polychlorinated biphenyls and pesticides in foods of animal origin 28

I. Hetman, I. Lukianchuk, O. Naumenko, V. Kovbasa, L. Tkachenko
Investigation of wheat gluten properties and its effect on bread quality 46

U. Pastsart, S. Salam, J. Rattanawut, P. Permpoonpattana, C. Chotimarkorn
Effect of durian rind pectin with basil and oregano essential oils on beef patty quality 60

N.A.F. Putri, N.A. Noerrosadi, Y.A. Rachma, Y.B. Pramono
Physicochemical properties and sensory acceptance
of honey-enriched chickpea (*Cicer arietinum* L.) yoghurt 77

V. Fedorov, O. Kepko, O. Trus, V. Kepko
Rationalisation of commercial and technological features
of biscuit semi-finished products during baking 93

ЗМІСТ

Технології харчових продуктів

М. Мадані, А. Макаринська, Н. Ворона
Комплексна переробка відходів пивоваріння з отриманням кормової добавки 9

М. Гиль, В. Посухін, Д. Чоботарь
Газо- та тонкошарова хроматографія в контролі залишкових кількостей поліхлорованих біфенілів і пестицидів у харчових продуктах тваринного походження 28

І. Гетьман, І. Лук'янчук, О. Науменко, В. Ковбаса, Л. Ткаченко
Дослідження властивостей глютену пшеничного
та його впливу на формування якості хліба 46

У. Пастсарт, С. Салам, Д. Раттанавут, П. Пермпунпаттана, Ч. Чотімаркорн
Вплив пектину зі шкірки дуріана у поєднанні
з ефірними оліями базиліку та орегано на якість яловичих котлет 60

Н. А. Ф. Путрі, Н. А. Ноерросаді, Я. А. Рачма, Й. Б. Прамоно
Фізико-хімічні властивості
та сенсорна привабливість йогурту з нуту (*Cicer arietinum* L.), збагаченого медом 77

В. Федоров, О. Кепко, О. Трус, В. Кепко
Раціоналізація товарних і технологічних властивостей бісквітних напівфабрикатів
під час випікання..... 93

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 17(1), 9-27
Received 01.10.2025 Revised 24.01.2026 Accepted 26.02.2026
Published 26.03.2026

UDC 663.4:628.4:636.085
DOI: 10.31548/animal.1.2026.9

ISSN 2706-8331
e-ISSN 2706-834X

Complex processing of brewery waste for obtaining feed additives

Mariya Madani*
PhD in Technical Sciences, Associate Professor
Odesa National University of Technology
65039, 112 Kanatna Str., Odesa, Ukraine
https://orcid.org/0000-0001-9386-7364

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

Nina Vorona
PhD in Technical Sciences, Associate Professor
Odesa National University of Technology
65039, 112 Kanatna Str., Odesa, Ukraine
https://orcid.org/0000-0001-6903-9016

Abstract. One of the key directions in the development of the modern brewing industry in Ukraine is the rational utilisation of organic waste generated during beer and malt production. The article aimed to investigate the composition, physicochemical properties, and utilisation methods of the main brewing wastes, using Mykulynetsky Brewery as a case study. Quantitative and qualitative characteristics of solid (brewer's spent grain, malt sprouts, barley screenings, trub) and liquid (transport water suspensions of spent grain, protein sediment, residual yeast, lager sediments, wastewater) wastes were determined. It was established that brewer's spent grain accounts for up to 85% of all solid production residues and represents a valuable raw material for feed production due to its high content of protein, fibre, and minerals. Experimental studies were conducted on the processes of spent grain dewatering and drying, malt sprout and barley screenings grinding, and filtration of concentrated suspensions from the fermentation-brewing workshop. Optimal conditions for phase separation of suspensions were determined, yielding a concentrated protein

Suggested Citation:
Madani, M., Makarynska, A., & Vorona, N. (2026). Complex processing of brewery waste for obtaining feed additives. *Animal Science and Food Technology*, 17(1), 9-27. doi: 10.31548/animal.1.2026.9.

*Corresponding author

fraction with 73% moisture content and a filtrate with a low concentration of suspended solids (0.1-0.45 g/dm^3). A technology for the comprehensive processing of all major brewing wastes was proposed, resulting in two types of feed products: dewatered brewer's spent grain and granulated protein-vitamin feed containing 35% crude protein, 5-7% fibre, and 8-10% moisture. The developed technology also provides for the neutralisation of acidic and alkaline wastewater to standard parameters and their reuse in the production cycle. Practical implementation of the proposed technology significantly reduces environmental impact, decreases waste generation, increases resource efficiency of production, and creates a closed-loop system for the utilisation of secondary resources in the brewing industry

Keywords: brewing industry; waste utilisation; wastewater; feed products; technology; technical ecology

Introduction

One of the key challenges facing the modern brewing industry in Ukraine is the rational management of production waste of organic origin. During the production of beer and malt, a significant amount of solid and liquid waste is generated, which has high potential as secondary raw material. According to O. Servetnyk & O. Piven (2024), an average Ukrainian brewery produces up to 10-15 thousand tons of solid and several hundred thousand cubic meters of liquid residues annually. However, only a small portion of these materials is effectively utilised. This issue has become particularly relevant in the context of the ongoing full-scale war, when food security, the stability of the agro-industrial sector, and the preservation of environmental balance are of strategic importance for the country. The processing of brewing waste into high-quality protein-vitamin feed products for livestock and poultry can significantly strengthen the national feed base and reduce dependence on imported resources. The main types of waste generated by Ukrainian breweries include barley screenings, malt sprouts, brewer's spent grain, yeast residues, lager and protein sediments, as well as technical wastewater. These wastes contain valuable nutrients – proteins, fibre, carbohydrates, vitamins, and minerals – which make them promising for secondary use, particularly in feed production. In Ukraine, there is no single technological approach to the comprehensive processing of these wastes. Most enterprises are forced to store or dispose of them as ordinary waste, leading to the loss of potentially valuable resources and increased environmental pressure. Therefore, the development of an integrated technology for the processing of all major brewing wastes, aimed at using their components for feed production and environmental protection, remains an urgent scientific and practical task.

The rational utilisation of food industry waste, particularly that generated in brewing, has been the focus of active research within both the global and Ukrainian scientific and technical communities. Numerous studies, like those by S. Chattaraj *et al*. (2024a), A. Soceanu *et al*. (2024), emphasised the high nutritional value of brewing by-products, which contain significant amounts of proteins, fibre, amino acids, B-group vitamins, minerals (Ca, Mg, P, Fe), and enzymes. The main wastes generated during beer production include residues from barley and malt polishing (cleaning) prior to milling, brewer's spent grain, residual brewing yeast, hop trub, as well as protein sediment and solid residues formed after wort cooling and clarification. A significant amount of wastewater is generated during the washing and disinfection of production facilities, equipment, containers,

and pipelines. As stated by M. Verhuelsdonk *et al*. (2021), brewing waste also includes defective labels, crown corks, and glass breakage. However, not all brewing industry wastes can be considered as secondary material resources suitable for the production of commercial products. The main types of brewery waste of practical interest due to their quantity and composition suitable for feed production include: solid wastes (brewer's spent grain, barley screenings, malt sprouts, and grain husks); highly concentrated liquid wastes (transport water from spent grain, protein sediment, residual yeast, and lager sediment). Because the main brewing wastes retain the nutritional components of the raw materials, their processing is of great importance to both the food and agricultural industries.

Due to its high moisture content (up to 88%), wet spent grain has limited storage stability; therefore, various stabilisation and processing technologies have been developed (Terefe, 2022). The main methods include drying, ensiling, and preservation. According to L. Nyhan *et al*. (2023), S. Chattaraj *et al*. (2024b), dried brewer's spent grain is used in animal feed, baking, and as an additive to biogas substrates. Other brewing by-products also have processing potential. Malt sprouts contain proteins, B-group vitamins, and enzymes, enabling their use in feed production, baking, microbiological media, and enzyme preparation manufacturing. Protein sediment can be hydrolysed to obtain protein hydrolysates used in brewing and compound feed production (Karlsen & Skov, 2022). Under modern conditions, integrated processing of brewing waste – combining several technological approaches – has gained special importance, as it minimises the loss of valuable substances and yields value-added products. Studies by M. Pires Maria *et al*. (2023), G. Wei *et al*. (2024) demonstrated the effectiveness of such methods: drying and granulation of spent grain and yeast, biogas or ethanol production from brewing residues, and the use of protein sediment in feed concentrate manufacturing.

Thus, literature analysis shows that brewing waste represents a promising biotechnological raw material. Its rational utilisation contributes to reducing environmental impacts, increasing production efficiency, and creating new types of feed and food products. Despite the wide range of existing methods for the utilisation of malt and beer production waste, there is still no systemic, integrated technology for complete processing of secondary brewing resources. Existing solutions are often complex, multi-stage, and costly. The most promising direction is the production of protein-vitamin feed products based on all major brewing wastes. The purpose of the study was to develop a comprehensive technology for processing solid and liquid wastes of the Ukrainian brewing industry to obtain feed products and reduce environmental impact. To achieve this goal, the following objectives were set: to investigate the processes of processing solid and liquid brewing waste and study the possibilities of cleaning and reusing industrial wastewater; to develop a technology for obtaining a feed product; to develop a technology for the complex processing of brewing waste.

Materials and Methods

The main object of the study was solid and liquid waste generated during the beer production process at Mykulynetsky Brewery LLC, the production facilities of which are located in the town of Mykulyntsi, Ternopil region, Ukraine. The research period was January-September 2025. The research was conducted on the basis of the laboratories of the Odesa National University of Technology.

Methods of processing the main brewing waste. Beer grains were processed by dehydration and drying to a humidity of 10%. Dehydration was aimed at removing excess liquid, improving transportability and extending the shelf life of the product. In order to study the process of beer grains dehydration and choosing the optimal method in laboratory conditions, static and dynamic methods of its processing on

polyethylene and metal meshes were tested. To ensure static dehydration conditions, the raw grain was placed in an even layer on a stationary filter material (according to the principle of a Nutsche filter). A metal mesh with square holes measuring 1.5 x 1.5 mm or a polyethylene mesh with rectangular holes measuring 0.5 x 1.5 mm was used as the filter elements. The process lasted for 2 hours, while the formed filtrate was collected in a receiving container (Fig. 1).

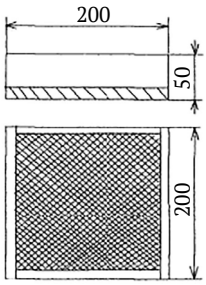

Figure 1. Dewatering of beer grains under stationary conditions
Note: materials: (1) metal mesh with square holes measuring 1.5 × 1.5 mm; (2) polyethylene mesh with rectangular holes measuring 0.5 × 1.5 mm
Source: authors' drawing

During the dynamic process, a cylindrical drum with the following main dimensions was made from the above-mentioned filter materials: diameter = 145 mm, length = 175 mm. The drum was equipped with a central axis for connection to the drive, which ensured its rotation around the axis at a speed of 0.35 rpm (Fig. 2). The moisture content of the material was determined after 1 and 2 hours during the dehydration process.

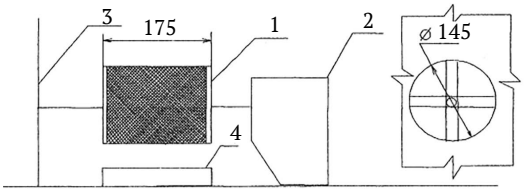

Figure 2. Dewatering of beer grains under dynamic conditions
Note: 1 – drum made of metal mesh with square holes measuring 1.5 × 1.5 mm; 2 – electric drive; 3 – tripod with holder; 4 – receiving tank
Source: authors' drawing

Drying of dehydrated grains. In laboratory conditions, hot air drying was carried out in a SESH-3M (Ukraine) drying cabinet (220 V, 50 Hz, 1,200 W) with the ability to regulate the temperature. To ensure uniform heating of the entire volume of material, it was periodically stirred. The drying temperature did not exceed +60°C, according to Y. Bulii *et al*. (2022), which did not impair the quality of the product.

Grinding of grain waste. The particle size composition of malt production waste was determined by sieving. For this purpose, a laboratory set of sieves with hole sizes from 2 to 0.5 mm was used. Grinding of dry malt production waste (barley bran and sprouts) was carried out using a laboratory mill OLISLAB 2100 (Olis, Ukraine), and the grinding efficiency was assessed by the particle size composition of the

grinding products (Tertyshny *et al.*, 2022). The flour production process included the preparation of raw materials, grinding and sorting of the resulting product. Given that the separate grinding of the product, previously sorted into fractions of a certain size, contributes to a more complete use of the grain, the barley screenings and malt sprouts were pre-sifted through sieves with opening sizes of 2.0; 1.0 and 0.5 mm. The grinding of fractions larger than 0.5 mm of dry malt waste was carried out in a laboratory mill for 3 minutes.

Separation of highly concentrated liquid waste. In order to isolate protein-containing substances from highly concentrated liquid waste of the fermentation and brewing plant, their phase separation was carried out by filtration on a laboratory installation (Fig. 3).

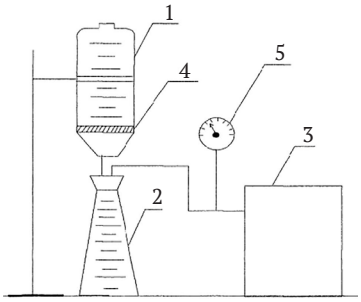

Figure 3. Laboratory setup for phase separation of highly concentrated liquid wastes
Note: 1 – filtering cylinder (volume – 2,000 mL, diameter – 80 mm); 2 – receiving conical flask (volume – 1,000 mL); 3 – vacuum pump: model VN-46 (Ukraine), Q = 45 l/min, P = 5 · 10-3 mm Hg, n = 500 rpm, m = 40 kg; 4 – filter: cotton belting (article 2073, Ukraine), BECO KD 7 filter cardboard (Eaton Filtration LLC, Germany); 5 – OBV1-100 vacuum gauge (model VT-100, Ukraine)
Source: authors' drawing

As filter materials, cardboard intended for filtering beer and cotton belting were used alternately. The pressure difference on the filter partition was created using a VN-46 vacuum pump, which provided a vacuum of up to 0.9 kgf/cm^2. Vacuum (rarefaction) was measured using a VT-100 vacuum gauge, the scale of which was graduated to -1 kgf/cm^2. Table 1 shows the parameters of the suspension filtration processes.

Table 1. *Parameters of the suspension filtration process of the fermentation and brewing plant*

Filtering material	Suspension volume, m^3	Sediment layer height (Hoc), m	Mass of solid particles (mth), kg	Sediment humidity, %	Filtrate volume (Vf), m^3	Concentration of suspended solids in the filtrate, g/dm^3
Belting	190×10^{-4}	2×10^{-3}	5.92×10^{-3}	73	174×10^{-3}	0.45
Cardboard	190×10^{-4}	2×10^{-3}	6.9×10^{-3}	73	174×10^{-3}	0.1

Note: the concentration of suspended solids in the mixture before filtration is 8.2 g/dm^3
Source: compiled by the authors

Purification of spent alkaline solution. In order to study the possibility of purifying the spent alkaline solution of the bottle washing machine from suspended substances and reusing it in beer bottling technology, it was filtered on a laboratory filter (Fig. 4). To purify and reuse the spent alkaline solution, laboratory tests were conducted using filtration through layers of activated carbon and sand of various grain sizes (0.7-3 mm).

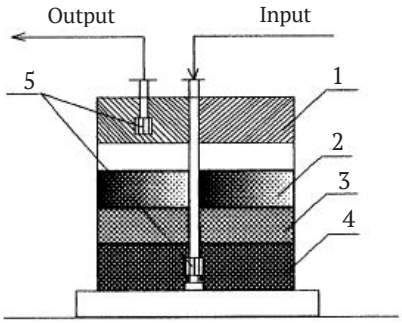

Figure 4. Laboratory filter for cleaning spent alkaline solution of bottle washing machine
Note: D = 100 mm; H = 800 mm; 1 – expanded polystyrene (0.8-2.5 mm); 2 – activated carbon AG-3 (1.0-2.8 mm); 3 – crushed quartz (0.7-1.2 mm); 4 – crushed quartz (2.0-3.5 mm); 5 – slotted nozzles
Source: authors' drawing

The principle of operation of the installation is the passage of liquid through a layer of bulk material. The filter materials for loading the installation consisted of four bulk layers. The choice of these materials and their arrangement in layers (from fine to coarse) is typical for multilayer filters. Crushed quartz (two fractions) provides mechanical filtration and acts as a supporting layer due to its high mechanical strength and insolubility. Activated carbon AG-3 is added to adsorb organic contaminants, colour, odour and chlorine, which is critical in the purification of technological solutions. Expanded polystyrene is used as a lightweight filter material that helps in the removal of suspended solids. Slotted nozzles in the laboratory filter provide a large contact area between the phases, creating optimal conditions for filtration.

Granulation and evaluation of granule quality. In laboratory conditions, a press granulator of the OPG 150 brand (Ukraine) was used to obtain beer grains granules (Fig. 5), a granulator with the following technological characteristics and modes: drive with a power of 1.5 kW; flat matrix; number of pressing rollers – 2 pcs, matrix diameter – 4.7 mm; mass fraction of moisture of the mixture fed for granulation – 16...18%; matrix heating temperature – +90 ± 5°C; temperature of granules at the exit of the press – +70 ± 3°C. The obtained granules with a length of 5-15 mm were dried in a SESH-3M cabinet dryer at a temperature not higher than +60°C for 15-45 minutes.

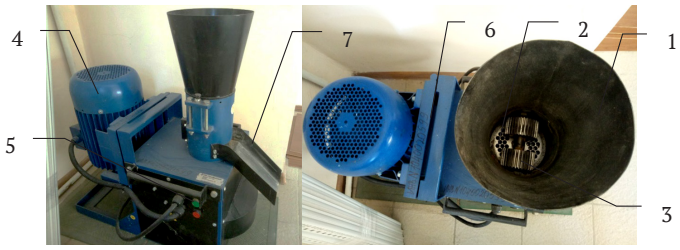

Figure 5. Press granulator OPG-150
Note: 1 – receiving hopper; 2 – matrix; 3 – pressing rollers; 4 – engine; 5 – control panel; 6 – housing; 7 – pellet tray
Source: authors' photo

Methods of physical and chemical analysis. During the experiments to study the composition and properties of brewing waste and their processing products, mainly methods of

physical and chemical analysis were used. The study of the chemical composition of malting waste and beer grains was based on methods intended for the analysis of compound feeds and raw materials for compound feeds, in particular: method for determining moisture content (DSTU ISO 6496:2005, 2006); method for determining the content of nitrogen and crude protein (DSTU 7169:2010, 2011); method for determining crude fibre (DSTU 8844:2019, 2020). During the study of the composition of liquid brewery waste, the following main indicators were determined: COD (chemical oxygen demand) – dichromate method; BOD_5 (biological oxygen demand for 5 days) – iodometric method; concentration of suspended solids – gravimetric method (Hryn *et al.*, 2019). The viscosity (coefficient of internal friction) of the liquid phase of the suspensions was determined using a capillary viscometer.

After cooling the granules, three single samples were selected, from which a general sample was formed. Then the average sample was selected for analysis. The sizes (diameter and length) of the granules were measured using a calliper. The volumetric mass was determined using a litre funnel with a falling load and laboratory scales of the 2nd accuracy class according to DSTU 8024:2015 (2017). The angle of natural slope was determined according to the degrees applied to the side surface of a special device by R. Zenkov by pouring the product from a funnel according to DSTU 8024:2015 (2017). The flowability of the granules is characterised by the speed of the product flowing out through a hole of a certain diameter according to DSTU 8482:2015 (2017). Granule friability was determined by shaking the granules in a device for one hour and measuring the mass before and after shaking in accordance with DSTU 8482:2015 (2017).

Processing of research results. The reliability of the results of the analysis of the composition of feed products was determined by the Student criterion (Danilov, 2019) (1):

$$t = Mean\ X/m, \quad (1)$$

where t – the reliability of the results; *Mean X* – the arithmetic mean of a series of indicators; m – the error of the arithmetic mean. The arithmetic Mean X was calculated by the formula (2):

$$Mean\ X = \sum X_i/n, \quad (2)$$

where *Xi* – individual variants of the series; n – the number of values in the series.

The error of the arithmetic mean was determined by the formula (3):

$$m = \frac{\sigma}{\sqrt{n}}, \quad (3)$$

where σ – the standard deviation, which was calculated as follows (4):

$$\sigma = \sqrt{\frac{\Sigma(Xi - Mean\ X)^2}{n-1}}. \quad (4)$$

The obtained value of the reliability of the results t (according to formula 1) was compared with the standard values of the Student's t-test. If $t > t_{table}$, a conclusion was made about the reliability of the results with the appropriate level of probability. The reliability of the detected differences between the averages was calculated by the Student's t-test. Statistical processing of the obtained data was carried out using the MS Excel 2019 package using generally accepted algorithms.

Results and Discussion

Composition and physico-chemical properties of the main brewing wastes and priority directions for their processing. Solid wastes – barley screenings, malt sprouts, trub, and brewer's spent grain – are generated during the malting and brewing processes. The main component of these wastes is brewer's spent grain (maximum up to 8,600 tons per year) with a moisture content of 87%, protein content of 5.2%, and fibre content of 18% (Table 2). After dewatering (to 80%), it is used as feed for cattle. The

highest protein content was found in malt sprouts (23%), which makes their use as feedstock reasonable. The crude fibre content in solid wastes ranged from 10% to 18%.

Table 2. *Composition and quantity of solid wastes from Mykulynetskyi Brewery for 2024*

Type of waste	Place of formation	Quantity, t/day	Quantity, t/year	Main indicators	Value, %
Brewer's spent grain	Lauter tuns of the brewhouse	24.0	8,000	Moisture Protein Fibre	87 5.2 18
Barley screenings	Sorting equipment of the malt house	0.17	57.3	Moisture Protein Fibre	10 15 11
Malt sprouts	Rootlet removal machine of the malt house	0.18	60.1	Moisture Protein Fibre	11 23 15
Barley trub	Steeping section of the malt house	0.1	18.2	Moisture Protein Fibre	29 10 10

Note: the daily quantity of wastes was calculated assuming 330-340 working days per year
Source: compiled by the authors according to the production balance sheet at the enterprise

Liquid wastes, or wastewater, are generated at virtually all stages of the production process. Depending on the degree of contamination, they can be divided into highly concentrated and low-concentrated types; according to the pH reaction of the medium, they are classified as acidic, alkaline, or neutral. The highest content of organic substances was observed in the wastewater from the fermentation and brewhouse departments (Table 2), which includes transport water from the spent grain, protein sediment, residual yeast, and lager sediments. The COD and BOD_5 values for these waters were 3-45 g O_2/dm^3 and 2-10 g O_2/dm^3, respectively – dozens of times higher than the permissible discharge standards for pollutants. The content of suspended solids in these wastewaters also exceeded the norms, ranging from 5 to 30 g/dm^3. All suspensions from the fermentation and brewhouse departments had an acidic reaction, with pH values between 4.5 and 5.5. In contrast, alkaline effluents from the bottling department exhibit pH levels exceeding the allowable limit – particularly the alkaline disinfectant solution from the bottle-washing machine, which is up to five units above the norm. Meanwhile, rinsing waters from the malt house and water treatment facilities are considered conditionally clean (Table 3).

Table 3. *Characteristics of liquid brewing wastes from Mykulynetskyi Brewery*

Type of waste	Place of formation	Quantity, m^3/day	Quantity, m^3/year	Indicators	Value	Exceedance of approved discharge standards
Transport water from spent grain	Spent grain dewatering section of the brewhouse	74	24,420	COD BOD_5 Suspended solids pH	3-8 g $O_2/dm^3$2-4.5 g $O_2/dm^3$4-6 g/$dm^3$4.5-5.0	1.5-4 × 1.5-3.5 × 5.7-8.5 × 1-1.5 units
Protein sediment	Whirlpool of the brewhouse	6	1,980	COD BOD_5 Suspended solids pH	18-30 g $O_2/dm^3$6-10 g $O_2/dm^3$8-15 g/$dm^3$5.0-5.5	9.2-15.4 × 4.6-7.7 × 11.4-21 × 0.5-1 unit

Table 3. Continued

Type of waste	Place of formation	Quantity, m^3/day	Quantity, m^3/year	Indicators	Value	Exceedance of approved discharge standards
Residual yeast	Fermentation tanks	6	1,980	COD BOD_5 Suspended solids pH	21-45 g O_2/dm^3 4-10 g O_2/dm^3 5-30 g/dm^3 4.5-5.5	10-23× 3.0-7.7× 7-14× 0.5-1.5 units
Lager sediments	Fermentation tanks	6	1,980	COD BOD_5 Suspended solids pH	21-45 g O_2/dm^3 4-10 g O_2/dm^3 5-30 g/dm^3 4.0-5.5	10-23× 3.0-7.7× 7-14× 0.5-2 units
Alkaline disinfectant solution from bottle washer	Bottle-washing machine in the bottling department (discharge once every 6 days, V = 40 m^3)	–	–	Suspended solids pH	0.8 g/dm^3 14	1.1× 5 units
Rinsing water from bottle washer	Bottle-washing machine in the bottling department	59	19,470	pH	11.0-11.5	2-2.5 units
Barley rinsing water	Steeping vessel of the malt house	60	19,800	COD BOD_5 Suspended solids pH	1 g O_2/dm^3 0.2 g O_2/dm^3 – 6.0-7.0	None None None None
Rinsing water from water treatment department	Water treatment filters	90	29,700	COD BOD_5 Suspended solids pH	0.4 g O_2/dm^3 0.006 g O_2/dm^3 0.05 g/dm^3 7.0-8.0	None None None None

Note: the daily quantity of wastes was calculated assuming 330-340 working days per year
Source: compiled by the authors according to the production balance sheet at the enterprise

Based on the study of the composition and physicochemical properties of the main wastes from Mykulynetskyi Brewery, three priority directions for their processing were identified: (1) dewatering and drying of brewer's spent grain for further use in animal husbandry or as secondary raw material; (2) utilisation of protein compounds contained in concentrated liquid wastes as components of feed additives; (3) neutralisation of alkaline wastewater generated in the bottling department to achieve standard quality parameters.

Dewatering and drying of brewer's spent grain. Processing of brewer's spent grain was carried out by dewatering and drying it to a moisture content of 10%. The purpose of dewatering was to remove excess liquid, improve transportability, and extend storage life. Experiments were performed using static and dynamic methods on polyethylene and metal meshes (Table 4).

Table 4. *Dewatering of raw spent grain*

Filtering material	Time, h	Amount of raw spent grain, kg	Moisture of raw spent grain, %	Moisture of dewatered spent grain, %
On polyethylene mesh				
Static	2	0.3	87.7	86.4
Dynamic	2	0.3	87.7	80.0
On metal mesh				
Static	1 2	0.3	87.7	82.7 82.0
Dynamic	1 2	0.3	87.7	80.2 75.2

Source: compiled by the authors

The most effective method was dynamic dewatering on a metal mesh, where the moisture content decreased from 87.7% to 75.2%. Under dynamic conditions, a rotating drum ensures continuous mixing of the material, while the metal mesh allows better liquid drainage due to larger openings and a reduced risk of clogging. Dynamic dewatering also offers several advantages: shorter processing time; lower final moisture content; improved storage stability; enhanced efficiency of subsequent drying. The resulting product (dehydrated pellets), with a moisture content of 10%, had a loose, granular consistency, suitable for use as a feed component. The disadvantage of this method is the aspiration loss of fine particles during drying in an amount of up to 8%.

Processing of malt production wastes (barley screenings and malt sprouts). Processing of malt production wastes – barley screenings and malt sprouts – was carried out to study the possibility of producing flour-based materials from them. The fractional composition of these wastes is presented in Table 5.

Table 5. *Fractional composition of malt production wastes*

Type of waste	Fractional composition, %			
	> 2 mm	2-1 mm	1-0.5 mm	< 0.5 mm
Barley screenings (moisture 10%)	86.7	9.0	1.0	3.2
Malt sprouts (moisture 8%)	12.0	41.1	4.8	42.1

Note: production of flour from barley trub is economically impractical due to its high moisture content (about 30%), which would require an additional drying stage
Source: compiled by the authors

As shown in Table 5, malt sprouts contain 13.6 times more fine fraction (<0.5 mm) than barley screenings, which indicates lower energy consumption required for their grinding. The grinding efficiency was evaluated based on the granulometric composition of the resulting products (Table 6), determined by sieving through a mesh with square openings of 0.5 mm.

Table 6. *Granulometric composition of ground malt production wastes*

Type of waste	Proportion of obtained products, %	
	Flour (< 0.5 mm)	Screenings (> 0.5 mm)
Barley screenings	86.9	13.0
Malt sprouts	95.9	4.0

Source: compiled by the authors

The obtained results indicate a high efficiency of grinding dry malt production wastes. The yield of the fine fraction (<0.5 mm) was 86.9% for barley screenings and 95.9% for malt sprouts. The resulting flour had a homogeneous structure: flour from malt sprouts exhibited a yellowish-straw tint, while that from barley screenings was white. The moisture content of the products was 8.8% and 9.15%, respectively.

Processing of highly concentrated liquid brewing wastes. The purpose of processing highly concentrated liquid wastes from brewing production – represented by the suspensions of the fermentation and brewhouse departments (Table 2) – is the utilisation of protein substances. For this purpose, it is advisable to separate the suspensions of transport water from spent grain, protein sediment, residual yeast, and lager sediments into solid and liquid phases. The study showed that filtration of suspensions through cardboard proceeds slightly slower than through cotton belting, due to a 1.03 times higher hydraulic resistance. However, cardboard provides a much cleaner filtrate: the concentration of suspended solids in it is 4.5 times lower, and the transparency is significantly higher. The mass of the sediment formed on the cardboard was 1 g greater than that formed on the belting cloth (Table 6). Thus, the use of cardboard for phase separation of concentrated liquid wastes is more efficient, providing clear separation into a filtrate with a low content of suspended solids and a thickened protein product with a moisture content of about 73%, which can be used as a component of feed additives. The tests on cleaning the spent alkaline solution from the bottle-washing machine demonstrated high purification efficiency (Table 7). The quality of the treated alkaline solution (28-112 mg/dm^3 of suspended solids) allows its reuse in the bottle-washing machine. Process water should be additionally purified from suspended solids to meet the standard limit of 900 mg/dm^3 established for Mykulynetskyi Brewery.

Table 7. *Treatment of spent alkaline solution*

Mode	Concentration of suspended solids, mg/dm^3		Purification efficiency, %	Filter parameters
	before	after		
Filtration	360	112	69	S_f = 0.008 m^2 Vss = 8 dm^3

Source: compiled by the authors

Production of feed products from brewing wastes. The dewatered brewer's spent grain is used separately as feed for cattle. Other wastes – spent grain filtrate, protein sediment, residual yeast, lager sediments, barley screenings, and malt sprouts – are processed into a protein-vitamin feed product. A conceptual flowchart of the feed production process was developed, which is presented in Figure 6. The annual yield of feed products will depend on the starting raw materials, daily productivity of the plant, and the number of working days per year. 30-35% of the malt mass goes into waste and by-products. The average balance of the enterprise consists of: brewer's grains – 75-80%, contact water (organic loads) – 15-20%, yeast – 3-5%, sediments and other losses – 1-2%. On average, when processing 1 ton of barley malt, the yield of by-products is: brewer's grains (moisture) 280-320 kg, brewer's yeast (moisture) 35-45 kg, protein sediments 6-10 kg, extract losses 15-25 kg, fermentation CO_2 28-35 kg, wastewater 3.5-5.0 m^3.

According to the developed scheme, highly concentrated wastewater from the fermentation and brewing plant was separated by filtration, obtaining a concentrated protein product (moisture content up to 73%) and filtrate. Solid waste from malt production was crushed and sieved through a 0.5 × 0.5 mm sieve; the fine fraction (flour) was added to the feed product,

and the coarse fraction was crushed again. The concentrated protein product and flour were mixed until the moisture content ⩽18% was reached, after which the mixture was granulated. The resulting dried product, with a moisture content of about 10%, became loose, and the granules acquired significant hardness. The characteristics of the feed product obtained from brewery waste, according to the main indicators, are presented in Table 8 and Table 9.

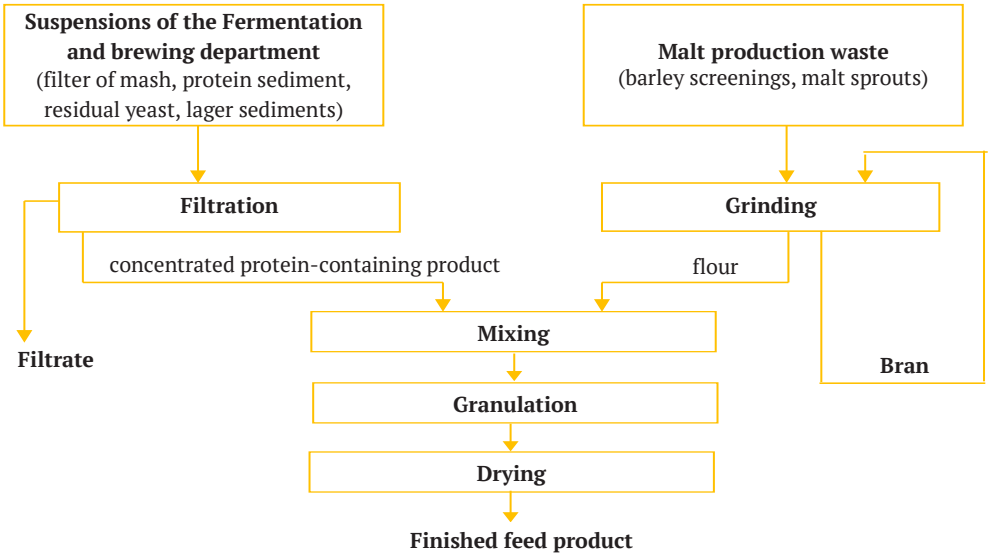

Figure 6. Flow chart of feed product production based on brewing waste

Source: developed by the authors

Table 8. *Chemical composition of the feed product based on brewing waste*

Indicator	Value, %
Moisture	8-10
Crude protein	35
Crude fibre	5-7
Crude fat	4
Ash	10

Source: compiled by the authors

Table 9. *Physical properties of granulated feed product based on brewery waste*

Indicator	Value, %
Moisture	8-10
Granule diameter, mm	7.7
Granule length, mm	7.7-11.5
Bulk weight, g/dm^3	400-420
Angle of natural slope, deg.	50-55
Flowability, cm/s	12-15
Fragility, %	4.6-5.0

Source: compiled by the authors

The developed scheme (Fig. 6) enabled the utilisation of all main wastes generated during malt and beer production to create a novel feed product with a high crude protein content of 35%. The developed granulated protein-vitamin feed product can be incorporated into animal diets at varying inclusion rates depending on species and production goals. Based on its high crude protein content and nutritional composition, recommended inclusion rates are: 10-25% in cattle feed formulations (up to 50% in protein concentrates for cattle), 10-11% for swine diets, and up to 800 g/t for poultry feed. These inclusion levels ensure optimal nutritional balance whilst maintaining feed palatability and digestibility. This achievement aligns with the growing recognition of brewery by-products as valuable resources rather than waste materials. S. Mussatto *et al*. (2006) emphasised that brewer's spent grain (BSG), which constitutes approximately 85% of total brewing by-products, contains around 20% protein and 70% fibre on a dry basis, making it an attractive raw material for various applications beyond traditional animal feeding. The protein content achieved in the present study (35%) represents a significant improvement over untreated BSG, demonstrating the effectiveness of the comprehensive processing approach. The practical application of brewery waste in feed formulations has been extensively documented. F. Karlsen & P. Skov (2022) highlighted that BSG's relatively high protein content, low market price, and stable annual availability position it as a promising protein source for aquaculture feeds, though they noted that appropriate refinement methods are essential to remove anti-nutritional factors such as lignin and fibre. This observation supports the necessity of the processing technology developed in the current study, which incorporates dewatering, drying, and granulation steps to enhance digestibility and nutritional value.

Recent advances in bioprocessing have demonstrated even greater potential for protein enrichment in brewery wastes. C. Eliopoulos *et al*. (2022) reported that solid-state fermentation of BSG using *Pleurotus ostreatus* resulted in a 49.49% increase in protein content and a ten-fold increase in β-glucans, whilst simultaneously reducing cellulose by 11.42%. Although the present study did not employ fermentation, the achieved protein concentration of 35% through mechanical and physical processing methods represents a cost-effective alternative that can be readily implemented at industrial scale without requiring specialised microbial cultivation. The versatility of brewery waste extends beyond animal feed applications. A. Chetrariu & A. Dabija (2023) reviewed the incorporation of spent grain into various food products, including bakery goods, pasta, and plant-based alternatives, noting that such applications align with circular economy principles by reducing waste and promoting sustainable development. Whilst the current study focused primarily on feed production, the developed processing technology – particularly the grinding and granulation steps – could potentially be adapted for food industry applications, thereby expanding the market opportunities for brewery waste valorisation.

The comprehensive approach presented in this study addresses several practical challenges identified in previous research. The granulated form of the final product overcomes the perishability issues associated with high-moisture BSG, as noted by F. Karlsen & P. Skov (2022), whilst the incorporation of multiple waste streams (spent grain, malt sprouts, barley screenings, protein sediment, and yeast residues) maximises resource utilisation. This integrated processing strategy not only produces a nutritionally enhanced feed product but also contributes to environmental sustainability by reducing the overall waste burden of brewing operations, thereby supporting the transition towards a circular bio-economy in the brewing industry.

Neutralisation of wastewater. According to the pH value, the wastewater of the enterprise

is divided into: acidic (pH 4.0-5.5) – suspensions from the fermentation and brewing shop; alkaline (pH 11-14) – disinfectant solutions and washing waters from the finished product bottling shop; neutral (pH about 7) – washing waters from the malt and water treatment shops (Table 3). Acidic wastewater of the enterprise, after phase separation of the suspensions from the fermentation and brewing shop, is represented by a filtrate containing a small amount of suspended solids – from 0.1 to 0.45 g/dm³ – and has a pH of 4.0-5.5 (Table 6). The alkaline wastewater from the finished product bottling shop exceeds the established discharge limits for Mykulynetskyi Brewery in two indicators: pH – by up to 5 units, and suspended solids – by 1.1 times (Table 3). Neutralisation of the alkaline wastewater from the bottling shop can be carried out using the filtrate obtained during the processing of suspensions from the fermentation and brewing shop. As previously noted, the washing waters from the malt and water treatment shops do not exceed the established discharge limits for pollutants in any indicator (Table 2). Therefore, they can be used as diluting solutions. Thus, the neutralisation of acidic and alkaline wastewater followed by dilution with conditionally clean washing waters from the malt and water treatment shops makes it possible to ensure compliance with all discharge standards for pollutants (Hryn *et al.*, 2019) established for Mykulynetskyi Brewery into the municipal sewage system.

Technology for integrated processing of solid and liquid brewing waste. Based on the generalisation of previous studies and the results obtained during experimental investigations, a comprehensive technology for the integrated processing of all major wastes generated during malt and beer production has been developed. To obtain feed products, the components of brewer's grains, malt sprouts, barley screenings, protein sediment, residual yeast, and lager sediments were utilised. The technology also provides for the treatment, neutralisation, and reuse of wastewater. The basic technological scheme for the integrated processing of solid and liquid brewing waste is shown in Figure 7.

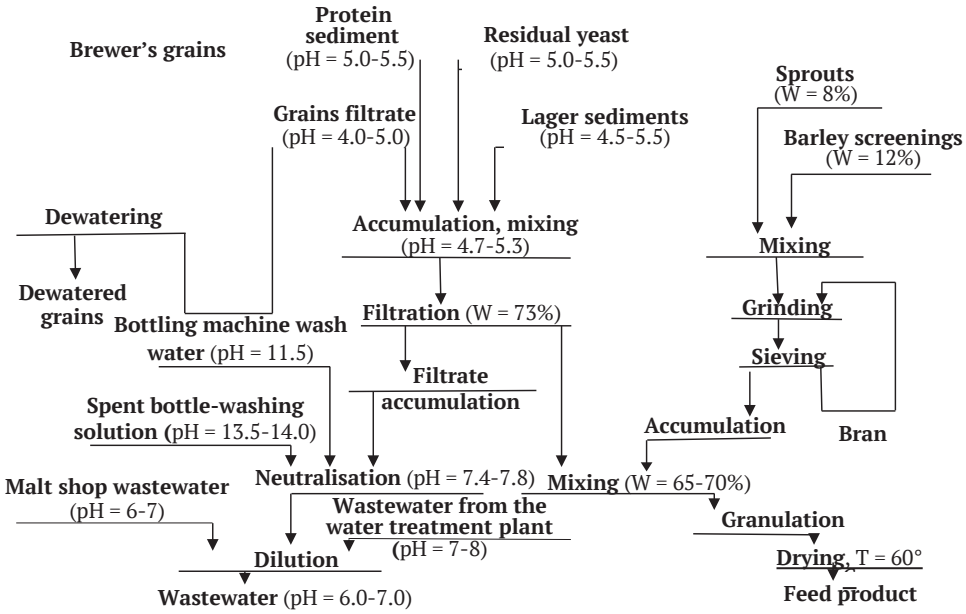

Figure 7. Basic technological scheme for the integrated processing of waste at Mykulynetskyi Brewery
Source: developed by the authors

According to the proposed technology, all highly concentrated liquid waste from the fermentation and brewing plant, consisting of suspensions of grain transport water (grain filtrate), protein sediment, residual yeast and lager sediment, are mixed for further phase separation by filtration. The resulting filtrate is sent to neutralise alkaline wastewater from the finished product bottling plant, and the concentrated protein fraction of the suspensions with a humidity of 73% is used as a basis for the production of a feed product. Solid waste from the malting plant (sprouts and screenings) can be added to the feed composition after grinding and sieving through a 0.5 mm sieve (Tymchuk & Stasyuk, 2010). The ground fraction that has passed through the sieve is added to the filtered product until the mixture reaches a moisture content of 65-70%, and the coarse fraction is returned for re-grinding. The resulting feed mixture is then mixed, granulated and dried. The spent alkaline solution from the bottle washing machine, after being cleaned of suspended matter by filtration, can be reused, and the washing solution containing insoluble label fibres and other impurities can be neutralised with the acidic filtrate of the fermentation and brewing plant suspensions. The resulting neutral mixture should then be diluted with conditionally clean washing waters from the malting and water treatment plants. After these technological operations, the wastewater after analysis met all the standards for the discharge of pollutants established for Mykulynetskyi Brewery. According to the developed technology, the beer grains were dehydrated to a moisture content of 75-80%. The dehydrated product is suitable for use as feed for cattle. The developed technology allows for the comprehensive processing of all major liquid and solid wastes of Mykulynetskyi Brewery, resulting in two types of feed products – granulated feed and dehydrated beer grains, as well as providing for the neutralisation, reuse and treatment of wastewater in accordance with established environmental standards.

The development of this technology was informed by extensive research into brewery waste valorisation strategies. S. Aliyu & M. Bala (2011) emphasised that brewer's spent grain, representing approximately 85% of total brewing by-products, possesses strong potential for recycling due to its rich content of cellulose and non-cellulosic polysaccharides. They highlighted the global pressure towards green environmental technology as a driving force for academic and industrial researchers to explore alternative uses beyond conventional animal feed applications. This perspective aligns with the objectives of the present study, which seeks to maximise the utilisation of brewery waste through integrated processing approaches. The methodological framework for waste valorisation in the food and processing industries has been well established. G. Krusir *et al*. (2014) provided comprehensive methodologies for determining quantitative and qualitative indicators of secondary raw material formation, utilisation, and consumption in production processes. Their work on the ecological and economic efficiency of processing secondary resources in the food industry informed the environmental and economic considerations underlying the technology developed in the current study, particularly regarding waste reduction and resource efficiency optimisation. Recent advances in extraction and concentration technologies have expanded the possibilities for brewery waste utilisation. K. Silva *et al*. (2023) reviewed emerging technologies and alternative solvents that enable higher extraction yields of phytochemical compounds, particularly bitter acids and polyphenols from hot trub and spent hops. Whilst the present study focused primarily on feed production rather than phytochemical extraction, the purification and concentration principles discussed by K. Silva *et al*. informed the phase separation and filtration methodologies employed for processing highly concentrated liquid wastes. Their emphasis on the importance of scale-up and economic feasibility

studies resonates with the practical, industrially applicable approach adopted in this research.

The specific context of Ukrainian brewing operations provided additional insights. L. Telezhenko & A. Dubyna (2025) systematically reviewed modern technologies for secondary processing of grain residues in beer production, highlighting the potential for implementing circular economy principles in the brewing industry. Their analysis of drying, fermentation, and bioprocessing technologies, alongside comparative data demonstrating significant protein (up to 30%) and fibre (up to 50%) concentrations in dried brewer's spent grain, supported the selection of processing parameters in the present study. Furthermore, their emphasis on adapting foreign technological experience to Ukrainian realities – considering technical capabilities, climatic conditions, and market demand – proved particularly relevant for the development of a technology suitable for implementation at Mykulynetskyi Brewery. The integrated approach presented in this study synthesises these various perspectives, combining traditional mechanical processing methods (dewatering, drying, grinding) with modern separation technologies (filtration under vacuum) and granulation techniques. This comprehensive strategy addresses the multiple challenges of brewery waste management whilst creating economically viable feed products and ensuring environmental compliance through wastewater neutralisation and reuse.

Conclusions

Comprehensive research on the processing of brewing industry waste has led to several key findings. Three priority directions for the utilisation of brewery waste were identified: recovery of protein compounds from concentrated liquid wastewater for use as valuable components in feed formulations in the amount of 10-25% (up to 50% protein feed) for cattle, 10-11% for pigs and up to 800 g/t for poultry; neutralisation of alkaline wastewater to achieve compliance with discharge regulations pH 6.5-8.5; dewatering and drying of brewer's grains up to 10% to reduce waste volume and facilitate their further application in animal husbandry or as secondary raw materials. It was determined that dynamic dewatering using a rotating drum with a metal mesh surface is the most efficient method for drying brewer's grains. Studies of malt production waste demonstrated that the milled product obtained from this material possesses a uniform composition (the yield of the fine fraction (<0.5 mm)), with a moisture content of 9.15% for flour from barley screenings and 8.8% for flour from malt sprouts.

For effective protein recovery from highly concentrated liquid wastewater, separation into solid and liquid phases is recommended, which gives a concentrated protein product with a crude protein content of 35% and a filtrate containing a low concentration of suspended solids 0.1-0.45 mg/dm^3. Neutralisation of acidic and alkaline wastewater with subsequent dilution with conditionally clean washing waters from the malting and water treatment plants ensures that the concentrations of all pollutants in the final wastewater comply with the established standards for discharge into the environment. A comprehensive technology for the integrated processing of all major solid and liquid wastes generated during malt and beer production was developed. This technology enables the efficient conversion of brewery by-products into two types of feed products – granulated feed and dewatered brewer's grains – while simultaneously providing for the neutralisation, reuse, and purification of wastewater in accordance with environmental standards. In the future, with an increase in production capacity, it is promising to create a protein-vitamin granulated feed additive with the inclusion of waste from the brewing industry.

Acknowledgements

To the management of Mykulynetskyi Brewery LLC for providing production data.

Funding

None.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Aliyu, S., & Bala, M. (2011). Brewer's spent grain: A review of its potentials and applications. *African Journal of Biotechnology*, 10(3), 324-331.

[2] Bulii, Y., Mukoid, R., Olshakovskii, I., Mihailov, I., Ivanchuk, V., & Ivanchuk, V. (2022). Technology of complex processing of post-alcoholic grain bard. *Food Industry*, 31-32, 84-92. doi: 10.24263/2225-2916-2022-31-32-11.

[3] Chattaraj, S., Mitra, D., Chattaraj, M., Ganguly, A., Thatoi, H., Pradeep K., & Mohapatra, P.K.D. (2024a). Brewers' spent grain as fish feed ingredient: Evaluation of bio-safety and analysis of its impact on gut bacteria of *Cirrhinus reba* by 16S Metagenomic sequencing. *Current Research in Microbial Sciences*, 7, article number 100286. doi: 10.1016/j.crmicr.2024.100286.

[4] Chattaraj, S., Mitra, D., Ganguly, A., Thatoi, H., & Mohapatra, P.K.D. (2024b). A critical review on the biotechnological potential of brewers' waste: Challenges and future alternatives. *Current Research in Microbial Sciences*, 6, article number 100228. doi: 10.1016/j.crmicr.2024.100228.

[5] Chetrariu, A., & Dabija, A. (2023). Spent grain: A functional ingredient for food applications. *Foods*, 12(7), article number 1533. doi: 10.3390/foods12071533.

[6] Danilov, V.Ya. (2019). *Statistical data processing*. Kyiv: Taras Shevchenko National University of Kyiv.

[7] DSTU 7169:2010. (2011). *Feed, compound feeds, compound feed raw materials. Methods for determining nitrogen and crude protein content*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=89230.

[8] DSTU 8024:2015. (2017). *Granulated compound feeds. General technical conditions*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=81111.

[9] DSTU 8482:2015. (2017). *Animal feed. Feed briquettes and pellets. Technical specifications*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page?id_doc=76885.

[10] DSTU 8844:2019. (2020). *Feed, compound feeds, compound feed raw materials. Methods for determining crude fibre*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=82156.

[11] DSTU ISO 6496:2005. (2006). *Animal feeds. Determination of moisture content and other volatile substances*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page?id_doc=86445.

[12] Eliopoulos, C., Arapoglou, D., Chorianopoulos, N., Markou, G., & Haroutounian, S.A. (2022). Conversion of brewers' spent grain into proteinaceous animal feed using solid state fermentation. *Environmental Science and Pollution Research*, 29, 29562-29569. doi: 10.1007/s11356-021-15495-w.

[13] Hryn, H.I., *et al*. (2019). *Methods for measuring environmental parameters*. Sievierodonetsk: V. Dahl East Ukrainian National University.

[14] Karlsen, F., & Skov, P.V. (2022). Review – potentials and limitations of utilising brewer's spent grain as a protein source in aquaculture feeds. *Journal of Cleaner Production*, 357, article number 131986. doi: 10.1016/j.jclepro.2022.131986.

[15] Krusir, G.V., *et al*. (2014). *Technologies of food production waste management*. Odesa: Odesa National Academy of Food Technologies.

[16] Mussatto, S.I., Dragone, G., & Roberto, I.C. (2006). Brewer's spent grain: Generation, characteristics and potential applications. *Journal of Cereal Science*, 43(1), 1-14. doi: 10.1016/j.jcs.2005.06.001.

[17] Nyhan, L., Sahin, A.W., Schmitz, H.H., Siegel, J.B., & Arendt, E.K. (2023). Brewers' spent grain: An unprecedented opportunity to valorize a brewing by-product. *Journal of Agricultural and Food Chemistry*, 71(28), 10543-10564. doi: 10.1021/acs.jafc.3c02489.

[18] Pires Maria, M., Torres, N.H., Nascimento, V.R.S., Chagas, T.S.A., Dattatraya Saratale, G., Mulla, S.I., Bharagava, R.N., Cavalcanti, E.B., & Romanholo Ferreira, L.F. (2023). Current advances in the brewery wastewater treatment from anaerobic digestion for biogas production: A systematic review. *Environmental Advances*, 13, article number 100394. doi: 10.1016/j.envadv.2023.100394.

[19] Servetnyk, O.P., & Piven, O.M. (2024). Justification of the rational use of secondary raw materials in brewing. In *Theoretical and practical studies of young scientists: Collection of abstracts of the 18th international scientific and practical conference of Masters and PhD students* (pp. 610-611). Kharkiv: NTU "KhPI".

[20] Silva, K.F.C., Strieder, M.M., Pinto, M.B.C., Rostagno, M.A., & Hubinger, M.D. (2023). Processing strategies for extraction and concentration of bitter acids and polyphenols from brewing by-products: A comprehensive review. *Processes*, 11(3), 921-931. doi: 10.3390/pr11030921.

[21] Soceanu, A., Dobrinas, S., Popescu, V., Buzatu, A., & Sirbu, A. (2024). Sustainable strategies for the recovery and valorization of brewery by-products – a multidisciplinary approach. *Sustainability*, 16(1), article number 220. doi: 10.3390/su16010220.

[22] Telezhenko, L., & Dubyna, A. (2025). Analysis of existing technologies for secondary processing of grain raw materials in beer production. *Grain Products and Mixed Fodder's*, 25(3), 26-34. doi: 10.15673/gpmf.v25i3.3208.

[23] Terefe, G. (2022). Preservation techniques and their effect on nutritional values and microbial population of brewer's spent grain: A review. *CABI Agriculture and Bioscience*, 3, article number 51. doi: 10.1186/s43170-022-00120-8.

[24] Tertyshny, O.O., Pivovarov, O.A., & Koshulko, V.S. (2022). *Mechanical processes and equipment of food production*. Dnipro: DDAEU.

[25] Tymchuk, S.S., & Stasiuk, O.K. (2010). Use of malt sprouts – brewing waste in feeding cows and fattening bulls. *Feed and Feed Production*, 66, 309-313.

[26] Verhuelsdonk, M., Glas, K., & Parlar, H. (2021). Economic evaluation of the reuse of brewery wastewater. *Journal of Environmental Management*, 281, article number 111804. doi: 10.1016/j.jenvman.2020.111804.

[27] Wei, G., Shang, W., Xie, Z., Zhang, M., Dan, M., Zhao, G., & Wang, D. (2024). Unlocking high-value components from brewer's spent yeast for innovative food applications. *Food Bioscience*, 59, 235-258. doi: 10.1016/j.fbio.2024.104047.

Комплексна переробка відходів пивоваріння з отриманням кормової добавки

Марія Мадані
Кандидат технічних наук, доцент
Одеський національний технологічний університет
65039, вул. Канатна, 112, м. Одеса, Україна
https://orcid.org/0000-0001-9386-7364

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

Ніна Ворона
Кандидат технічних наук, доцент
Одеський національний технологічний університет
65039, вул. Канатна, 112, м. Одеса, Україна
https://orcid.org/0000-0001-6903-9016

Анотація. Одним із ключових напрямків розвитку сучасної пивоварної промисловості в Україні є раціональне використання органічних відходів, що утворюються під час виробництва пива та солоду. Метою статті було дослідити склад, фізико-хімічні властивості та методи утилізації основних відходів пивоваріння на прикладі ТОВ «Микулинецький пивоварний завод». Визначено кількісні та якісні характеристики сухих (пивна дробина, солодові паростки, ячмінний відсів, шлам) та рідких (транспортні води, суспензії дробини, білковий осад, залишкові дріжджі, лагерні осади, стічні води) відходів. Встановлено, що пивна дробина становить до 85 % усіх твердих залишків виробництва та є цінною сировиною для кормовиробництва завдяки високому вмісту білка, клітковини та мінеральних речовин. Були проведені експериментальні дослідження процесів зневоднення та сушіння дробини, подрібнення солодових паростків та ячмінного відсіву, а також фільтрації концентрованих суспензій з бродильно-пивоварного цеху. Визначено оптимальні умови для фазового розділення суспензій, що забезпечують отримання концентрованої білкової фракції з вмістом вологи 73 % та фільтрату з низькою концентрацією завислих речовин (0,1-0,45 г/дм^3). Запропоновано технологію комплексної переробки всіх основних відходів пивоваріння, в результаті якої отримано два типи кормових продуктів – зневоднену пивну дробину та гранульований білково-вітамінний корм, що містить 35 % сирого протеїну, 5–7 % клітковини та 8-10 % вологи. Розроблена технологія також передбачає нейтралізацію кислих та лужних стічних вод до стандартних параметрів та їх повторне використання у виробничому циклі. Практичне впровадження запропонованої технології значно зменшує вплив на навколишнє середовище, зменшує утворення відходів, підвищує ресурсоефективність виробництва та створює замкнуту систему утилізації вторинних ресурсів у пивоварній промисловості

Ключові слова: пивоварна промисловість; утилізація відходів; стічні води; кормові продукти; технологія; технічна екологія

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 17(1), 28-45
Received 23.09.2025 Revised 01.02.2026 Accepted 26.02.2026
Published 26.03.2026

UDC 637[608.3:543.544
DOI: 10.31548/animal.1.2026.28

ISSN 2706-8331
e-ISSN 2706-834X

Gas and thin-layer chromatography in the control of residual levels of polychlorinated biphenyls and pesticides in foods of animal origin

Michael Gill*
Doctor of Agricultural Sciences, Professor
Mykolaiv National Agrarian University
54008, 9 Georgy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0001-7353-9865

Vadim Posukhin
Assistant
Mykolaiv National Agrarian University
54008, 9 Georgy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0001-6757-260X

Diana Chobotar
Higher Education Student, Master's Degree
Mykolaiv National Agrarian University
54008, 9 Georgy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0009-0007-4581-7048

Abstract. The biological safety of food products of animal origin is closely related to the socio-economic development of society and the state of the environment. The study aimed to verify the presence of residual amounts of polychlorinated biphenyls and pesticides in food products of animal origin by gas and thin-layer chromatography methods. For the analysis of powdered milk samples, gas chromatography was used on a Bruker Scion 456-GC device equipped with an electron capture detector (ECD), an autosampler, and high-precision temperature control. Extraction of polychlorinated biphenyls was conducted with hexane followed by purification using a hexane-acetonitrile system and adsorption chromatography on silica gel. Calibration was performed with a Supelco PCB Mix standard in the concentration range of 0.001-0.250 μg/cm³ with a correlation coefficient of $R^2 = 0.9999$. For the study of fish samples, thin-layer chromatography was used with preliminary acid hydrolysis of the sample and extraction with diethyl ether.

Suggested Citation:
Gill, M., Posukhin, V., & Chobotar, D. (2026). Gas and thin-layer chromatography in the control of residual levels of polychlorinated biphenyls and pesticides in foods of animal origin. *Animal Science and Food Technology*, 17(1), 28-45. doi: 10.31548/animal.1.2026.28.

*Corresponding author

Visualisation of chromatographic zones was conducted by treating the plates with a silver nitrate solution followed by ammonia and UV irradiation at 254 nm. The percentage of analyte recovery was within the range of 70-120%, which confirms the reliability and accuracy of the methods used. In the samples of the studied fish, the detection limit of pesticides was 0.6 mg/kg, which ensured efficient detection of the residual amount of toxicants even at their low content. The main peaks on the milk chromatogram appeared in the interval from 9 to 14 minutes and corresponded to the standards of polychlorinated biphenyl components, and their retention time correlated with respective standard substances, which confirmed the presence of polychlorinated biphenyls in the sample and ensured quantitative determination. The significance of the study is determined by modern, reliable and effective practice-oriented information on food safety assessment to both relevant laboratories and institutions, as well as educational institutions specialising in the training of specialists in the specialty "Biotechnology and Bioengineering"

Keywords: biosafety; analyte; standards; temperature; instrument

Introduction

The issue of quality and safety of food products of animal origin becomes increasingly relevant both for consumers producers. Since proper control over the preparation, processing, storage and further sale of livestock products ensures not only monitoring of the presence of residual amounts of veterinary drugs, but also reliable control of compliance with hygiene requirements at all stages of product production. In total, compliance with each of the listed elements makes it possible to prevent the occurrence of a number of food poisonings, and deliver an exceptionally high-quality and safe product to the consumer.

The problem of food quality and safety is an issue that is actively discussed by a range of scientific communities. For instance, according to the works of Italian scientists A. Arienzo *et al.* (2022) on the monitoring the number of international publications devoted to the issue of microbiological safety of food products, an annual increase in their number (2 thousand and more) over the last decade is notable. This, in turn, confirms the significant interest in this topic from the international scientific community. Ukrainian scientists O. Mateyuk *et al*. (2025) also substantially addressed problems related to the quality and safety of food products, noting that to ensure a high level of microbiological safety of food products, it is necessary to systematically improve control and regulation systems. To minimise the risks of microbiological contamination, it is necessary to ensure systematic monitoring and compliance with control at all stages of the food chain, as well as create proper hygienic conditions at all stages of the technological process.

As T. Romanovska *et al*. (2022) highlighted, the principles of producing a safe food product ensure the quality and safety of the product at all stages of production, from the receipt of raw materials and packaging materials at the enterprise to the arrival of finished products on the market. As indicated by O. Kuzmin *et al.* (2022), increasing the efficiency of food production, while ensuring compliance with high standards of their quality and safety, are relevant issues for the competitiveness of restaurant establishments. S. Prache *et al.* (2022) emphasised that the quality of food products of animal origin is determined by the following seven main attributes: safety, commercial, sensory, nutritional, technological, convenient and image. Image encompasses ethical, cultural and environmental aspects related to the origin of food products and the method of their production and

processing. This framework highlighted the priority of different quality attributes.

According to G. Richard *et al.* (2022) the quality of food products usually depends on those properties that satisfy all the needs of the consumer. The concept of quality is becoming more complex, especially regarding products of animal origin. These changes occur in parallel and are to some extent due to concerns related to their impact on human health and the environment, agricultural methods and food processing, as well as animal welfare. Consumer habits are also changing, and this, in turn, is accompanied by a range of (sometimes contradictory) expectations. There is a demand not only for convenient and ready-to-eat meals, which are often (but not always) highly processed, but also for quality labels (in particular organic), healthy eating, etc. As the Omani scientists I. Al-Bulushi *et al*. (2026) highlighted, the quality and safety of ready-to-eat foods, which are widely consumed worldwide due to their convenience, wide availability, and relatively low prices compared to other traditionally prepared foods, must be strictly monitored. K. Hilgendorf *et al.* (2024) noted that maintenance and improvement of the quality of food products are the main objectives of research in the field of food science and technology.

Although chemical and physical approaches were always substantial in improvement of the quality of food products, while biotechnological methods have become the most innovative solutions to overcome the limitations of traditional approaches. Therefore, the primary goal of study was to verify the presence of harmful pesticides and other foreign chemicals in food products of animal origin using modern biotechnological methods.

Materials and Methods

The research was conducted from 12.05.2025 to 14.06.2025 at the Mykolaiv Regional State Laboratory of the State Service for Food Supervision and Consumer Protection of the city of Mykolaiv, Ukraine, in the relevant analytical unit, namely the gas chromatography sector, which is equipped with chromatographic equipment. Scientific processing of the results and generalisation of the obtained data were conducted at the Mykolaiv National Agrarian University.

The gas chromatography method was used to determine the residual amounts of polychlorinated biphenyls in powdered milk samples, as well as the thin-layer chromatography (TLC) method to identify the herbicide 2,4 dichlorophenoxyacetic acid in fish (carp) samples. The research was conducted based on relevant regulatory documents and methodological recommendations, following the requirements of analytical accuracy and reproducibility of results (SANTE 11312/2021 v2, 2024). The objects of the research were received for research in the laboratory through the sample registration department. Each sample was assigned an individual registration number upon receipt, which ensured identification within the laboratory records and the anonymity of the origin of the product during analytical research. The objects of analysis were samples of powdered milk and fish, to which controlled amounts of hazardous chemical compounds were added. For each type of product, the study was conducted in triplicate.

Gas chromatographic studies were performed using a Bruker Scion 456-G gas chromatograph, serial number BR 1308M149. The device was manufactured by Bruker Chemical Analysis B.V. (Netherlands) in 2013 and is owned by the Mykolaiv Regional State Laboratory of the State Service for Food Safety and Consumer Protection. The gas chromatograph has passed state metrological certification, which is confirmed by certificate No. 130-05-FKh dated December 19, 2014, issued by the State Enterprise "Odesa Regional Centre for Standardisation, Metrology and Certification". The metrological characteristics of the gas chromatograph were defined and described in the relevant technical documentation, and the inter-verification interval is 12 months. During

the metrological certification, standard samples were used, in particular, lindane for chromatography, standard sample DSZU 042.7-96, methyl parathion and GSO 1854. The research was conducted under standard environmental conditions: air temperature 20°C, relative humidity 55%, atmospheric pressure 760 mm Hg, supply voltage 220 V, frequency 50 Hz.

Characteristics of gas chromatography. In the study of powdered milk samples, a mixture of polychlorinated biphenyls (PCBs), in particular PCB congeners No. 28, 52, 101, 138, 153, 180, was used as model contaminants. For calibration and identification, a certified analytical standard Supelco PCB Mix, manufactured by Merck KGaA (Darmstadt, Germany), was used. The standard was a solution of PCBs in isooctane with a nominal concentration of each congener of approximately 10 µg/mL. According to the certificate of analysis, the solution is a colourless liquid, the identity of the components was confirmed by gas chromatography with mass spectrometric detection, and the refractive index values corresponded to the regulatory range. The standard was stored at a temperature of 2-8°C, the shelf life is until September 2028, which guaranteed its stability throughout the entire study period.

To determine polychlorinated biphenyls in food samples, the method of extraction with an organic solvent was used with subsequent purification of the extract. PCB extraction was conducted with a non-polar solvent – hexane, which ensures the effective extraction of lipophilic compounds from the sample matrix. In the presence of a significant lipid content, the extract was purified using liquid-liquid distribution in the hexane-acetonitrile system, which reduced the content of accompanying fats and ballast substances. Further purification was conducted by adsorption column chromatography on silica gel, with elution of PCBs with a mixture of hexane and diethyl ether in a ratio typical for the determination of organochlorine compounds.

The analysis was conducted on 2 g of a sample of powdered milk. After the extraction and purification stages, the extract was concentrated to a final volume of 3 cm^3. For gas chromatographic determination of polychlorinated biphenyls, 1 µL of the prepared extract was injected into the chromatograph. According to the presented calibration protocol for the Bruker Scion 456-GC instrument, the quantitative analysis procedure was based on the following parameters:

- Concentration range. Calibration solutions were prepared by serial dilution of the stock standard with isooctane. According to the calibration table for PCB congener 28, the range of concentrations tested was from 0.001 µg/cm^3 to 0.250 µg/cm^3.
- Mathematical model. The calibration dependence was described by a linear equation of the form $y = bx + a$ (forced zero crossing: $a = 0$).
- Coefficient of approximation. The obtained value of the correlation coefficient is 0.9999, which indicates the high precision of the method and the linearity of the detector response in the specified range.

The analysis was initiated at 80°C, which was maintained for 1.00 min to stabilise the solvent front and focus the components in the initial part of the column. Then, a linear temperature increase was conducted at a rate of 20°C/min until the target value of 280°C was reached. The duration of the heating step was 10 min. After reaching 280°C, the system was maintained in isothermal mode for 5 min (for a total time of 16 min) to completely elute high-molecular-weight congeners and clean the column from matrix residues (Table 1, Fig. 1). The CompassCDS software was used for full control of the gas chromatograph operation: adjustment of temperature programmes for the column thermostat, injector, and detector, control of carrier gas flows, and configuration of sample injection modes, and support of the detector. For data collection, CompassCDS was used for stable and accurate registration

of the chromatographic signal with the ability to visualise chromatograms in real time. The programme automatically performs peak integration, determines the retention time, area, and height of peaks, and can also be used for manual adjustment of the integration to increase the accuracy of the results, which is relevant in the analysis of complex mixtures or low concentrations of analytes. Compass-CDS was also used for results documentation. The software provides data archiving, standardised report generation, export of results to common formats, and traceability of all stages of the analysis.

Table 1. *Programmable temperature mode of the column thermostat*

Heating rate, °C/min	Temperature, °C	Time, min	Total time, min
Primary	80	1.00	1.00
20	280	5.00	16.00
			16.00

Source: Scion 436-GC and 456-GC gas chromatographs: Workplace requirements (2021)

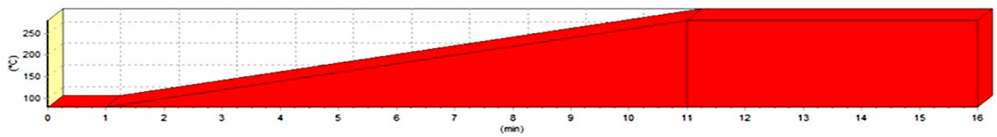

Figure 1. Programme for programming the temperature of the Bruker Scion 456-GC column thermostat

Source: Gas chromatographs Scion models 436-GC and 456-GC: Maintenance instructions (2013)

Characteristics of thin layer chromatography. For the study of fish samples, the herbicide 2,4-dichlorophenoxyacetic acid in the form of an amine salt (2,4-D) was used as a model pollutant. For quantitative determination, a standard sample of the composition of 2,4 D amine salt was used, certified following the requirements of the national metrological system. The standard was manufactured by the Special Design and Technological Bureau for Experimental Production of the O.V. Bogatsky Institute of Physics and Chemistry of the National Academy of Sciences of Ukraine (Odesa, Ukraine). The certified value of the mass concentration of 2,4-D amine salt was 0.100 mg/cm^3 with a relative error of ±1.4% at p = 0.95. Furthermore, the study used reference material (DSanPiN 8.8.1.2.3.4-000-2001, 2001).

Extraction of the analysed compounds from fish samples was conducted after acid hydrolysis of the matrix. A portion of the crushed sample weighing 30 g was treated with a 6% hydrochloric acid solution in a volume of 100 cm^3 with subsequent heating in a water bath at a temperature of 100°C for 1 hour. After cooling, 15 cm^3 of a 40% aqueous solution of phosphorotungstic acid and 100 cm^3 of distilled water were added to the mixture, after which filtration was performed. Extraction of the target components from the filtrate was conducted by liquid-liquid extraction with diethyl ether three times 50 cm^3, with a total volume of extractant of 150 cm^3. The combined ether extract was dried over anhydrous sodium sulphate and concentrated on a rotary evaporator to a final volume of 2 cm^3 for further chromatographic analysis. A standard 2,4-D solution with a concentration of 100 µg/cm^3 was used to create a model concentration of 1 mg/kg of 2,4-dichlorophenoxyacetic acid in fish samples. Incorporating the mass of the crushed sample of 30 g, the estimated amount of analyte that needed to

be added was calculated, based on the instructions registered in the quality system in the laboratory, through the proportion:

1 mg/kg – 1,000 g
X mg – 30 mg,

where 1 mg/kg – conditional calculated concentration adopted to determine the amount of analyte that must be added to a sample of a given mass; 1,000 g – mass of product corresponding to a concentration of 1 mg/kg; X – mass of 2,4-D per 30 g sample; 30 g – mass of crushed sample.

Calculation: X = (30 × 1)/1,000 = 0.03 mg. Conversion to micrograms: 0.03 mg × 1,000 = 30 mcg. Therefore, to create a concentration of 1 mg/kg in a 30 g sample, it is necessary to add 30 µg of 2,4-D. After adding the calculated volume of standard solution, the sample was thoroughly homogenised and incubated for uniform distribution of the analyte in the matrix, after which acid hydrolysis, extraction, and subsequent analysis by thin-layer chromatography were performed. The limit of quantification of the method – 0.6 mg/kg – was experimentally determined during the validation of the methods.

Visualisation of chromatographic zones was conducted following the laboratory's internal methodology for the determination of pesticides by thin-layer chromatography (Regulation (EC) No 396/2005, 2005). All computational processes met the requirements of standards and regulatory documents for laboratory quality control (Chechet *et al.*, 2023). Calculations were conducted based on the formulas and examples:

$$W = \frac{\text{QTY} \cdot \text{V}}{\text{m}}, \quad (1)$$

where QTY – concentration according to the calibration graph; V – volume of the obtained extract, cm^3; m – sample weight, g.

$$W = \frac{0{,}0994 \cdot 3}{2{,}072} = 0.144 \text{ mg/kg}.$$

Analyte recovery is the percentage of the true concentration of analyte that is detected by an analytical method. It is a key indicator of the accuracy and reliability of an analytical method, particularly during its validation. The determination of the recovery of the analyte additive to the matrix was calculated as follows (% recovery) (2):

$$\text{RP} = \frac{Xhpov}{D}, \quad (2)$$

where *Xhpov* – the amount of analyte returned; *D* – added amount of analyte, mg/kg.

$$\text{RP} = \frac{0.144\ mg/kg}{0.2\ mg/kg} \times 100\% = 72\%.$$

The range of return percentage for trials was 70-120%, hence the experiment was done correctly (Voytsitsky *et al.*, 2024). A full description of formulas, calculation algorithms, and examples of quantitative determination of pesticides based on the results of an experimental study are demonstrated below. Irregular shapes were traced with a pencil to form a square or rectangle. The area of the spot is calculated using the formula (3):

$$S = a \cdot b, \quad (3)$$

where *a* – width of the rectangle (mm); *b* – height of the rectangle (mm).

For instance, when determining pesticides at a standard concentration of 0.5 mg/kg, a spot with dimensions of 6 mm × 16 mm was obtained, which corresponds to the area:

$$S_{0.5} = 16 \cdot 6 = 96 \text{ mm}^2$$

Similarly, using a standard with a concentration of 1 mg/kg, with a spot size of 7 mm × 18 mm:

$$S_{1.0} = 18 \cdot 7 = 126 \text{ mm}^2$$

The area of the sample spot was:

$$S_{samples} = 18 \cdot 4 = 72 \text{ mm}^2$$

The quantitative determination of pesticides in the sample was conducted based on the following formula (4):

$$X = \frac{A \cdot S2}{m \cdot S1}, \quad (4)$$

where X – amount of pesticides in the test sample; A – amount of standard applied to the plate, μg; m – sample weight, g; S1 – area of the standard spot, mm^2; S2 – area of the sample spot, mm^2. Substituting the values:

$$X = \frac{50 \cdot 72}{30 \cdot 126} = 0.95 \text{ mg/kg}.$$

The processing of gas chromatographic analysis results was performed using Compass-CDS software, which was used for instrument control, chromatographic signal recording, peak integration, component identification by retention time, and quantitative determination of polychlorinated biphenyls based on calibration curves. The obtained analytical data were exported and summarised in Microsoft Excel, which was used to systematise the results, calculate descriptive statistics, and present data as mean ± standard deviation. The validation of gas and thin-layer chromatography methods was conducted by evaluating key performance characteristics, including linearity, precision within the same analysis conditions (convergence), and accuracy, which was determined by the percentage of analyte recovery. The results of experimental studies confirmed the necessary level of accuracy and reliability for food product biosafety indicator monitoring.

For the gas chromatography method for the determination of PCB congeners, high linearity of the detector response was established, and the convergence and percentage recovery indicators fully met the regulatory requirements for the selected concentration range. Similarly, for the thin-layer chromatography method for the study of fish for the content of 2,4-D amine salt, the selectivity and accuracy values obtained confirmed the possibility of effective use of this technique for quantitative and qualitative analysis. All calculated validation parameters are within the permissible deviations provided for by the laboratory's internal regulations and methodological guidelines (DSTU ISO/IEC 17025:2019, 2021). To ensure the objectivity of the assessment of the biological safety of food products, the conditions and operating rules that provide stable functionality of the devices, thus accurate results, were established. These conditions were described in detail in Table 2.

Table 2. *Study parameters*

Parameter	Value
Ambient temperature	+20 °C
Atmospheric pressure	100.79 kPa (756 mm Hg)
Relative humidity (at +20 °C)	65%
Voltage in the electrical supply network	220 V

Source: Scion 436-GC and 456-GC gas chromatographs: Workplace requirements (2021)

The ambient temperature was +20°C. This temperature was most often maintained during the study, as it ensures stability of biological samples and reagents and provides comfortable working conditions for personnel. The atmospheric pressure in the conducted study was also within the normal range and was 100.79 kPa or 756 mm Hg. Constant maintenance of stable atmospheric pressure is a prerequisite for the operation of devices that use gas. An equally relevant metric that indicates the content of water vapor in the air is the humidity. In the present case, this parameter was 65%, which is a level. This level does not interfere with the operation

of the devices and, most notably, does not cause condensation on the surfaces. Continuous operation of the devices was ensured by the voltage in the electrical power supply network, which is supplied to all laboratory devices. In this experiment, it was 220 volts. The supply voltage is constantly verified and stabilisers were utilised. The advantage of the Bruker Scion 456-GC is the high-precision of the analytical instrument, which can separate complex mixtures of substances with high resolution. In addition, it operates stably during long series of analyses, has simple programming of temperature regimes and is compatible with various types of detectors. The operating mode of the device is described in Table 3.

Table 3. *Device configuration and operating mode*

Configuration	Operating mode
Gas chromatograph	Bruker Scion 456 GC
Autosampler	With automatic sample introduction
Capillary column	Rxi-5ms, Restek, 30 m × 0.25 mm × 0.25 µm
Stationary phase	5% phenyl, 95% dimethylpolysiloxane
Injector temperature	250°C
Detector	Front electronic capture detector
Detector temperature	300°C
Column temperature	80C – 1 min; 20°C – min to 200°C; 6 min – 280°C
Sample input mode	Split 1:10
Injection volume (sample introduction)	1 µl
Carrier gas	Nitrogen, 99.999% purity (1.0 mL/min)

Source: Gas chromatographs Scion models 436-GC and 456-GC: Maintenance instructions (2013)

All parameters that were set for effective chromatographic separation and subsequent quantitative determination of substances are described below. An autosampler is a device that introduces samples into the instrument in automatic mode. Therefore, operator errors were prevented, and continuous measurements without human intervention were performed. A capillary column is a thin tube located inside the chromatograph for high-quality separation of components, the diameter is 0.25 mm – a thin column, used for analytical tasks; the layer thickness of 0.25 µm determines the speed at which the substance moves through the column. The column has a polarity that is suitable for the analysis of organochlorine substances and provides high-quality peaks without overlapping them. The composition of the stationary phase was represented by 5% phenyl, 95% dimethylpolysiloxane. This is a special coating inside the column, which separates substances by their properties. Phenyl 5% ensures improved interaction with non-polar or aromatic compounds. Stable and versatile operation is provided by 95% dimethylpolysiloxane. This phase ensures effective separation of PCBs following their volatility and structure.

The injector introduces the sample. The injector temperature is 250°C, which is the temperature at which the sample evaporates after being introduced into the column, turns into a gaseous state and enters the gas stream. In front, an electron capture detector (ECD) is a type of detector, sensitive to chlorinated compounds such as PCBs. It records the change in electric current caused by the capture of electrons by analytes. In this experiment, the detector was used to detect PCBs at the milligram level. To avoid the precipitation of volatile substances and ensure signal stability of the experiment, the detector was constantly maintained at a high temperature of 300°C. At this temperature, sensitivity and reliability of measurement are ensured without loss of analytes. The column temperature was

gradually heated: the initial temperature of 80°C was held for 1 min, then at a rate of 20 degrees per minute it rose to 200°C, held for 6 min, and then increased to 280°C. This mode was used to gradually remove substances with different levels of volatility from the column and separatee with maximum efficiency.

The Split 1:10 sample injection mode was the next criterion, hence only one tenth of the sample that was injected entered the column, everything else was routed to the outlet. All the above elements are performed in order not to overload the column with a large amount of substance, reduce the risk of peak deformation and ensure uniform distribution of the substance. The injection volume was 1 µL. This volume of liquid is automatically introduced into the chromatograph for further analysis. The carrier gas was nitrogen with a purity of 99.999% (1.0 mL/min). After the gas chromatograph was set up and all sample preparation steps were completed, a chromatographic analysis of the powdered milk sample was performed. For this procedure, a Bruker Scion 456-GC was used, which is highly sensitive to organochlorine compounds, including pesticide residues. The limit of qualitative determination of the method for 2,4-Dichlorophenoxyacetic acid is 0.04-0.8 mg/kg, the mass of the sample required for the work is 30 g. According to the control card of the condition of the premises during the study, the following conditions were observed throughout the experiment (Table 4).

Table 4. *Conditions for conducting the study*

Condition	Value
Ambient temperature	+22.8°C
Atmospheric pressure	101.4 kPa (761 mm Hg)
Relative humidity (at +20°C)	61%
Voltage in the electrical supply network	220 V
Frequency	50/60 Hz
Lighting	Natural, without direct sunlight on thin-layer chromatography plates
Elements required for maintaining biological safety	Availability of a hood for handling of organic solvents (acetone, ether)
Detection limit of pesticides in fish	0.6 mg/kg

Source: C. Poole (2023)

In the experiment room, the temperature was maintained at +22.8°C. This indicator is substantial for the stability of the physical and chemical properties of reagents, including standards and solvents, as well as for prevention of the influence of temperature fluctuations on the processes of extraction, sample application and migration of substances along the sorbent. The next indicator was atmospheric pressure. At the time of the study in the laboratory room, it was 101.4 kPa (which corresponds to 761 mm Hg). This pressure is standard for ensuring uniform evaporation of phases and normal volatility of organic solvents, which is a substantial prerequisite for effective chromatographic separation. For the successful completion of the study, the next condition was the relative humidity of the air. Its level was kept at 61% at a temperature of +20°C. This indicator is substantial for processing of sorbents that can absorb moisture from the air. Excessive humidity can affect the polarity of the stationary phase, reducing the clarity of the image on the chromatographic plate, causing blurring of spots and changing the nature of the distribution of substances. The electrical power supply of chromatographic equipment, auxiliary devices and lighting are also relevant factors for

the correctness of the studies. It was conducted from a standard electrical network with a voltage of 220 V and a frequency of 50/60 Hz. Throughout the entire period of analytical procedures, the parameters of the electrical network were stable, which negated possible interruptions in the operation of equipment, including drying and fume hoods, lighting devices and other equipment in the laboratory. The lighting in the laboratory was natural, but at the same time the requirements were met for the absence of direct sunlight on the surface of the chromatographic plates. This is due to the fact that direct sunlight has a negative effect on the photostability and thermal stability of the substances being analysed, especially regarding pesticides, the structure of which is photosensitive. The organisation of the workplace during the experiment was emphasised. The operating surface of the table on which the analytical equipment was placed was stable and sufficiently strong. All this was necessary to prevent even minor vibrations, which can negatively affect the reproducibility of the results and the accuracy of measurements, especially during handling of highly sensitive equipment.

Regarding compliance with biological safety requirements, all actions with reagents and samples were performed exclusively in a ShV-1 fume hood (Ukrainian company "Zapovit"). This was crucial during handling of organic solvents characterised by high toxicity and volatility (ether and acetone). The parameters and requirements of the work are presented in Table 5.

Table 5. *Parameters and operating requirements*

Parameter	Requirements
Plate	Silica gel 60 (thickness 250 microns)
Activation	110°C, 30 min
Sample application	Micropipette, distance between samples ⩾1 cm
Mobile phase	Hexane-diethyl ether-formic acid (50:50:2)
Development time	30-40 min in a closed chamber
Visualisation	Spraying $AgNO_3$ + NH_3, UV (254 nm)

Source: P. Malhotra (2023)

In the samples of the studied fish, the detection limit of pesticides was 0.6 mg/kg. This level of sensitivity of the method ensured efficient detection of the residual amount of toxicants, even incorporating their low content, which crucial for both food safety and the sanitary and epidemiology. The experiment was conducted following recommendations using Silica gel 60 (CAC/GL 40-1993, 2010). This sorbent is one of the most common in thin-layer chromatography applications and provides high resolution during the analysis of complex matrices. Uniform movement of the mobile phase and stability of the chromatographic process were guaranteed by plates with a silica gel layer 250 µm thick.

The sorbent was activated at a temperature of 110°C for 30 min before use to remove impurities and residual moisture that could affect the analysis results. Sample application was conducted manually using a micropipette. To achieve a separation of the components in the sample and avoid mixing of spots, the interval between applications of at least 1 cm was ensured. A mixture of organic solvents was used as the mobile phase: formic acid, diethyl ether and hexane in a ratio of 50:50:2. This composition provides optimal separation of the analysed substances due to compatibility with the sample matrix and effective elution ability. The development of the chromatogram was conducted in a hermetically sealed chromatographic

chamber, which ensured stable conditions for the movement of the mobile phase. The optimal development time was 30-40 min.

After the completion of chromatography, the chromatograms were visualised. For this purpose, a method was used in which the plate was sprayed with a solution of silver nitrate (AgNO3) followed by treatment with ammonia (NH3). Such treatment can detect compounds with high efficiency, which include functional groups capable of interacting with silver ions. To detect invisible spots in the study and final fixation of the results, ultraviolet radiation was used, the wavelength reached 254 nm. After preliminary sample preparation, chromatography and visualisation of pesticide spots on chromatographic plates, a quantitative assessment of the content of the analysed substances was performed. For this purpose, a planimetric method was used, based on measuring the area of the spots for both the samples under study and standard solutions.

To ensure the reliability of the results obtained and to control the accuracy of the analysis, the recovery coefficient of the analytical standard added to the sample was additionally calculated. This indicator was used to assess the accuracy of quantitative determination by the selected method and the efficiency of extraction. When validating the method for determining pesticides in food samples, one of the most relevant stages is the assessment of the efficiency and accuracy of the extraction, purification, and subsequent quantitative analysis process. For this purpose, the percentage of recovery of the analytical standard was calculated, which was used to determine what part of the introduced pesticide was detected after passing through the entire analytical process. This indicator is relevant for assessment of the reliability of the results obtained, since in the case of low analyte recovery, it is possible to state that certain substances were lost during analytical procedures (chromatography, concentration, filtration) or the extraction was ineffective. On the contrary, a high percentage of recovery indicates the correctness of the method and its suitability for further use in routine analysis. The application of samples was conducted manually, using a micropipette in the form of narrow or dotted lines, while maintaining a distance between them to effectively prevent mixing or overflow of components. After the chromatogram development process was completed, the plate was treated with a special reagent – a silver nitrate solution, followed by treatment with ammonia, which revealed traces of the substances under investigation.

Results and Discussion

Result of the gas chromatography method

One of the main food products studied in the experiment was powdered milk. For this purpose, a matrix of powdered milk with an additive was prepared (the additive was a standard of polychlorinated biphenyls). The results obtained in the process of scientific research are presented in the form of a chromatogram, which records signals from the target analytes and auxiliary components that were part of the sample (Fig. 2).

The chromatogram from the analysis of a powdered milk sample, to which a polychlorinated biphenyl standard was previously added to control the accuracy of the research results, is demonstrated above. This additive was used to verify the effectiveness of the method and calculate the percentage of analyte recovery during the analysis process. The study by B. Güzel & O. Canli (2022) confirmed that the gas chromatography method is sufficient for the rapid, selective and correct determination of indicator PCBs not only in food products, but also in solid samples of waste oils. Figure 2 demonstrates that the retention time (set in minutes) is plotted on the X axis, which characterises the moment the substance leaves the column, and the detector signal (expressed in microvolts) is plotted on the Y axis, which indicates the concentration of the corresponding compound.

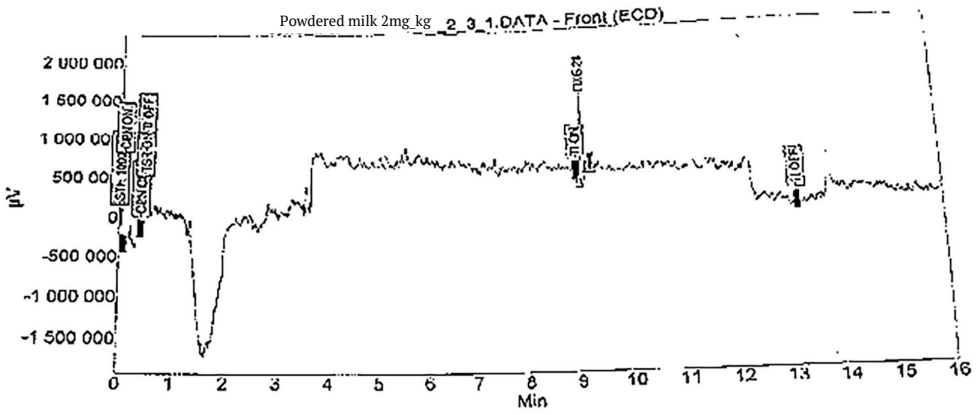

Figure 2. Results of the experiment on powdered milk

Source: compiled by the authors using CompassCDS software

At the beginning of the chromatographic analysis, at approximately 1.5 min, a pronounced hexane peak is notable. This is the solvent that was used during the extraction of the analysed sample. Despite the fact that hexane is not the target analyte, presence is normal, since it is part of the sample that was introduced into the chromatograph. Its presence is also due to the fact that hexane was used not only as an extractant during sample preparation, but also for washing the chromatograph liner before each sample injection. As an analytical substance, hexane does not interact with the electron-capture detector, therefore its presence is manifested in the form of a negative (downward) signal. This is a normal phenomenon for the used detectors, which does not affect the analysis results. This peak then reduces, hence, has a negative signal value recorded by the detector. This direction of the peak is determined by the specifics of the detector operation and the physical properties of hexane.

The main peaks, which appeared in the interval from approximately 9 to 14 min, correspond to the standards of the PCB component. Their retention time coincided with the corresponding standard substances, which made it possible to confirm the presence of PCBs in the sample and perform quantitative determination. The peak is symmetrical, well distributed and has a clearly defined shape, which indicates optimally selected chromatography conditions and correct operation of the column. The intensity of the peak was used for quantitative determination of the analyte content by comparison with calibration standards. The performed chromatographic analysis confirms the effectiveness of the sample preparation method and ensures confident conclusions on the quality and safety of the milk powder samples that were examined. The obtained level of chromatographic separation could only be achieved if the controlled analysis conditions were carefully observed. F. Wang *et al.* (2022), in their studies of dairy products, also confirmed the satisfactory linearity, sensitivity, accuracy, and precision of the gas chromatography method.

The stable temperature of the chromatograph oven ensured linear evaporation of the sample components, which in turn prevented the displacement and superposition of the analytes on each other. In this experiment, a temperature programme was used that consolidated the initial isothermal heating with a gradual increase in temperature in the future, which ensured efficient separation of both heavy and lighter chlorinated components. In addition to temperature control, a substantial factor that

ensured the clarity of the signal was the stability of the carrier gas (flow) – nitrogen. Even a slight fluctuation in the flow rate or pressure could cause their deformation or shift the time of peak emergence. However, in the chromatogram image presented above, exceptional symmetry is observed, which in turn indicates the absence of turbulence in the column and the accuracy of the system calibration.

The low level of the background line shown in the graph, which is almost noise-free, is notable. This is evidence that the sample introduction liners were thoroughly cleaned, the column phase was maintained in proper condition, and the system was free of contaminants. Background noise is often a positive indicator of the quality of the analytical process: under conditions of temperature fluctuations or uncontrolled humidity, it increases, which significantly complicates the detection of small concentrations. In this case, there is no such effect in the image, so the analysis was conducted under conditions that are maximally conducive to analytical reliability and accuracy. After the procedure for preparing a powdered milk sample with the addition of a standard was performed and gas chromatographic analysis was performed using an electron capture detector, one main peak was recorded in the processed chromatogram file, which corresponds to the substance PCB-28 (Table 6).

Table 6. *Peak study results*

Index	Name	Time, min	Quantity, mkg/mL	Area, pV min	Height, uV	Signal/noise	Result, mg/kg
1	PCB 28	9.12	0.0994	43,838.7	990,555.0	1.25	0.144
Total			0.0994	43,838.7	990,555.0		

Source: compiled by the authors

Table 6 demonstrates that the retention time of the substance on the column was 9.12 min, which confirms its identification based on comparison with the standard sample. According to the software CompassCDS used for analysis, the following peak parameters were determined:

➢ Analyte concentration Quantity (μg/mL) – this is the calculated concentration of the analyte in the extract, which was 0.0994 μg/mL.

➢ Area (peak area) – 43,838.7 pKV min, which is proportional to the amount of substance in the sample. The larger the peak area – the higher the concentration.

➢ Height (peak height) – 990,555.0 μV, which indicates the intensity of the signal recorded by the detector.

➢ Signal/noise – 1.25, i.e. the signal is more than 1 time higher than the noise level. This is an acceptable ratio for low concentrations, although ideally it should be higher.

➢ Result, mg/kg – recalculation of the amount of analyte considering the volume of extract and the mass of the sample. In this case, the result was 0.144 mg/kg.

The peak characteristics of PCB-28 fully corresponded to the expected parameters of the standard. The accuracy and reliability of the analyte detection were clearly indicated by the presence of a clearly pronounced peak, the correspondence of the retention time and the stability of the signal.

The results of the work by thin layer chromatography method

The establishment of biological safety and norms compliance of the thin-layer chromatography is also a priority of the experiment. In the

specified subsection of the experiment, the main sample (matrix) was a food product in the form of fish (carp), and the main additive was 2,4-D amine salt in the amount of 1 mg/kg. The results obtained indicate that the method is highly efficient and sufficiently accurate, since the level of permissible return in the experiment is from 70 to 120%, depending on the type of research being conducted and the sample matrix.

Figure 3 shows a chromatographic plate after the completion of the analysis of a fish sample by thin-layer chromatography. This image demonstrates the practical result of the study performed and confirms the efficiency of the applied method for detection of residual amounts of the pesticide 2,4-D amine salt. Traces of chromatographic separation are evident on the surface of the plate, namely grey or dark spots that were formed as a result of the movement of substances along the sorbent layer under the influence of the mobile phase. Thanks to these manipulations, characteristic spots appeared on the plate, which differed in size and colour depending on the amount of analyte in the sample. The figure demonstrates the difference between the spots of the test and standard samples, which highlights the presence of traces of PCBs in the sample, and can be used for quantitative determination based on the ratio of areas. The shape of the spots is clear, mostly oval or elliptical, with even contours, which indicates the quality of the sorbent, the correctness of the plate preparation and stable conditions for conducting the analysis. The spot of the test sample is located at the same level with the spot of the standard, with an identical Rf value, i.e. the ratio of the migration distance of the substance to the front of the mobile phase. This, in turn, confirms the identity of the substance detected in the sample under study with the specified standard 2,4-D.

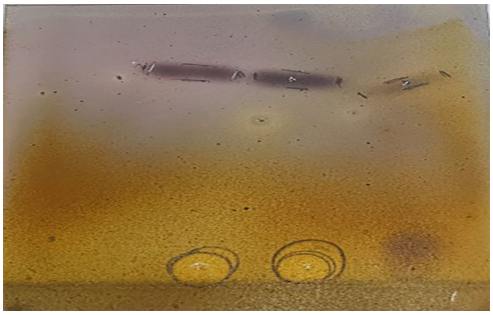

Figure 3. Result of the thin layer chromatography method
Source: compiled by the authors

The stability of the conditions of the experiment was confirmed by the visual appearance of the chromatographic plate. Thus, the clarity and shape of the spots indicated that the temperature and humidity remained within the permissible values, and the absence of smearing or blurring indicated the effective avoidance of excess moisture. The spots did not lose their colour intensity due to the absence of direct sunlight, and this moment is relevant when visualising with silver nitrate. Summarising the above, it is possible to state that the resulting image is not only the result of the reaction but also an indicator of the correctness of the entire process. In general, Figure 3 illustrates the final stage of the experiment, which shows not only the detected target analyte in the food sample, but also the quality of compliance with the methodological instructions. The resulting visual result provides not

only analytical, but also visual confirmation of the reliability of the research conducted. Therefore, it is possible to conclude that the applied methodology was sensitive, reliable, and effective for controlling pesticide residues in fish products. Similar results were obtained by A. Tan *et al*. (2025) in an analysis of the detection of residues of banned substances in shrimp based on the combined method of thin-layer chromatography and surface-enhanced Raman spectroscopy. The results demonstrated the effectiveness of the TLC-SERS method (combines thin-layer chromatography with surface-enhanced Raman spectroscopy) for the rapid, sensitive and accurate detection of residues of banned substances in seafood, which has significant implications for monitoring the quality and safety of food products.

Relevant areas such comparative analysis of the two applied research methods – thin-layer chromatography and gas chromatography, is notable. Both methods are quite effective, but at the same time they differ in sensitivity, accuracy and principle of operation, which makes it possible to assess compliance with the standards of biological safety of food products more fundamentally. Similar findings were reported in other studies. For instance, Y. Li *et al.* (2023) confirmed that liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-MS/MS (GC-MS/MS) are the main methods with excellent selectivity, sensitivity and specificity used for target analysis. LC-MS/MS and GC-MS/MS can also be used simultaneously to determine several HCPs with instrumental detection limits down to 0.010 ng mL −1, such as PBDEs, polychlorinated biphenyls and organochlorine pesticides.

Gas chromatography was used to analyse milk powder samples, which is a method used to detect even microscopic levels of harmful substances with high reproducibility of results with high accuracy. This subsequently prevented the presence in products even in minimal concentrations. Y. Shao *et al.* (2023) also confirmed that approaches such as thin-layer chromatography, high-performance liquid chromatography, gas chromatography, and high-performance liquid chromatography/gas chromatography-mass spectrometry are successfully used for pesticide detection. This method was described as highly efficient in the analysis of chlorine-containing organic compounds due to the high sensitivity of the detector, modern equipment and automated sample introduction. Thin-layer chromatography, which was used at that time to examine fish samples, is an accessible method that can quickly assess the presence of pesticides and does not require complex laboratory equipment. However, this method had a lower sensitivity and depended largely on visual assessment of the result. Therefore, the use of both methods in one scientific study ensure an integrated approach to assessing the safety and quality of food products.

Conclusions

Based on the conducted scientific research, using gas and thin-layer chromatography methods, residual amounts of polychlorinated biphenyls and pesticides were detected in food products of animal origin, namely powdered milk and fish. For the analysis of powdered milk samples, gas chromatography was used – a method that can detect even microscopic levels of harmful substances with high reproducibility of results and high accuracy. This subsequently prevented their presence in products even in minimal concentrations. This method was highlighted as a significant level of efficiency for the analysis of chlorine-containing organic compounds due to the high sensitivity of the detector, modern equipment and automated sample introduction. To verify the accuracy and reliability of the method, the percentage of analyte recovery was calculated, which was within the range of 70-120%, which, in turn, confirms the reliability and accuracy of the method. Thin-layer chromatography, which was used at that time to study fish

samples, is an accessible method that can quickly assess the presence of pesticides and does not require complex laboratory equipment. In the samples of fish studied, the detection limit of pesticides was 0.6 mg/kg. The main peaks that appeared in the time interval from approximately 9 to 14 min correspond to the standards of the PCB component. Their retention times coincided with the corresponding standard substances, which confirmed the presence of PCBs in the sample and was used for a quantitative determination. Further research on this topic should continue the study and use chromatographic methods to determine the presence of any harmful substances not only in animal products, but also in other food products.

Acknowledgements

The authors would like to express their gratitude to the Main Directorate of the State Service for Food and Consumer Protection in Mykolaiv region for the opportunity to conduct scientific research.

Funding

None.

Conflict of Interest

None.

References

[1] Al-Bulushi, I.M., Al-Kharousi, Z.S., Al-Khusaibi, M.K., Al-Sarmi, K.N., & Albloushi, M. (2026). Ready-to-eat sandwich microbiota: Diversity, antibiotic resistance, and strategies to enhance food safety. *Foods*, 15(2), article number 251. doi: 10.3390/foods15020251.

[2] Arienzo, A., Gallo, V., Fanali, C., & Antonini, G. (2022). Introduction to the special issue: Microbiological safety and quality of foods. *Foods*, 11(5), article number 673. doi: 10.3390/foods11050673.

[3] CAC/GL 40-1993. (2010). *Codex Alimentarius guideline. Recommendations for good laboratory practice. Analysis of pesticide residues*. Retrieved from https://www.scribd.com/document/389575849/cxg-040e.

[4] Chechet, O.M., Shulyak, S.V., Pishchansky, O.V., Omelchun, Yu.A., Kobysh, A.I., & Klochkova, N.P. (2023). *Simultaneous detection of pesticides and non-dioxin-like polychlorinated biphenyls in food and feed, in particular beekeeping products, by capillary gas chromatography: Methodological recommendations*. Kyiv: DNDILDVSE.

[5] DSanPiN 8.8.1.2.3.4-000-2001. (2001). *Permissible doses, concentrations, quantities and levels of pesticides in agricultural raw materials, food products, air in the working area, atmospheric air, water in reservoirs, and soil*. Retrieved from https://zakon.rada.gov.ua/rada/show/v0137588-01#Text.

[6] DSTU ISO/IEC 17025:2019. (2021). *General requirements for the competence of testing and calibration laboratories (EN ISO/IEC 17025:2017, IDT; ISO/IEC 17025:2017, IDT)*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=88724.

[7] Gas chromatographs Scion models 436-GC and 456-GC: Maintenance instructions. (2013). Retrieved from https://www.nrc.gov/docs/ML1407/ML14072A218.pdf.

[8] Güzel, B., & Canlı, O. (2022). An environmental friendly and stable analytical method for the determination of indicator polychlorinated biphenyls (PCBs) in solid and waste oil samples by gas chromatography-electron capture detector (GC-ECD). *Microchemical Journal*, 178, article number 107325. doi: 10.1016/j.microc.2022.107325.

[9] Hilgendorf, K., Wang, Y., Miller, M.J., & Jin, Y.S. (2024). Precision fermentation for improving the quality, flavor, safety, and sustainability of foods. *Current Opinion in Biotechnology*, 86, article number 103084. doi: 10.1016/j.copbio.2024.103084.

[10] Kuzmin, O.V., Stukalska, N.M., Labenska, N.V., Derysh, A.D., Berladyn, P.V., & Hrushevska, I.O. (2022). Determination of the balance of nutritional rations. *International Scientific Journal "Internauka"*, 1. doi: 10.25313/2520-2057-2022-1-7864.

[11] Li, Y., Wang, L., Zheng, M., Lin, Y., Xu, H., Liu, A., Hua, Y., Jiang, Y., Ning, K., & Hu, S. (2023). Thin-layer chromatography coupled with HPLC-DAD/UHPLC-HRMS for target and non-target determination of emerging halogenated organic contaminants in animal-derived foods. *Food Chemistry*, 404(Part B), article number 134678. doi: 10.1016/j.foodchem.2022.134678.

[12] Malhotra, P. (2023). Thin layer chromatography. In *Analytical chemistry: Basic techniques and methods* (pp. 223-234). Cham: Springer. doi: 10.1007/978-3-031-26757-4_14.

[13] Mateyuk, O., Efremova, O., & Mironova, N. (2025). Current threats to microbiological food safety and food safety regulation. *Herald of Khmelnytskyi National University. Technical Sciences*, 353(3.2), 426-437. doi: 10.31891/2307-5732-2025-353-59.

[14] Poole, C. (Ed.). (2023). *Instrumental thin-layer chromatography*. Amsterdam: Elsevier.

[15] Prache, S., *et al*. (2022). Review: Quality of animal-source foods. *Animal*, 16(S1), article number 100376. doi: 10.1016/j.animal.2021.100376.

[16] Regulation (EC) No 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. (2005, February). Retrieved from https://surl.li/beetqt.

[17] Richard, G., De Smet, S., Font-i-Furnois, M., Leroy, F., & Lind, V. (2022). Quality of animal-source foods related to their production and processing conditions. *Animal*, 16(S1), article number 100440. doi: 10.1016/j.animal.2021.100440.

[18] Romanovska, T.I., Romanovska, N.I., & Romanovsky, N.O. (2022). Principles of safe food production. *Scientific Bulletin of International Association of Scientists. Series: Economy, Management, Security, Technology*, 1(1). doi: 10.56197/2786-5827/2022-1-1-6.

[19] SANTE 11312/2021 v2. (2024). *Analytical quality control and method validation procedures for pesticide residues analysis in food and feed*. Retrieved from https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE-11312_2021-V2.pdf.

[20] SCION 436-GC and 456-GC gas chromatographs: Workplace requirements. (2021). Retrieved from https://surl.li/vnbove.

[21] Shao, Y., Wang, M., Cao, J., She, Y., Cao, Z., Hao, Z., Jin, F., Wang, J., & Abd El-Aty, A.M. (2023). A method for the rapid determination of pesticides coupling thin-layer chromatography and enzyme inhibition principles. *Food Chemistry*, 416, article number 135822. doi: 10.1016/j.foodchem.2023.135822.

[22] Tan, A., He, Y., Wang, H., Zhang, Z., Zhao, R., Ma, W., & Zhao, Y. (2025). Research on residue detection of prohibited drugs in shrimp based on the thin-layer chromatography – surface-enhanced Raman spectroscopy combined method. *Food Additives & Contaminants: Part A*, 42, 925-939. doi: 10.1080/19440049.2025.2512879.

[23] Voytsitsky, V.M., Kornienko, V.I., Khyzhnyak, S.V., Samkova, O.P., Vyshnivsky, P.S., & Altanova, A.B. (2024). *Analytical methods of research of raw materials and food products (spectrophotometric, chromatographic, electrochemical, electrophoretic, immunological): Manual*. Kyiv: Publishing house "Scientific Capital".

[24] Wang, F., Chen, M., Luo, R., Huang, G., Wu, X., Zheng, N., Zhang, Y., & Wang, J. (2022). Fatty acid profiles of milk from Holstein cows, Jersey cows, buffalos, yaks, humans, goats, camels, and donkeys based on gas chromatography-mass spectrometry. *Journal of Dairy Science*, 105(2), 1687-1700. doi: 10.3168/jds.2021-20750.

Газо- та тонкошарова хроматографія в контролі залишкових кількостей поліхлорованих біфенілів і пестицидів у харчових продуктах тваринного походження

Михайло Гиль

Доктор сільськогосподарських наук, професор
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0001-7353-9865

Вадим Посухін

Асистент
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0001-6757-260X

Діана Чоботарь

Здобувач вищої освіти, ступень магістр
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0009-0007-4581-7048

Анотація. Біологічна безпека харчових продуктів тваринного походження тісно пов'язана з соціально-економічним розвитком суспільства та станом навколишнього середовища. Метою проведеного дослідження було здійснити перевірку наявності залишкової кількості поліхлорованих біфенілів і пестицидів в харчових продуктах тваринного походження методами газової та тонкошарової хроматографії. Для аналізу зразків сухого молока використовували газову хроматографію на приладі Bruker Scion 456-GC з електронно-захоплюючим детектором (ECD), автосемплером і високоточним температурним контролем. Екстракцію поліхлорованих біфенілів проводили гексаном з наступним очищенням системою hexane-acetonitrile та адсорбційною хроматографією на силікагелі. Калібрування виконували стандартом Supelco PCB Mix у діапазоні концентрацій 0,001-0,250 мкг/см^3 з коефіцієнтом кореляції R^2 = 0,9999. Для дослідження зразків риби застосовували тонкошарову хроматографію з попереднім кислотним гідролізом проби та екстракцією діетиловим ефіром. Візуалізацію хроматографічних зон проводили обробкою пластин розчином нітрату срібла з наступною обробкою аміаком та УФ-опроміненням при 254 нм. Відсоток повернення аналіту перебував у межах 70-120 %, що підтверджує надійність і точність проведених методів. У зразках досліджуваної риби межа виявлення пестицидів становила 0,6 мг/кг, що дозволило ефективно знаходити залишкову кількість токсикантів навіть при їх низькому вмісті. Основні піки на хроматограмі молока з'являлися в інтервалі від 9 до 14 хвилин і відповідали стандартам компонентів поліхлорованих біфенілів, а час їх утримання співпадав із відповідними стандартними речовинами, що дало можливість підтвердити наявність поліхлорованих біфенілів у зразку та здійснити кількісне визначення. Цінність виконаної роботи полягає в забезпеченні сучасною, достовірною та ефективною практико-орієнтованою інформацією з оцінки безпечності харчової продукції як відповідних лабораторій і установ, так і освітніх закладів, що спеціалізуються на підготовці фахівців за спеціальністю «Біотехнологія та біоінженерія»

Ключові слова: біобезпека; аналіт; стандарти; температура; прилад

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 17(1), 46-59
Received 09.10.2025 Revised 27.01.2026 Accepted 26.02.2026
Published 26.03.2026

UDC 664.664.6
DOI: 10.31548/animal.1.2026.46

ISSN 2706-8331
e-ISSN 2706-834X

Investigation of wheat gluten properties and its effect on bread quality

Inna Hetman
PhD, Senior Researcher
Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine
02002, 4-A Ye. Sverstyuk Str., Kyiv, Ukraine
https://orcid.org/0000-0002-9448-9956

Ivanna Lukianchuk
Postgraduate Student
Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine
02002, 4-A Ye. Sverstyuk Str., Kyiv, Ukraine
https://orcid.org/0009-0007-4876-0470

Oksana Naumenko
Doctor of Technical Sciences, Professor
Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine
02002, 4-A Ye. Sverstyuk Str., Kyiv, Ukraine
https://orcid.org/0000-0002-1691-1381

Volodymyr Kovbasa
Doctor of Technical Sciences, Professor
National University of Food Technologies
01033, 68 Volodymyrska Str., Kyiv, Ukraine
https://orcid.org/0009-0001-8876-3373

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

Suggested Citation:
Hetman, I., Lukianchuk, I., Naumenko, O., Kovbasa, V., & Tkachenko, L. (2026). Investigation of wheat gluten properties and its effect on bread quality. *Animal Science and Food Technology*, 17(1), 46-59. doi: 10.31548/animal.1.2026.46.

*Corresponding author

Abstract. Enriching bakery products with hemp flour increases their nutritional value, but the absence of gluten requires the addition of structure-forming additives, such as wheat gluten. However, technological indicators and the impact on the dough system of various samples of wheat gluten presented on the Ukrainian market remain unexplored. The purpose of the study was to analyse the effectiveness of wheat gluten from various manufacturers to improve the rheological properties of dough and the quality of wheat-hemp bread. It was shown that in the gluten samples "Viten-Vital" and "Gluvital 21020", the largest fraction was prolamins, 69.2% and 68.59%, respectively, and a smaller amount contained glutelins: 21.91% and 30.45%, respectively. In the "BeneoPro VWG 75" and "NVG-Vital" samples, the average distribution of these fractions was approximately 50:50. It was found that the introduction of wheat gluten in the amount of 3% by weight of flour increased the amount of raw gluten by 25.5-27.6%, depending on the sample. Based on the results of trial laboratory baking, it was established that wheat-hemp bread with gluten "BeneoPro VWG 75" or "NVG-Vital" had the best quality in terms of a combination of indicators: volume, porosity, and freshness retention. Bread with the gluten sample "Gluvital 21020" was characterised by a 7.7% higher specific volume than in the control, but its porosity increased slightly. The established differences in the technological indicators of wheat gluten from different manufacturers can help in the correct choice of this raw material for use in the production of wheat bread varieties, in particular, with the addition of hemp flour

Keywords: hemp flour; sourdough; quality indicators; nutritional value; bakery products

Introduction

The consumer value of bakery products is determined by many factors, among which a significant place is occupied by physical and chemical indicators, terms of freshness preservation, and nutritional value. These characteristics depend primarily on the recipe ingredients and how the dough is prepared (Sciacca *et al*., 2023). One of the most promising additives for enriching bakery products is non-narcotic cannabis seeds and processed products. The high interest in using cannabis seeds in the food industry is conditioned by its valuable chemical composition. According to I. Švec & M. Hrušková (2015) and S. Kornpointner *et al*. (2021), hemp products can contain up to 25-35% oil, 20-25% protein, 20-30% carbohydrates, 20-30% dietary fibre, vitamins and minerals (calcium, zinc, magnesium, phosphorus, potassium, sulphur, and iron), omega-3 and omega-6 fatty acids, which are also in an optimal ratio for the human body. Most non-conventional ingredients, having low technological properties, can reduce the volume and porosity of bread, hold the shape of products, etc. This is especially true if a certain percentage of wheat flour is replaced in the recipe with a functional ingredient. A significant deterioration in the physical and chemical parameters of finished products in this case leads to restrictions in the dosage of functional ingredients and, as a result, the nutritional value of bread does not improve significantly. Thus, the research conducted by S. Gunko *et al*. (2024) showed that the inclusion of grain processing products, alternative types of flour, in particular, from hemp seeds in bread recipes worsened the structural and mechanical properties of dough and bread, but positively affected the organoleptic parameters and nutritional value of products.

An effective technological solution can be the use of structure-forming additives, among which wheat gluten (WG) deserves special attention. Dry wheat gluten, or wheat gluten, is a complex of protein components that do not show solubility in aqueous or saline solutions. According to M. Schopf *et al*. (2021), this substance retains its native protein properties,

since its direct isolation from grain is carried out by separation, followed by mechanical processing (grinding) and dehydration (drying). The technological process includes pre-cleaning and hydration of wheat grains, after which they are ground to produce a whole kernel. The next step involves crushing the kernel to maximise the release of starch. The resulting suspension containing protein and starch is centrifuged (separated) to concentrate the protein fraction. The final product – wheat gluten – is dried and packaged.

One of the reasons for using WG is the unsatisfactory quality of wheat grain flour grown in too hot or rainy regions, and therefore, it contains a reduced amount of protein (Kryzhanovskiy, 2022). This problem is even more acute, as with the beginning of Russia's full-scale invasion of Ukraine, the geography of wheat cultivation has changed, and the number of acreage has decreased due to the occupation of certain regions and the threat of military operations. Data by D. Zhygunov *et al.* (2023) regarding the quality of wheat grain presented on the market, it is shown that only 15-20% of wheat can produce varietal flour, in particular the highest grade. One of the key motivations for using wheat gluten is the lack of protein in the modern diet, which is critical for human health. Accordingly, enriching food products with gluten is a simple and effective method of increasing their protein value. As stated by X. Zhang *et al*. (2021), WG is widely used not only in baking, but also in other sectors of the food industry, such as meat processing and dairy.

The Ukrainian market offers WG samples only of foreign production, which differ in cost. However, there is no scientific data on their technological characteristics and impact on the quality of finished bread, which requires research. The purpose of the study was to investigate the technological properties of millet gluten from various producers and their impact on the development of consumer characteristics of bread with functional vegetable raw materials. To achieve this goal, the following tasks were set: to determine the effect of WG on the quantity and quality of gluten; to investigate the effect of WG on the quality of dough (gas-forming ability, mass fraction of moisture, titrated acidity, lifting force, gas-holding capacity of the dough for the period of fermentation and proofing, shape-holding capacity, fermentation duration, proofing duration); to study the effect of WG on physical and chemical parameters, and the preservation of freshness of wheat-hemp bread.

Materials and Methods

The research was conducted between June and August 2025 at the Department of Bread Technology and Biotransformation of Grain Products of the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine (IFR NAAS). Raw materials were used for the test laboratory baking: wheat flour of the highest grade (LLC "Nash Mlyn", Ukraine), whole grain hemp flour (LLC "Agrosnab", Ukraine), hemp seeds (LLC "Agrosnab", Ukraine), pressed baking yeast (PJSC "Enzim Company", Ukraine), sugar (LLC "Sarkara-Group", Ukraine), table salt (SE "Artemsil", Ukraine), sunflower oil (SE "Santrade", Ukraine). The following manufacturers of WG were used: sample No. 1 – "BeneoPro VWG 75" (manufacturer "Beneo", Belgium); sample No. 2 – "Viten-Vital" (manufacturer "Roquette", France); sample No. 3 – "NVG-Vital" (manufacturer "Viresol", Hungary); sample No. 4 – "Gluvital 21020" (manufacturer "Cargill", Poland).

Physical and chemical parameters of wheat flour of the highest grade: mass fraction of moisture – 12.8%; whiteness – 55 units of the device; number of drops – 220 seconds; ash content – 0.4% to dry matter (DM). Physical and chemical parameters of whole-grain hemp flour: mass fraction of moisture – 9%; acidity – 4.8 degrees; mass fraction of metal and magnetic impurities – no more than 0.0003%; no foreign impurities. Physical and chemical parameters of hemp seeds: mass fraction of moisture – 10.2%; seed purity – 99.0%; mass

fraction of oil – 38.7% per DM; no impurities of castor seeds, poisonous weeds. Physical and chemical parameters of WG samples are presented in Table 1.

Table 1. *Physical and chemical parameters of wheat gluten samples*

Indicator	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
Mass fraction of moisture, %	5.5	8.0	8.0	5.9
Mass fraction of protein, %	83.7	83.0	83.0	83.5
Ash content, %	1.0	2.0	1.0	1.0
Granulometry, particles >200 μm	2.0	1.0	3.0	1.0
Water binding capacity, %	156.0	160.0	155.0	154.0

Source: compiled by the authors

Bread was made according to the recipe of "Wheat-hemp" bread according to TU 00419880.068:2023, IFR NAAS. Recipe for a control sample of bread, g: premium wheat flour – 240; whole-grain hemp flour – 30; hemp seeds – 30; pressed baking yeast – 4.5; table salt – 4.5; sugar – 6.0; water – 180. In prototypes of bread (4 pcs.), WG was added in the amount of 3% by weight of the flour mixture (9 g), the remaining ingredients and the amount of water remained unchanged. The preparation of the samples consisted of sifting bulk raw materials and mixing flour with seeds (in prototypes also with GP) to form a dry flour mixture. Yeast was introduced as a yeast suspension by mixing with water in a ratio of 1:3. Salt and sugar were added as solutions with concentrations of 26% and 50%, respectively. Water for the preparation of solutions and suspensions was subtracted from the total amount of water intended for kneading. The further technological process of production took place in the same way for both the control sample of bread and prototypes.

The dough was prepared in a non-paired way, mixing solutions, suspension with flour mixture for 5-7 minutes in a KVL4100S dough mixing machine (China). Next, the dough was left to ferment. The fermentation duration was (100 ± 2) min at (32 ± 2)°C until the volume increased by 1.5 times. The dough was divided into dough pieces weighing (250 ± 2) g, shaping was carried out manually. Proofing of samples was carried out for (36 ± 2) minutes at a temperature of (35 ± 2) °C in a proofing cabinet XLT 133-UNOX (Italy), the weight of dough pieces was (290 ± 10) g. The readiness of dough pieces during the proofing process was determined by their volume. Then the dough pieces were sent to the Unox XFT133 oven (Italy), where they were baked at a temperature of 180-200°C for (32 ± 2) minutes.

To determine the raw gluten content of the flour mixture, a dough was kneaded, which consisted of 40 g of premium wheat flour, 5 g of whole-grain hemp flour, 5 g of hemp seeds, 1.5 g of WG for each of the samples and 28 cm^3 drinking water at temperature (20 ± 2)°C. The results were compared with a control sample of the dough without WG, the amount of other ingredients and water remained unchanged. Gluten was taken at a concentration of 3% by weight of flour. Raw gluten was washed from the kneaded dough manually and the raw gluten content was determined in accordance with DSTU ISO 21415-1:2009 (2011). In raw gluten, hydration capacity, elasticity (quality index), and stretchability were determined according to the generally accepted methods described by V. Ionescu *et al.* (2010). The obtained results of gluten elasticity and stretchability were the basis for assessing the quality of gluten, assigning gluten to a certain quality group according to the method developed by V. Drobot (2019).

The fractional composition of proteins was determined by the Osborne method, which is

based on extraction in various solvents, followed by firing of fractions by the Kjeldahl method, which was described by R. Horax *et al.* (2011). The mass fraction of dough moisture was determined by express drying on the Chizhov device ("Olis" LLC, Ukraine) according to V. Drobot (2019). Gas-forming ability of the dough – by volumetric method on the AG-1M device, which is described in the paper by G. Munteanu *et al.* (2019). The gas retention capacity of the dough from flour mixtures was based on the change in the volume of samples from the beginning of fermentation to the moment when the dough falls off in measuring cylinders at a temperature of 30°C, which is described by A. Shevchenko *et al*. (2023). The shape-holding capacity of the dough was determined by the method of spreading the dough ball, by changing its diameter during fermentation at a temperature of 30°C for 180 minutes, which was described in the paper by C. Verheyen *et al.* (2015). The lifting force of the dough was determined by the surfacing of the dough ball, the titrated acidity of the dough was determined by the titrimetric method, according to I. Hetman *et al*. (2021).

Determination of organoleptic and physico-chemical indicators of the quality of finished bread (mass fraction of moisture, acidity, specific volume, shape stability, porosity) was carried out 4 hours after baking in accordance with DSTU 7045-2009 (2010). The moisture content of bread was determined by the standard method of drying the suspension in a drying cabinet SESH-3M (LLC "UkrAnalityka", Ukraine) at a temperature of 130°C, described in DSTU 7045-2009 (2010). Porosity was studied using the Zhuravlev device (LLC NVF "Standard-M", Ukraine), described by C. Verheyen *et al*. (2015). Shape stability (ratio of the height of the loaf (H) to its diameter (D) was measured on the IFC device; the volume of bread was determined using the OHL brand device, according to the methods described by I. Hetman *et al*. (2021). The acidity of titrated bread was determined by the titrimetric method according to DSTU 7045-2009 (2010). The duration of preservation of bread freshness was evaluated during its storage of 24, 48, 72 hours by changes in the fragility of bread and the amount of water absorbed by the crumb (water absorption capacity). These indicators characterise the freshness of bread or the degree of staleness.

To assess the crumbiness of bread, two samples were taken in the form of parallelepipeds, each weighing 5 g. These pieces were placed in a conical flask with a volume of 250 cm^3 and stirred on a vibrating mixer for 5 minutes. Crumbs formed as a result of mutual friction of the samples were carefully collected and weighed with an accuracy of 0.01 g. The final calculations were performed according to the method described by V. Drobot (2019). Analysis of the water absorption capacity of the crumb began with its preliminary grinding. A sample weighing 3 g was selected from the crushed mass, which was transferred to a special sieve (with 12 holes per 1 cm^2). Within five minutes, 17 cm^3 of distilled water was added to the sample using a pipette. The moistened crumb was collected from a sieve and re-weighed for further calculations. Statistical processing of the obtained data was performed using Microsoft Excel 2016 software suite. The number of repetitions for each experiment was $n = 3$. The results obtained were considered reliable at the significance level of $p \leq 0.05$.

Results and Discussion

According to V. Drobot (2019), the composition of gluten fractions plays an important role in determining its strength and effectiveness in ensuring the necessary rheological characteristics of dough. Prolamine fractions, in particular, gliadin protein, and glutelins (glutenin protein) are involved in the development of the gluten framework. The fractional protein composition of 4 wheat gluten samples was studied, the results are presented in Table 2.

Table 2. *Fractional composition of wheat gluten*

Indicator, unit of measurement	Wheat gluten			
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
Albumins (water fraction), g	3.20 ± 0.02	0.08 ± 0.02	0.17 ± 0.02	1.14 ± 0.02
Prolamins (alcohol fraction), g	47.20 ± 0.95	69.20 ± 0.95	53.44 ± 0.1	67.59 ± 0.05
Globulins (salt fraction), g	1.20 ± 0.02	8.81 ± 0.02	2.02 ± 0.02	0.82 ± 0.02
Glutelins (alkaline fraction), g	48.50 ± 0.95	21.91 ± 0.05	44.38 ± 0.05	30.45 ± 0.02
"Prolamins/glutelins" ratio	0.97	3.2	1.2	2.2

Note: sample No. 1 – WG "BeneoPro VWG 75" (Belgium); sample No. 2 – WG "Viten-Vital" (France); sample No. 3 – WG "NVG-Vital" (Hungary); sample No. 4 – WG "Gluvital 21020" (Poland)
Source: compiled by the authors

It was found that in samples No. 2 and No. 4, the determining fraction was prolamins (69.20 g and 67.59 g, respectively) and a smaller amount of glutelins (21.91 g and 30.45 g). As a result, the "prolamine/glutelin" ratios for these samples had the highest values, 3.2 and 2.2, respectively (Table 2). That is, such gluten, due to the dominant prolamins, will work perfectly in a dough system with elastic gluten to weaken it or for categories of products in which the dough needs greater stretchability (for example, for pita bread). In samples No. 1 and No. 3, there was no significant predominance of any of the fractions, in general, the distribution of gluten fractions was 50:50 with minimal values of the "prolamine/glutelin" ratio, respectively, 0.97 and 1.2. This means the equilibrium effect of such gluten on the stretchability and elasticity of the dough, it is more suitable for a dough system with low elasticity, medium or long gluten stretchability, which needs to be increased. It should be noted that researchers and manufacturers have not sufficiently substantiated what the ratio of "prolamins/glutelins" should be for the best effectiveness of gluten. M. Schopf & K. Scherf (2020) reported in their studies that "ideal gluten" should contain 60-70% prolamins and 40-30% glutelins. According to V. Drobot *et al*. (2005), gluten quality determines the "prolamins/glutelins" ratio, which should have a minimum value that ensures high gluten quality. However, a stable correlation between quality and the "prolamine/glutelin" ratio has not been established, since V. Dhaka & B. Khatkar (2015) suggest that this is also directly affected by the nature of the gluten proteins glutenin and gliadin.

Based on the results of the fractional composition of WG from different manufacturers, it was established that for a wheat-hemp dough system, which is characterised by stretchy, weak gluten with low elasticity, it is better to add gluten No. 1 or No. 3. According to C. Verheyen *et al*. (2015), wheat dough is a complex system in which processes such as hydration, swelling, structure formation, and protein peptisation occur continuously. These dynamic changes are crucial for the development of its structural and mechanical characteristics. Flour proteins have the ability to absorb a significant amount of water, mainly by an osmotic mechanism. This leads to their intense swelling and, as a result, to the establishment of an internal gluten framework in the dough. The strength of this protein structure directly depends on the amount and quality of gluten, which ultimately affects the elastic properties of the dough, its consistency, and ability to retain gas. It is the gas-retaining function, together with the gas-forming one, that is critical for the volume of finished bread and the porosity of its crumb. To determine the effectiveness of WG from different manufacturers, its effect on the quantity and quality of gluten was determined. Table 3 shows the results of the conducted studies.

Table 3. *Effect of wheat gluten on the quantity and quality of gluten in the dough*

Indicator, unit of measurement	Samples of wheat-hemp dough				
	Control	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
Raw gluten content, %	25.4 ± 0.5	35.1 ± 0.5	34.1 ± 0.5	34.2 ± 0.5	35.0 ± 0.5
Hydration capacity, %	168.1 ± 2.0	162.0 ± 2.0	169.8 ± 2.0	164.6 ± 2.0	170.5 ± 2.0
Dry gluten content, %	10.2 ± 0.2	11.1 ± 0.2	11.1 ± 0.2	12.9 ± 0.2	13.3 ± 0.2
Stretchability, cm	13 ± 0.5	10 ± 0.5	14 ± 0.5	11 ± 0.5	14 ± 0.5
IDC (quality index), conventional units	80.9 ± 2.0	68.6 ± 2.0	70.5 ± 2.0	67.0 ± 2.0	78.1 ± 2.0

Note: control – without WG; sample No. 1 – WG "BeneoPro VWG 75" (Belgium); sample No. 2 – WG "Viten-Vital" (France); sample No. 3 – WG "NVG-Vital" (Hungary); sample No. 4 – WG "Gluvital 21020" (Poland)
Source: compiled by the authors

It was found that the addition of WG significantly affected the state of gluten in the dough in all samples of wheat-hemp dough. The amount of raw gluten increased by 25.5-27.6%, depending on the sample, and was 34-35% compared to 25.4% in the control. In samples No. 2 and No. 4, an increase in the hydration capacity of gluten was observed, which made the dough more stretchable and less elastic (Table 3). This is not suitable for the wheat-hemp dough system under investigation, as it may impair the quality of the finished products. Samples No. 1 and No. 3 by stretchability and elasticity (quality index), gluten can be characterised as a quality group "Good" according to classification by V. Drobot (2019). The use of WG in these samples resulted in a 15.4-23.0% reduction in gluten stretchability. This should have a positive impact on the quality of finished products. The results obtained are consistent with the previous ones (Table 2) and indicate that gluten samples No. 1 and No. 3 are the most suitable for use in the formulation of wheat-hemp bread. R. Kaushik *et al.* (2015) made similar conclusions that the use of WG in the amount of 1 to 3% gluten by weight of wheat flour allows controlling the level of stretchability of the dough, improve other rheological characteristics of wheat dough, obtaining flour of a given quality, and enhancing the ability of the dough to absorb water. To finally determine the effect of WG on the dough system, trial laboratory baking of wheat-hemp bread was carried out with a gluten dosage of 3% of the flour weight. Table 4 shows the results of studies of wheat-hemp bread from WG of various manufacturers.

Table 4. *Influence of wheat gluten on the technological process, bread quality*

Indicator, unit of measurement	Samples of wheat-hemp dough, bread				
	Control	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
	Dough				
Fermentation time, min.	100 ± 2				
Proofing time, min.	36 ± 2				
Baking time, min.	32 ± 2				
Mass fraction of moisture, %	43.8 ± 0.2	44.3 ± 0.2	43.9 ± 0.2	44.3 ± 0.2	43.7 ± 0.2
Accumulation of CO_2, cm^3	1,088.0 ± 5.0	1,098.0 ± 5.0	1,080.0 ± 5.0	1,070.0 ± 5.0	1,080.0 ± 5.0
Gas retention capacity, cm^3	138 ± 1.0	130 ± 1.0	136 ± 1.0	131 ± 1.0	133 ± 1.0
Shape-holding capacity, mm	72 ± 1.0	67 ± 1.0	74 ± 1.0	68 ± 1.0	74 ± 1.0

Table 4. Continued

Indicator, unit of measurement	Samples of wheat-hemp dough, bread				
	Control	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
	Bread				
Mass fraction of moisture, %	43.4 ± 0.2	43.8 ± 0.2	43.2 ± 0.2	43.8 ± 0.2	43.1 ± 0.2
Specific volume, g/cm^3	2.6 ± 0.1	2.9 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	2.8 ± 0.1
Porosity, %	66.0 ± 1.0	72.0 ± 1.0	74.0 ± 1.0	70.0 ± 1.0	67.0 ± 1.0
Shape stability (H/D)	0.64 ± 0.1	0.62 ± 0.1	0.66 ± 0.1	0.61 ± 0.1	0.65 ± 0.1
Acidity, deg	2.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2

Note: control – without WG; sample No. 1 – WG "BeneoPro VWG 75" (Belgium); sample No. 2 – WG "Viten-Vital" (France); sample No. 3 – WG "NVG-Vital" (Hungary); sample No. 4 – WG "Gluvital 21020" (Poland)
Source: compiled by the authors

The data in Table 4 show that in all samples with the introduction of WG, the gas retention capacity of the dough improved by 1.4-5.8% compared to the control. The shape-holding capacity improved only in samples No. 1 and No. 3 – by 5.6-7.0%, compared to the control. In dough samples No. 2 and No. 4, shape retention worsened by 2.8%. This is probably conditioned by differences in the fractional composition of proteins of different types of gluten and the specifics of the effect of fractions on the gluten framework of wheat-hemp dough. In terms of gas formation, no significant differences were recorded between the samples. Analysis of finished products showed that bread with gluten sample No. 1 had a specific volume increased by 11.5%, porosity by 9.0% compared to the gluten-free control. The shape stability of bread with gluten No. 1 and No. 3 was better by 3.1-4.8% compared to the control. Gluten-free bread sample No. 2 had a high porosity index – 6% more than in the control, but had a lower volume and shape stability of 8% and 3.2%, respectively. Bread sample No. 4 had a high volume, 7.7% more than in the control, but the porosity increased slightly and did not meet the regulatory requirements of DSTU 7517:2024 (2025). Overall, bread with gluten samples No. 1 and No. 3 had the best quality among all the products under study. It should be noted that samples No. 1 and No. 2 differed from other bread samples with a better porosity structure. It was uniform, the pores were small, which is consistent with higher porosity indicators compared to other samples. All bread samples with WG had a more elastic crumb compared to the control bread sample without WG addition.

The results obtained are consistent with the data provided by S. Li *et al*. (2020), concluding that if there is a need to improve the porosity and increase the volume of the finished product from low-quality flour, the amount of WG can be increased from 2% to 4%, in some cases up to 6%. M. Zhang *et al*. (2022) proved that the inclusion of WG in the recipe of bakery products in the amount of up to 3% can not only improve the rheological properties of the dough, but also enrich the products with protein, reduce the content of easily digestible carbohydrates. According to data by I. Pohorielov & L. Mykonik (2021), to improve the quality of both the flour itself and bread, it is necessary to use no more than 2% WG by weight of flour; V. Drobot *et al*. (2005) suggest that the best quality indicators of bakery products are achieved if 4% WG to the weight of flour is added. However, the conducted studies show that the introduction of WG in the amount of 3% by weight of flour allows fully compensating for the lack of wheat flour in the recipe, preventing the spread of dough, and simultaneously not making the gluten strong and short-lived. The results obtained regarding the

quality of the finished bread are consistent with the indicators of rheological properties of the dough with WG (gas-holding and shape-holding capacity) (Table 4). Along with the analysis of structural and mechanical properties, changes in crumb fragility and changes in the hydrophilic properties of bread during 72 hours of storage were studied (Table 5).

Table 5. *Effect of wheat gluten on the fragility of bread crumb*

Duration of storage	Fragility, % by weight of the crumb				
	Control	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
24 hours	0.7 ± 0.05	0.6 ± 0.06	0.7 ± 0.05	0.5 ± 0.05	0.5 ± 0.05
48 hours	0.98 ± 0.10	0.96 ± 0.10	0.99 ± 0.11	0.95 ± 0.10	0.94 ± 0.10
72 hours	1.4 ± 0.12	1.1 ± 0.11	1.2 ± 0.12	1.3 ± 0.12	1.2 ± 0.12

Note: control – without WG; sample No. 1 – WG "BeneoPro VWG 75" (Belgium); sample No. 2 – WG "Viten-Vital" (France); sample No. 3 – WG "NVG-Vital" (Hungary); sample No. 4 – WG "Gluvital 21020" (Poland)
Source: compiled by the authors

It was found that during storage, due to a decrease in the strength of the bread pore walls, the brittleness index increased evenly in all samples, which is explained by a gradual decrease in the mass fraction of moisture. Bread samples with different WG after 72 hours of storage had a lower brittleness by 7.1-21.4% compared to the control. The best indicator was characterised by bread sample No. 1 (Table 5). Determination of the hydrophilic properties of bread crumb showed that after 72 hours of storage, bread with WG absorbed more water compared to the control (Table 6).

Table 6. *Effect of wheat gluten on the water absorption capacity of bread crumb*

Duration of storage	Water absorption capacity of the crumb, % of water absorbed by crumb				
	Control	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
24 hours	460.8 ± 2.0	500.3 ± 2.0	505.0 ± 2.2	500.4 ± 2.1	498.2 ± 2.0
48 hours	404.8 ± 2.0	484.6 ± 2.2	420.9 ± 2.0	473.0 ± 2.0	456.2 ± 2.1
72 hours	360.5 ± 1.5	365.3 ± 1.5	383.6 ± 1.7	376.3 ± 1.6	370.0 ± 1.5

Note: control – without WG; sample No. 1 – WG "BeneoPro VWG 75" (Belgium); sample No. 2 – WG "Viten-Vital" (France); sample No. 3 – WG "NVG-Vital" (Hungary); sample No. 4 – WG "Gluvital 21020" (Poland)
Source: compiled by the authors

The obtained data indicate a decrease in staling of products with the addition of WG. All samples with gluten absorbed 2.2-5.2% more water compared to the control. The best indicator among all the samples under study was characterised by bread sample No. 1 (Table 6). Thus, based on the results of the study of the effect of each type of gluten on the dough system and the quality of bread made from a mixture of wheat and hemp flour, it was concluded that gluten samples No. 1 and No. 3 are best suited for adjusting the quality indicators of this bread. The technology of wheat-hemp bread was tested in the bakery workshop of "Pravo" LLC, Kyiv region. The effectiveness of the developed technological solutions for obtaining high-quality wheat and hemp bread has been established (Approval Certificate No. 1 dated 16.10.2023). Regulatory documentation for wheat-hemp bread TU 00419880.068:2023 was approved by the specialised tasting Commission of "Ukrhlibprom" (Act No. 5 of 31.10.2023). Scientific data on the use of WG in bakery products technologies were implemented at "Azelis" LLC in Kyiv; agreement No. 08/24/1 of 01.02.2024.

Conclusions

It was established that the use of WG tends to increase against the background of climatic and technological challenges in the bakery industry. Gluten is not produced by Ukrainian manufacturers, but there are samples of foreign manufacturers on the Ukrainian market, which causes interest in studying their properties. An analysis of the quality and impact on the dough system of WG from different manufacturers was carried out: sample No. 1 – "BeneoPro VWG 75" (Belgium); sample No. 2 – "Viten-Vital" (France); sample No. 3 – "NVG-Vital" (Hungary); sample No. 4 – "Gluvital 21020" (Poland). It was found that in samples No. 2 and No. 4, the largest fraction was prolamins – 69.2 and 68.59 g, respectively. In smaller amounts, samples No. 2 and No. 4 contained glutelins, 21.91 and 30.45 g, respectively, and the "prolamins/glutelins" ratio had the highest values among all the samples presented. In samples No. 1 and No. 3, there was no significant predominance of any of the fractions, in general, the distribution of gluten fractions was 50:50.

It was found that with the addition of WG, the amount of raw gluten in the dough samples increased by 25.5-27.6%, depending on the sample, and amounted to 34-35% against 25.4% in the control. Samples No. 1 and No. 3 for the elasticity and stretchability of gluten can be described as the quality group "Good". Due to the introduction of gluten No. 1 and No. 3, the stretchability of gluten decreased by 15.4-23.0% compared to the control, and the elasticity increased by 15.2-17.2%, respectively. Bread with gluten sample No. 1 had a higher volume of 11.5%, porosity was better by 9% compared to the gluten-free control. Bread sample No. 2 with gluten had a high porosity index – 6% higher than in the control, but the remaining indicators were low. Sample No. 4 had a high volume, 7.7% larger than in the control, but porosity did not increase significantly. All bread samples with added WG retained freshness longer in terms of water absorption and brittleness, but bread with gluten No. 1 was best preserved. Thus, it was found that gluten samples No. 1 and No. 3 are best suited for correcting the quality indicators of wheat-hemp bread. The results obtained can be used in research papers, and in industrial conditions of bakeries, bakeries, in particular, in the technological process of production of wheat bread varieties with the addition of hemp flour and hemp seeds. They will help flour milling technologists to adjust the quality of wheat flour, and technologists at bread factories and bakeries to model recipe compositions and the technological process of producing wheat bread varieties. The prospect of further research is to find other technological ways to improve dough systems with a weak gluten system and low technological properties.

Acknowledgements

None.

Funding

The study was conducted as part of state research project No. 39.00.04.01F, "Establish criteria for selecting functional ingredients of various origins for the development of technologies for bread and bakery products with high consumer value", No. DR 0121U108540.

Conflict of Interest

None.

References

[1] Dhaka, V., & Khatkar, B.S. (2015). Gluten proteins, dough rheology and bread quality. *Journal of Food Quality*, 38, 71-82. doi: 10.1111/jfq.12122.

[2] Drobot, V.I., Silchuk, T.A., & Udvorgeli, L.I. (2005). Influence of dry wheat gluten on technological parameters and quality of rye-wheat bread. *Grain Products and Mixed Fodder's*, 2, 53-55.

[3] Drobot, V. I. (2019). *Handbook on baking technology*. Kyiv: Professional Book.
[4] DSTU 7045-2009. (2010). *Bakery products. Methods for determining physicochemical parameters*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=83710.
[5] DSTU 7517:2024. (2025). *Bread made from wheat flour. General technical conditions*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=112017.
[6] DSTU ISO 21415-1:2009. (2011). *Wheat and wheat flour. Gluten content. Part 1. Determination of grey gluten by manual method*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=84541.
[7] Gunko, S.M., Naumenko, O.V., Hetman, I.A., Korolyuk, K.Ye., Lukianchuk, I.V., & Kuznietsova, I.V. (2024). Use of products of hemp seed processing for bread production. *Food Resources*, 12(22), 50-60. doi: 10.31073/foodresources2024-22-06.
[8] Hetman, I., Mykhonik, L., Kuzmin, O., & Shevchenko, A. (2021). Influence of spontaneous fermentation leavens from cereal flour on the indicators of the technological process of making wheat bread. *Ukrainian Food Journal*, 10(3), 492-506. doi: 10.24263/2304-974X-2021-10-3-6.
[9] Horax, R. Hettiarachchy, N., Kannan, A., & Chen, P. (2011). Protein extraction optimisation, characterisation, and functionalities of protein isolate from bitter melon (*Momordica Charantia*) seed. *Food Chemistry*, 124(2), 545-550. doi: 10.1016/j.foodchem.2010.06.068.
[10] Ionescu, V., Stoenescu, G., Vasilean, I., Aprodu, I., & Banu, I. (2010). Comparative evaluation of wet gluten quantity and quality through different methods. *Annals of the University Dunarea de Jos of Galati. Fascicle VI: Food Technology*, 34(2), 44-48.
[11] Kaushik, R., Kumar, N., Sihag, M.K., & Ray, A. (2015). Isolation, characterization of wheat gluten and its regeneration properties. *Journal of Food Science and Technology*, 52(9), 5930-5937. doi: 10.1007/s13197-014-1690-2.
[12] Kornpointner, C., Martinez, A.S., Marinovic, S., Haselmair-Gosch, C., Jamnik, P., Schröder, K., Löfke, C., & Halbwirth, H. (2021). Chemical composition and antioxidant potential of *Cannabis sativa* L. roots. *Industrial Crops and Products*, 165, article number 113422. doi: 10.1016/j.indcrop.2021.113422.
[13] Kryzhanovskiy, V. (2022). Peculiarities of grain quality formation of winter wheat varieties in the right bank forest steppe. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 18(1). doi: 10.31548/dopovidi2022.01.008.
[14] Li, S., Qu, Z., Feng, J., & Chen, Y. (2020). Improved physicochemical and structural properties of wheat gluten with konjac glucomannan. *Journal of Cereal Science*, 95, article number 103050. doi: 10.1016/j.jcs.2020.103050.
[15] Munteanu, G.-M., Voicu, G., Ferdeș, M., Ștefan, E.-M., Constantin, G.-A., & Tudor, P. (2019). Dynamics of fermentation process of bread dough prepared with different types of yeast. *Scientific Study and Research: Chemistry and Chemical Engineering*, 20(4), 575-584.
[16] Pohorielov, I., & Mykhonik, L. (2021). The use of dry wheat gluten in the technology of bakery products with cereal processing products. In *Proceedings of the 1st international scientific and practical conference "Problems and practical approaches to the production and regulation of the use of food additives in the European Union countries and in Ukraine"* (pp. 124-125). Kyiv: NUFT.
[17] Schopf, M., & Scherf, K.A. (2020). Predicting vital wheat gluten quality using the gluten aggregation test and the microscale extension test. *Current Research in Food Science*, 3, 322-328. doi: 10.1016/j.crfs.2020.11.004.

[18] Schopf, M., Wehrli, M.C., Becker, T., Jekle, M., & Scherf, K.A. (2021). Fundamental characterization of wheat gluten. *European Food Research and Technology*, 247, 985-997. doi: 10.1007/s00217-020-03680-z.

[19] Sciacca, F., Virzì, N., Pecchioni, N., Melilli, M.G., Buzzanca, C., Bonacci, S., & Di Stefano, V. (2023). Functional end-use of hemp seed waste: Technological, qualitative, nutritional, and sensorial characterization of fortified bread. *Sustainability*, 15(17), article number 12899. doi: 10.3390/su151712899.

[20] Shevchenko, A., Litvynchuk, S., & Koval, O. (2023). The influence of rice protein concentrate on the technological process of wheat bread production. *EUREKA: Life Sciences*, 4, 65-72. doi: 10.21303/2504-5695.2023.003031.

[21] Švec, I., & Hrušková, M. (2015). Properties and nutritional value of wheat bread enriched by hemp products. *Potravinarstvo Slovak Journal of Food Sciences*, 9(1), 304-308. doi: 10.5219/487.

[22] Verheyen, C., Albrecht, A., Elgeti, D., Jekle, M., & Becker, T. (2015). Impact of gas formation kinetics on dough development and bread quality. *Food Research International*, 76(3), 860-866. doi: 10.1016/j.foodres.2015.08.013.

[23] Zhang, M., Jia, R., Ma, M., Yang, T., Sun, Q., & Li, M. (2022) Versatile wheat gluten: Functional properties and application in the food-related industry. *Critical Reviews in Food Science and Nutrition*, 63(30), 10444-10460. doi: 10.1080/10408398.2022.2078785.

[24] Zhang, X., Mu, M., Tian, Y., Fu, J., Jia, F., Wang, Q., Liang, Y., & Wang, J. (2021). Aggregative and structural properties of wheat gluten during post-harvest maturation. *Journal of Stored Products Research*, 94, article number 101897. doi: 10.1016/j.jspr.2021.101897.

[25] Zhygunov, D., Chumachenko, Y., Kovalov, M., & Kovalova, V. (2023). Wheat flour quality correction by using enzyme preparations. *Grain Products and Mixed Fodder's*, 23(4), 12-18. doi: 10.15673/gpmf.v23i4.2811.

Дослідження властивостей глютену пшеничного та його впливу на формування якості хліба

Інна Гетьман

Доктор філософії, старший науковий співробітник
Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
https://orcid.org/0000-0002-9448-9956

Іванна Лук'янчук

Аспірант
Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
https://orcid.org/0009-0007-4876-0470

Оксана Науменко

Доктор технічних наук, професор
Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
https://orcid.org/0000-0002-1691-1381

Володимир Ковбаса

Доктор технічних наук, професор
Національний університет харчових технологій
01033, вул. Володимирська, 68, м. Київ, Україна
https://orcid.org/0009-0001-8876-3373

Любов Ткаченко

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

Анотація. Збагачення хлібобулочних виробів конопляним борошном підвищує їхню харчову цінність, проте відсутність у ньому клейковини вимагає додавання структуроутворюючих добавок, наприклад, пшеничного глютену. Водночас технологічні показники та вплив на тістову систему різних зразків глютену пшеничного, представлених на ринку України, залишаються недослідженими. Мета досліджень – проаналізувати ефективність глютену пшеничного різних виробників для покращення реологічних властивостей тіста та якості пшенично-конопляного хліба. Показано, що у зразках глютену «Viten-Vital» та «Gluvital 21020» найбільшою фракцією були проламіни, відповідно, 69,2 та 68,59 %, і в меншій кількості містились глютеліни: відповідно, 21,91 та 30,45 %. У зразках «BeneoPro VWG 75» та «NVG-Vital» усереднений розподіл цих фракцій становив близько 50:50. Досліджено, що внесення глютену пшеничного в кількості 3 % до маси борошна збільшувало кількість сирої клейковини на 25,5-27,6 %, залежно від зразка. За результатами пробних лабораторних випікань встановлено, що найкращою якістю за сукупністю показників: об'єм, пористість та збереження свіжості характеризувався хліб пшенично-конопляний з глютеном «BeneoPro VWG 75» або «NVG-Vital». Хліб зі зразком

глютену «Gluvital 21020» характеризувався на 7,7 % вищим питомим об'ємом, ніж у контролі, але пористість його збільшилась несуттєво. Встановлені відмінності у технологічних показниках глютену пшеничного різних виробників можуть допомогти у правильному виборі даної сировини для використання у виробництві пшеничних сортів хліба, зокрема з додаванням борошна конопляного

Ключові слова: борошно конопляне; закваска; показники якості; харчова цінність; хлібобулочні вироби

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 17(1), 60-76
Received 15.09.2025 Revised 22.01.2026 Accepted 26.02.2026
Published 26.03.2026

UDC 664.91:664.292:577.115:637.5
DOI: 10.31548/animal.1.2026.60

ISSN 2706-8331
e-ISSN 2706-834X

Effect of durian rind pectin with basil and oregano essential oils on beef patty quality

Umaporn Pastsart*
PhD in Applied Biological Sciences, Associate Professor
Faculty of Innovative Agriculture, Fisheries and Food,
Prince of Songkla University Surat Thani Campus
84000, 31 Moo 6 Str., Surat Thani, Thailand
https://orcid.org/0000-0003-1844-1085

Saneeta Salam
Master
Faculty of Innovative Agriculture, Fisheries and Food,
Prince of Songkla University Surat Thani Campus
84000, 31 Moo 6 Str., Surat Thani, Thailand
https://orcid.org/0009-0008-1023-3410

Jessada Rattanawut
PhD in Animal Sciences, Associate Professor
Faculty of Innovative Agriculture, Fisheries and Food,
Prince of Songkla University Surat Thani Campus
84000, 31 Moo 6 Str., Surat Thani, Thailand
https://orcid.org/0000-0003-1967-1114

Patima Permpoonpattana
PhD in Biology, Assistant Professor
Faculty of Innovative Agriculture, Fisheries and Food,
Prince of Songkla University Surat Thani Campus
84000, 31 Moo 6 Str., Surat Thani, Thailand
https://orcid.org/0000-0002-0320-7184

Chatchawan Chotimarkorn
PhD in Food and Technology Sciences, Assistant Professor
Faculty of Innovative Agriculture, Fisheries and Food,
Prince of Songkla University Surat Thani Campus
84000, 31 Moo 6 Str., Surat Thani, Thailand
https://orcid.org/0000-0002-7522-4549

Suggested Citation:
Pastsart, U., Salam, S., Rattanawut, J., Permpoonpattana, P., & Chotimarkorn, C. (2026). Effect of durian rind pectin with basil and oregano essential oils on beef patty quality. *Animal Science and Food Technology*, 17(1), 60-76. doi: 10.31548/animal.1.2026.60.

*Corresponding author

Abstract. This study aimed to evaluate the effects of durian rind pectin (DP) in combination with basil or oregano essential oils on the quality and shelf life of beef patties during refrigerated storage, as natural alternatives to synthetic additives. Six treatments were formulated: T1 (control), T2 (0.02% BHT), T3 (1% DP + 1% basil oil), T4 (1% DP + 1% oregano oil), T5 (1% DP + 2% basil oil), and T6 (1% DP + 2% oregano oil). Physicochemical, microbiological, and antioxidant properties were monitored over 8 days at 4°C. The incorporation of pectin and essential oils significantly ($p < 0.05$) affected pH, colour (L*, a*, b*), cooking loss, and texture. Basil oil treatments lowered pH values, while oregano oil increased them. Treated samples showed higher initial lightness, redness, and yellowness, although a* and b* values declined by day 8. Cooking loss was significantly reduced, and tenderness improved in all treated groups, as indicated by decreased hardness, gumminess, chewiness, and springiness, with no significant changes in cohesiveness or adhesiveness. Patties supplemented with pectin and essential oils exhibited higher DPPH radical scavenging activity (63-80% vs. 27% in control) and lower TBARS values (0.08-0.10 µg MDA/g vs. 0.19 µg MDA/g in control) ($p < 0.05$), indicating improved oxidative stability. Microbiological analysis showed significantly reduced total viable counts and *E. coli* levels in treated samples across all time points. No *Salmonella* spp. was detected. Overall, the combination of DP with basil or oregano essential oils enhanced the quality and extended the shelf life of beef patties ($p < 0.05$), offering a promising natural alternative to synthetic preservatives in meat products. The results of this study can be used by meat processing industries to produce safer and longer-lasting refrigerated minced meat products using natural preservatives

Keywords: minced meat products; natural preservatives; oxidative stability; shelf-life extension; agro-waste utilisation

Introduction

The stability of minced meat products during refrigerated storage represents a persistent technological and safety challenge in modern meat processing. Beef patties are widely consumed due to their convenience and sensory appeal; however, the grinding process disrupts muscle structure, increases oxygen exposure, and accelerates oxidative and microbial deterioration. These changes lead to discolouration, lipid oxidation, textural degradation, and microbial proliferation, ultimately limiting shelf life and affecting consumer acceptance. As global demand for minimally processed and clean-label foods continues to grow, developing effective natural preservation strategies for fresh meat systems has become both an industrial necessity and a scientific priority.

In recent years, researchers have increasingly explored plant-derived compounds as alternatives to synthetic antioxidants. S. Salam *et al*. (2024) demonstrated that incorporating pectin powder into beef patties improved oxidative stability and texture during retail display, although the antimicrobial effect was limited. Similarly, C. Srikamwang *et al*. (2024) reported that mango peel pectin enhanced physicochemical properties and functional performance in meat systems, emphasising the structural role of polysaccharides in water retention and matrix stabilisation. These findings suggest that plant-derived pectins can positively influence technological properties, yet their contribution to microbial control remains insufficient. Durian rind has attracted attention as a sustainable source of pectin due to its abundance as an agro-industrial by-product in Southeast Asia. M. Unhasirikul *et al*. (2021) characterised pectin extracted from durian

husks and reported distinct physicochemical properties compared to commercial pectin. Their work highlighted the potential of durian rind pectin as a value-added ingredient; however, its application in meat preservation systems was not extensively evaluated. This indicates a gap between raw material characterisation and functional validation in real food matrices.

Parallel to polysaccharide research, essential oils have been widely investigated for their bioactive properties in meat systems. A. Mohammed & A. Alrefiee (2021) found that basil and oregano essential oils improved physicochemical stability and delayed spoilage in meat burgers during chilled storage. K. Zheng *et al*. (2023) further confirmed that oregano essential oil incorporated into edible coatings significantly reduced microbial growth and maintained quality in refrigerated poultry meat. More recently, M. Walasek-Janusz *et al*. (2024) analysed the chemical composition of oregano essential oil and concluded that its high carvacrol and thymol content contributes to strong antioxidant and antimicrobial activity. In addition, A. Javid *et al*. (2024) developed a basil essential oil–incorporated preservation system for ground beef and observed effective suppression of total viable counts during storage. Collectively, these studies confirm the potential of essential oils to inhibit lipid oxidation and microbial proliferation in meat products.

Despite these promising findings, most existing studies have evaluated pectin and essential oils separately. Polysaccharides primarily enhance water-holding capacity and texture, whereas essential oils contribute antimicrobial and antioxidant effects. However, limited research has investigated the combined application of waste-derived pectin and essential oils within the same meat matrix under identical storage conditions. Furthermore, direct comparison between such natural combinations and conventional synthetic antioxidants remains scarce in current literature. The absence of integrated approaches combining structural stabilisers and bioactive compounds represents a significant research gap. Addressing this gap is particularly relevant in the context of sustainable meat processing. Utilising durian rind pectin not only offers functional advantages but also contributes to waste valorisation and environmental sustainability. At the same time, optimising essential oil concentration is necessary to balance antimicrobial efficacy with product quality. An integrated preservation strategy that simultaneously improves texture, oxidative stability, and microbial safety could provide a practical solution aligned with clean-label trends and circular economy principles.

Therefore, the purpose of this study was to examine whether the integration of durian rind pectin with basil or oregano essential oils could produce complementary effects on physicochemical stability, oxidative resistance, and microbial dynamics in beef patties during refrigerated storage, and to evaluate its performance relative to a conventional synthetic antioxidant system.

Materials and Methods

In this study, durian rinds were obtained from mature 'Mon Thong' fruits harvested from the tree and subsequently ripened under ambient conditions for 6-9 days to achieve full ripeness, in accordance with the Thai Agricultural Standard (TAS 3-2013), issued by the National Bureau of Agricultural Commodity and Food Standards. No size grading was applied during selection. Beef Longissimus dorsi (LD) muscle and subcutaneous fat used for patty preparation were procured from a local meat vendor in Mueang Surat Thani District, Surat Thani Province, Thailand. Prior to use, visible fat and connective tissues were removed from the lean meat. Both meat and fat were stored at 4 ± 1°C under refrigeration. Commercial food-grade basil and oregano essential oils were purchased from Lapis Tropical Spa Product Co., Ltd. (Thailand) for use in treatments.

The inner white portion of 'Mon Thong' durian rinds was chopped into small fragments and weighed. These fragments were dried in a hot-air oven at approximately 55°C until a constant weight was achieved. Once dried, the material was ground into a fine powder and stored in vacuum-sealed pouches until further use. Pectin extraction was performed based on the method adapted from M. Unhasirikul *et al.* (2021), with minor modifications. Briefly, durian rind powder was mixed with 0.1 M hydrochloric acid at a 1:2 (w/v) solid-to-liquid ratio. The mixture was incubated under controlled conditions, and pectin was subsequently precipitated using 80% ethanol. The precipitate was collected by centrifugation at 8,000 rpm for 30 minutes. To purify the extract, the pectin was washed three times with ethanol and re-dried in a hot-air oven at 55°C until a constant weight was obtained. The final dried product was milled into a fine powder and stored in air-tight containers for later use in the experiment.

The antibacterial activity of durian rind pectin, basil essential oil, and oregano essential oil was assessed using the agar well diffusion method, as described by M. Balouiri *et al.* (2016). Briefly, *E. coli* and *Salmonella* spp. were cultured in nutrient broth and incubated at 37°C for 24 hours until reaching the logarithmic growth phase. The bacterial suspensions were adjusted to match a 0.5 McFarland standard (approximately 1×10^8 CFU/mL). Müller-Hinton agar (MHA) plates were prepared and inoculated evenly with the bacterial suspension using sterile cotton swabs. Wells of 6 mm in diameter were created using a sterile cork borer, and 100 µL of each test solution, durian rind pectin, basil essential oil, and oregano essential oil, was introduced into the wells separately. The plates were then incubated at 37°C for 24 hours. After incubation, the diameters of the inhibition zones (in mm) surrounding the wells were measured using a digital caliper. All tests were conducted in triplicate, and results were reported as mean ± standard deviation.

The antioxidant activity of durian rind pectin, basil essential oil, and oregano essential oil was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the method described by O. Sharma & T. Bhat (2009), with slight modifications. Briefly, a 0.1 mM DPPH solution was prepared by dissolving 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol. For sample preparation, 2.5 mL of each test solution (pectin, basil essential oil, or oregano essential oil) was mixed with 7.5 mL of ethanol and vortexed to ensure homogeneity. Then, 1.5 mL of this solution was transferred into a test tube, followed by the addition of 10.5 mL of 0.1 mM DPPH solution. The mixture was vortexed and incubated in the dark at room temperature for 20 minutes. Absorbance was measured at 517 nm using a spectrophotometer, with ethanol serving as the blank. The DPPH scavenging activity (%) was calculated using the formula (1):

$$\text{DPPH Scavenging activity (\%)} = \\ = (1 - As/Ac) \times 100, \quad (1)$$

where Ac – the absorbance of the control (DPPH in ethanol without the sample); As – the absorbance of the sample.

Each sample was analysed in triplicate, and the results were expressed as mean ± standard deviation. Beef patties were prepared using lean beef (LD) and subcutaneous beef fat at a ratio of 90:10 (w/w). The meat and fat were ground and mixed thoroughly, then divided into six treatment groups, with five replicates (n = 5) per treatment. The experiment followed a completely randomised design (CRD) with the following treatments: 1) Control (ground beef), 2) BHT 0.02% (ground beef + 0.02% BHT), 3) ground beef + durian rind pectin (DP) 1% + basil essential oil 1%, 4) ground beef + DP 1% + oregano essential oil 1%, 5) ground beef + DP 1% + basil essential oil 2%, and 6) ground beef + DP 1% + oregano essential oil 2%. The concentrations of 1% and 2%

essential oils were selected based on previous studies that demonstrated their effectiveness in improving meat quality and storage stability (Falowo *et al.*, 2019; Zheng *et al.*, 2023). Essential oils were first mixed with a small amount of ground meat, followed by gradual incorporation of the remaining meat to ensure uniform distribution. The mixtures were then formed into round flat patties (approximately 100 g each) using a standard mold. Patties were wrapped in plastic film and stored at 4°C for 0, 4, and 8 days for subsequent quality analyses.

The internal pH of beef patties was measured on days 0, 4, and 8 of refrigerated storage. A portable pH meter (Mettler Toledo) equipped with a penetration-type electrode was used. The electrode was carefully inserted into the center of each patty to obtain accurate pH values. Colour attributes, including lightness (L*), redness (a*), and yellowness (b*), were evaluated according to the CIE system on days 0, 4, and 8. A HunterLab Miniscan colourimeter was used, calibrated with a white reference tile and set to a D65 illuminant, 10° standard observer, and 45°/0° geometry. Measurements were taken at three different points on each sample to ensure accuracy and account for surface variability.

Cooking loss, an indicator of water-holding capacity, was measured on days 0, 4, and 8 according to the method described by U. Pastsart *et al.* (2024). Beef patties were vacuum-sealed in plastic bags and cooked in a water bath at 90°C for 15 minutes. Each sample was weighed before and after cooking. Cooking loss (%) was calculated based on the difference between the pre- and post-cooking weights, providing an estimate of moisture retention in the patties. Texture profile analysis (TPA) was performed on days 0, 4, and 8 of storage using a Brookfield CT3 texture analyser equipped with a 10 kg load cell. Patties were cooked in a water bath at 90°C for 15 minutes and then cut into uniform cubes (1 × 1 × 1 cm). Each treatment was analysed in triplicate. Textural parameters including hardness, springiness, cohesiveness, adhesiveness, gumminess, and chewiness were measured to assess structural changes during storage.

The antioxidant capacity of beef patties was assessed on days 0, 4, and 8 of storage using the DPPH radical scavenging assay, following the method of L. Wang *et al.* (2019) as described above. Briefly, 2.5 g of each sample was homogenised in 7.5 mL of ethanol and extracted on a shaker for 10 minutes. The homogenate was then centrifuged at 1,800 rpm for 10 minutes. A 1.5 mL aliquot of the supernatant was mixed with 10.5 mL of 0.1 mM DPPH solution in ethanol. The mixture was incubated in the dark at room temperature for 20 minutes, after which the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Lipid oxidation was evaluated on days 0, 4, and 8 during the retail display period using the thiobarbituric acid reactive substances (TBARS) assay, following B. Tarladgis *et al.* (1960). The assay quantifies malondialdehyde (MDA) as a marker of oxidative degradation. Absorbance was measured at 532 nm, and results were reported as micrograms of MDA per gram of meat sample (μg MDA/g).

Beef patty samples were analysed on days 0, 4, and 8 of storage at 4°C for total plate count (TPC), *Salmonella* spp., and *E. coli*. A 5 g portion of each sample (n = 3) was homogenised with 45 mL of 0.85% NaCl solution, and serial dilutions (10^{-1} to 10^{-6}) were prepared. TPC was determined using Plate Count Agar (PCA), *E. coli* on Eosin Methylene Blue (EMB) agar, and *Salmonella* spp. were detected through pre-enrichment in Buffered Peptone Water (BPW), selective enrichment in Rappaport-Vassiliadis (RV) broth, and plating on Xylose Lysine Deoxycholate (XLD) agar. All plates were incubated at 37°C for 24 hours. Results were expressed as log CFU/g of sample. All collected data were subjected to analysis using the General Linear Model (GLM) procedure in SPSS. Differences between treatments were assessed using Duncan's multiple range test, and statistical significance was considered at $p < 0.05$.

Results and Discussion

The antibacterial activity of durian rind pectin, basil essential oil, and oregano essential oil against *Salmonella* spp. and *E. coli* is summarised in Table 1. Oregano essential oil showed the highest inhibition against both *Salmonella* spp. (20.00 ± 1.00 mm) and *E. coli* (14.67 ± 2.08 mm). Basil essential oil exhibited moderate activity, with inhibition zones of 7.67 ± 0.57 mm and 8.00 ± 1.00 mm, respectively. In contrast, durian rind pectin and the negative control showed no inhibition (0.00 ± 0.00 mm). *Bacillus subtilis*, used as a positive control, produced inhibition zones of 8.67 ± 0.58 mm against *Salmonella* spp. and 13.66 ± 1.53 mm against *E. coli*.

Table 1. *Zone of inhibition of durian rind pectin, basil and oregano essential oils against Salmonella spp. and E. coli. (mean ± SD)*

Test substance	Zone of inhibition (mm) against *Salmonella* spp.	Zone of inhibition (mm) against *E. coli*
Negative control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Pectin from durian rind	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Basil essential oil	7.67 ± 0.57^{b}	8.00 ± 1.00^{b}
Oregano essential oil	20.00 ± 1.00^{a}	14.67 ± 2.08^{a}
Bacillus subtilis	8.67 ± 0.58^{b}	13.66 ± 1.53^{a}

Note: $^{a-c}$ different superscript letters in the same column indicate statistically significant differences among groups ($p < 0.05$)

Source: authors' own research

The results clearly demonstrate that both basil and oregano essential oils exhibit antibacterial activity against *E. coli* and *Salmonella* spp., with oregano oil showing greater efficacy. This higher inhibition may be associated with differences in chemical composition between the two essential oils. These findings align with those of M. Hossain *et al*. (2010), who reported that basil essential oil was effective against *E. coli* and *Salmonella typhi*, attributing its activity to compounds such as methyl chavicol, linalool, and eugenol. Supporting this mechanism, M. Tajkarimi *et al*. (2010) explained that phenolic components in essential oils can disrupt membrane integrity, increase membrane permeability, and impair essential metabolic processes, ultimately suppressing bacterial growth. The moderate inhibition observed for basil oil in the present study is therefore likely related to the concentration and proportion of these active compounds. The stronger antibacterial effect of oregano essential oil may be attributed to its higher content of carvacrol, a potent antimicrobial compound. According to D. Pérez-Conesa *et al*. (2011), carvacrol disrupts cytoplasmic membrane structure and interferes with energy metabolism, which may explain the larger inhibition zones recorded in the present study. This interpretation is consistent with the findings of C. Semeniuc *et al*. (2017), who demonstrated broad-spectrum antibacterial activity of oregano oil against both Gram-positive and Gram-negative bacteria, including *E. coli* and *Salmonella* spp. In contrast, durian rind pectin did not exhibit antibacterial activity against either *E. coli* or *Salmonella* spp. This lack of inhibition may be attributed to the absence of intrinsic antimicrobial compounds within purified pectin. However, although pectin alone showed no direct antibacterial effect, it may function as a film-forming or stabilising agent and potentially act synergistically with essential oils in food preservation systems. The antioxidant activity of durian rind pectin, basil essential oil, and oregano essential oil was evaluated using the DPPH assay, as shown in Table 2. Basil essential oil exhibited the highest free radical

scavenging activity (91.88 ± 0.28%), followed by oregano essential oil (90.19 ± 0.28%) and durian rind pectin (81.48 ± 0.73%), with all values showing significant differences ($p < 0.05$).

Table 2. *Antioxidant activity (% free radical scavenging) by DPPH assay of durian rind pectin, basil and oregano essential oils (mean ± SD)*

Test substance	DPPH (% free radical scavenging)
Pectin from durian rind	81.48 ± 0.73^{c}
Basil essential oil	91.88 ± 0.28^{a}
Oregano essential oil	90.19 ± 0.28^{b}

Note: $^{a\text{-}c}$ different superscript letters in the same column indicate statistically significant differences among groups ($p < 0.05$)

Source: authors' own research

These results are consistent with previous findings demonstrating the strong radical scavenging activity of essential oils in the DPPH assay. A. Ahmed *et al.* (2019) demonstrated that basil essential oil from various sources possesses potent antioxidant activity in the DPPH assay. A. Sahu *et al.* (2023) also reported strong antioxidant capacity in basil oil, attributed to active compounds such as methyl chavicol, linalool, eugenol, and flavonoids, which function as free radical scavengers and protect against oxidative damage. The antioxidant activity observed in the present study may therefore be attributed to the concentration and composition of these phenolic constituents. Similarly, oregano essential oil has been widely recognised for its antioxidant properties. M. Walasek-Janusz *et al.* (2024) identified carvacrol and thymol as major contributors to its high radical scavenging activity. Z. Suntres *et al.* (2015) further emphasised the biological and pharmacological significance of carvacrol, particularly its role in reducing oxidative stress. The moderate antioxidant activity of durian rind pectin suggests the presence of natural antioxidants, although to a lesser extent than in essential oils. As shown in Table 3, there were no significant differences ($p > 0.05$) in the initial pH values among all treatment groups on day 0, indicating that the samples started with similar acidity. However, significant differences emerged during storage. On day 4, patties treated with durian rind pectin and essential oils particularly T4 (1% pectin + 1% oregano oil) and T6 (1% pectin + 2% oregano oil) had significantly higher pH values than other treatments ($p < 0.05$). By day 8, T1 (control) and T6 maintained the highest pH values, while T3 (1% pectin + 1% basil oil) and T5 (1% pectin + 2% basil oil) recorded the lowest.

The increase in pH during storage, particularly in the control group, is consistent with the findings of M. Triki *et al.* (2018), who observed rising pH in refrigerated meats due to the formation of nitrogenous compounds such as ammonia and amines. These are generated by proteolytic enzymes released by microorganisms during protein degradation. Interestingly, treatments containing basil essential oil (T3 and T5) consistently showed lower pH values, especially at higher concentrations. This may be attributed to a synergistic acidifying effect between durian rind pectin and basil oil. Durian pectin, rich in galacturonic acid, naturally lowers pH, while basil essential oil contains phenolic compounds such as linalool and eugenol, which possess mild acidity and antimicrobial properties. This pH-lowering effect aligns with the findings of G. Khaled *et al.* (2023), who reported similar trends in basil oil-treated goat meat stored under refrigeration.

The effect of durian rind pectin combined with basil and oregano essential oils on beef patty colour parameters (L*, a*, b*) during refrigerated storage is shown in Table 3. On day 0, treatments significantly influenced lightness (L*) and yellowness (b*), with T6 (1% pectin + 2% oregano oil) showing the highest values, while redness (a*) peaked in T4 and was lowest in T6. By day 4, L* and b* remained highest in T6 and T3, while a* was highest in T3 (1% pectin + 1% basil oil). On day 8, T6, T4, and T5 continued to show increased L*, while redness was highest in T2 (BHT), and lowest in control. Yellowness (b*) decreased across all treatments but remained significantly higher in essential oil-treated samples than the control.

Overall, the addition of durian rind pectin and essential oils significantly enhanced lightness and yellowness compared to the control across all storage days ($p < 0.05$). These results are consistent with A. Falowo *et al*. (2019), who reported increased L*, a*, and b* values in beef patties treated with basil essential oil during cold storage. I. Karabagias *et al.* (2011) also observed improved lightness and yellowness in lamb meat stored in oregano- and thyme-oil-infused packaging, attributing the effect to the antioxidant properties of the oils, which help reduce oxidative discolouration. Similarly, C. Srikamwang *et al.* (2024) found that mango peel pectin enhanced colour values in meat products due to its natural pigments and structural properties. However, a notable reduction in redness (a*) was observed by day 8 in treatments containing higher levels of essential oils, particularly T3 and T5. This may be due to the dilution or masking of meat pigments by plant-based components, as previously reported by S. Salam *et al.* (2024), who found that excessive incorporation of plant-derived ingredients can lead to undesired colour changes in meat products. The effect of durian rind pectin combined with basil and oregano essential oils on cooking loss of beef patties during refrigerated storage is shown in Table 3. All treated groups exhibited significantly lower cooking loss than the control (T1) and BHT group (T2) throughout the storage period ($p < 0.05$). On day 0, the highest cooking loss was recorded in T1 (47.10%), while the lowest was observed in T6 (42.72%). This trend persisted through days 4 and 8, with T6 consistently showing the greatest reduction, indicating enhanced water retention.

This reduction in cooking loss may result from the synergistic effects of durian rind pectin and essential oils. Pectin serves as a gelling agent that retains water within the meat matrix (Rolin, 1993), a function supported by M. Namir *et al.* (2015), who reported that tomato-derived pectin reduced cooking loss in minced meat. Additionally, basil and oregano essential oils may contribute to improved water-holding capacity by stabilising muscle protein structures and enhancing water binding, as noted by K. Zheng *et al.* (2023). A. Mohammed & A. Alrefiee (2021) similarly observed reduced cooking loss in camel meat burgers treated with these essential oils during chilled storage, attributing the effect to their antioxidant and protein-stabilising properties. In summary, the incorporation of durian rind pectin with basil or oregano essential oils effectively minimised cooking loss in beef patties, supporting better moisture retention and cooking yield during refrigerated storage.

Table 3. *Effect of durian rind pectin (DP) and basil and oregano essential oils (EOs) supplementation on quality of beef patties during refrigerated storage (mean ± SD)*

Item	T1 (Control)	T2 (BHT 0.02%)	T3 (DP 1% + Basil EO 1%)	T4 (DP1% + Oregano EO 1%)	T5 (DP 1% + Basil EO 2%)	T6 (DP 1% + Oregano EO 2%)
pH, day 0	5.48 ± 0.01	5.50 ± 0.01	5.48 ± 0.01	5.48 ± 0.02	5.49 ± 0.02	5.49 ± 0.01
pH, day 4	5.50^{b} ± 0.02	5.50^{b} ± 0.03	5.44^{c} ± 0.01	5.58^{a} ± 0.01	5.46^{c} ± 0.01	5.60^{a} ± 0.01
pH, day 8	5.66^{a} ± 0.03	5.60^{b} ± 0.02	5.21^{d} ± 0.01	5.56^{c} ± 0.03	5.19^{d} ± 0.01	5.64^{a} ± 0.01

Table 3. Continued

Item	T1 (Control)	T2 (BHT 0.02%)	T3 (DP 1% + Basil EO 1%)	T4 (DP1% + Oregano EO 1%)	T5 (DP 1% + Basil EO 2%)	T6 (DP 1% + Oregano EO 2%)
L*, day 0	$71.18^{d} \pm 0.93$	$69.60^{e} \pm 0.93$	$79.75^{c} \pm 0.93$	$82.58^{b} \pm 1.21$	$80.33^{c} \pm 0.31$	$86.40^{a} \pm 0.84$
L*, day 4	$79.85^{c} \pm 0.56$	$79.13^{c} \pm 1.23$	$90.53^{a} \pm 2.08$	$90.68^{a} \pm 0.67$	$87.60^{b} \pm 1.34$	$90.85^{a} \pm 0.91$
L*, day 8	$73.90^{c} \pm 0.94$	$72.70^{c} \pm 0.74$	$93.43^{b} \pm 1.00$	$98.20^{a} \pm 1.31$	$98.13^{a} \pm 1.45$	$99.53^{a} \pm 0.43$
a*, day 0	$13.35^{cd} \pm 0.25$	$14.85^{ab} \pm 0.57$	$14.05^{bc} \pm 0.79$	$15.05^{a} \pm 0.91$	$13.13^{cd} \pm 0.26$	$12.75^{d} \pm 0.57$
a*, day 4	$11.75^{d} \pm 0.10$	$11.93^{d} \pm 0.67$	$15.35^{a} \pm 0.35$	$12.73^{c} \pm 0.21$	$14.55^{b} \pm 0.37$	$11.85^{d} \pm 0.58$
a*, day 8	$0.15^{f} \pm 0.06$	$14.48^{a} \pm 0.42$	$1.33^{e} \pm 0.17$	$4.73^{b} \pm 0.29$	$2.88^{d} \pm 0.17$	$3.45^{c} \pm 0.21$
b*, day 0	$19.70^{d} \pm 1.45$	$20.55^{d} \pm 1.19$	$24.33^{b} \pm 0.52$	$25.93^{a} \pm 1.14$	$22.30^{c} \pm 0.53$	$26.60^{a} \pm 0.26$
b*, day 4	$24.10^{cd} \pm 0.38$	$21.33^{e} \pm 0.59$	$26.28^{a} \pm 0.39$	$23.65^{d} \pm 1.05$	$24.78^{bc} \pm 0.60$	$25.25^{b} \pm 0.37$
b*, day 8	$11.03^{f} \pm 0.22$	$18.78^{a} \pm 0.59$	$13.73^{e} \pm 0.17$	$17.23^{b} \pm 1.03$	$14.93^{d} \pm 0.39$	$15.55^{c} \pm 0.42$
% cooking loss, day 0	$47.10^{a} \pm 2.80$	$45.44^{ab} \pm 1.00$	$44.80^{abc} \pm 1.01$	$45.07^{abc} \pm 1.15$	$43.14^{bc} \pm 0.77$	$42.72^{c} \pm 1.25$
% cooking loss, day 4	$46.14^{a} \pm 0.60$	$44.16^{b} \pm 1.73$	$39.45^{c} \pm 1.92$	$40.58^{c} \pm 0.84$	$41.25^{c} \pm 0.52$	$40.20^{c} \pm 0.85$
% cooking loss, day 8	$45.80^{a} \pm 0.84$	$45.13^{a} \pm 1.63$	$40.38^{b} \pm 1.32$	$42.04^{b} \pm 1.96$	$40.72^{b} \pm 0.78$	$39.75^{b} \pm 1.62$

Note: $^{a-c}$ different superscript letters in the same row indicate statistically significant differences among groups ($p < 0.05$)

Source: authors' own research

The effect of durian rind pectin combined with basil and oregano essential oils on the texture properties of beef patties during refrigerated storage is presented in Table 4. Significant differences were observed in hardness, springiness, gumminess, and chewiness ($p < 0.05$), while cohesiveness and adhesiveness remained unaffected throughout storage ($p > 0.05$). On day 0, the control (T1) and BHT-treated patties (T2) exhibited the highest hardness, gumminess, and chewiness. In contrast, T3-T6 showed the lower values, suggesting that the addition of pectin and essential oils contributed to a softer texture, likely due to enhanced moisture retention and reduced protein cross-linking. By day 8, T6 exhibited the lowest hardness (9.86 N) and chewiness (289.25 mJ), while the control remained significantly firmer. This softening effect may be attributed to the water-binding and gelling properties of pectin, along with the interaction of essential oils with muscle proteins, which can alter structural integrity and reduce firmness (Rolin, 1993; Mohammed & Alrefiee, 2021; Zheng *et al.,* 2023). In summary, the combined use of durian rind pectin with basil or oregano essential oils significantly reduced hardness and chewiness of beef patties during storage, without compromising cohesiveness. These changes may enhance tenderness and improve consumer acceptability in cooked meat products.

Table 4. *Effect of durian rind pectin (DP) and basil and oregano essential oils (EOs) supplementation on the textural profile of beef patties during refrigerated storage (mean ± SD)*

Item	T1 (Control)	T2 (BHT 0.02%)	T3 (DP 1% + Basil EO 1%)	T4 (DP1% + Oregano EO 1%)	T5 (DP 1% + Basil EO 2%)	T6 (DP 1% + Oregano EO 2%)
			Day 0			
Hardness (N)	$18.92^{a} \pm 0.95$	$18.09^{a} \pm 1.23$	$13.78^{b} \pm 0.75$	$14.05^{b} \pm 0.73$	$14.20^{b} \pm 0.68$	$14.69^{b} \pm 1.03$
Cohesiveness	0.52 ± 0.08	0.53 ± 0.02	0.53 ± 0.06	0.50 ± 0.01	0.53 ± 0.03	0.50 ± 0.04
Adhesiveness (mJ)	1.75 ± 0.50	1.75 ± 0.50	2.00 ± 0.82	1.75 ± 0.50	1.50 ± 0.58	1.75 ± 0.50

Table 4. Continued

Item	T1 (Control)	T2 (BHT 0.02%)	T3 (DP 1% + Basil EO 1%)	T4 (DP1% + Oregano EO 1%)	T5 (DP 1% + Basil EO 2%)	T6 (DP 1% + Oregano EO 2%)
			Day 0			
Springiness (mm)	$21.68^{a} \pm 0.71$	$17.34^{b} \pm 0.45$	$6.16^{c} \pm 0.11$	$6.16^{c} \pm 0.13$	$6.13^{c} \pm 0.15$	$6.16^{c} \pm 0.20$
Gumminess (N)	$15.16^{a} \pm 1.02$	$11.98^{b} \pm 0.88$	$8.11^{c} \pm 0.62$	$7.96^{c} \pm 0.69$	$8.10^{c} \pm 0.48$	$8.27^{c} \pm 0.43$
Chewiness (mJ)	$2,467.25^{a} \pm 80.39$	$2,305.50^{b} \pm 47.96$	$944.25^{c} \pm 30.03$	$754.25^{d} \pm 26.08$	$632.50^{e} \pm 6.56$	$455.25^{f} \pm 35.53$
			Day 4			
Hardness (N)	$27.69^{a} \pm 0.86$	$26.07^{a} \pm 1.34$	$18.72^{b} \pm 1.20$	$18.53^{b} \pm 0.83$	$17.70^{bc} \pm 1.83$	$15.66^{c} \pm 2.36$
Cohesiveness	0.41 ± 0.03	0.40 ± 0.01	0.41 ± 0.03	0.41 ± 0.03	0.41 ± 0.02	0.41 ± 0.02
Adhesiveness (mJ)	1.50 ± 0.58	1.50 ± 0.58	1.25 ± 0.50	1.50 ± 0.58	1.50 ± 0.58	1.50 ± 0.58
Springiness (mm)	$6.16^{ab} \pm 0.16$	$5.75^{c} \pm 0.11$	$5.85^{bc} \pm 0.41$	$6.24^{a} \pm 0.17$	$5.97^{abc} \pm 0.14$	$5.96^{abc} \pm 0.03$
Gumminess (N)	$15.99^{a} \pm 1.02$	$13.40^{b} \pm 0.69$	$11.98^{b} \pm 0.75$	$11.92^{b} \pm 1.18$	$12.29^{b} \pm 1.16$	$12.42^{b} \pm 0.34$
Chewiness (mJ)	$1,041.75^{a} \pm 72.80$	$819.25^{b} \pm 35.91$	$607.50^{c} \pm 52.44$	$562.25^{cd} \pm 51.94$	$610.50^{c} \pm 33.31$	$488.00^{d} \pm 68.15$
			Day 8			
Hardness (N)	$23.19^{a} \pm 0.75$	$19.99^{b} \pm 2.52$	$15.75^{c} \pm 0.93$	$13.29^{d} \pm 1.51$	$11.39^{de} \pm 0.95$	$9.86^{e} \pm 0.55$
Cohesiveness	0.44 ± 0.01	0.45 ± 0.03	0.45 ± 0.02	0.45 ± 0.03	0.44 ± 0.01	0.44 ± 0.03
Adhesiveness (mJ)	1.75 ± 0.96	1.75 ± 0.96	2.00 ± 0.82	1.75 ± 0.96	1.75 ± 0.96	2.00 ± 0.82
Springiness (mm)	$6.81^{a} \pm 0.64$	$6.49^{ab} \pm 0.76$	$5.78^{bc} \pm 0.39$	$5.83^{bc} \pm 0.25$	$5.80^{bc} \pm 0.35$	$5.62^{c} \pm 0.28$
Gumminess (N)	$11.09^{a} \pm 1.29$	$10.64^{a} \pm 0.68$	$5.99^{b} \pm 0.47$	$5.94^{b} \pm 0.42$	$5.91^{b} \pm 0.30$	$5.92^{b} \pm 0.29$
Chewiness (mJ)	$775.25^{a} \pm 30.51$	$595.75^{b} \pm 38.08$	$463.00^{c} \pm 37.07$	$480.75^{c} \pm 29.84$	$340.75^{d} \pm 18.98$	$289.25^{e} \pm 23.98$

Note: $^{a-c}$ different superscript letters in the same row indicate statistically significant differences among groups ($p < 0.05$)

Source: authors' own research

Table 5 presents the oxidative stability of beef patties evaluated via DPPH radical scavenging activity and TBARS values during refrigerated storage. All treated samples (T2-T6) showed significantly higher DPPH activity and lower TBARS values compared to the control (T1) at every time point ($p < 0.05$). The strongest antioxidant capacity was observed in T6 (1% pectin + 2% oregano oil) and T5 (1% pectin + 2% basil oil), with DPPH scavenging activities of 67.95 ± 2.60% and 62.03 ± 3.25%, respectively, on day 0. This pattern persisted through day 8, indicating a sustained antioxidant effect.

Table 5. *Effect of durian rind pectin (DP) and basil and oregano essential oils (EOs) supplementation on the textural profile of beef patties during refrigerated storage (mean ± SD)*

Item	T1 (Control)	T2 (BHT 0.02%)	T3 (DP 1% + Basil EO 1%)	T4 (DP1% + Oregano EO 1%)	T5 (DP 1% + Basil EO 2%)	T6 (DP 1% + Oregano EO 2%)
			DPPH scavenging activity (%)			
Day 0	$34.92^{e} \pm 0.94$	$42.91^{d} \pm 2.14$	$50.53^{c} \pm 2.29$	$55.58^{c} \pm 4.32$	$62.03^{b} \pm 3.25$	$67.95^{a} \pm 2.60$

Table 5. Continued

Item	T1 (Control)	T2 (BHT 0.02%)	T3 (DP 1% + Basil EO 1%)	T4 (DP1% + Oregano EO 1%)	T5 (DP 1% + Basil EO 2%)	T6 (DP 1% + Oregano EO 2%)
	DPPH scavenging activity (%)					
Day 4	$31.69^{d} \pm 4.75$	$51.88^{c} \pm 5.65$	$81.69^{b} \pm 2.38$	$86.39^{ab} \pm 1.01$	$90.74^{a} \pm 0.67$	$87.60^{a} \pm 1.16$
Day 8	$27.18^{e} \pm 0.83$	$41.87^{d} \pm 2.52$	$63.93^{c} \pm 4.0$	$75.25^{b} \pm 2.88$	$80.19^{a} \pm 1.29$	$80.93^{a} \pm 1.32$
	TBARS (µg MDA/g meat)					
Day 0	$0.038^{a} \pm 0.002$	$0.033^{b} \pm 0.002$	$0.032^{b} \pm 0.001$	$0.034^{b} \pm 0.002$	$0.033^{b} \pm 0.001$	$0.033^{b} \pm 0.001$
Day 4	$0.097^{a} \pm 0.005$	$0.087^{b} \pm 0.001$	$0.066^{c} \pm 0.003$	$0.066^{d} \pm 0.004$	$0.064^{d} \pm 0.004$	$0.059^{e} \pm 0.002$
Day 8	$0.190^{a} \pm 0.003$	$0.146^{b} \pm 0.004$	$0.102^{c} \pm 0.003$	$0.097^{d} \pm 0.002$	$0.087^{e} \pm 0.002$	$0.080^{f} \pm 0.002$

Note: $^{a-f}$ different superscript letters in the same row indicate statistically significant differences among groups ($p < 0.05$)

Source: authors' own research

Lipid oxidation, indicated by increasing TBARS values over time, was significantly suppressed in all treated groups compared to the control. On day 8, T6 exhibited the lowest TBARS, followed by T5 and T4, demonstrating the effective inhibitory role of pectin-essential oil combinations on lipid peroxidation. Notably, these natural treatments outperformed BHT (T2), a common synthetic antioxidant. The improved oxidative stability likely stems from synergistic interactions between durian rind pectin and the bioactive components of essential oils. Previous studies have shown similar effects of pectin from Jerusalem artichoke, mangosteen peel, and other vegetables (Liu *et al.*, 2016; Wathoni *et al.*, 2019). The antioxidant activity of basil oil is attributed to compounds like methyl chavicol, linalool, and eugenol, while oregano oil owes its effect to carvacrol and thymol (Hossain *et al.*, 2010; Suntres *et al.*, 2015; Ahmed *et al.*, 2019). Comparable outcomes were reported in beef products by A. Falowo *et al.* (2019). Although BHT is widely used, its comparatively lower performance reinforces the growing interest in natural alternatives (Yehye *et al.*, 2015). These findings support the application of durian rind pectin combined with basil or oregano oil as promising clean-label preservatives to enhance oxidative stability in meat products.

Table 6 presents the microbial counts of beef patties supplemented with durian rind pectin and basil or oregano essential oils during refrigerated storage. Across all time points, samples treated with pectin and essential oils exhibited significantly lower ($p < 0.05$) total viable counts (TVC) and *E. coli* levels compared to the control and BHT-treated samples. On day 0, the TVC of treated samples ranged from 6.51 ± 0.02 to 6.63 ± 0.02 log CFU/g, significantly lower than the control (6.73 ± 0.03) and BHT group (6.75 ± 0.02). The lowest count was recorded in T6 (durian pectin + 2% oregano EO). *E. coli* counts followed a similar trend, with T6 exhibiting the lowest value (4.67 ± 0.02 log CFU/g). During storage, microbial growth increased in all samples; however, the rate of increase was significantly suppressed in treatments containing essential oils. On day 4, the TVC in the control reached 8.06 ± 0.02 log CFU/g, while T6 remained significantly lower at 6.66 ± 0.03 log CFU/g. By day 8, T6 again showed the lowest bacterial load (6.83 ± 0.02 log CFU/g), followed closely by T4 and T5. In contrast, the control and BHT groups exceeded 9.30 log CFU/g. *E. coli* counts also remained consistently lower in all pectin-essential oil treatments across storage. On day 8, the lowest *E. coli* level was found in T6 (5.16 ± 0.01 log CFU/g), compared to 7.15 ± 0.13 log CFU/g in the control. No *Salmonella spp.* was detected in any sample throughout the storage period.

Table 6. *Effect of durian rind pectin (DP) and basil and oregano essential oils (EOs) supplementation on bacteria counts (log10 CFU/g) of beef patties during refrigerated storage (mean ± SD)*

Item	T1 (Control)	T2 (BHT 0.02%)	T3 (DP 1% + Basil EO 1%)	T4 (DP1% + Oregano EO 1%)	T5 (DP 1% + Basil EO 2%)	T6 (DP 1% + Oregano EO 2%)
			Day 0			
Total bacteria	$6.73^{a} \pm 0.03$	$6.75^{a} \pm 0.02$	$6.63^{b} \pm 0.02$	$6.62^{b} \pm 0.02$	$6.57^{c} \pm 0.02$	$6.51^{c} \pm 0.02$
E. coli	$4.86^{a} \pm 0.04$	$4.86^{a} \pm 0.02$	$4.83^{ab} \pm 0.02$	$4.74^{c} \pm 0.03$	$4.80^{b} \pm 0.03$	$4.67^{d} \pm 0.02$
Salmonella spp.	none	none	none	none	none	none
			Day 4			
Total bacteria	$8.06^{a} \pm 0.02$	$8.03^{a} \pm 0.02$	$6.88^{b} \pm 0.03$	$6.71^{c} \pm 0.05$	$6.87^{b} \pm 0.05$	$6.66^{c} \pm 0.03$
E. coli	$6.40^{a} \pm 0.02$	$6.41^{a} \pm 0.02$	$4.98^{b} \pm 0.03$	$4.92^{cd} \pm 0.02$	$4.94^{c} \pm 0.02$	$4.88^{d} \pm 0.02$
Salmonella spp.	none	none	none	none	none	none
			Day 8			
Total bacteria	$9.31^{a} \pm 0.01$	$9.30^{a} \pm 0.03$	$7.17^{b} \pm 0.04$	$6.90^{d} \pm 0.02$	$7.11^{c} \pm 0.04$	$6.83^{e} \pm 0.02$
E. coli	$7.15^{a} \pm 0.13$	$7.05^{b} \pm 0.02$	$5.30^{b} \pm 0.02$	$5.24^{bc} \pm 0.02$	$5.26^{bc} \pm 0.01$	$5.16^{c} \pm 0.01$
Salmonella spp.	none	none	none	none	none	none

Note: $^{a-e}$ – different superscript letters in the same row indicate statistically significant differences among groups ($p < 0.05$). None – not found in 5 g of beef patties

Source: authors' own research

These results demonstrate that the combination of durian rind pectin with basil or oregano essential oils significantly reduced total viable counts (TVC) and Escherichia coli levels in beef patties during refrigerated storage compared to both the control and BHT-treated groups ($p < 0.05$), with the strongest effect observed in the group containing 2% oregano essential oil (T6). The antimicrobial activity is largely attributed to the active compounds present in the essential oils. Basil essential oil contains methyl chavicol, linalool, and eugenol, which have been reported to disrupt bacterial membranes and inhibit microbial metabolism (Hossain *et al*., 2010). A. Javid *et al*. (2024) developed a basil oil-infused bacterial cellulose foam for meat preservation and observed effective suppression of microbial growth. Oregano essential oil, rich in carvacrol and thymol, has been shown to damage bacterial cell membranes and induce leakage of cellular components, explaining the strong antimicrobial activity observed in the present study. No *Salmonella* spp. was detected in any of the beef patties throughout storage. This result complies with Thai food safety regulations, which require the absence of *Salmonella* spp. in 25 g of chilled meat products. Although microbial testing in this study used 5 g samples, the outcome remains within regulatory limits. The combined use of durian rind pectin and essential oils, particularly oregano oil at 2%, effectively suppressed spoilage and pathogenic bacteria during cold storage. These findings highlight the potential of these natural additives as effective antimicrobial agents for improving microbial safety and extending the shelf life of meat products.

Conclusions

This study demonstrated that durian rind pectin (DP), when combined with basil or oregano essential oils (EOs), significantly improved the quality and storage stability of beef patties during refrigerated storage. Among all treatments, the combination of 1% DP and 2% oregano oil (T6) showed the most consistent and pronounced effects. Oxidative stability was markedly enhanced in treated samples. On day 8, DPPH radical scavenging activity reached 80.93% in T6 compared to 27.18% in the control.

Lipid oxidation, measured by TBARS, was significantly reduced from 0.190 µg MDA/g in the control to 0.080 µg MDA/g in T6 ($p < 0.05$), indicating substantial inhibition of oxidative deterioration. Microbiological quality was also significantly improved. After 8 days of storage, total viable counts (TVC) increased to 9.31 log CFU/g in the control, whereas T6 maintained a significantly lower level of 6.83 log CFU/g. Similarly, *Escherichia coli* counts were reduced from 7.15 log CFU/g in the control to 5.16 log CFU/g in T6. No *Salmonella* spp. were detected in any treatment throughout storage. Technological properties were positively affected. Cooking loss decreased from 45.80% in the control to 39.75% in T6 on day 8. Texture analysis revealed substantial reductions in hardness (23.19 N vs. 9.86 N) and chewiness (775.25 mJ vs. 289.25 mJ), indicating improved tenderness. Overall, the incorporation of durian rind pectin combined with oregano essential oil at 2% effectively enhanced oxidative stability, suppressed microbial growth, and improved texture, supporting its potential as a natural alternative to synthetic preservatives such as BHT in beef patties. Future research prospects include investigating consumer sensory acceptance, optimising pectin and essential oil concentrations for industrial application, elucidating the mechanisms of antimicrobial action in meat systems, and evaluating the effectiveness of this combination in other types of meat products and under extended storage conditions.

Acknowledgements

None.

Funding

This research was financially supported by Prince of Songkla University, Surat Thani Campus, 2024 Grant No. IAF6703032S.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Ahmed, A.F., Attia, F.A.K., Liu, Z., Li, C., Wei, J., & Kang, W. (2019). Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. *Food Science and Human Wellness*, 8(3), 299-305. doi: 10.1016/j.fshw.2019.07.004.

[2] Balouiri, M., Sadiki, M., & Ibnsouda, S.K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71-79. doi: 10.1016/j.jpha.2015.11.005.

[3] Falowo, A.B., Mukumbo, F.E., Idamokoro, E.M., Afolayan, A.J., & Muchenje, V. (2019). Phytochemical constituents and antioxidant activity of sweet basil (*Ocimum basilicum* L.) essential oil on ground beef from Boran and Nguni cattle. *International Journal of Food Science*, 2019, article number 2628747. doi: 10.1155/2019/2628747.

[4] Hossain, M.A., Kabir, M.J., Salehuddin, S.M., Rahman, S.M.M., Das, A.K., Singha, S.K., Alam, K., & Rahman, A. (2010). Antibacterial properties of essential oils and methanol extracts of sweet basil (*Ocimum basilicum*) occurring in Bangladesh. *Pharmaceutical Biology*, 48(5), 504-511. doi: 10.3109/13880200903190977.

[5] Javid, A.L., Almasi, H., Molaei, R., & Moradi, M. (2024). Basil essential oil-incorporated bacterial nanocellulose foam: Fabrication, characterization, and application in extending ground beef shelf life. *Journal of Agriculture and Food Research*, 18, article number 101453. doi: 10.1016/j.jafr.2024.101453.

[6] Karabagias, I., Badeka, A., & Kontominas, M.G. (2011). Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. *Meat Science*, 88(1), 109-116. doi: 10.1016/j.meatsci.2010.12.010.

[7] Khaled, G., Galil, H.A., Salama, H., Bkear, N., & Ali, E. (2023). Effect of basil essential oil on caprine meat quality and shelf-life. *Damanhour Journal of Veterinary Sciences*, 9(2), 26-32. doi: 10.21608/djvs.2023.182014.1105.

[8] Liu, Y., Dong, M., Yang, Z., & Pan, S. (2016). Anti-diabetic effect of citrus pectin in diabetic rats and potential mechanism via PI3K/Akt signaling pathway. *International Journal of Biological Macromolecules*, 89, 484-488. doi: 10.1016/j.ijbiomac.2016.05.015.

[9] Mohammed, A.A.A., & Alrefiee, A.A. (2021). Effect of adding basil and oregano essential oils on the physicochemical characteristics of camel meat burger during cold storage. *American Journal of Food Technology*, 16(1), 38-46. doi: 10.3923/ajft.2021.38.46.

[10] Namir, M., Siliha, H., & Ramadan, M.F. (2015). Fiber pectin from tomato pomace: Characteristics, functional properties and application in low-fat beef burger. *Food Measure*, 9, 305-312. doi: 10.1007/s11694-015-9236-5.

[11] Pastsart, U., Sresomjit, F., Bochuai, R., & Pimpa, O. (2024). Texture profile, water holding capacity, antioxidant activity and lipid oxidation of beef during retail display from cattle fed total mixed ration supplemented with *Capsicum frutescens* L. and *Curcuma longa* L. powders. *Online Journal of Animal and Feed Research*, 14(1), 40-46. doi: 10.51227/ojafr.2024.5.

[12] Pérez-Conesa, D., Cao, J., Chen, L., McLandsborough, L., & Weiss, J. (2011). Inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 biofilms by micelle-encapsulated eugenol and carvacrol. *Journal of Food Protection*, 74(1), 55-62. doi: 10.4315/0362-028X.JFP-08-403.

[13] Rolin, C. (1993). Pectin. In R.L. Whistler & J.N. Bemiller (Eds.), *Industrial gums: Polysaccharides and their derivatives* (3rd ed.) (pp. 257-293). Cambridge: Academic Press. doi: 10.1016/B978-0-08-092654-4.50014-0.

[14] Sahu, A., Nayak, G., Bhuyan, S.K., Bhuyan, R., Kar, D., & Kuanar, A. (2023). Antioxidant and antimicrobial activities of *Ocimum basilicum* var. thyrsiflora against some oral microbes. *Multidisciplinary Science Journal*, 6(3), article number e2024026. doi: 10.31893/multiscience.2024026.

[15] Salam, S., Kwanonin, K., Nakawiroj, P., & Pastsart, U. (2024). Quality, textural profiles, oxidative stability and bacterial content during retail display of beef patties formulated with pectin powder. *Food Agricultural Sciences and Technology*, 10(2), 43-54. doi: 10.14456/fast.2024.11.

[16] Semeniuc, C.A., Pop, C.R., & Rotar, A.M. (2017). Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *Journal of Food and Drug Analysis*, 25(2), 403-408. doi: 10.1016/j.jfda.2016.06.002.

[17] Sharma, O.P., & Bhat, T.K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, 113(4), 1202-1205. doi: 10.1016/j.foodchem.2008.08.008.

[18] Srikamwang, C., Willats, W.G.T., Bakshani, C.R., Sommano, S.R., & Wongkaew, M. (2024). Potentials of Mahachanok mango peel pectin in modulating glycaemic index in simulated *in vitro* carbohydrate digestion of meat product. *Journal of Agriculture and Food Research*, 18, article number 101304. doi: 10.1016/j.jafr.2024.101304.

[19] Suntres, Z.E., Coccimiglio, J., & Alipour, M. (2015). The bioactivity and toxicological actions of carvacrol. *Critical Reviews in Food Science and Nutrition*, 55(3), 304-318. doi: 10.1080/10408398.2011.653458.

[20] Tajkarimi, M.M., Ibrahim, S.A., & Cliver, D.O. (2010). Antimicrobial herb and spice compounds in food. *Food Control*, 21(9), 1199-1218. doi: 10.1016/j.foodcont.2010.02.003.

[21] Tarladgis, B.G., Watts, B.M., Younathan, M.T., & Dugan, L. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society*, 37(1), 44-48. doi: 10.1007/BF02630824.

[22] Triki, M., Herrero, A.M., Jiménez-Colmenero, F., & Ruiz-Capillas, C. (2018). Quality assessment of fresh meat from several species based on free amino acid and biogenic amine contents during chilled storage. *Foods*, 7(9), article number 132. doi: 10.3390/foods7090132.

[23] Unhasirikul, M., Unhasirikul, K., Wisuttipat, S., Wutthanasikunchai, H., & Suwanposri, A. (2021). Extraction of pectin from durian husks for use in the production of jams from low prices of Salacca zalacca and Salacca wallichiana Mart in Chanthaburi province. *Journal of Science and Technology, Ubon Ratchathani University*, 23(1), 81-90.

[24] Walasek-Janusz, M., Grzegorczyk, A., Malm, A., Nurzyńska-Wierdak, R., & Zalewski, D. (2024). Chemical composition and antioxidant and antimicrobial activity of oregano essential oil. *Molecules*, 29(2), article number 435. doi: 10.3390/molecules29020435.

[25] Wang, L., Guo, H., Liu, X., Jiang, G., Li, C., Li, X., & Li, Y. (2019). Roles of *Lentinula edodes* as the pork lean meat replacer in production of the sausage. *Meat Science*, 156, 44-51. doi: 10.1016/j.meatsci.2019.05.016.

[26] Wathoni, N., Shan, C.Y., Shan, W.Y., Rostinawati, T., Indradi, R.B., Pratiwi, R., & Muchtaridi, M. (2019). Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garcinia mangostana* L.) rind. *Heliyon*, 5(8), article number e02299. doi: 10.1016/j.heliyon.2019.e02299.

[27] Yehye, W.A., Rahman, N.A., Ariffin, A., Hamid, S.B.A., Alhadi, A.A., Kadir, F.A., & Yaeghoobi, M. (2015). Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review. *European Journal of Medicinal Chemistry*, 101, 295-312. doi: 10.1016/j.ejmech.2015.06.026.

[28] Zheng, K., Li, B., Liu, Y., Wu, D., Bai, Y., & Xiang, Q. (2023). Effect of chitosan coating incorporated with oregano essential oil on microbial inactivation and quality properties of refrigerated chicken breasts. *LWT*, 176, article number 114547. doi: 10.1016/j.lwt.2023.114547.

Вплив пектину зі шкірки дуріана у поєднанні з ефірними оліями базиліку та орегано на якість яловичих котлет

Умапон Пастсарт
Доктор філософії з прикладних біологічних наук, доцент
Факультет інноваційного сільського господарства, рибальства та харчових технологій, Університет Принца Сонгкли, кампус Суратхані
84000, вул. 31 Му, 6, м. Суратхані, Таїланд
https://orcid.org/0000-0003-1844-1085

Саніта Салам
Магістр
Факультет інноваційного сільського господарства, рибальства та харчових технологій, Університет Принца Сонгкли, кампус Суратхані
84000, вул. 31 Му, 6, м. Суратхані, Таїланд
https://orcid.org/0009-0008-1023-3410

Джессада Раттанавут
Доктор тваринницьких наук, доцент
Факультет інноваційного сільського господарства, рибальства та харчових технологій, Університет Принца Сонгкли, кампус Суратхані
84000, вул. 31 Му, 6, м. Суратхані, Таїланд
https://orcid.org/0000-0003-1967-1114

Патіма Пермпунпаттана
Кандидат біологічних наук, асистент професора
Факультет інноваційного сільського господарства, рибальства та харчових технологій, Університет Принца Сонгкли, кампус Суратхані
84000, вул. 31 Му, 6, м. Суратхані, Таїланд
https://orcid.org/0000-0002-0320-7184

Чатчаван Чотімаркорн
Доктор харчових та технологічних наук, асистент професора
Факультет інноваційного сільського господарства, рибальства та харчових технологій, Університет Принца Сонгкли, кампус Суратхані
84000, вул. 31 Му, 6, м. Суратхані, Таїланд
https://orcid.org/0000-0002-7522-4549

Анотація. Метою дослідження було оцінити вплив пектину з шкірки дуріану (DP) у поєднанні з ефірними оліями базиліку або орегано на якість і термін зберігання яловичих котлет під час холодильного зберігання як натуральної альтернативи синтетичним добавкам. Було сформовано шість варіантів: T1 (контроль), T2 (0,02 % BHT), T3 (1% DP + 1 % олії базиліку), T4 (1 % DP + 1 % олії орегано), T5 (1 % DP + 2 % олії базиліку) та T6 (1 % DP + 2 % олії орегано). Фізико-хімічні, мікробіологічні та антиоксидантні показники контролювали протягом 8 діб за температури 4 °C. Внесення пектину та ефірних олій достовірно ($p < 0,05$) впливало на pH, колір (L*, a*, b*), втрати під час термічної обробки та текстурні характеристики. Обробки з олією базиліку знижували значення pH, тоді як олія орегано сприяла його підвищенню. Дослідні зразки характеризувалися вищими початковими показниками світлоти, червоності та жовтизни, однак значення a* та b* зменшувалися до 8-ї доби зберігання. Втрати під час

термічної обробки суттєво зменшилися, а ніжність покращилася в усіх дослідних групах, що підтверджувалося зниженням твердості, гумоподібності, жувальності та пружності, без істотних змін у показниках когезивності та адгезивності. Котлети з додаванням пектину та ефірних олій продемонстрували вищу DPPH-активність (63-80 % проти 27 % у контролі) та нижчі значення TBARS (0,08-0,10 мкг МДА/г проти 0,19 мкг МДА/г у контролі) ($p < 0,05$), що свідчить про підвищену окисну стабільність. Мікробіологічний аналіз показав достовірне зниження загальної кількості життєздатних мікроорганізмів і рівня *E. coli* у дослідних зразках протягом усього періоду зберігання. *Salmonella* spp. не виявлено. Загалом поєднання пектину з шкірки дуріану з ефірними оліями базиліку або орегано покращило якість та подовжило термін зберігання яловичих котлет ($p < 0,05$), демонструючи перспективність використання натуральних консервантів як альтернативи синтетичним у м'ясних продуктах. Результати дослідження можуть бути використані м'ясопереробними підприємствами для виробництва безпечніших і більш стабільних під час зберігання охолоджених продуктів із подрібненого м'яса з використанням натуральних консервантів

Ключові слова: продукти з подрібненого м'яса; натуральні консерванти; окисна стабільність; подовження терміну зберігання; використання агровідходів

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 17(1), 77-92
Received 11.10.2025 Revised 25.01.2026 Accepted 26.02.2026
Published 26.03.2026

UDC 631.831:664.8:579.6
DOI: 10.31548/animal.1.2026.77

ISSN 2706-8331
e-ISSN 2706-834X

Physicochemical properties and sensory acceptance of honey-enriched chickpea (*Cicer arietinum* L.) yoghurt

Naila Amani Fatiha Putri
Bachelor
Diponegoro University
50275, Prof. H. Soedarto Str., Semarang, Indonesia
https://orcid.org/0009-0005-2323-0452

Naila Ar-rifdah Noerrosadi
Bachelor
Diponegoro University
50275, Prof. H. Soedarto Str., Semarang, Indonesia
https://orcid.org/0009-0008-3167-2383

Yasmin Aulia Rachma
Master, Lecturer
Diponegoro University
50275, Prof. H. Soedarto Str., Semarang, Indonesia
https://orcid.org/0000-0002-1543-005X

Yoyok Budi Pramono*
Professor
Diponegoro University
50275, Prof. H. Soedarto Str., Semarang, Indonesia
https://orcid.org/0000-0002-0550-545X

Abstract. The rising demand for functional plant-based foods has driven the use of legumes as a viable alternative to traditional dairy-based yoghurt. Chickpea (*Cicer arietinum* L.) offers high protein, fibre, and bioactive compounds, while honey may improve fermentation, texture, and antioxidant properties. This study aimed to evaluate the effect of honey addition (0%,

Suggested Citation:
Putri, N.A., Noerrosadi, N.A., Rachma, Y.A., & Pramono, Y.B. (2026). Physicochemical properties and sensory acceptance of honey-enriched chickpea (*Cicer arietinum* L.) yoghurt. *Animal Science and Food Technology*, 17(1), 77-92. doi: 10.31548/animal.1.2026.77.

*Corresponding author

2%, 4%, and 6%) on the physicochemical characteristics, antioxidant activity, and sensory acceptance of chickpea-based yoghurt. Chickpea milk supplemented with skimmed milk was fermented using lactic acid bacteria and analysed for pH, titratable acidity, total soluble solids (TSS), syneresis, antioxidant activity, and consumer acceptance. Honey addition significantly improved the physicochemical and sensory properties of chickpea yoghurt by enhancing fermentation performance (higher titratable acidity, total soluble solids, and antioxidant activity), lowering pH and syneresis values. Sensory evaluation demonstrated increased taste and overall acceptance with increasing honey concentration, whereas aroma and texture were not significantly affected. Overall, 6% honey provided the best balance of stability, functionality, and consumer preference, indicating its potential as an optimal formulation for plant-based yoghurt development. These findings highlighted the role of honey as a natural functional ingredient that enhanced both technological quality and consumer acceptability in legume-based fermented products. The findings of this study can be utilised by plant-based food manufacturers and small-scale producers to develop functional yoghurt alternatives with improved nutritional and sensory profiles, addressing the growing market demand for fermented products

Keywords: plant-based fermentation; lactic acid bacteria; prebiotic sweetener; antioxidant activity; sensory evaluation

Introduction

The increasing global demand for functional foods reflects a shift in consumer awareness toward products that promote health beyond basic nutrition. At the same time, plant-based dietary patterns are gaining momentum due to lactose intolerance prevalence, sustainability concerns, and the environmental impact associated with conventional dairy production. Fermented foods play an important role within this development because fermentation enhances digestibility, improves nutrient bioavailability, and introduces beneficial microbial activity. Yoghurt, traditionally produced from milk, is widely recognised as a carrier of probiotic bacteria and bioactive compounds. However, replacing dairy substrates with plant-based materials introduces technological challenges, particularly in achieving stable gel formation, acceptable texture, and balanced sensory characteristics. These limitations highlight the need for systematic formulation strategies to improve the quality of plant-based fermented products.

The fundamental mechanism of yoghurt formation relies on lactic acid bacteria metabolism. R. Rahmawati *et al.* (2022) explained that lactic acid production during fermentation decreases pH and induces protein coagulation, resulting in gel structure development and characteristic flavour. R. Masriatini *et al*. (2023) indicated in their work that *Lactobacillus bulgaricus* and *Streptococcus thermophilus* can survive acidic gastrointestinal conditions and potentially contribute to intestinal microbial balance. In plant-based substrates, the absence of lactose becomes a limiting factor during fermentation. S. Pannerchelvan *et al*. (2024) demonstrated that plant milks lack sufficient fermentable sugars to support optimal bacterial growth, often leading to weak gel structures and lower acidification rates. These findings emphasise that carbohydrate availability plays a crucial role in determining fermentation efficiency in non-dairy systems.

Chickpea is a plant-based raw material containing protein, dietary fibre, and phenolic

compounds. N. Kumar *et al.* (2025) observed that chickpea proteins can form a protein matrix during fermentation, although the resulting structure is generally less compact than that of dairy yoghurt. The same study also reported that fermentation of chickpea-based substrates may produce a beany flavour associated with lower consumer acceptance. In addition, Y. Sari *et al.* (2024) indicated that the addition of complementary ingredients is required to improve flavour and structural stability in legume-based yoghurt systems. These findings show that chickpea-based yoghurt requires formulation adjustment to obtain appropriate physicochemical and sensory characteristics. Honey has also received attention as a multifunctional ingredient in fermented foods. T. Baltić *et al.* (2025) reported that the glucose and fructose present in honey serve as readily fermentable substrates that stimulate lactic acid bacteria growth. M. Rahardjo *et al.* (2022) further demonstrated that phenolic and flavonoid compounds in honey contribute antioxidant activity and may influence microbial stability during fermentation. While previous research has mainly focused on honey supplementation in dairy yoghurt systems, its interaction with plant proteins during fermentation has received limited scientific attention. In particular, the role of honey concentration in influencing gel formation, acidity development, viscosity, and sensory perception in chickpea-based yoghurt remains insufficiently clarified.

The concentration of honey may significantly affect both microbial metabolism and physicochemical stability. Excessive sugar addition can increase osmotic pressure, potentially altering bacterial activity and fermentation kinetics. Conversely, inadequate carbohydrate supplementation may limit acid production and weaken protein aggregation. In legume-based systems where protein network formation is inherently weaker than in dairy matrices, the balance between carbohydrate availability and protein interactions becomes especially critical. In addition, determining the appropriate sweetener level is important from a technological perspective because small variations in sugar concentration can modify water binding capacity, gel firmness, and whey separation in plant protein systems. Therefore, evaluating concentration-dependent effects is necessary to ensure that improvements in fermentation performance are accompanied by stable physical properties and acceptable sensory perception. Despite growing interest in plant-based yoghurt innovation, few studies have systematically examined how varying levels of honey influence fermentation performance and product quality in chickpea-based substrates.

This study addressed that gap by investigating the influence of graded honey concentrations on the fermentation behaviour, physicochemical characteristics, and sensory acceptance of chickpea-based yoghurt to determine an optimal formulation that balances microbial activity, structural stability, and consumer preference.

Materials and Methods

The experimental work was conducted in 2025 at the Food Engineering and Agricultural Products Laboratory, Food Chemistry and Nutrition Laboratory, Faculty Animal Husbandry and Agriculture, Diponegoro University, and Cendekia Nanotech Hutama Laboratory, Semarang. Physicochemical analysis including the analysis of pH, total titratable acidity, syneresis, and total soluble solids were performed at the Food Engineering and Agricultural Products Laboratory, Food Chemistry and Nutrition Laboratory, Faculty Animal Husbandry and Agriculture, Diponegoro University and the antioxidant activity were performed at the Cendekia Nanotech Hutama Laboratory, Semarang. The materials used in this study were high-quality peeled chickpeas, skimmed milk, yoghurt starter, pure honey, baking soda, water, distilled water, pH 4 and 7 buffer solutions, 0.1 N NaOH, and 1% Phenolphthalein (PP) indicator. The equipment

used in this study included digital scales, filter cloth, blender, Erlenmeyer flasks, measuring cups, beaker glass, thermometer, spatula, glass jar, pot, refractometer, Ohaus centrifuge, Brookfield viscometer, pH meter (Ohaus, USA), burette stands, and plastic cups.

Chickpea (*Cicer arietinum* L.) were sorted and washed with fresh water, then soaked in water at a ratio of 1:5 with 0.25% added baking soda. Chickpea were soaked for 24 hours to reduce the "beany" taste and aroma, and also to soften the texture of chickpea. After soaked, chickpeas were rinsed, then blended with added water (1:5, w/v), and filtered through filter cloth to obtain the chickpea milk. The filtrate then pasteurised before being processed into yoghurt. Chickpea milk was added with 5% (w/v) skimmed milk and honey according to the treatment level, where the treatments are P0 (0% honey), P1 (2% honey), P2 (4% honey), and P3 (6% honey), then homogenised and heated at 72°C for 10 minutes. The mixture is cooled down to 40°C and is inoculated with yoghurt starter culture, then incubated at 37°C for 10 hours.

The pH value was determined using a calibrated digital pH meter (Ohaus, USA). Titratable acidity was measured by titrating 10 mL of yoghurt sample with 0.1 N NaOH using phenolphthalein as an indicator until a persistent pink equivalent was reached. Results were expressed as percentage of lactic acid and calculated using the following formula (1):

$$\text{total Asam} = \frac{V1 \times N \times B}{V2 \times 1{,}000 \times 100} \times 100\%, \qquad (1)$$

where V1 – volume NaOH (mL); V2 – volume sample (mL); N – normality NaOH (0.1 N); B – molecular weight of lactic acid (90).

Total soluble solids were measured using a digital hand refractometer at 25°C and expressed as Brix. Syneresis was determined using a centrifugation method. A total of 15 grams of yoghurt samples was centrifuged at 3,000 rpm for 10 minutes. The separated whey was collected and weighed, and syneresis was expressed as the percentage of whey released relative to the initial sample weight. The percentage of syneresis was calculated using the formula (2):

$$\text{syneresis (\%)} = \frac{W1 - W2}{W1} \times 100\%, \qquad (2)$$

where W1 – initial weight (g); W2 – final weight (g).

Antioxidant activity was evaluated using the DPPH radical scavenging method. Approximately 0.2 grams of the sample was dissolved in ethanol, mixed with 0.1 mM DPPH solution, and incubated in the dark for 30 min. Absorbance was measured at 515 nm using a UV-Vis spectrophotometer. Results were expressed as percentage inhibition of DPPH radicals and was calculated using the following formula (3):

$$\text{\% inhibition} = \left(\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%\right). \qquad (3)$$

Consumer acceptance was evaluated by 30 untrained panellists using a five-point hedonic scale (1 = extremely disliked until 5 = extremely liked) for colour, aroma, taste, texture, and overall acceptance. The sensory evaluation was conducted in accordance with the ethical principles of WMA (1964). Informed consent was obtained from all participants prior to the study, and they were fully briefed on the nature of the samples being evaluated. Data of physicochemical properties (pH, titratable acidity, total soluble solids (TSS), and syneresis) were analysed using one-way analysis of variance (ANOVA) at a 5% significance level followed by Duncan's Multiple Range Test (DMRT). Antioxidant activity was analysed using the descriptive method. Hedonic data were analysed using the non-parametric Kruskal-Wallis test followed by Mann-Whitney test.

Results and Discussion

To better understand the effects of honey enrichment on chickpea yoghurt, the following sections present the detailed physicochemical,

functional, and sensory characteristics of the product. The study evaluated how varying honey concentrations influence key quality parameters, including acidity, soluble solids, pH, syneresis, and antioxidant capacity, as well as consumer acceptance. This approach allows for a comprehensive assessment of honey's role as a natural functional ingredient in enhancing both the technological and sensory qualities of chickpea-based fermented products.

The physicochemical properties of chickpea yoghurt enriched with different levels of honey, including pH, titratable acidity, total soluble solids, and syneresis, are presented in Table 1. Honey addition significantly affected all parameters observed ($p < 0.05$). Higher honey concentrations showed an increase in total titratable acidity and total soluble solids, and a decrease in pH and syneresis value. The antioxidant activity of chickpea yoghurt enriched with different concentrations of honey is presented in Figure 1. Antioxidant activity was evaluated using the DPPH radical scavenging assay to determine the contribution of honey supplementation to the functional properties of the fermented product. And the consumer acceptance test results of chickpea yoghurt are presented in Table 2.

Table 1. *Physicochemical test results of chickpea yoghurt with additional honey*

% Honey	Parameters			
	Titratable acidity (%)	pH	TSS (°Brix)	Syneresis (%)
0	0.20 ± 0.01[a]	4.52 ± 0.01[a]	8.68 ± 0.15[a]	51.27 ± 0.49[a]
2	0.57 ± 0.02[b]	4.43 ± 0.02[b]	9.24 ± 0.02[b]	44.03 ± 0.02[b]
4	0.66 ± 0.03[c]	4.32 ± 0.02[c]	12.76 ± 0.02[c]	43.90 ± 0.02[c]
6	0.81 ± 0.03[d]	4.09 ± 0.02[d]	13.84 ± 0.02[d]	41.67 ± 0.02[d]

Note: [a-d] – values with different lowercase superscript letters in the same column indicate significant differences ($p < 0.05$). Data are presented as mean values from 5 replicates ± standard deviation
Source: developed by the authors

Table 2. *Consumer acceptance test results*

% Honey	Colour	Aroma	Texture	Taste	Overall
0	3.73 ± 0.94[a]	2.67 ± 0.96	3.03 ± 0.93	1.77 ± 0.77[a]	2.33 ± 0.92[a]
2	3.67 ± 0.80[b]	3.03 ± 0.72	3.27 ± 1.01	2.57 ± 1.14[b]	2.67 ± 0.96[b]
4	3.23 ± 0.82[c]	3.03 ± 0.85	2.90 ± 1.09	3.27 ± 1.05[c]	3.20 ± 0.66[c]
6	3.03 ± 0.89[d]	3.13 ± 0.97	3.27 ± 0.83	3.97 ± 0.89[d]	3.87 ± 0.68[d]

Note: [a-d] – values with different lowercase superscript letters in the same column indicate significant differences ($p < 0.05$). Data are presented as mean values from 5 replicates ± standard deviation
Source: developed by the authors

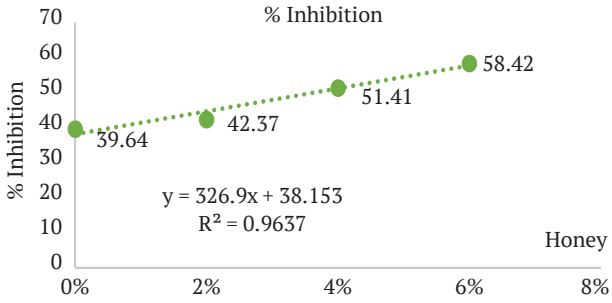

Figure 1. Antioxidant activity (% inhibition)
Source: developed by the authors

Total Titratable Acidity (TTA) values of chickpea yoghurt are presented in Table 1. Honey addition significantly affected chickpea yoghurt ($p < 0.05$). Titratable acidity showed an increasing trend with increasing honey concentration. This result suggests that honey influenced the acidification process during fermentation, which is associated with enhanced metabolic activity of lactic acid bacteria leading to increased organic acid production. P. Ningrum *et al*. (2022) demonstrated that honey supplementation provides additional energy for lactic acid bacteria and supports acid formation during yoghurt fermentation. Based on the result obtained in this study, the titratable acidity of chickpea yoghurt showed lowest value at 0% honey addition (0.20%) and the highest value at 6% honey addition (0.81%). M. Nugroho *et al*. (2023) stated that these values were higher than those observed for cow's milk yoghurt (0.63-0.73%). The higher titratable acidity observed in this study may be attributed to differences in raw materials and fermentation characteristics as chickpea yoghurt addition with honey exhibits a carbohydrate profile distinct from yoghurt produced solely from milk as a lactose source. J. Wang *et al.* (2021) confirm that the presence of fermentable carbohydrate components in chickpea may further enhance organic acid formation during fermentation. R. Rizkyanto & M. Ulfah (2025) emphasised that lactic acid production is driven by the fermentation of available sugars by lactic acid bacteria with substrate availability determining the extent of acid formation. The increased titratable acidity observed in the present study indicates that honey addition, together with the inherent carbohydrate content of chickpea, contributed to enhanced acidification during yoghurt fermentation.

The pH values of chickpea yoghurt are presented in Table 1. Honey addition significantly affected chickpea yoghurt. The pH decreased from 4.52 at 0% honey addition to 4.09 at 6% honey addition, indicating effective fermentation. The reduction in pH reflects increased lactic acid production during fermentation, as lactic acid bacteria (LAB) metabolise available sugars into lactic acid, thereby acidifying the yoghurt matrix. Research by I. Kumalasari & I. Fajriyanti (2024) confirms that sugar availability enhances LAB growth and promotes lactic acid formation, which directly contributes to pH reduction. In the work of Y. Putri *et al.* (2024), it was determined that the lower pH values are associated with improved yoghurt quality and shelf life because acidic conditions suppress spoilage microorganisms and enhance product stability relative to fresh milk. The lower pH observed at higher honey concentrations indicates that honey served as an additional fermentable carbohydrate source during fermentation. This is consistent with the findings of S. Fatimah & R. Agustini (2024) where higher honey levels increased sugar availability with fructose being efficiently metabolised by lactic acid bacteria (LAB), thereby enhancing lactic acid formation and reducing pH. Based on the result obtained in this study, the lowest pH value obtained (4.09) was lower than that reported for cow's milk yoghurt (4.20) by M. Nugroho *et al.* (2023) which may be attributed to differences in raw materials and formulation.

The TSS values of chickpea yoghurt are presented in Table 1. Honey addition significantly affected the TSS values of chickpea yoghurt. Higher honey concentration showed an increase in TSS values, with the highest value observed at 6% honey (13.84 °Brix) and the lowest value observed at 0% honey (8.68 °Brix). The increase in °Brix value indicates that the addition of honey contributes significantly to the accumulation of soluble solids in the yoghurt matrix. It was observed that honey fortification increased the soluble solids in fermented dairy products. The increase in TSS value along with the increase in honey addition is due to the sugar content in honey, especially glucose and fructose, which contribute significantly to the increase in the amount of soluble solids in

yoghurt. According to Z. Albay *et al.* (2025), honey mostly contains sugar and has a high total soluble solids content that can increase the value of the TSS in yoghurt. Sugar plays an important role in the fermentation process of yoghurt by helping the growth of lactic acid bacteria (LAB) that break down fructose during fermentation and are calculated as TSS. Authors D. Kartika *et al*. (2025) indicated in their work that the main component of TSS is sugar which during fermentation, sugar that has not been completely fermented by LAB will be counted as total soluble solids. The nutritional components of chickpea milk can also contribute to the TSS values during processing, particularly during heating and fermentation. Research by A. Sitohang *et al.* (2024) shows that heating especially with high temperatures may facilitate carbohydrate degradation into simple sugars and partial starch gelatinisation, which enhances solid solubility in the yoghurt. Furthermore, the increase in TSS occurs along with a decrease in syneresis value, which occurs because high sugar content can bind water. This is consistent with the findings of A. Famuji *et al.* (2023) that the water-binding capacity of sugars may reduce free water release, which explains the inverse relationship between TSS and syneresis.

The percentage syneresis of chickpea yoghurt is presented in Table 1. The analysis of variance showed that the additional honey significantly affected the syneresis value of chickpea yoghurt ($p < 0.05$). Increasing the concentration of honey showed a decrease in syneresis values, with the highest values observed at 0% honey (51.27%) and the lowest values observed at 6% honey (41.67%). Previous studies by T. Singh *et al.* (2024) have also reported that the reduced syneresis of yoghurt may come from honey. A similar effect was reported by T. Brčina *et al.* (2022) that showed a positive effect of adding honey to yoghurt on viscosity and water retention and may cause the reduction of syneresis. The decrease in syneresis values along with an increase in honey concentration may be due to the ability of honey in yoghurt to bind water. A. Anwar *et al*. (2025) emphasised that fructose content in honey can improve the texture of yoghurt and prevent the syneresis because of its water-binding properties. The fructose in honey which can bind water, increases viscosity and total soluble solids. Research by A. Famuji *et al.* (2023) stated that high total soluble solids values are inversely proportional to syneresis and cause syneresis values to decrease. Gel formation during fermentation also plays an important role. Lactic acid bacteria such as *Streptococcus thermophillus* and *Lactobacillus bulgaricus* promote protein coagulation and are capable of entrapping water. L. Campos *et al*. (2024) reported that protein denaturation and aggregation during acidification strengthen this gel structure and improve water retention which contributes to lower syneresis.

The percentage antioxidant activity of chickpea yoghurt is presented in Figure 1. The linear regression analysis showed a strong relationship between honey concentration and the antioxidant activity of chickpea-based yoghurt, with a coefficient of determination (R^2) of 0.96. N. Shaharom *et al*. (2024) explained that the R^2 value close to 1 suggests that the regression model provides highly accurate predictions of the optimal value. The increase in honey concentration significantly contributes to an increase in DPPH radical inhibition and indicates a higher antioxidant capacity in yoghurt. The antioxidant activity was evaluated using the DPPH method, which is widely applied to determine the free radical scavenging capacity of food samples. DPPH inhibition activity correlates with the ability of antioxidant compounds in yoghurt to reduce the properties of free radicals and increase the percentage of inhibition as the concentration of honey increases, thus indicating an increase in antioxidant activity. Previous study by I. Gulcin & S. Alwasel (2023) stated that this method is commonly used as an indicator to measure antioxidant capacity.

In this study, the inhibition values ranged from 39.64% to 58.42%, where yoghurt with 6% honey exhibited the highest antioxidant activity, while the 0% honey showed the lowest value. These findings indicate that honey serves as the main contributor to antioxidant compounds in the yoghurt. The fermentation process may also enhance antioxidant activity. Previous studies by G. Budryn & J. Grzelczyk (2024) state that during fermentation, the metabolic activity of microorganisms may be an additional source of antioxidants and will affect the release of flavonoids. In addition, the antioxidant activity of chickpea yoghurt may be related to TSS values, because the amounts of soluble components such as sugar during the heating process will go through a Maillard reaction and produce compounds that are indicated to have an antioxidant activity. Additionally, F. Nirwana *et al*. (2025) reported that the Maillard reaction has the potential to produce the brown-coloured melanoidin as the antioxidant compounds.

Consumer acceptance was evaluated for colour, aroma, taste, texture, and overall acceptance. Hedonic quality evaluation was carried out to assess the acceptance and preference levels of chickpea-based yoghurt with different concentrations of honey, using a scoring-based assessment. The evaluation comprised five hedonic quality parameters. Based on the results presented in Table 2, the mean hedonic scores for colour preference of chickpea yoghurt ranged from 3.03-3.73. Chickpea yoghurt with 0% honey addition received the highest score (3.73) whereas chickpea yoghurt with 6% honey addition obtained the lowest score (3.03). The hedonic values for all in terms of colour were classified as slightly liked by the panellist. The Mann-Whitney test result indicated that the 0% honey did not show a significant difference compared to the 2% honey, but differed significantly from the 4% and 6% honey. The colour of the resulting chickpea yoghurt appeared slightly yellowish-white, which was attributed to the addition of honey. According to A. Anwar *et al*. (2025), the yellow colour intensity in yoghurt increases due to the presence of natural golden yellow pigments derived from honey. Higher levels of addition tend to produce a more yellowish colour, as honey contains natural pigments such as carotenoids. I. Smetanska *et al.* (2021) stated that honey colour is influenced by various factors, including pigments such as carotenoids and flavonoids, total phenolic content, and handling methods. Another contributing factor is the occurrence of Maillard reaction during heating and fermentation processes which may contribute to the development of a slightly brownish colour in yoghurt. According to previous studies by M. Alief *et al.* (2025), Maillard reactions between fructose and glucose and amino acids during fermentation or heating leads to the formation of melanoidin compounds, which are responsible for yellowish-brown coloration in honey. Furthermore, honey colour is directly associated with its antioxidant content.

Based on the data presented in Table 2, the aroma parameter showed no significant difference among chickpea yoghurt with varying levels of honey addition, therefore the Kruskal-Wallis was not followed by a Mann-Whitney test. The data in Table 2 indicate that the mean hedonic scores for the aroma parameter ranged from 2.67-3.13 with preference scores increasing as the percentage of honey addition increased. Chickpea-based yoghurt containing 6% honey exhibited the highest hedonic score (3.13), which was classified as slightly liked by the panellists, whereas yoghurt without honey addition (0%) showed the lowest hedonic score (2.67), categorised as slightly disliked. Overall, variations in honey addition did not result in significant differences in the aroma preference of chickpea-based yoghurt. The aroma of chickpea yoghurt was characterised by contributions from honey and a slight beany odour originating from chickpea. Although increasing honey levels enhanced panellists' perception of aroma complexity, this effect was

not sufficient to produce significant differences in overall aroma preference, resulting in similar acceptance levels across samples. The characteristic aroma of honey introduces sweet and floral notes that may reduce the perception of undesirable beany odours, thereby contributing to improved hedonic scores. M. Al-Khalili *et al.* (2025) stated that the importance of volatile aroma compounds as key determinants of flavour perception and consumer acceptance in food products has been well documented. Honey contains aroma components commonly described as sweet, floral, acidic and woody. H. Mulheron *et al*. (2024) reported that the aroma of honey differs from that of common sweeteners and is characterised by distinctive notes such as sweet, floral, citrus, medicinal, and woody. More than 600 volatile organic compounds have been identified in various types of honey, including aldehydes, ketones, acids, alcohols, esters, hydrocarbons, and sulphur-containing compounds. Chickpea has a distinctive aroma and may produce a beany odour due to its volatile compounds. M. Tangyu *et al.* (2021) identified unfermented chickpeas contain aldehydes such as pentanal, hexanal, and heptanal, which originate from plant lipid oxidation and contribute to the characteristic beany odour. Panellists perceived the aroma differences among chickpea yoghurt samples as subtle, making them difficult to distinguish. This similarity in aroma acceptance may be attributed to comparable fermentation rates, resulting in similar formation of volatile compounds and lactic acid across samples. This finding is in consistent with E. Anggraini *et al.* (2018), who stated that acidic aroma is generated by volatile compounds and lactic acid formed during fermentation.

The taste attribute in the sensory test was significantly influenced by honey addition ($p < 0.05$). Hedonic scores increased along with the honey concentration, with the highest scores observed at 6% honey (3.97) and the lowest scores observed at 0% honey (1.77). Yoghurt generally has a distinctive sour taste caused by lactic acid. E. Suharto & F. Ferawati (2024) explained that the distinctive taste of yoghurt is caused by the content of lactic acid and small compounds such as diacetyl, acetaldehyde, and acetic acid produced by the symbiotic interaction between *Streptococcus thermophilus* and *Lactobacillus*. L. Campos *et al*. (2024) stated that honey is perceived to have the best taste by consumers. Honey acts as a natural sweetener. Enhancing the sweetness to balance the sweet and sour flavour in yoghurt. Fructose, in particular, plays a dominant role in enhancing sweetness perception due to its higher sweetness intensity compared to other sugars. According to previous studies by J. Wu *et al.* (2025), fructose exhibits a sweetness level approximately 1.73 higher than sucrose and 2.34 higher than glucose, which explains the marked increase in hedonic taste scores observed with higher honey concentrations. Consequently, the incorporation of honey effectively improves taste acceptance by moderating sourness and enhancing sweetness in chickpea yoghurt.

Texture. Based on the data presented in Table 2, honey addition did not significantly affect the texture preference of chickpea yoghurt ($p > 0.05$). Hedonic scores of the texture ranging from 2.90 to 3.27. Although differences were not statistically significant, a tendency toward improved smoothness was observed in this yoghurt. Higher levels of honey addition tended to increase the softness of the yoghurt texture, which may contribute to higher hedonic acceptance by the panellists. This improvement in texture perception is likely related to the role of honey as a natural sweetener containing sugars that interact with the yoghurt matrix. A previous study by A. Rashwan *et al.* (2023) reported that the addition of honey to soy yoghurt increased sensory acceptance, including texture attributes. A. Anwar *et al*. (2025) explained that sugar content in honey can improve the texture quality of yoghurt by its water-binding properties that can produce a more stable gel matrix in

yoghurt when combined with protein. According to T. Lubis & D. Ardila (2024) higher sugar content indicates the viscosity value becomes higher because it reduces the water content. An increase in honey concentration is related to the viscosity value because it produces a high total soluble solids content and makes the viscosity enhanced. This statement is in consistent with S. Susanti *et al*. (2023) that the higher the sweetener added, the higher viscosity produced.

The results presented in Table 2 indicate that the mean overall hedonic scores of the yoghurt ranged from 2.33 to 3.87. Chickpea-based yoghurt with 6% honey addition obtained the highest overall preference score (3.87), while yoghurt without honey addition (0%) showed the lowest score (2.33). Overall, panellists tended to prefer chickpea-based yoghurt with 6% honey addition, presumably due to the sweeter taste produced. M. Taufik *et al.* (2025) reported that honey has been shown to enhance consumer acceptance by masking undesirable flavours and balancing bitter, sour, and salty tastes. Similar findings were observed by M. Rahardjo *et al.* (2022), in which higher honey concentrations resulted in greater preference for plant-based soy yoghurt. Honey also contributes to masking the beany odour derived from chickpeas, resulting in a more pronounced acidic aroma typical of yoghurt combined with a slight sweet aroma. Panellists perceived the aroma differences among chickpea-based yoghurt samples as subtle, making them difficult to distinguish, which resulted in similar hedonic scores for the aroma parameter. Research by L. Xiang *et al.* (2023) explained that the beany off-flavours, generated by volatile compounds such as aldehydes and ketones derived from plant proteins, are recognised as sensory characteristics that influence overall flavour profiles and consumer perception in plant-based foods. Consequently, the improvement in overall acceptability observed at higher honey levels is likely driven by the combined effects of enhanced sweetness and moderated beany notes, rather than pronounced differences in aroma intensity.

In conclusion, the addition of honey to chickpea-based yoghurt significantly improved overall consumer acceptance, particularly at the 6% concentration, by enhancing sweetness and mitigating undesirable beany notes. While aroma differences among samples were subtle, the combination of improved taste and texture contributed to higher hedonic scores and overall preference. These findings highlight the potential of honey as a natural functional ingredient in plant-based fermented products, offering both sensory appeal and technological benefits, and providing valuable insights for the development of nutritionally enhanced, consumer-friendly yoghurt alternatives.

Conclusions

The addition of honey significantly influenced the physicochemical and sensory characteristics of chickpea-based yoghurt ($p < 0.05$). Increasing honey concentration from 0% to 6% enhanced fermentation performance, as shown by the increase in titratable acidity from 0.20% to 0.81% and total soluble solids from 8.68 to 13.84 °Brix. At the same time, pH decreased from 4.52 to 4.09, confirming intensified acid production by lactic acid bacteria. Product stability also improved, indicated by the reduction of syneresis from 51.27% to 41.67%. These findings demonstrate that honey promotes gel formation and water retention in chickpea yoghurt. Sensory analysis revealed that honey addition significantly improved taste and overall acceptance ($p < 0.05$), while aroma and texture were not significantly affected ($p > 0.05$). The formulation containing 6% honey produced the most balanced sensory profile and the highest consumer preference. The improvement in yoghurt quality is associated with the functional role of honey as both a fermentable carbon source and a bioactive ingredient. The glucose and fructose present in honey are easily metabolised by lactic acid bacteria, leading

to increased organic acid production and accelerated acidification. This process enhances protein coagulation and strengthens the gel matrix, thereby reducing whey separation. Furthermore, phenolic and flavonoid compounds in honey contribute to antioxidant activity and may stabilise the fermentation system through mild antimicrobial effects. However, concentrations higher than 6% may potentially generate osmotic pressure that inhibits bacterial activity, explaining the optimal performance observed at this level. From an industrial application perspective, incorporating 6% honey provides a natural sweetener that improves product stability, functional properties, and consumer acceptance without the need for artificial additives Therefore, chickpea-based yoghurt enriched with honey has strong potential for commercial development as a plant-based functional food suitable for lactose-intolerant and health-conscious consumers. Future studies should evaluate probiotic viability during storage, shelf-life stability, and the influence of different botanical origins of honey on product quality.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

References

[1] Albay, Z., Çelebi, M., & Şimşek, B. (2025). Physicochemical, rheological, and microbiological properties of honey-fortified probiotic drinkable yoghurt. *Foods and Raw Materials*, 13(2), 320-329. doi: 10.21603/2308-4057-2025-2-641.

[2] Alief, M., Triana, A., & Nuraeni. (2025). Goat milk yoghurt with added honey as a natural sweetener. *Journal of Multidisciplinary Research Studies*, 9(7), 92-103.

[3] Al-Khalili, M., Pathare, P.B., Rahman, S., & Al-Habsi, N. (2025). Aroma compounds in food: Analysis, characterization and flavor perception. *Measurement: Food*, 18, article number 100220. doi: 10.1016/j.meafoo.2025.100220.

[4] Anggraini, E.K., Kiranawati, T.M., & Mariana, R.R. (2018). Quality analysis of yoghurt with variations in the ratio of cowpea milk (*Vigna unguiculata* (L.) Walp. ssp.) and cow's milk. *Journal of Food Technology*, 1(1), 16-20.

[5] Anwar, A., Faiz, M.A., & Hou, J. (2025). Effect of honey concentration on the quality and antioxidant properties of probiotic yoghurt beverages from different milk sources. *Applied Sciences*, 15(4), article number 2210. doi: 10.3390/app15042210.

[6] Baltić, T., Đorđević, V., Ćirić, J., Velebit, B., Borović, B., Simunović, S., & Branković Lazić, I. (2025). Probiotic and prebiotic properties of honey: A review. *Meat Technology*, 66(3), article number 68. doi: 10.18485/meattech.2025.66.3.68.

[7] Brčina, T., Vilušić, M., & Zahirović, M. (2022). Influence of starter and sweetener on the syneresis intensity of fermented milk drink. *Technologica Acta*, 15(2), 15-18. doi: 10.51558/2232-7568.2022.15.2.15.

[8] Budryn, G., & Grzelczyk, J. (2024). Assessment of the nutritional value and antioxidant properties of plant-based yoghurt from chickpeas. *Applied Sciences*, 14(20), article number 9228. doi: 10.3390/app14209228.

[9] Campos, L., Tuma, P., Silva, T., Gomes, D., Pereira, C.D., & Henriques, M.H.F. (2024). Low fat yoghurt produced with different protein levels and alternative natural sweeteners. *Foods*, 13(2), article number 250. doi: 10.3390/foods13020250.

[10] Famuji, A., Zulaikhah, S.R., & Sidhi, A.H. (2023). Syneresis characteristics and moisture content of red dragon fruit (*Hylocereus polyrhizus* L.) yoghurt with the addition of coconut crystal sugar. *Journal of Animal Science*, 11(1), 9-14. doi: 10.21067/jsp.v11i1.8538.

[11] Fatimah, S.A., & Agustini, R. (2024). The effect of adding avocados (*Persea americana*) on microbiological and chemical qualities in yoghurt drinks. *Pijar MIPA Journal*, 19(3), 493-498. doi: 10.29303/jpm.v19i3.6601.

[12] Gulcin, I., & Alwasel, S.H. (2023). DPPH radical scavenging assay. *Processes*, 11(8), article number 2248. doi: 10.3390/pr11082248.

[13] Kartika, D.A., Legowo, A.M., & Nurwantoro, N. (2025). The effect of adding dates on the moisture content, acidity level, total dissolved solids, and viscosity of mung bean milk yoghurt. *Journal of Food Technology*, 9(2), 64-69. doi: 10.14710/jtp.2025.51501.

[14] Kumalasari, I.D., & Fajriyati, I. (2024). Physicochemical properties of mung bean (*Vigna radiata*) yoghurt with variations of bacterial starter types and sukkari date syrup (*Phoenix dactylifera*) as a natural sweetener and antioxidant source. *Agrointek: Journal of Agro-Industrial Technology*, 18(1), 49-56. doi: 10.21107/agrointek.v18i1.17556.

[15] Kumar, N., Hong, S., Zhu, Y., Garay, A., Yang, J., Henderson, D., Zhang, X., Xu, Y., & Li, Y. (2025). Comprehensive review of chickpea (*Cicer arietinum*): Nutritional significance, health benefits, techno-functionalities, and food applications. *Comprehensive Reviews in Food Science and Food Safety*, 24(2), article number e70152. doi: 10.1111/1541-4337.70152.

[16] Lubis, T.A.B., & Ardila, D. (2024). Formulation and quality evaluation of calamansi orange-based cordial from commodities in Bukit Kor, Terengganu. *Journal of Food and Nutrition*, 14(1), 77-87.

[17] Masriatini, R., Bakrie, M., Fatimura, M., Sefentry, A., Fitrianti, R., Nurlela, Husnah, & Wahyudi, A. (2023). Simple yoghurt production as an alternative entrepreneurship opportunity for students of SMK Kimia Yanitas Palembang. *KEMAS: Journal of Community Service*. doi: 10.31851/kemas.v1i1.11651.

[18] Mulheron, H., DuBois, A., & Mayhew, E.J. (2024). Quantifying the sweetness intensity and impact of aroma in honey from four floral sources. *Journal of Food Science*, 89(12), 9732-9741. doi: 10.1111/1750-3841.17461.

[19] Ningrum, P., Nazaruddin, & Amaro, M. (2022). The effect of trigona honey concentration on the quality of orange sweet potato (*Ipomoea batatas* L.) yoghurt. *Journal of Food Science and Technology*, 8(2), 93-106. doi: 10.29303/profood.v8i2.275.

[20] Nirwana, F.M., Putri, S.H., & Suparno, D.N. (2025). Physicochemical characteristics of pineapple peel extract. *Agroindustrial Engineering and Management Journal*, 13(3), 450-459. doi: 10.24843/JRMA.2025.v13.i03.p13.

[21] Nugroho, M.R., Wanniatie, V., Qishton, A., & Septinova, D. (2023). Physical properties and total lactic acid bacteria (LAB) of yoghurt made from different types of cow's milk. *Journal of Livestock Research and Innovation*, 7(2), 279-286. doi: 10.23960/jrip.2023.7.2.279-286.

[22] Pannerchelvan, S., Rios-Solis, L., Wasoh, H., Sobri, M.Z.M., Wong, F.W.F., Mohamed, M.S., Mohamad, R., & Halim, M. (2024). Functional yoghurt: A comprehensive review of its nutritional composition and health benefits. *Food & Function*, 15, 10927-10955. doi: 10.1039/D4FO03671A.

[23] Putri, Y.A., Fitri, F., & Rifa'i, M. (2024). Temperature control and pH monitoring in yoghurt fermentation using the PID method. *Journal of Mechanical and Electrical Technology*, 3(3), 149-156. doi: 10.70609/metrotech.v3i3.5196.

[24] Rahardjo, M., Sihombing, M., & Firdaus, V.P. (2022). Influence of honey addition on the physical properties and sensory attributes of soy yoghurt (soyghurt). *Journal of Tropical AgriFood*, 4(2), 96-104. doi: 10.35941/jtaf.4.2.2022.9165.96-104.

[25] Rahmawati, R., Mukarlina, M., & Engda, E.P. (2022). Antibacterial activity of yoghurt with added honey against the bacteria *Staphylococcus aureus* and *Escherichia coli*. *Journal of Food Technology and Nutrition*, 21(1), 12-18. doi: 10.33508/jtpg.v21i1.3366.

[26] Rashwan, A.K., Osman, A.I., & Chen, W. (2023). Natural nutraceuticals for enhancing yoghurt properties: A review. *Environmental Chemistry Letters*, 21, 1907-1931. doi: 10.1007/s10311-023-01588-0.

[27] Rizkyanto, R., & Ulfah, M. (2025). Health impacts and consumer acceptance of plant-based foods in Indonesia: A literature review. *Jompa Journal of Health*, 4(2), 658-663. doi: 10.57218/jkj.Vol4.Iss2.1844.

[28] Sari, Y.P., Candraruna, D.B., Imani, M.I., Maharani, R., Afifah, Z.U.F., & Rahman, A. (2024). Identification of adding skim milk effect on the physical, chemical and organoleptic properties of yoghurt. *Pro Food*, 10(1), 11-19. doi: 10.29303/profood.v10i1.354.

[29] Shaharom, N.F.M., Mutalib, S.R., Yusoff, A., & Seow, E.K. (2024). Enhancinig instant fortified rice congee (IFRC) for elderly nutrition: Collagen and curcumin optimisation using response surface methodology (RSM). *Indonesian Food Science and Technology Journal*, 8(1), 1-15. doi: 10.22437/ifstj.v8i1.32680.

[30] Singh, T.P., Verma, A.K., Rajkumar, V., Chatli, M.K., Arora, S., & Rai, B. (2024). Investigating the impact of milk protein, inulin, and honey on quality attributes of goat milk yoghurt. *Journal of Food Science and Technology*, 61, 1598-1608. doi: 10.1007/s13197-024-05932-2.

[31] Sitohang, A.C., Naibaho, N.M., & Sari, R.A. (2024). Effect of boiling time on nutritional value, total soluble solids, and sensory characteristics of jackfruit seed milk (*Artocarpus heterophyllus*). *Journal of Tropical AgriFood*, 6(1), 17-26. doi: 10.35941/jtaf.6.1.2024.12713.17-26.

[32] Smetanska, I., Alharthi, S.S., & Selim, K. (2021). Physicochemical, antioxidant capacity and color analysis of six honeys from different origin. *Journal of King Saud University – Science*, 33(1), article number 101447. doi: 10.1016/j.jksus.2021.101447.

[33] Suharto, E.L.S., & Ferawati, F. (2024). Exploration of consumer preferences: Sensory evaluation of synbiotic yoghurt with honey. *Andalasian Livestock*, 1(2), 144-149. doi: 10.25077/alive.v1.n2.p144-149.2024.

[34] Susanti, S., Kumoro, A.C., Suzery, M., & Oku, H. (2023). The effect of various sweeteners on the physical, chemical, and organoleptic characteristics of ginger leaf extract syrup. *Food Research*, 7(2), 164-169. doi: 10.26656/fr.2017.7(2).787.

[35] Tangyu, M., Fritz, M., Aragao-Börner, R., Ye, L., Bogicevic, B., Bolten, C.J., & Wittmann, C. (2021). Genome-based selection and application of food-grade microbes for chickpea milk fermentation towards increased L-lysine content, elimination of indigestible sugars, and improved flavour. *Microbial Cell Factories*, 20, article number 109. doi: 10.1186/s12934-021-01595-2.

[36] Taufik, M., Triana, A., Rasuli, N., Maruddin, F., Kristianti, N.D., & Nawangsari, D.N. (2025). Sensory characteristics of fermented dangke whey functional beverage with different levels of honey addition. *Indonesian Journal of Animal Science*, 35(1), 42-52. doi: 10.21776/ub.jiip.2025.035.01.5.

[37] Wang, J., Li, Y., Li, A., Liu, R.H., Gao, X., Li, D., Kou, X., & Xue, Z. (2021). Nutritional constituents and health benefits of chickpea (*Cicer arietinum* L.): A review. *Food Research International*, 150, article number 110790. doi: 10.1016/j.foodres.2021.110790.

[38] World Medical Association (WMA). (1964). *WMA Declaration of Helsinki – ethical principles for medical research involving human subjects*. Retrieved from https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/.

[39] Wu, J., *et al*. (2025). Advancements on the mechanism of soluble sugar metabolism in fruits. *Horticulturae*, 11(9), article number 1001. doi: 10.3390/horticulturae11091001.

[40] Xiang, L., Jiang, B., Xiong, Y.L., Zhou, L., Zhong, F., Zhang, R., Tahir, A.B., & Xiao, Z. (2023). Beany flavor in pea protein: Recent advances in formation mechanisms, analytical techniques, and microbial fermentation mitigation strategies. *Food Bioscience*, 56, article number 103166. doi: 10.1016/j.fbio.2023.103166.

Фізико-хімічні властивості та сенсорна привабливість йогурту з нуту (*Cicer arietinum* L.), збагаченого медом

Наїла Амани Фатіха Путрі
Бакалавр
Університет Діпонегоро
50275, вул. Проф. Х. Соедарто, м. Семаранг, Індонезія
https://orcid.org/0009-0005-2323-0452

Наїла Ар-Ріфда Ноерросаді
Бакалавр
Університет Діпонегоро
50275, вул. Проф. Х. Соедарто, м. Семаранг, Індонезія
https://orcid.org/0009-0008-3167-2383

Ясмін Аулія Рачма
Магістр, викладач
Університет Діпонегоро
50275, вул. Проф. Х. Соедарто, м. Семаранг, Індонезія
https://orcid.org/0000-0002-1543-005X

Йойок Буді Прамоно
Професор
Університет Діпонегоро
50275, вул. Проф. Х. Соедарто, м. Семаранг, Індонезія
https://orcid.org/0000-0002-0550-545X

Анотація. Зростаючий попит на рослинні функціональні продукти сприяє ширшому використанню бобових культур як альтернативи традиційній молочній сировині. Нут (*Cicer arietinum* L.) є багатим на білок, клітковину та біоактивні сполуки, тоді як мед може покращувати ферментацію, текстуру та антиоксидантні властивості. Метою цього дослідження було оцінити вплив додавання меду (0 %, 2 %, 4 % та 6 %) на фізико-хімічні характеристики, антиоксидантну активність та сенсорну привабливість йогурту на основі нуту. Молоко з нуту, збагачене знежиреним молоком, ферментували за допомогою молочнокислих бактерій і аналізували на pH, титровану кислотність, загальні розчинні речовини, синерезис, антиоксидантну активність та сприйняття споживачами. Додавання меду значно покращило фізико-хімічні та сенсорні властивості йогурту з нуту, підвищуючи ефективність ферментації (збільшення титрованої кислотності, загальних розчинних речовин та антиоксидантної активності) та знижуючи значення pH і синерезису. Сенсорна оцінка показала покращення смакових якостей та загальної привабливості з підвищенням концентрації меду, тоді як аромат і текстура істотно не змінювалися. В цілому, 6 % меду забезпечили оптимальний баланс стабільності, функціональності та сприйняття споживачами, що вказує на його потенціал як оптимальної формули для розробки рослинного йогурту. Ці результати підкреслили роль меду як природного функціонального інгредієнта, який покращує як технологічні властивості, так і прийнятність продукту серед споживачів у ферментованих продуктах на основі бобових.

Результати дослідження можуть бути використані виробниками рослинної продукції та малими підприємствами для розробки функціональних аналогів йогурту з покращеними поживними та органолептичними властивостями, що особливо актуально для ринків із високим попитом на ферментовані продукти

Ключові слова: рослинна ферментація; молочнокислі бактерії; пребіотичний підсолоджувач; антиоксидантна активність; сенсорна оцінка

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 17(1), 93-104
Received 18.09.2025 Revised 29.01.2026 Accepted 26.02.2026
Published 26.03.2026

UDC 664.66:664.644
DOI: 10.31548/animal.1.2026.93

ISSN 2706-8331
e-ISSN 2706-834X

Rationalisation of commercial and technological features of biscuit semi-finished products during baking

Volodymyr Fedorov
Doctor of Technical Sciences, Professor
Uman National University
20300, 1 Instytutska Str., Uman, Ukraine
https://orcid.org/0000-0002-4453-4122

Oleg Kepko
PhD in Technical Sciences, Associate Professor
Uman National University
20300, 1 Instytutska Str., Uman, Ukraine
https://orcid.org/0000-0003-1443-307X

Oleksandr Trus*
PhD in Agricultural Sciences, Assistant Professor
Uman National University
20300, 1 Instytutska Str., Uman, Ukraine
https://orcid.org/0000-0002-9493-5469

Valentyna Kepko
PhD in Economics, Associate Professor
Bila Tserkva National Agrarian University
09117, 8/1 Soborna Sq., Bila Tserkva, Ukraine
https://orcid.org/0000-0001-6432-7211

Abstract. Each type of flour-based confectionery requires specific thermophysical conditions to be achieved within the selected oven. Sponge cake semi-finished products have unique baking characteristics that must be taken into account when designing and adjusting baking equipment. This study aimed to examine heat flux densities across the surfaces and layers of sponge cake

Suggested Citation:
Fedorov, V., Kepko, O., Trus, O., & Kepko, V. (2026). Rationalisation of commercial and technological features of biscuit semi-finished products during baking. *Animal Science and Food Technology*, 17(1), 93-104. doi: 10.31548/animal.1.2026.93.

*Corresponding author

products using technical instrumentation, and to provide recommendations for industrial thermal regimes. Temperature measurements were carried out using thermocouples and compact, low-inertia heat flux sensors. Baking was performed in a laboratory oven using traditional metal moulds, as well as with anti-adhesive paper inserts placed inside the same moulds. The inserts were prepared by priming paper or cardboard with a 10% polyvinyl alcohol solution, followed by coating with a water-based emulsion of silicone fluid mixed with polyvinyl alcohol and a small amount of curing catalyst. The experiments were conducted at temperatures ranging from 140 to 200°C. The paper presented data on the baking kinetics of the upper layer of sponge semi-finished products, both in metal moulds and with paper inserts under optimal heating conditions. Two series of tests were carried out using a laboratory oven with combined convective and radiant heat input. The optimal baking regime was determined to be 170-180°C with equal heat supply from the top and bottom. The results confirmed the effectiveness of anti-adhesive paper inserts, which reduce the thickness of the bottom crust, minimise product breakage, and improve sanitary conditions during production and distribution. The study identified optimal baking regimes for sponge semi-finished products in industrial bakery ovens, based on technological, quality, and economic considerations. The practical value of this work lies in the applicability of its findings at baking and confectionery enterprises seeking to optimise the baking modes of biscuit semi-finished products and to improve the quality and efficiency of production

Keywords: kinetics of baking; thermometry; heat metering; thermal and physical characteristics; thermal resistance

Introduction

The production of semi-finished biscuit products is one of the most widespread segments of the flour confectionery industry, one that combines high demands for quality consistency, process energy efficiency, and the competitiveness of finished products. Modern market conditions necessitate the rationalisation of the commodity and technological properties of biscuits at the baking stage, since this process determines the formation of structure, volume, colour, crust texture, and crumb, whilst also affecting the keeping quality and consumer characteristics of the product. Inconsistency in thermal regimes or the design parameters of moulds leads to increased energy consumption, uneven baking, deformation, and product losses. In this regard, improving approaches to controlling heat and mass transfer processes during the baking of semi-finished biscuit products constitutes an urgent scientific and practical task.

In recent years, considerable research attention has been directed towards modelling thermal processes during baking. S. Simpson (2023) found that the distribution of heat flux between the upper and lower zones of the oven significantly affects the rate of crust formation and the final moisture content of the crumb, and that optimising the ratio of convective to radiant heating allows the duration of the process to be reduced without adversely affecting textural indicators. Studies by P. Jayapragasam *et al*. (2021) showed that controlling heat flux density makes it possible to minimise temperature gradients within the volume of the biscuit mass and to produce a more uniform porous structure. The influence of recipe factors on the formation of technological properties during baking was examined by B. Inanlar & F. Altay (2024), who demonstrated that altering the ratio of egg foam to flour significantly modifies the rheological characteristics

of the dough and determines the behaviour of gas bubble expansion during the initial period of heat treatment. D. Nekesa *et al*. (2024) found that the use of alternative protein ingredients modifies the mechanism of protein coagulation and requires adjustment of the temperature regime in order to preserve product volume.

A separate area of research concerns the energy efficiency of the baking process. S. Chakraborty & K. Dash (2023) concluded that adaptive temperature control during the baking cycle can reduce energy consumption by up to 12% by limiting overheating in the final phase. Similar results were obtained by A. Görgülü (2025), who demonstrated that the use of heat flux sensors in laboratory and industrial ovens contributes to more accurate determination of the end point of baking and a reduction in excess energy consumption. Research aimed at improving contact surfaces and mould materials is also noteworthy. W. Yang *et al*. (2025) demonstrated that the use of non-stick coatings based on silicone emulsions reduces the intensity of heat transfer through the base of the mould, which in turn affects the thickness of the bottom crust and reduces the risk of scorching. P. Zarzycki *et al*. (2024) showed that the use of paper liners with polymer treatment provides more uniform heating of the side layers and facilitates product removal without mechanical damage.

The structural formation of biscuits during baking was studied by H. Dizlek & A. Altan (2021), who established that the rate of dehydration of the surface layers correlates with the formation of an elastic crust and determines the subsequent redistribution of moisture within the product. N. Mattioli *et al*. (2025), by contrast, emphasised that excessive heat flux intensity at the initial stage leads to premature fixation of the structure and a reduction in specific volume, which adversely affects the commercial properties of the product. The analysis of the foregoing studies indicates significant progress in understanding the mechanisms of heat and mass transfer during the baking of biscuit semi-finished products, as well as growing interest in energy- and material-saving technologies. Nevertheless, most research focuses either on recipe aspects or on individual parameters of the thermal regime, without a comprehensive assessment of their combined impact on the commodity and technological characteristics of the product. The question of coordinating heat supply modes, the design features of moulds, and quality indicators from the standpoint of rationalising the production process remains insufficiently studied.

The purpose of the study was to substantiate and experimentally verify approaches to the rationalisation of the commodity and technological properties of biscuit semi-finished products during baking, based on the optimisation of thermal regimes and conditions of product formation.

Materials and Methods

The research was conducted in 2023 in an educational and scientific laboratory and under the experimental and industrial conditions of a small-scale enterprise. This approach made it possible to combine the controllability of a laboratory experiment with verification of the results obtained in a production environment. The research programme was designed according to a factorial scheme with variation of the heating medium temperature and heat supply conditions. The experimental design was a full factorial with two main factors – temperature and the presence or absence of an anti-adhesive insert – with fixed recipe parameters. Each experiment was carried out in at least three replications. The control conditions were defined as baking in traditional metal moulds without paper inserts at 170°C with uniform heat supply from above and below. Comparison with the control allowed the impact of design and regime changes on commodity and technological indicators to be assessed.

Baking regimes covered a temperature range of 140-200°C in increments of 10°C.

The duration of the process ranged from 18 to 30 min depending on the temperature and mass of the workpiece. The laboratory oven provided combined convective-radiant heat supply with the ability to adjust the ratio of heat fluxes from above and below. Relative humidity in the working chamber was maintained at 20-35%; in some experiments, the effect of short-term humidification at the initial stage of baking was additionally assessed. Air velocity in the working space was 0.5-1.2 m/s and was monitored by an anemometer. Biscuit semi-finished products were prepared according to a standardised recipe with a fixed ratio of the main ingredients. After mixing and aeration, the dough was divided into samples weighing 210 ± 1 g and placed in metal moulds of identical diameter. Some of the moulds were fitted with anti-adhesive paper inserts with a polymer coating. The geometric parameters of the workpieces (diameter and layer height) were measured prior to baking in order to correctly determine the characteristic dimension for thermophysical calculations.

Temperature at different points of the workpiece and the heating medium was recorded using chromel-alumel type K thermocouples connected to a multi-channel data logger. Heat flux density was measured using low-inertia heat flux sensors with dimensions of 10×10×1.2 mm. Prior to each experimental series, the thermocouples were calibrated in a water bath and a dry-block calibrator over the range of 20-200°C. The maximum absolute error of temperature measurement did not exceed ±0.5°C, and that of heat flux density ±5%. Calibration was rechecked after every 30 baking cycles. Heat fluxes were measured from the top, bottom, and through the side surface of the mould, and temperature profiles along the height of the workpiece were recorded at 5 mm intervals. Mass loss was determined by weighing on an electronic balance with an accuracy of ±0.01 g before and after baking and cooling. To assess the efficiency of the thermal process, specific heat consumption per unit mass of product and the time required to reach the protein coagulation temperature in the central zone were additionally calculated.

The quality of the finished products was assessed using standardised methods. The mass fraction of moisture was determined by drying to constant mass in accordance with current regulatory requirements for flour confectionery products. Specific volume was determined by the rapeseed displacement method. Crust thickness was measured with a calliper at four equidistant points. Textural profile was analysed using a texture analyser by the double-compression method, with determination of elasticity, cohesiveness, and crumb hardness. Ease of removal was assessed as the percentage of products showing no mechanical damage.

Statistical processing of the results was performed using an applied statistics software package. Mean values, standard deviations, and confidence intervals were calculated. The influence of factors was assessed by analysis of variance (ANOVA) at a significance level of $p \leqslant 0.05$. Correlation analysis was used to establish relationships between thermal process parameters and quality indicators. Errors were expressed as the standard error of the mean based on three or more replications. The combination of a controlled factorial design, metrologically verified measuring instruments, and statistically sound data processing ensured the reliability of the results obtained and the feasibility of their practical application in the rationalisation of baking modes for biscuit semi-finished products.

Results and Discussion

The need to bake biscuit semi-finished products in industrial ovens in the most rational manner – with respect to both energy and material resources and to the organoleptic and commercial characteristics of the product – necessitated preliminary laboratory investigation. The article by A. Dorohovich *et al*. (2012) represents an attempt to link thermal, physicochemical,

structural, and mechanical processes during the baking of flour confectionery products, and presents experimental data on baking kinetics and volume increase of cakes and biscuits in traditional metal moulds. Certain data on the thermal conductivity of flour confectionery products are also provided, though the conditions and methods under which these values were obtained are not specified.

S. Dudko *et al*. (2017) calculated the Biot number for baking "massive" or "thin" bodies as $Bi = \alpha l/\lambda$, where α, W/(m^2 · K) is the heat transfer coefficient at the surface of the flour confectionery product; l, m – the characteristic dimension of the product; λ, W/(m · K) – its thermal conductivity. The authors derived the values of α, l and λ from verified experimental data on the baking of flour confectionery products under laboratory and industrial conditions. Biscuit semi-finished products are appropriately classified as "massive" bodies. In the articles by S. Bagliuk *et al*. (2015) and A. Bolger *et al*. (2021), the quality of semi-finished biscuits made according to different recipes is characterised by the Biot (Bi) and Grashof (Gr) similarity numbers. However, the heat transfer coefficient α required for the calculation of Bi cannot be determined directly from the experimental setup described, since in this context α represents the total heat transfer coefficient (comprising convective, radiant, and mass transfer components, the last of which may take a negative value). The thermal conductivity of the dough cannot be derived from $q = -\lambda\, grad T$ either, since the heat flux density q, W/m^2 was not measured. The Grashof similarity number Gr is conventionally a hydrodynamic parameter and, in this context, relates to the leavening force acting on the dough.

Baking was carried out both in traditional metal tins and with inserts made of anti-adhesive paper, or in anti-adhesive cardboard cases. To impart anti-adhesive properties, paper or cardboard was primed with a 10% polyvinyl alcohol solution and then coated with an aqueous emulsion of organosilicon liquid mixed with polyvinyl alcohol solution and a small amount of curing catalyst. No adverse chemical interactions between these materials and the dough or finished biscuit were observed during baking or storage. The dough of confectionery workpieces has a low viscosity (0.4-0.7 Ns/m^2), which causes heat flux sensors placed on its surface to sink into the mass. A small piece of gauze placed on the surface of the product proved sufficient to carry the sensor upward along with the rising dough during baking. Other sensors were embedded entirely within the dough and fixed at the centre of the bottom and side surfaces of the tin.

As noted by A. Farisieiev *et al*. (2023) and S. Soleimanifard *et al*. (2024), for baking flour confectionery products in commercial catering settings, only steam convection ovens offer practical prospects among non-traditional baking equipment, whilst microwave ovens are appropriate for domestic use. The search for a rational baking mode for biscuit semi-finished products was conducted in a laboratory electric oven fitted with top and bottom heating elements; the influence of baking chamber temperatures in the range of 140-200°C was investigated. The temperature was either maintained constant or varied according to a prescribed pattern. The proportions of thermal energy supplied to the workpiece from above and below were also varied. Comparable findings regarding thermal conditions for baking semi-finished biscuit products at chamber temperatures of 160-200°C were reported by A. Cappelli *et al*. (2021) and I. Davidson (2024).

The heat balance was calculated by three independent methods in the experiments: from the equation of heat consumption for heating and physicochemical and phase transformations; from the sum of local heat fluxes multiplied by the area of the corresponding surfaces; and from the analysis of the temperature fields of the workpiece during baking. In all experiments, agreement between the three methods was satisfactory. The heat consumption

estimated from heat flux sensor signals was generally slightly higher, which is attributable to the sensors being positioned at the centre of each surface, where energy flux density is greatest. Since the discrepancy between heat balance estimates did not exceed 11%, no additional experiments were conducted in this series. On the basis of these studies, the following rational baking mode for biscuit semi-finished products is proposed: a constant baking chamber temperature of 170-180°C with equal intensity of heat supply from above and below.

In a number of experiments, heat flux sensors were positioned at different depths within the workpiece, also using gauze supports. Study of the kinetics of heat distribution through the layers of the workpiece revealed a wave-like pattern in each layer. This is explained by the fact that, in addition to heat transfer by conduction, various phase and physicochemical transformations occur simultaneously – evaporation, crystallisation, caramelisation, and others. As noted by O. Kepko *et al*. (2018), in certain cases the phase shift between the heat flux and temperature signals from a pair of sensors positioned on opposite sides of a layer allows the sensor pair to be treated as a flow calorimeter with directed energy transit or accumulation. This makes it possible to simultaneously characterise the technological and thermophysical properties of the material – as was demonstrated for cream in V. Fedorov *et al*. (2014) – directly during technological processing.

Figure 1 presents the results of one such experiment. The signal from heat flux sensor 1, positioned at the centre of the upper surface of the workpiece, first rises sharply and then gradually decreases as the temperature difference between the heating medium and the biscuit surface diminishes. The signal from sensor 2, located 5 mm below sensor 1 with a layer of dough between them, rises considerably more slowly (as part of the energy is consumed in heating this layer), and at the 7th minute equals the signal at the inlet of the layer; this equality persists until the 11th minute. In accordance with the theory of complex thermophysical characterisation, a transient cycle with temperature perturbation (highlighted area) is followed by a quasi-stationary process. This is confirmed by the temperature readings on the upper (3) and lower (4) surfaces of the dough layer. From these data, the effective volumetric heat capacity can be calculated: at a mean temperature of $t = 39$°C, it was $c = 1.2$ MJ/(m^3 · K). The effective thermal conductivity during the quasi-stationary process ranged from 0.19 to 0.21 W/(m · K), averaging $\lambda = 0.2$ W/(m · K). These results are in good agreement with the thermophysical characteristics of biscuit dough of the same recipe reported by A. Dorohovich *et al*. (2012) and V. Fedorov *et al*. (2014).

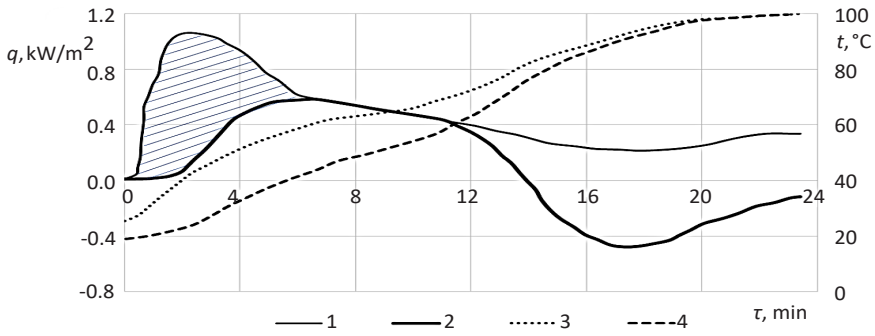

Figure 1. Kinetics of baking the upper layer of biscuit semi-finished product
Note: 1, 3 – heat flux density and temperature on the upper surface; 2, 4 – the same at the depth of 5 mm
Source: created by the authors

The kinetics of heat flux density 2 through the lower surface of the upper dough layer revealed an anomaly: during crust formation, the heat fluxes decrease sharply, and from the 14th minute onwards become negative. This indicates that heat is being conducted into the layer beneath the upper crust not from above, but from below. Consequently, the layer immediately beneath the upper crust is the last to be baked. It may therefore be appropriate to include an assessment of this zone in the technological criteria for product readiness and marketable quality. These experiments enabled a comparative study of baking biscuit semi-finished products in plain metal tins (the traditional method) and in identical tins fitted with anti-adhesive paper inserts. The use of such inserts reduces the rate of product breakage during demoulding and improves sanitary conditions. Furthermore, the inserts reduce the thickness of the bottom crust by lowering the intensity of heat flux at the base of the product.

The heating medium temperature was maintained at 165-175°C. Dough weighing 210 g was poured into tins to a height of 30 mm. Heat flux sensors with thermocouples were placed at the centre of the upper, lower, and one of the lateral surfaces of the dough. A comparison of the kinetics of heat fluxes and temperatures shows that, whilst the magnitude and variation of heat fluxes at the upper (curve 1) and lateral (curve 2) surfaces remained unchanged with the use of inserts, heat fluxes from below decreased and the thickness of the bottom crust was reduced, particularly during the first half of the process (Figs. 2, 3).

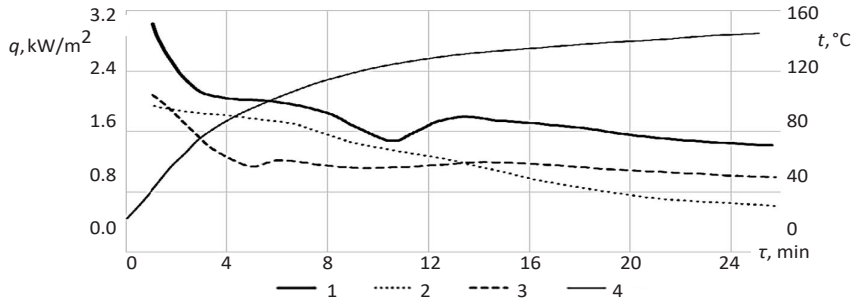

Figure 2. Kinetics of baking biscuit semi-finished products in metal tins under optimal heating medium temperature conditions
Note: 1, 2, 3 – heat flux density at the upper, lower, and lateral surfaces of the product; 4 – mean surface temperature of the product
Source: created by the authors

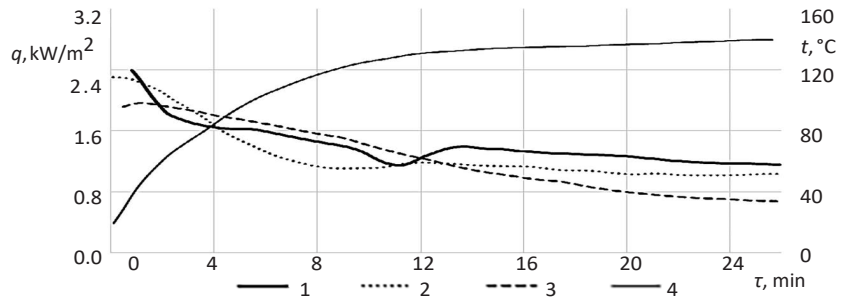

Figure 3. Kinetics of baking products with paper inserts
Note: 1, 2, 3 – heat flux density at the upper, lower, and lateral surfaces of the product; 4 – mean surface temperature of the product
Source: created by the authors

In both experimental series, the reduction in heat flux from below was moderate, which may be attributed to the increased thermal resistance of the product resulting from vapour generation. Once the product temperature reached 80°C, protein denaturation and starch gelatinisation began to occur intensively, causing a renewed rise in heat fluxes (curve 1). The cost of producing inserts for metal tins or cardboard cases is more than offset by savings in fat used for greasing tins and by reduced labour input; consequently, the retail price of the biscuits is not adversely affected.

Conclusions

Based on comprehensive laboratory studies employing thermocouples and low-inertia heat flux sensors, a rational baking regime for biscuit semi-finished products in industrial ovens was substantiated through integrated analysis of local heat fluxes, temperature fields, and heat balances. The discrepancy between heat balances calculated by three independent methods did not exceed 11%, confirming the reliability of the experimental data. During the quasi-stationary stage, the effective thermal conductivity of the biscuit dough averaged 0.20 W/(m·K), whilst the effective volumetric heat capacity at 39°C reached approximately 1.2 MJ/(m^3·K), consistent with reference thermophysical values and thereby validating the applied methodology. A wave-like pattern of heat propagation through the product layers was experimentally confirmed, arising from phase and physicochemical transformations including evaporation, protein denaturation, and starch gelatinisation. An anomaly in heat transfer beneath the upper crust was identified: from the 14th minute of baking onwards, the heat flux density through the lower boundary of the upper layer became negative, indicating that heat was supplied predominantly from below. This finding substantiates the need to assess product readiness specifically in the layer beneath the upper crust, as it constitutes the most thermally inert zone.

The optimal baking mode was found to be a constant chamber temperature of 170-180°C with equal heat input from the top and bottom. Under conditions of 165-175°C, a dough mass of 210 g, and a fill height of 30 mm, the use of non-stick paper or cardboard liners reduced the heat flux from below during the first half of baking, decreasing the thickness of the bottom crust and minimising mechanical damage during demoulding. Heat transfer through the upper and lateral surfaces remained unchanged, confirming the selective thermal effect of the liners. Their production costs are offset by reduced fat consumption and labour savings. The results are directly applicable to industrial oven design, energy optimisation, and quality stabilisation. Further research should focus on the scaling of baking regimes, the influence of recipe variations, and the development of real-time thermometric control systems.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

References

[1] Bagliuk, S.V., Romanovska, O.L., Forostyana, N.P., & Lazarenko, M.V. (2015). Effect of flour "health" and kerob powder on the heat, mass transfer and technological process during biscuit semi-finished biscuit baking. *ScienceRise*, 12(2(17)), 11-15. doi: 10.15587/2313-8416.2015.56317.

[2] Bolger, A.M., Rastall, R.A., Oruna-Concha, M.J., & Rodriguez-Garcia, J. (2021). Effect of D-allulose, in comparison to sucrose and D-fructose, on the physical properties of cupcakes. *LWT*, 150, article number 111989. doi: 10.1016/j.lwt.2021.111989.

[3] Cappelli, A., Lupori, L., & Cini, E. (2021). Baking technology: A systematic review of machines and plants and their effect on final products, including improvement strategies. *Trends in Food Science & Technology*, 115, 275-284. doi: 10.1016/j.tifs.2021.06.048.

[4] Chakraborty, S., & Dash, K.K. (2023). A comprehensive review on heat and mass transfer simulation and measurement module during the baking process. *Applied Food Research*, 3(1), article number 100270. doi: 10.1016/j.afres.2023.100270.

[5] Davidson, I. (2024). *Biscuit, cookie and cracker production: Process, production and packaging equipment* (2nd ed.). Cambridge: Academic Press. doi: 10.1016/C2023-0-01202-X.

[6] Dizlek, H., & Altan, A. (2021). The impacts of batter and baking temperatures and baking time on sponge cake characteristics. *Electronic Letters on Science & Engineering*, 17(2), 89-95.

[7] Dorohovich, A.N., Dorohovich, V.V., Telichkun, V.I., Tasheva, S., & Valchev, G. (2012). Reasoning of the regime firing of pasta products. In *Food science, engineering and technologies* (pp. 785-790). Plovdiv: University of Food Technology.

[8] Dudko, S., Malinovskyi, V., & Obolkina, V. (2017). Heat treatment of flour confectionery products. *Food Industry of the Agro-Industrial Complex*, 5, 22-24.

[9] Farisieiev, A., Matsuk, Y., & Oliynyk, N. (2023). Improving the technology of dry mixes for craft flour confectionery products. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Food Technologies*, 25(100), 60-66. doi: 10.32718/nvlvet-f10010.

[10] Fedorov, V.H., Skarboviichuk, O.M., Kepko, O.I., & Kravchuk, P.O. (2014). *Thermophysical characteristics of agricultural products and materials*. Uman: Uman National University of Horticulture.

[11] Görgülü, A. (2025). Real-time quality analysis of baked goods using advanced technologies. *Journal of Food Engineering*, 388, article number 112359. doi: 10.1016/j.jfoodeng.2024.112359.

[12] Inanlar, B., & Altay, F. (2024). Effects of egg on cake batter rheology and sponge cake texture. *Journal of Food Process Engineering*, 47(11), article number e14765. doi: 10.1111/jfpe.14765.

[13] Jayapragasam, P., le Bideau, P., & Loulou, T. (2021). Selection of better mathematical model describing cake baking for inverse analysis. *Food and Bioproducts Processing*, 126, 265-281.

[14] Kepko, V.M., Fedorov, V.H., Kepko, O.I., Pushka, O.S., & Lisovyi, I.O. (2018). Use of correlation of commodity and thermophysical characteristics of cream for their examination. In *Science and technology of the present time: Priority development directions of Ukraine and Poland* (pp. 102-106). Wolomin: Baltija Publishing.

[15] Mattioli, N.G., Olivera, D.F., Salvadori, V.O., Marra, F., & Goñi, S.M. (2025). Technological and quality features of gluten free sponge cakes baked by combined ohmic-hot air system. *Journal of Food Process Engineering*, 48(3), article number e70089. doi: 10.1111/jfpe.70089.

[16] Nekesa, D., Nalule, R.M., Kyomuhangi, A., Muhumuza, A.K., & Oyem, A.O. (2024). Optimization of heat transfer in cassava flour based cake baking: A computational fluid dynamics approach. *Wseas Transactions on Heat and Mass Transfer*, 19, 107-113. doi: 10.37394/232012.2024.19.12.

[17] Simpson, S. (2023). *Measurement of conduction, radiation, and convection thermal energy to assess baking performance in residential ovens*. (Doctoral dissertation, University of Louisville, Louisville, United States). doi: 10.18297/etd/4157.

[18] Soleimanifard, S., Emam-djomeh, Z., Askari, G., & Shahedi, M. (2024). Multidimensional comparative analysis of three baking methods of the cupcake – thermophysical approach. *Acta Scientiarum Polonorum Technologia Alimentaria*, 23(2), 179-186. doi: 10.17306/J.AFS.001206.

[19] Yang, W., Long, L., Zhang, L., Xu, K., Huang, Z., & Ye, H. (2025). Heat and mass transfer and deformation during chiffon cake baking. *Journal of Food Engineering*, 388, article number 112361. doi: 10.1016/j.jfoodeng.2024.112361.

[20] Zarzycki, P., Wirkijowska, A., & Pankiewicz, U. (2024). Functional bakery products: Technological, chemical and nutritional modification. *Applied Sciences*, 14(24), article number 12023. doi: 10.3390/app142412023.

Раціоналізація товарних і технологічних властивостей бісквітних напівфабрикатів під час випікання

Володимир Федоров
Доктор технічних наук, професор
Уманський національний університет
20305, вул. Інститутська, 1, м. Умань, Україна
https://orcid.org/0000-0002-4453-4122

Олег Кепко
Кандидат технічних наук, доцент
Уманський національний університет
20305, вул. Інститутська, 1, м. Умань, Україна
https://orcid.org/0000-0003-1443-307X

Олександр Трус
Кандидат сільськогосподарських наук, доцент
Уманський національний університет
20305, вул. Інститутська, 1, м. Умань, Україна
https://orcid.org/0000-0002-9493-5469

Валентина Кепко
Кандидат економічних наук, доцент
Білоцерківський національний аграрний університет
09117, пл. Соборна, 8/1, м. Біла Церква, Україна
https://orcid.org/0000-0001-6432-7211

Анотація. Кожен вид борошняних кондитерських виробів потребує визначення власних теплофізичних характеристик, дотримання яких має бути забезпечене в обраній печі. Випікання бісквітних напівфабрикатів має особливості, які необхідно враховувати при проєктуванні та налагоджуванні обладнання. Метою досліджень було вивчення за допомогою технічних засобів густини теплових потоків крізь поверхні та по шарах бісквітних напівфабрикатів, а також формулювання рекомендацій щодо теплового режиму для промислових умов. Для вимірювання температури використовували термопари та малогабаритні малоінерційні вимірювачі густини теплового потоку. Випікання бісквітних напівфабрикатів проводили у лабораторній печі в традиційних металевих формах, а також у формах із використанням вкладишів з антиадгезійного паперу. Для надання антиадгезійних властивостей папір або картон ґрунтували розчином полівінілового спирту 10 %, після чого покривали водною емульсією кремнійорганічної рідини у суміші з розчином полівінілового спирту та невеликою кількістю каталізатора затвердіння. Дослідження проводили в інтервалі температур 140-200°С. У статті наведено результати вивчення кінетики випікання верхнього прошарку бісквіта, процесу в металевих формах за раціональної температури гріючого середовища та кінетики випікання виробів із паперовими вкладишами. За допомогою лабораторної печі з підведенням конвективної та променистої теплоти встановлено раціональний режим: температура гріючого середовища 170-180°С, інтенсивність підведення енергії зверху та знизу – однакова.

Доведено доцільність випікання виробів у вкладишах з антиадгезійного паперу, що дозволяє зменшити товщину нижньої скоринки, знизити ломкість продукту та покращити санітарні умови виготовлення й реалізації. На основі результатів визначено раціональні за технологічними, товарними та економічними ознаками режими випікання бісквітних напівфабрикатів у промислових печах. Практична цінність роботи полягає у можливості застосування її результатів на підприємствах для оптимізації процесів випікання та підвищення якості й економічності виробництва

Ключові слова: кінетика випікання; термометрія; теплометрія; теплофізичні характеристики; термічний опір

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Scientific Journal

Volume 17, No. 1. 2026

Founded in 2010. Published four times per year

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

Managing Editor:
N. Shevchenko

Signed for print of February 26, 2026
Format 70*100/16
Conventional printed pages 8.6
Circulation 50 copies

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

ТВАРИННИЦТВО ТА ТЕХНОЛОГІЇ ХАРЧОВИХ ПРОДУКТІВ

Науковий журнал

Том 17, № 1. 2026

Заснований у 2010 р. Виходить чотири рази на рік

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

Відповідальний редактор:
Н. Шевченко

Підписано до друку 26 лютого 2026 р.
Формат 70*100/16
Умов. друк. арк. 8,6
Наклад 50 прим.

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