CHAPTER 5

FERMENTATION OF VICIA FABA BEAN PROTEIN CONCENTRATE FOR THE DEVELOPMENT OF PLANT-BASED BEVERAGES

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INTRODUCTION

The contemporary transformation of food systems is driven by global challenges associated with ensuring food security, population growth, climate change, and the imperative for rational natural resource management. Under these conditions, identifying alternative protein sources capable of providing the population with high-quality food products while simultaneously mitigating the environmental burden on ecosystems becomes of paramount importance. One of the most promising avenues entails utilizing plant proteins, specifically those derived from grain legume crops, to manufacture next-generation functional food products[1].

Over the past decade, the global market for plant-based alternatives to traditional dairy products has demonstrated consistent growth. The heightened demand for plant-derived commodities is driven not only by the popularization of vegan, vegetarian, and flexitarian dietary models but also by an increasing cohorts of consumers exhibiting lactose intolerance or allergies to bovine milk proteins, alongside a general orientation toward a healthy lifestyle[2]. Fermented plant-based beverages are garnering exceptional interest, as they seamlessly combine the inherent benefits of plant matrices with the positive impacts of biotechnological fermentation on the nutritional and biological value of the finished product.

[1] Prevalence of lactose intolerance and nutrients intake in an older population regarded as lactase non-persistent / E. Dewiasty, S. Setiati, R. Agustina et al. *Clinical Nutrition ESPEN*. 2021. Vol. 43. P. 317–321. DOI: https://doi.org/10.1016/j.clnesp.2021.03.033.

[2] A review on current scenario and key challenges of plant-based functional beverages / N. Sharma, N. Yeasmen, L. Dube, V. Orsat. *Food bioscience*. 2024. Vol. 60. Art. 104320. DOI: https://doi.org/10.1016/j.fbio.2024.104320.

Among a broad spectrum of grain legumes, faba beans (*Vicia faba L.*) attract substantial scientific attention. They are characterized by a high protein content that can reach 30–35%, a favorable amino acid profile, and substantial amounts of dietary fibers, vitamins, and minerals. Compared to alternative plant protein sources, notably soybeans, *Vicia faba* possesses several distinct advantages, including lower allergenicity, suitability for cultivation in temperate climatic zones, high crop yields, and a positive impact on soil fertility due to its nitrogen-fixing capacity. Furthermore, the manufacturing of protein ingredients from faba beans exhibits a lower carbon footprint and reduced resource consumption compared to the production of animal-derived proteins[3].

Advanced processing of *Vicia faba* beans yields protein concentrates and isolates with elevated protein concentrations, which can be utilized as functional ingredients across diverse food matrices. Due to their capacity for emulsification, foam formation, water binding, and the development of structured networks, faba bean proteins represent a highly viable raw material for alternative dairy production. Concurrently, the widespread integration of legume protein concentrates into food technologies is constrained by several restrictive factors, including a native "beany" off-flavor and aroma, limited protein solubility, and insufficiently explored mechanisms governing the development of consumer properties in the finished products.

Biotechnological fermentation stands out as one of the most effective methods for modifying the functional, technological, and sensory characteristics of plant proteins. Microbial action triggers complex biochemical transformations that facilitate partial protein and carbohydrate hydrolysis, reduction of antinutritional factor concentrations, improvement of protein digestibility, and the generation of a more appealing flavor and aroma profile[4]. Moreover, fermentation is capable of enhancing the bioavailability of specific nutritional components and promoting the synthesis of biologically active compounds, thereby expanding options for designing health-promoting functional commodities.

Despite a substantial volume of literature dedicated to the fermentation of soy-based products, utilizing *Vicia faba* proteins for the manufacturing of fermented alternative beverages remains an under-researched area. Investigating the effects of the fermentation process on the properties of faba bean protein concentrates, the formation of beverage texture and sensory characteristics, as

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[3] Fortification of yogurt with bioactive functional foods and ingredients and associated challenges: A review / I. Ahmad, M. Hao, Y. Li et al. *Trends in food science & technology*. 2022. Vol. 129. P. 558–580. DOI: https://doi.org/10.1016/j.tifs.2022.11.003.

[4] A review of nutritional profile and processing of faba bean (Vicia faba L.) / S. B. Dhull, M. K. Kidwai, R. Noor et al. *Legume Science*. 2022. Vol. 4. No. 3. Art. e129. DOI: https://doi.org/10.1002/leg3.129.

well as establishing optimal microbial cultivation parameters to achieve high-quality yields, is of particular relevance.

Consequently, exploring the fermentation processes of *Vicia faba* bean protein to design alternative beverages constitutes a timely scientific and practical task. This research is directed toward diversifying the assortment of functional plant-derived commodities, enhancing the utilization efficiency of domestic plant raw materials, and satisfying modern consumer demands for healthy nutrition.

The aim of this work is to investigate the technological conditions for the fermentation of *Vicia faba L.* bean protein and to evaluate the feasibility of its application in developing fermented plant-based dairy analogues with enhanced organoleptic, nutritional, and functional properties.

The object of the research encompasses the regularities of the lactic acid fermentation process of *Vicia faba L.* protein concentrate for the development of plant-based beverages.

The subject of the research comprises *Vicia faba L.* bean protein concentrate, a bacterial α-amylase enzyme preparation, starter cultures, and the physical-chemical and organoleptic indicators of the fermented beverage.

To evaluate the fermentation process, a protein concentrate manufactured from *Vicia faba L.* beans via a “dry processing method” was utilized. The hydrolytic enzyme preparation used was a thermostable α-amylase, Termamyl (produced by “Novozymes”), while the bacterial starter cultures included Iprovit-Kefir, Iprovit-Symbilact, Iprovit-Vitalact, Iprovit-BTA (supplemented with bifidobacteria), Iprovit-Yogurt, and Iprovit-Streptosan, all of which were standardized in accordance with state regulatory requirements[5]. The hydrolysis of starch, which occurs as an impurity within the protein concentrate structure, was conducted under laboratory conditions using a conventional thermostat as well as within an ultra-high-frequency electromagnetic field using a Panasonic microwave oven operating at a maximum power of 900 W. The total content of reducing substances (RS) expressed in g/dm^3 of the product was quantified using the Willstätter-Schudl method; total acidity was determined via standard titrimetric analysis, and the free amino nitrogen (FAN) content was measured using the ninhydrin method[6].

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[5] ТУ У 15.5-00419880-10:2010. Культури заквашувальні сухі та рідкі. НААН України, Інститут продовольчих ресурсів. Київ, 2010.

[6] Free Amino Nitrogen (International Method) / Technical Committee A. ASBC Methods of Analysis. St. Paul, MN : American Society of Brewing Chemists, 2011.

1. Vicia faba L. beans as a promising raw material for the production of plant-based fermented products

The projected increase in the global population to 9 billion people by 2050 necessitates a profound revision of protein nutrition structures and the discovery of innovative technological paths for processing renewable raw materials. Currently, the primary foundation for plant-based milk analogues is established by soy and various nuts (such as almond, cashew, and coconut). However, nut raw materials involve high initial production costs, and their concentration in finished beverages typically does not exceed 5%, which keeps the final protein level critically low (under 0.2 g per 100 g)[7]. Soy protein, while nutritionally complete and functional, encounters consumer skepticism due to its high allergenicity and the mass utilization of genetically modified lines of this crop[8].

In this regard, faba beans (*Vicia faba L.*, also known as broad or field beans) emerge as a highly viable alternative. Due to their large seed size, ease of dehulling compared to chickpeas or lentils, and minimal lipid content, this crop represents an ideal object for advanced deep processing technologies aimed at extracting protein concentrates. The chemical composition of dry matter in faba beans demonstrates clear advantages in protein content compared to other widely distributed legumes (Table 1).

Table 1

Approximate composition of faba beans compared to peas and soybeans (g/100 g dry matter)[9]

Beans	Proteins	Carbohydrates	Fats	Ash
Faba beans	27.6	66	1.4	3.4
Peas	23.4	63.5	1.6	3
Soybeans	40	28.6	19.7	5.2

The fatty acid spectrum of *Vicia faba L.* lipids is predominantly composed of mono- and polyunsaturated fatty acids, specifically oleic and linoleic acids. These fractions exert a beneficial effect on the human blood lipid profile, notably stimulating the synthesis of high-density lipoproteins (HDL), which serves as an

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7 Технологічні та економічні аспекти застосування бобових в технологіях харчової продукції: міні-огляд / В. В. Дегтяр, А. Е. Радченко, Н. Г. Гринченко, О. О. Гринченко. *Journal of Chemistry and Technologies*. 2023. Vol. 31. № 4. С. 886–896. DOI: https://doi.org/10.15421/jchemtech.v31i4.287753.

8 Nutritional quality of protein flours of fava bean (Vicia faba L.) and in vitro digestibility and bioaccesibility / V. A. Ayala-Rodríguez, A. A. López-Hernández, M. L. C. Lomelí et al. *Food Chemistry: X*. 2022. Vol. 14. Art. 100303. DOI: https://doi.org/10.1016/j.fochx.2022.100303.

9 Labba I. C. M. Nutritional and antinutritional composition of fava bean (Vicia faba L., var. minor) cultivars / I. C. M. Labba, H. Frøkiær, A. S. Sandberg. *Food Research International*. 2021. Vol. 140. Art. 110038. DOI: https://doi.org/10.1016/j.foodres.2020.110038.

essential factor in preventing cardiovascular pathologies. The mineral complex of the beans is characterized by significant concentrations of potassium, iron, and zinc, combined with a low sodium level, presenting an added benefit for specialized dietary nutrition. Beyond starch, the carbohydrate fraction contains a substantial amount of dietary fibers. They exhibit a pronounced prebiotic effect: passing transit through the upper sections of the gastrointestinal tract, these fibers are fermented by the colon microflora to generate short-chain fatty acids, which effectively suppress the proliferation of pathogenic microorganisms.

According to Osborne's classification, the protein fraction of *Vicia faba L.* consists primarily of salt-soluble globulins (69.5%–78.1%) and alkali-soluble glutelins (12.0%–18.4%), whereas the proportions of water-soluble albumins and alcohol-soluble prolamins are negligible[10]. The amino acid profile of the total protein is characterized by elevated levels of lysine, leucine, threonine, and arginine; however, consistent with most legumes, it exhibits a deficit in sulfur-containing amino acids (methionine and cysteine) as well as tryptophan (Table 2). This nutritional limitation is easily overcome by designing composite formulations with cereal crops that possess a highly complementary amino acid spectrum.

Table 2

Amino acid composition of faba beans compared to peas and soybeans (g/100 g protein)[11]

Amino acids	Faba beans	Peas	Soybeans
1	2	3	4
Histidine	2.41	2.52	2.91
Isoleucine	3.94	3.33	4.6
Leucine	7.47	6.58	7.76
Lysine	7.08	6.84	7.08
Methionine	0.87	1.03	1.29
Cysteine	1.33	1.55	1.19
Phenylalanine	4.19	4.19	5.87
Tyrosine	2.78	3.16	3.65
Threonine	3.4	3.59	3.69
Tryptophan	0.87	0.94	1.38

[10] Proximate composition and anti-nutritional factors of fava-bean (Vicia faba), green-pea and yellow-pea (Pisum sativum) flour / K. A. Millar, E. Gallagher, R. Burke et al. *Journal of Food Composition and Analysis.* 2019. Vol. 82. Art. 103233. DOI: https://doi.org/10.1016/j.jfca.2019.103233.

[11] Oluwajuyitan T. D. Amino acid profile, peptide size distribution, antioxidant effect, and enzyme inhibitory activities of fava bean (Vicia faba) protein hydrolyzates / T. D. Oluwajuyitan, D. Kadam, R. E. Aluko. *International Journal of Food Properties.* 2025. Vol. 28. No. 1. Art. 2466575. DOI: https://doi.org/10.1080/10942912.2025.2466575.

Continuation of table 2

1	2	3	4
Valine	4.31	3.89	4.64
Arginine	9.46	6.84	8.86
Alanine	4.15	4.27	4.39
Aspartic acid	10.74	10.68	11.98
Glutamic acid	16.51	16.92	17.88
Glycine	4.73	4.32	4.2
Proline	3.94	3.76	4.92
Serine	4.69	4.79	4.77

Based on the sedimentation coefficient, the dominant globulins are classified into hexameric legumins (11S), comprising up to 55% of the total seed protein, and trimeric glycosylated vicilins (7S). The ratio of the 11S/7S fractions directly establishes the functional and technological attributes of the resulting concentrates. Legumins exhibit a higher denaturation temperature (95.4 °C) than vicilins (83.8 °C), which surpasses the corresponding values of soy and pea proteins, thereby ensuring excellent thermal stability of colloid systems formulated from these beans[12]. *Vicia faba L.* proteins display strong potential for gelation, emulsification, and the stabilization of disperse systems, which is critically important for forming a smooth, uniform texture in plant-based yogurts, fermented dairy alternatives, and beverages.

However, the successful realization of this structural potential requires overcoming several biochemical and technological barriers. The primary flaw of the raw material is its specific "beany" or herbaceous aroma and a bitter aftertaste. These arise from the oxidation of linoleic acid residues catalyzed by the lipoxygenase enzyme, yielding volatile aldehydes and ketones (predominantly hexanal). A secondary obstacle involves the presence of antinutrients (tannins, phytic acid, and protease inhibitors), along with the glycosides vicine and convicine, which impair the absorption of essential micronutrients and lower the efficiency of gastric proteolysis.

The adoption of traditional processing methodologies (such as soaking, germination, and extended thermal treatment) only partially minimizes the concentration of antinutritional compounds, while potentially inducing

[12] Milk Analog: Plant based alternatives to conventional milk, production, potential and health concerns / A. A. Paul, S. Kumar, V. Kumar, R. Sharma. *Critical reviews in food science and nutrition*. 2020. Vol. 60. No. 18. P. 3005–3023. DOI: https://doi.org/10.1080/10408398.2019.1674243.

protein denaturation and severely reducing solubility[13]. Consequently, directed lactic acid fermentation serves as the most effective strategy for targeted biotechnological modification. Driven by the enzymatic apparatus of the starter cultures, partial macromolecular hydrolysis, breakdown of antinutritional components, and a transformation of the volatile compound profile occur. This minimizes the intensity of the unwanted beany flavor, enhances the rheological properties of the protein matrix, and results in a next-generation plant-based product characterized by superior sensory attributes and heightened nutrient bioavailability.

2. Investigation of the fermentation of Vicia faba bean protein concentrate

The development of scientific and practical foundations for utilizing plant-based raw materials in fermented beverage technologies is anchored in the foundational works of numerous domestic and international scientists, including O. Vitriak, V. Hnitsevych, S. Nevmyvanyis, A. Petryna, N. Prytulska, I. Romanchuk, A. Trubnikova, Yu. Savchuk, A. Serenko, T. Yudina, A. Almnuri, M. Tangyu, M. Youssef, and others.

Despite a substantial volume of scientific research, plant-derived proteins remain underutilized and have limited industrial applications in the food sector. Currently, systematic scientific investigations focusing specifically on the technology of fermented plant-based beverages utilizing legume protein concentrates are lacking in Ukraine.

The innovative concept of this study lies in establishing a scientifically substantiated technology for fermented beverages using Vicia faba processing products, specifically a protein concentrate that serves as the protein base for forming a plant dispersed system. It is anticipated that the integration of this ingredient will facilitate the creation of a product with elevated plant protein concentrations while establishing a structural matrix highly susceptible to further targeted biotechnological modification during fermentation.

The essence of the proposed innovative approach consists in coupling specialized technological methods of plant substrate preparation with controlled microbial fermentation, thereby enabling precise control over the physical-chemical and organoleptic transformations of the finished matrix. This strategy supports the development of novel functional plant-based dairy analogues that align with modern nutritional safety and consumer standards.

[13] Fermentation of plant-based milk alternatives for improved flavour and nutritional value / M. Tangyu, J. Muller, C. J. Bolten, C. Wittmann. *Applied microbiology and biotechnology*. 2019. Vol. 103. No. 23. P. 9263–9275. DOI: https://doi.org/10.1007/s00253-019-10175-9.

The operational framework of this innovative strategy is grounded in three fundamental principles. The first principle is the rational utilization of plant-derived protein raw materials, which mandates the maximum preservation of their native nutritional and biological value throughout the technological cycle. This is achieved by determining the optimal limits of processing parameters, preventing excessive, destructive thermal denaturation while preserving the key functional properties of the protein assemblies.

The second principle is the adaptation of processing modes of the plant protein system to the distinct operational conditions required for fermented food production. Thermal treatment, specifically pasteurization, represents a critical phase that ensures microbiological safety and early structural stabilization. The selection of these thermal limits must strictly accommodate the specific structural boundaries of the plant protein matrix, as excessive thermal exposure induces non-reversible aggregation and impairs the final texturological behavior.

The third principle of the innovative strategy implementation is the exploitation of fermentation processes as a highly efficient biotechnological instrument for altering the macro-composition and properties of the plant matrix. Fermentation drives the deep biochemical transformation of raw substrate constituents, accompanied by the accumulation of organic acids, free amino nitrogen compounds, and volatile metabolites that establish the target flavor, aroma, and bioavailability profiles[14]. Beyond this, fermentation can facilitate enhanced protein digestibility and heightened nutrient bioavailability.

Based on the synthesis of prior scientific literature and preliminary experimental trials, a working hypothesis was formulated, according to which utilizing Vicia faba bean protein concentrate as the foundation for plant-based beverages, when coupled with optimized enzymatic and microbial fermentation parameters, enables the generation of a stable system with significantly enhanced physical-chemical, structural-mechanical, and organoleptic properties.

To validate this hypothesis, a conceptual technological matrix for producing a fermented beverage based on Vicia faba L. protein concentrate was engineered. The generalized technological scheme of the process is shown in Figure 1.

The generalized technological architecture comprises the preparation of a protein suspension, treatment with a hydrolytic thermostable bacterial alpha-amylase preparation, stabilization via sterilization, cooling to the target inoculation temperature, culture inoculation, and subsequent multi-hour

[14] Jiang L. Unlocking the microbial mechanisms in fermented bean products: Advances and future directions in multi-omics and machine learning approaches / L. Jiang, Y. Lu, H. Qu. *Critical Reviews in Food Science and Nutrition*. 2026. Vol. 66. No. 7. P. 1364–1386. DOI: https://doi.org/10.1080/10408398.2025.2544764.

fermentation. The primary focus was directed toward identifying the optimal processing configurations for raw suspension preparation.

The baseline plant substrate was formulated as an aqueous dispersion with a dry matter mass fraction of 6% by blending the dry-processed Vicia faba L. bean protein concentrate with water. Following mechanical homogenization, the suspension was inoculated with 1 mL of a bacterial alpha-amylase enzyme preparation (per 500 ml of total volume) to initiate the targeted enzymatic hydrolysis of the native starch fractions.

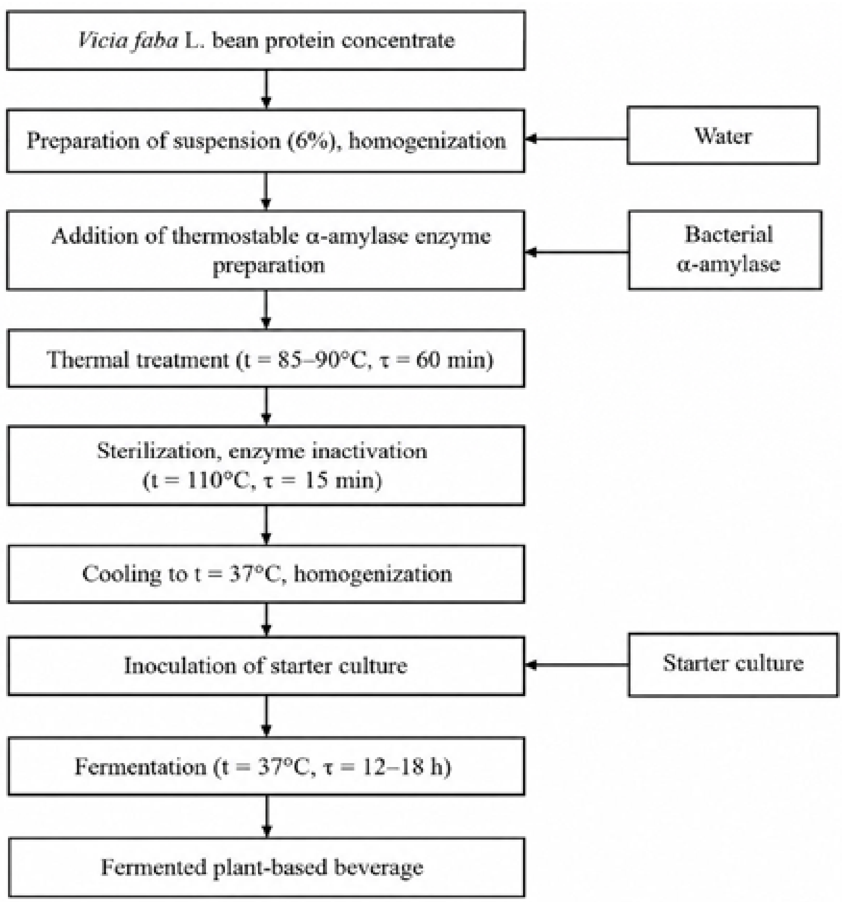

Fig. 1. Process flow diagram for the production of a fermented plant-based beverage from *Vicia faba* protein concentrate

The native faba bean protein concentrate contains approximately 10% starch as a structural impurity, which undergoes intense gelatinization at elevated temperatures in the presence of protein macro-assemblies, resulting in a highly viscous, paste-like consistency that seriously impedes downstream liquid processing. Furthermore, the development of a highly viscous, sticky starch gel network acts as a steric hindrance, disrupting proper protein-protein interactions and inhibiting the formation of a clean, acid-induced protein gel matrix. Consequently, such unrefined, gelatinized protein-starch systems display far lower texturological properties than traditional emulsion-based food gels. Therefore, the thermal processing of the protein suspension in the presence of alpha-amylase was targeted at reducing system viscosity via starch liquefaction into reducing sugars, optimizing the fermentation environment for the starter cultures, and reducing the native legume aroma.

The initial experimental stage involved a comparative validation of two distinct thermal processing setups for the protein-enzyme mixture: conventional heating using a water bath at 80–85 °C for 60 minutes versus electromagnetic microwave treatment performed in an ultra-high-frequency microwave field. The microwave treatment was applied to a 200 g aliquot of the suspension inside a glass vessel at an operating power of 900 W for a total duration of 6 minutes, utilizing discrete exposure cycles (30 seconds of heating followed by a 10 seconds mechanical stirring phase). During the microwave exposure, a continuous increase in foam generation was monitored once temperatures surpassed 7 °C, followed by an abrupt alteration in the structural aggregation state of the protein system within the 80–85 °C range. Aliquots were periodically sampled throughout both thermal operations to evaluate the accumulation kinetics of reducing substances (Table 3).

Table 3

Kinetics of reducing substance accumulation during the enzymatic hydrolysis of the faba bean protein suspension

№	Water Bath Thermal Treatment		Microwave Field Thermal Treatment	
	Hydrolysis Time, min	Reducing Substances, g/mL	Hydrolysis Time, min	Reducing Substances, g/mL
1	20	4.8	2	5.2
2	40	6.3	4	7.2
3	60	8.9	6	8.15

The experimental data indicated that both the water bath processing at 80 °C for 60 minutes and the microwave treatment were equally viable regarding their starch liquefaction efficiency; however, the drastic kinetic acceleration achieved within the ultra-high-frequency microwave field was severely counterbalanced by negative physical phenomena, specifically uncontrolled foam destabilization and macro-protein aggregation.

Following the enzymatic liquefaction via alpha-amylase, the liquefied suspension was subjected to sterilization in an autoclave at 110 °C for 15 minutes. This phase achieved complete microbiological sterilization, enzyme inactivation, and the chemical thermal degradation of the key volatile compounds responsible for the native helical legume off-flavor. As a direct outcome of the sequential enzyme-thermal treatment, the visual profile of the suspension shifted from a yellowish cast to a milk-white appearance, accompanied by a major drop in the intensity of the beany odor. The subsequent microbial fermentation phase further transforms the organoleptic profile, as specific lactic acid bacteria utilize and metabolize native volatile aldehydes and ketones, notably reducing their concentrations. Strains such as Lactiplantibacillus plantarum, Lacticaseibacillus casei, Limosilactobacillus fermentum, and Streptococcus thermophilus possess metabolic pathways capable of converting volatile green aldehydes into low-odor alcohols or organic acids, naturally refining the flavor profile[15]. Moreover, fermentation triggers structural modifications within the protein-polysaccharide complex, enhancing the matrix's capacity to bind volatile flavor components and reducing their overall volatility.

The prepared white protein base was subsequently cooled to 37 °C, inoculated with selected starter cultures, and subjected to an 18-hour incubation period.

The selection of appropriate bacterial ecosystems represents a core challenge in plant-based dairy alternative design, as the specific microflora directly governs the rate of biochemical transformations, the kinetics of acidification, carbohydrate utilization, proteolytic protein cleavage, and the sensory attributes of the final product.

The experimental matrix evaluated six commercial freeze-dried bacterial starter cultures: Iprovit-Kefir, Iprovit-Symbilact, Iprovit-Vitalact, Iprovit-BTA with bifidobacteria, Iprovit-Yogurt, and Iprovit-Streptosan. An uninoculated sample incubated under identical thermal conditions served as the control. The cultures demonstrated universal viability within the legume-based medium, though their metabolic efficacy varied considerably. The parameters monitored during the fermentation process included titratable acidity, free amino nitrogen content, and the accumulation of reducing substances.

Acidification kinetics served as a primary gauge of fermentation intensity, with all inoculated matrices exhibiting a steady increase in titratable acidity

[15] Effect of lactic acid bacteria on the level of antinutrients in pulses: A case study of a fermented faba bean–oat product / M. Kahala, I. Ikonen, L. Blasco et al. *Foods*. 2023. Vol. 12. No. 21. Art. 3922. DOI: https://doi.org/10.3390/foods12213922.

relative to the control, verifying the progression of active lactic acid fermentation (Figure 2). The highest acidity values were recorded in samples driven by symbiotic multicomponent starter cultures, an outcome supported by their complex microbial consortia and metabolic synergy.

The highest metabolic rates and superior adaptability to the Vicia faba matrix were displayed by Iprovit-BTA and Iprovit-Symbilact. This intensive acid production allowed the system to rapidly shift toward the target pH value of 4.7.

Consequently, investigating the fermentation processes of Vicia faba bean protein concentrate using various cultures of microorganisms, tracking the acidification kinetics, and monitoring the improvement of sensory parameters, namely color and aroma, confirms the positive effect of fermenting raw plant proteins from Vicia faba beans.

Carbohydrate conversion dynamics further illustrated the metabolic activity of the inoculated microflora, as the reducing sugar concentration dropped steadily throughout the fermentation period (Figure 3). The initial reducing sugar concentration of 5.01 mg/mL within the control medium dropped sharply to 1.0 mg/mL across the fermented samples by the end of the incubation cycle. This rapid sugar consumption correlated directly with the surge in titratable acidity, confirming the active conversion of mono- and disaccharides into organic acids. The most intense drop in this parameter was recorded in samples with elevated enzymatic activity, correlating with the acid profile.

Proteolytic transformations were verified by monitoring the accumulation of free amino nitrogen (FAN) (Figure 4), with inoculated samples displaying

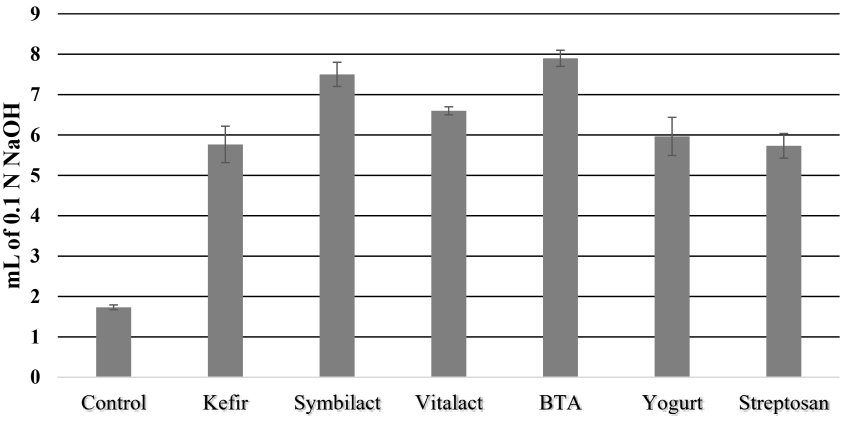

Fig. 2. The effect of bacterial starter cultures on the titratable acidity of fermented samples

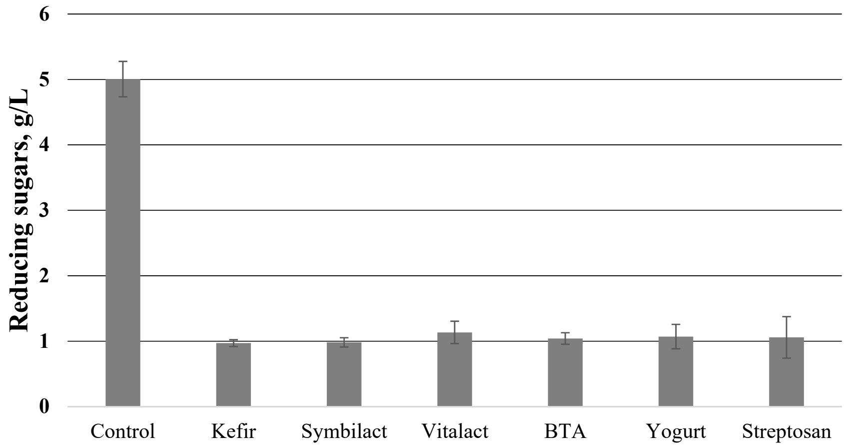

Fig. 3. Dynamics of reducing sugar content during the fermentation process depending on the starter culture type

a significant increase in FAN concentrations, reflecting active bacterial proteolysis. This enzymatic cleavage of dense legume globulins into low-molecular-weight nitrogenous fractions significantly enhances both the biological value and the metabolic digestibility of the finished product.

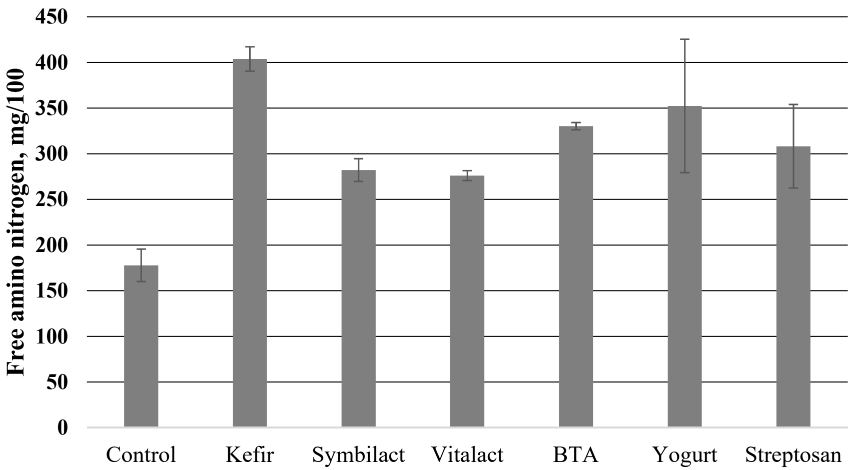

Fig. 4. The effect of starter cultures on the accumulation of free amino nitrogen

The multi-species consortia proved most effective at driving both deep carbohydrate conversion and advanced protein proteolysis, ensuring intensive acidification, sugar depletion, and structural transformation.

The ultimate sensory quality and texture of the fermented beverage were found to be highly dependent on the strain profile of the starter culture used (Table 4). The highest scores were secured by the samples driven by the Iprovit-BTA, Iprovit-Symbilact, and Iprovit-Yogurt consortia, which were distinguished by a pleasant taste, smooth consistency, and a well-balanced aroma. Crucially, the intense enzymatic activity of these cultures managed to completely mask or transform the volatile compounds responsible for the native "beany" off-flavor, which represents a substantial breakthrough for consumer acceptance. Conversely, the unfermented control exhibited the lowest properties, characterized by a weak, unbalanced flavor profile, a coarse, non-homogeneous texture, and a strong, unpleasant herbaceous aroma and aftertaste.

To systematically evaluate these sensory metrics, a specialized scale was designed across core indices: taste, aroma, consistency, and appearance. An expert evaluation method was used to calculate weight significance coefficients for establishing a 20-point sensory scoring framework. Each specific descriptor was scored on a scale where 1.5 represented highly significant, 1 stood for significant, 0.5 meant minor significance, and 0 was evaluated as unnecessary for inclusion.

Based on the optimization of these parameters, a structured 20-point scoring database was compiled for the sensory profiling of the fermented plant-based beverages, as detailed in Table 4.

Table 4

Weight coefficients and score distribution for the sensory evaluation of fermented plant-based beverages within a 20-point framework

Sensory Attribute	Significance Coefficient	Maximum Score Allocation (Raw / Weighted)
Taste	1	5/5
Aroma	1.5	5/7.5
Consistency	1	5/5
Appearance	0.5	5/2.5
Total Value	–	20

Based on the quantitative panel scores collected during the sensory trials, a database was compiled to track individual sample performance across the weighted criteria (Table 5).

Table 5

Sensory profile evaluation of the fermented Vicia faba L. protein beverage samples

Sample No.	Starter Culture Type	Taste	Aroma	Consistency	Appearance	Total Weighted Score
1	Iprovit-Kefir	3.9	4.1	4.0	4.0	16.05
2	Iprovit-Symbilact	4.5	4.4	4.6	4.5	17.95
3	Iprovit-Vitalact	4.1	4.0	4.2	4.1	16.35
4	Iprovit-BTA	4.8	4.7	4.9	4.8	19.15
5	Iprovit-Yogurt	4.3	4.2	4.4	4.3	17.15
6	Iprovit-Streptosan	4.0	4.1	4.1	4.0	16.25

The sensory matrix demonstrated that fermenting the legume protein concentrate with diverse starter microflora yields products with comparable physical characteristics. Regarding structural consistency and surface appearance, the fermented plant matrices closely mirrored traditional dairy-based yogurts. However, despite these favorable texturological profiles, the unflavored fermented bases require further fine-tuning through the incorporation of targeted flavorings and natural aromatic modifiers. Thus, the current matrices serve as a functional, structural baseline for the subsequent design of fully finished consumer plant-based functional beverages.

CONCLUSIONS

The experimental findings confirm that legume protein concentrate represents a highly viable and technologically sustainable raw material for manufacturing next-generation functional plant-based dairy analogues.

Preliminary enzyme-thermal processing of the raw protein suspension using a bacterial alpha-amylase successfully drives the partial liquefaction of structural starch impurities into reducing sugars, which effectively prevents uncontrolled starch gelatinization, improves the rheological properties of the raw suspension prior to inoculation, and optimizes the liquid substrate for subsequent microbial fermentation by starter cultures.

Evaluation of different starter cultures demonstrated that Iprovit-BTA, Iprovit-Symbilact, and Iprovit-Yogurt exhibit superior metabolic activity, acid production, and sensory design capacity within the legume matrix. Lactic acid fermentation plays a transformative role in product design, stabilizing the physical-chemical structure, eliminating the native “beany” off-flavor, and yielding a smooth, uniform yogurt-like matrix with elevated nutrient bioavailability.

As an outcome of fermentation, the sensory indicators of the matrix are markedly refined: the characteristic beany aroma disappears, the color improves, and a dense, uniform, yogurt-like structure is formed, confirming that the developed product represents a stable baseline for constructing commercial alternatives to traditional milk beverages.

SUMMARY

This study addresses the relevant challenge of expanding the assortment of plant-based milk alternatives through the biotechnological modification of non-deficient domestic raw materials. It presents the experimental results on the topic "Fermentation of *Vicia faba* bean protein concentrate for the development of plant-based beverages" characterized by enhanced biological value. The parameters for the preliminary enzyme-thermal treatment of the protein suspension using α-amylase were substantiated, enabling a targeted reduction in system viscosity via starch liquefaction. It was established that both water bath processing and microwave heating are equally effective regarding starch hydrolysis efficiency, though the kinetic acceleration within the ultra-high-frequency field is accompanied by intense foaming and partial protein aggregation. Based on the evaluation of six "Iprovit" commercial starter cultures, the "Iprovit-BTA" and "Iprovit-Symbilact" consortia demonstrated the superior acidification kinetics, effectively driving the system to the protein isoelectric point at pH 4.7. Microbial carbohydrate utilization was verified by a sharp decline in reducing sugars from the baseline 5.01 mg/mL down to 1.0 mg/mL. The maximum proteolytic activity was displayed by the "Iprovit-Kefir" culture, which triggered a 2.2-fold increase in free amino nitrogen compared to the unfermented control. Sensory profiling performed via a developed 20-point expert framework confirmed that directed lactic acid fermentation successfully masks the specific native beany off-flavor, establishing a smooth cream-like consistency and a well-balanced flavor profile.

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