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BIOCHEMICAL VARIABILITY OF VEGETABLE JUICE POWDERS: A KEY FACTOR IN MODULATING THE PHYSICOCHEMICAL PROPERTIES AND SAFETY PROFILE OF VEGAN FERMENTED SAUSAGES

This research focuses on developing Thai mushroom vegan sausages (Naem Het) with improved quality and safety profiles by incorporating pumpkin seed protein isolate (PSMPI) and vegetable juice powders (yellow beet, celeriac, yellow carrot, radish) as functional ingredients. Their effects on sensory and microbiological parameters, nutritional value, and stability were evaluated. The growing demand for plant-based meat analogues necessitates the development of high-quality fermented products. A key challenge remains the achievement of desirable characteristics without synthetic additives; therefore, the use of vegetable juices as natural nitrite precursors in mushroom-based systems is a promising but under-researched area. Five formulations (A0 and 4 experimental ones) were analyzed for their chemical composition, microbiological parameters (aerobic mesophilic bacteria, lactic acid bacteria, coliforms, pH, NO_3^-/NO_2^-), and sensory properties during fermentation and 14 days of storage. It was found that PSMPI significantly increased the protein content (from 8.50% in A0 to 10.75% in A1) and improved the texture. The vegetable powders effectively served as sources of NO_3^-, modulating microbial activity. Based on a comprehensive evaluation, the samples with beetroot (A1) and carrot (A3) were identified as the best. They exhibited superior sensory profiles compared to the control A0 (7.87), receiving the highest overall scores on a 9-point scale – 8.22 (A1) and 8.12 (A3) – and demonstrated optimal microbiological stability (pH 4.49–4.51, LAB ≈ 8.3–8.5 log_{10} CFU/g). This comprehensive positive effect was attributed to the high content of pigments with antioxidant properties in these vegetables, which facilitated a controlled fermentation. In contrast, in the samples with celery and radish, a significantly lower pigment content impaired the bacterial reduction of their extremely high initial NO_3^- content (270–290 mg/kg), leading to a unique dynamic and maximal accumulation of NO_2^-. Thus, this research confirms that the judicious selection of vegetable juice powder is an effective tool for creating high-quality, safe, and sensorially appealing vegan fermented products.

Keywords: *meat analogues, natural curing, ethnic cuisine, organic ingredients, future foods.*

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1. Introduction

Modern humanity faces significant global challenges in ensuring food security for a constantly growing population [1]. Concurrently, the issue of climate change and other negative environmental impacts is becoming increasingly critical [2]. These environmental pressures, including greenhouse gas emissions and resource consumption, are largely driven by traditional animal husbandry practices [3, 4]. In this context, the search for and implementation of alternative protein sources, particularly of plant origin, is of crucial importance. This trend is vividly illustrated by the functional beverage market, where a significant share is occupied by plant-based milk alternatives for coffee drinks [5], and fortified "green" beverages based on sprouts and other plant materials [6]. Consequently, a broad spectrum of plant-based products – from meat and dairy analogues to egg substitutes – is regarded as a healthier and more ecologically sustainable alternative to traditional animal products amidst climate change [7]. Another promising area is the exploration of effective and eco-friendly alternative protein sources, notably from invertebrates like mollusks. The use of invertebrates not only provides a high content of protein and other nutrients but also helps to reduce the environmental load due to lower resource consumption compared to traditional livestock farming [8]. The transition to plant-based diets demonstrates advantages in terms of environmental impact and protein quality, which is important for overall health and physical activity [9].

Alongside this, there is a growing interest in natural food ingredients and their health effects. This is particularly true for nitrates and nitrites, the natural sources of which offer significant health benefits [10]. Dietary nitrates, found in green leafy vegetables and beetroot, positively affect the vascular system through the nitrate-nitrite-nitric oxide pathway [11]. Root and tuber vegetables are also important dietary sources of health-promoting compounds [12]. Therefore, the use of plant extracts as sources of natural nitrates is a promising approach for replacing artificial additives in the food industry [13]. However, the movement towards natural and functional products is not limited to replacing auxiliary ingredients but also involves a fundamental revision of core formulation components, particularly proteins. A key direction in the modernization of food systems is the careful integration of alternative protein sources – from plants, fungi, algae, and invertebrates – into traditional culinary practices. For instance, one manifestation of this trend is the modernization of classic recipes, such as the French dish Brandade de morue, by enriching it with sustainable invertebrate protein (snails) [14]. The same trend is observed in the production of traditional meat products, where the improvement of recipes for well-known white sausages (Weißwurst, Biała kiełbasa) is aimed at enhancing their nutritional value and improving structural characteristics within the framework of food sustainability [15]. These examples illustrate the modernization of meat-containing products; however, a logical extension of this global trend is the creation of products where animal-derived raw materials are completely replaced by alternative, primarily plant-based, components. The creation of plant-based meat analogues is a promising avenue for providing the population with healthy and sustainable meat substitutes [16]. The development of such products involves addressing challenges in their production and quality control to achieve desired characteristics [17]. Experience in enhancing traditional meat recipes with functional plant-based raw materials can serve as a guide for further innovations in the production of vegan alternatives [18]. Plant-based food science is actively developing approaches to create nutritious and sustainable analogues for meat, seafood, milk, and eggs [19]. Such innovative approaches contribute to the formation of healthy dietary patterns and the promotion of sustainable food systems, which could potentially help address the problems of hunger and malnutrition on a global scale [20].

The adaptation of these approaches to traditional products, such as Thai fermented sausages *Naem Het*, is of particular interest. The combination of a mushroom base, plant protein, and vegetable powders to create a vegan version of this product opens up new possibilities. However, the comprehensive impact of such ingredients, their interactions, and their influence on the final properties of the product remain insufficiently studied. Therefore, research in this direction is highly topical. *The aim of this research* was to evaluate the comprehensive impact of pumpkin seed protein isolate (PSMPI) and dried organic vegetable juices on the key quality parameters of vegan fermented *Naem Het* sausages.

2. Materials and Methods

2.1. Object of research and hypothesis

The object of this research was the technology and quality parameters of vegan fermented sausages, developed based on the Thai dish "*Naem Het*". The comprehensive impact of functional ingredients – namely, pumpkin seed protein isolate (PSMPI) and powders from dried organic vegetable juices (yellow beet, celeriac, yellow carrot, radish) – on the organoleptic, physicochemical, and microbiological characteristics of the final product was investigated.

The research hypothesis was that the combined use of pumpkin seed protein isolate (PSMPI) and vegetable juice powders would allow for the targeted management of the quality of vegan fermented sausages. It was postulated that PSMPI would act as a structural and nutritional component, improving texture and increasing protein content, while the vegetable powders would serve as natural sources of nitrates (NO_3^-) to ensure controlled fermentation. Simultaneously, it was anticipated that the biochemical characteristics of each powder (specifically, the content of pigments and antioxidants) would determine the efficiency of nitrate-to-nitrite (NO_2^-) conversion and, consequently, shape the unique sensory profile and stability of the final product.

2.2. Materials

Organic pumpkin (*Cucurbita maxima*) was purchased from a farm in the Odesa region, Ukraine, with certificate number (MAOC standard) 24-1306-06-02 and certificate number (Ukrainian organic product) 24-1306-01-UA-02.

Organic yellow beet (*Beta vulgaris subsp. vulgaris* (*Conditiva Group*)), organic celeriac (*Apium graveolens var. rapaceum*), organic yellow carrot (*Daucus carota subsp. sativus*), organic radish (*Raphanus sativus var. radicula Pers.*), organic cayenne pepper (*Capsicum annuum* (*Cayenne Group*)), and organic garlic (*Allium sativum*) were purchased from a farm in the Sumy region, Ukraine, with certificate number (MAOC standard) 24-0511-09-01 and certificate number (Ukrainian organic product) 24-0511-02-UA-01.

King bolete mushrooms (*Boletus edulis*) were collected in an ecologically clean forest, remote from urbanized areas in the Sumy region, Ukraine. Due to their mycorrhizal mode of nutrition, *Boletus edulis* mushrooms are not subject to industrial cultivation. The mushrooms were stored at 2°C for subsequent use in the recipe.

Organic glutinous rice (*Oryza sativa var. glutinosa*) from Tasty Bite (USDA Organic), India; Organic Green Banana Powder from MRM Nutrition (USDA Organic), Ecuador; Organic Vegetable Glycerine from NOW Foods, Solutions (USDA Organic), USA; and Organic Tapioca Maltodextrin from Nutricost (USDA Organic) were purchased from a specialized organic food store. *Pediococcus pentosaceus* bacteria, *Staphylococcus xylosus* flavor bacteria, salt, carrageenan (kappa-carrageenan and iota-carrageenan in a 1:1 ratio), and red algae powder (*Lithothamnium calcareum*) were purchased from a specialized store for food semi-finished products. All reagents used for laboratory tests were of analytical grade. Deionized water was used in all experiments.

2.3. Preparation of freeze-thaw pre-treated vegetable juice powders as natural nitrite sources

Organic vegetables (yellow beet, yellow carrot, celeriac, radish) were cleaned of mechanical debris and washed. The vegetables were prepared based on a freeze-thaw pre-treatment principle to disrupt cellular structures [21]. After thawing, juice was extracted from the vegetables using a screw juicer and passed through an 80-mesh sieve. Before drying, organic tapioca maltodextrin was added to the juice as a carrier at a concentration of 10% (w/w) of the total juice mass. The organic vegetable juice was spray-dried using a fine-particle atomization technology (inlet air temperature 125 ± 5°C, outlet air temperature 65 ± 5°C; feed rate 5 ± 2 ml/min). The resulting vegetable juice powder was sifted through an 80-mesh sieve and milled if necessary.

2.4. Preparation of pumpkin seed meal protein isolate (PSMPI)

2.4.1. Preparation of pumpkin seed meal

Pumpkin seeds (*Cucurbita maxima*) were dried in a solar dryer. The seeds, pre-heated to 40°C, were defatted using a screw oil press to obtain a press cake. For further defatting, the ground press cake was extracted with hexane at a 1:5 (w/v) ratio at 40°C for 1 hour with constant stirring. After centrifugation to separate the miscella, the resulting meal was dried in a fume hood at 40°C for 8 hours until a constant weight was achieved, indicating complete solvent removal.

2.4.2. Alkaline extraction with isoelectric precipitation and pH-shift treatment of pumpkin seed meal

The pumpkin seed meal was ground in a laboratory mill and sifted through an 80-mesh sieve. The protein isolate was obtained from the pumpkin seed meal (*Cucurbita maxima*) using alkaline extraction with isoelectric precipitation and a pH-shift treatment to maximize the functional properties of the protein isolate [22], but with some modifications. Specifically, after the alkaline extraction and isoelectric precipitation process, the resulting protein isolate slurry was not dried but was immediately advanced to the pH-shift treatment step at pH 2.

2.4.3. Hydrated PSMPI

The PSMPI was hydrated in water (t = 35°C) at a 1:3 ratio (25% PSMPI) to a homogeneous consistency and used immediately in the *Naem Het* formulation.

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2.5. Preparation of *Naem Het* with organic nitrite sources and alternative proteins

2.5.1. Imitation of banana leaves with a vegan banana casing based on resistant starch

To create the vegan banana casing, 15% organic green banana powder, 1% carrageenan, and 0.03% red algae powder (to optimize the gelling process) were thoroughly mixed. This mixture was then dissolved in 80% distilled water (20°C) until a homogeneous, lump-free suspension was formed, after which 4% glycerin was added. The solution was heated in a water bath (80–85°C) with constant stirring until the components were fully dissolved and a thick, transparent, homogeneous gel had formed. The hot gel was poured evenly in a thin layer (2 mm) onto a smooth, non-stick surface. The gel was then dried in a drying oven at 40°C for 24–48 hours until completely dry, which was verified by achieving a constant weight. The thickness of the dried vegan banana casing sheets was 0.2–0.4 mm.

2.5.2. *Naem Het* technology

King bolete mushrooms were thoroughly cleaned of dirt and damage, the lower part of the stipe was trimmed, and they were washed several times. A head of organic garlic was separated into cloves, peeled, and the hard bottom part was trimmed. Organic cayenne pepper was washed, and the stem, seeds, and internal white membranes were removed. The prepared king bolete mushrooms were finely chopped and manually pressed to remove excess liquid. The prepared organic garlic and cayenne pepper were finely minced. Steamed organic glutinous rice was cooked until done (4 minutes) and cooled (t = 25°C). The glutinous rice was mixed with the hydrated PSMPI. Starter cultures (*Staphylococcus xylosus* and *Pediococcus pentosaceus*) at 0.05 g per 100 g of the mixture were activated in water (t = 30°C, V = 1 mL) for 15 min. The recipe ingredients (Table 1) were thoroughly mixed by hand until a homogeneous, sticky mass was obtained. This mass was then formed into sausages 5–6 cm long and 3–4 cm in diameter and wrapped very tightly in the vegan banana casing, ensuring all air was expelled to create anaerobic conditions. Fermentation was carried out in a fermentation chamber for 5 days at 85% relative humidity, an air velocity of 0.1 m/s, and a temperature of 20°C. The finished *Naem Het* was stored in the vegan banana casing for 14 days at 2°C. *Naem Het* is served either lightly grilled (without the casing) or raw. Grilled *Naem Het* is served hot (65–75°C), while raw is served cold (4–8°C).

2.6. Determination of pH, nitrates, and nitrites in *Naem Het*

The pH of the samples was measured using a calibrated laboratory pH meter (model pH-305). Nitrite (NO_2^-) content was determined according to AOAC official method 973.31 [23]. Nitrate (NO_3^-) content was determined after its reduction to nitrite on a cadmium column (AOAC 993.03), followed by spectrophotometric analysis [23].

2.7. Determination of nutritional value and microbiological parameters of *Naem Het*

The chemical composition was determined by standard methods: protein content by the Kjeldahl method (AOAC 981.10), fat content by the Soxhlet method (AOAC 920.39), ash content by incineration in a muffle furnace (AOAC 942.05), and moisture by drying at 105°C (AOAC 950.46) [23]. Carbohydrate content was calculated by difference. Energy value (EV) was calculated based on the macronutrient content. Total aerobic mesophilic bacteria (AMB) and coliforms were determined according to standard methods described in the Compendium of Methods for the Microbiological Examination of Foods [24]. The count of lactic acid bacteria (LAB) was determined by the plate count method on appropriate media according to ISO 15214:1998 [25].

2.8. Sensory analysis of *Naem Het*

The sensory evaluation of *Naem Het* (lightly grilled, t = 65–75°C) was conducted using the Scorecard method on a 9-point scale. The panel consisted of 10 experts who were previously familiarized with the sensory evaluation method for cooked sausage products [26]. Nine sensory attributes were evaluated: external appearance, color, aroma, taste, sour taste, pungency, aftertaste, texture, and overall score. The overall score was calculated as the average of the other sensory attributes. Color, sour taste, and pungency were highlighted as additional sensory parameters. The color of *Naem Het* at its maximum score should be free from hues attributable to the pigments of the organic juice powders and should be characteristic of the traditional dish. The sour taste and pungency should be pleasant and consistent with traditional *Naem Het*.

2.9. Statistical analysis

Measurements were performed in five replicates (n = 5), and the results are presented as mean ± standard deviation, unless another number of replicates is specified. Latin letters were used to denote statistically significant differences ($p < 0.05$) between groups. Analysis of variance (ANOVA) and Tukey's test were used to measure significant differences ($p < 0.05$) between independent variables. Statistical analysis of the data was performed using R (4.5.0) with the RStudio (2025.05.0+496) integrated development environment.

3. Results and Discussion

3.1. Traditional and adapted formulation of *Naem Het* for healthy nutrition

Naem Het is a traditional Thai fermented mushroom dish, part of Thailand's rich culinary culture where fermentation has long been used as a method of preservation and for imparting unique flavor profiles to products. The first known printed Thai cookbook is considered to be "Mae Khrua Hua Pa". This book played a key role in preserving and standardizing Thai culinary recipes of that era and contains the first documented references to fermented sausages. Ancient recipes involved the simple mixing of local mushrooms with salt, garlic, and cooked rice for spontaneous lactic acid fermentation, which gave the mushrooms their characteristic sourness and texture. *Naem Het* emerged as a vegan interpretation of the ancient dish *Naem Moo* (made with pork and pork skin) and continues to evolve as part of Thailand's traditional cuisine [27]. The formulation was enhanced by adding hydrated PSMPI and powders from organic vegetable juices (yellow beet, yellow carrot, celeriac, radish). The authentic control formulation of *Naem Het* A0 (without $NaNO_3$ and $NaNO_2$), and its adapted-for-a-healthy-diet experimental samples: A1 (1% yellow beet juice powder), A2 (1% celeriac juice powder), A3 (1% yellow carrot juice powder), and A4 (1% radish juice powder), are presented in Table 1.

Standard preparation methods for *Naem Het* need to be improved: modern approaches to preparing traditional food are focused on healthy nutrition and compliance with strict quality standards. This includes the use of controlled starter cultures to ensure a consistent result, guarantee microbiological safety, and highlight the probiotic properties of the fermented product, which meets modern consumer demands for healthy and safe food. Using organic vegetable juice powders instead of chemical nitrites in fermented sausages requires a careful approach. This is because the significantly different content of natural nitrates in them directly determines the fermentation efficiency, nitrite formation, and final organoleptic characteristics of the product. In vegan *Naem Het* sausages, which are an alternative to traditional *Naem Moo*, the use of 10% hydrated PSMPI helps to form the desired texture and significantly increases the nutritional value. It is worth noting that in traditional *Naem Moo*, this role, and a similar proportion in the recipe, is usually fulfilled by cooked minced pork skin. Hydrated PSMPI is added to the formulation at a 10% level to improve organoleptics, nutritional value, and to perform functional roles. These properties ensure the product's juiciness, prevent fat and moisture separation, form a dense and elastic texture, improve consistency, and stabilize the sausage batter system. Although PSMPI may be inferior to WPI in some functional aspects, it is a valuable vegan alternative, free from the allergens inherent in WPI (Whey), SPI (Soy), and PPI (Peanut) [28]. The king bolete mushroom, which is the base of *Naem Het*, contains the enzyme nitrite reductase, which can affect the microbiological parameters and nitrite content in the final food product. The formed sausages are wrapped in a vegan banana casing, which aims to mimic banana leaves and give *Naem Het* its traditional sensory characteristics. Although banana leaves are an authentic and traditional material, edible casings and films are the most common and practical solution for preparing *Naem* in modern conditions. The developed *Naem Het* formulations closely reflect the traditional recipe and are well-suited to demonstrate the impact of dried organic juices and PSMPI on their quality characteristics.

3.2. Nutritional value of *Naem Het* with natural nitrite sources and alternative proteins

The nutritional value of *Naem Het*, including its protein, fat, ash, moisture, carbohydrate (calculated by difference), and energy content, is presented in Table 2.

The analysis of the nutritional value of *Naem Het* demonstrated a significant impact of the formulation modification, particularly the introduction of hydrated PSMPI and various vegetable powders, on the final chemical composition of the product compared to A0. All modified samples (A1–A4) showed a statistically significant ($p < 0.05$) increase in protein content. This increase is likely a direct consequence of the addition of PSMPI, which replaced a portion of the king bolete mushrooms and rice, consequently reducing their proportion in the A1–A4 formulations. As a result, the energy value was also higher in the modified samples. Although the moisture content did not differ significantly between the samples, certain variations in fat and ash content were observed, which may indicate the different mineral and lipid compositions of the initial vegetable powders. The carbohydrate content was highest in A3 and A0, which is explained by the higher amount of rice in A0 and the lower moisture content in A3. Overall, the results indicate that the use of PSMPI effectively enhances the protein and energy value of the product, while the type of vegetable powder can further modulate the content of minerals, fat, and carbohydrates in the fermented sausages.

3.3. Microbiological investigation of *Naem Het* with natural nitrite sources and alternative proteins and the effect of juice powder on bacterial counts

The results of the microbiological investigation, which include the counts of aerobic mesophilic bacteria (AMB), lactic acid bacteria (LAB), coliforms, pH, and the content of nitrates and nitrites in *Naem Het*, are presented in Table 3.

The microbiological analysis and nitrate/nitrite content underscore the significant impact of the fermentation process and formulation composition on the final characteristics of the sausages, which is a multifactorial process dependent on a complex of interactions. In all samples, after 5 days of fermentation, typical signs of a successful process were observed: a substantial decrease in pH, effective elimination of coliforms, and a significant increase in AMB and LAB populations. This indicates not only the activity of the added starter cultures but also a favorable environment for their growth. The initial nitrate content varied significantly, and in A1–A4, it was high due to the contribution of the vegetable juice powders, which, in addition to nitrates, could have introduced additional microelements or specific carbohydrates that influenced the initial microbial dynamics. In contrast, the nitrates in A0 originated mainly from the base ingredients, such as king bolete mushrooms, garlic, and pepper. It is important to note that the king bolete mushrooms, present in all formulations, contain endogenous enzymes capable of breaking down nitrates/nitrites. This enzymatic activity, along with microbial activity, likely contributed to the overall reduction of nitrates in all samples during fermentation, although its relative contribution could have depended on substrate concentration and the activity of competing microbial pathways. Additionally, the initial microbial contamination of the vegetable powders themselves could have introduced some diversity into the initial fermentation conditions, affecting the competition among microorganisms.

Table 1

Authentic formulation and its adaptations for a healthy diet

Formulation Ingredients, g/100 g	A0 without $NaNO_3$ and $NaNO_2$	A1 1% yellow beet juice powder	A2 1% celeriac juice powder	A3 1% yellow carrot juice powder	A4 1% radish juice powder	Nitrate content, mg/kg
King bolete mushroom (*Boletus edulis*)	65	60	60	60	60	30.80 ± 4.87[d]
Hydrated PSMPI	–	10	10	10	10	–
Cooked organic glutinous rice	26	20	20	20	20	–
Organic garlic	4	4	4	4	4	25.20 ± 3.35[d]
Organic cayenne pepper	2	2	2	2	2	40.60 ± 6.50[d]
Salt	2	2	2	2	2	–
Activated starter cultures	1	1	1	1	1	–
Organic yellow beet juice powder	–	1	–	–	–	8950.40 ± 751.85[b]
Organic celeriac juice powder	–	–	1	–	–	9878.40 ± 541.71[ab]
Organic yellow carrot juice powder	–	–	–	1	–	2116.00 ± 210.84[c]
Organic radish juice powder	–	–	–	–	1	10483.40 ± 856.78[a]

Notes: data are presented as mean ± standard deviation (n = 5). Values in the same column with different superscript letters are significantly different ($p < 0.05$)

Table 2

Nutritional value of *Naem Het* after 5 days of fermentation

Naem Het Samples	Chemical composition, %					
	Moisture	Protein	Fat	Ash	Carbohydrates	EV, kJ
A0	71.14 ± 1.22[a]	8.50 ± 0.07[d]	1.28 ± 0.05[c]	3.65 ± 0.11[ab]	15.43	251.2
A1	70.75 ± 1.06[a]	10.75 ± 0.11[a]	1.37 ± 0.07[abc]	3.54 ± 0.15[b]	13.59	290.3
A2	69.81 ± 1.85[a]	10.02 ± 0.16[b]	1.42 ± 0.05[ab]	3.83 ± 0.14[ab]	14.92	284.8
A3	69.18 ± 1.59[a]	9.33 ± 0.09[c]	1.50 ± 0.08[a]	3.62 ± 0.24[ab]	16.37	272.8
A4	70.28 ± 1.29[a]	9.95 ± 0.11[b]	1.32 ± 0.10[bc]	3.93 ± 0.17[a]	14.52	281.6

Notes: data are presented as mean ± standard deviation (n = 5). Values in the same column with different superscript letters are significantly different ($p < 0.05$)

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Table 3

Microbiological Investigation of *Naem Het*

Naem Het Samples	Microbiological parameters					
	Aerobic mesophilic bacteria, $\log_{10}$ CFU/g	Lactic acid bacteria, $\log_{10}$ CFU/g	Total coliforms, MPN/g	pH	Nitrate content, mg/kg	Nitrite content, mg/kg
Start of fermentation, raw product						
A0	7.35 ± 0.09^{e}	6.94 ± 0.12^{d}	49.2 ± 2.3^{d}	5.77 ± 0.01^{a}	22.17 ± 2.48^{d}	0.00^{f}
A1	7.57 ± 0.11^{de}	6.78 ± 0.11^{d}	245.2 ± 7.3^{b}	5.69 ± 0.02^{c}	156.53 ± 17.90^{b}	0.00^{f}
A2	7.45 ± 0.12^{de}	6.92 ± 0.12^{d}	212.6 ± 14.6^{c}	5.74 ± 0.02^{ab}	270.53 ± 23.73^{a}	0.00^{f}
A3	7.51 ± 0.10^{de}	6.96 ± 0.15^{d}	231.4 ± 15.8^{b}	5.72 ± 0.01^{bc}	50.31 ± 8.84^{c}	0.00^{f}
A4	7.64 ± 0.12^{d}	6.82 ± 0.09^{d}	275.0 ± 17.4^{a}	5.75 ± 0.02^{ab}	289.97 ± 26.14^{a}	0.00^{f}
5 days of fermentation, final product						
A0	9.37 ± 0.11^{c}	9.11 ± 0.12^{c}	$< 3^{e}$	4.60 ± 0.02^{d}	0.00^{d}	1.26 ± 0.17^{e}
A1	9.75 ± 0.09^{a}	10.53 ± 0.07^{a}	$< 3^{e}$	4.49 ± 0.02^{e}	5.00 ± 0.70^{d}	4.38 ± 0.23^{c}
A2	9.58 ± 0.08^{abc}	9.74 ± 0.10^{b}	$< 3^{e}$	4.52 ± 0.02^{e}	16.13 ± 1.92^{d}	10.43 ± 0.60^{b}
A3	9.52 ± 0.12^{bc}	9.68 ± 0.07^{b}	$< 3^{e}$	4.51 ± 0.01^{e}	0.00^{d}	2.18 ± 0.25^{d}
A4	9.63 ± 0.09^{ab}	9.81 ± 0.10^{b}	$< 3^{e}$	4.57 ± 0.03^{d}	18.48 ± 2.15^{d}	11.18 ± 0.74^{a}

Notes: data are presented as mean ± standard deviation ($n = 5$). Values in the same column with different superscript letters are significantly different ($p < 0.05$)

A more detailed analysis of the impact of individual ingredients reveals interesting trends, indicating synergistic and antagonistic effects. A1 demonstrated the highest LAB level and the lowest pH. This may indicate the creation of particularly favorable conditions for the growth of certain lactic acid bacteria (LAB) strains, such as *Pediococcus pentosaceus*. Presumably, such conditions are formed under the influence of specific beet pigments (betalains) and its other bioactive compounds, such as saponins or phenolic components, through their ability to selectively stimulate these microorganisms or inhibit competing microflora. A3, which also contains carotenoid pigments known for their antioxidant properties, showed a slightly lower LAB level compared to A1. Notably, in A3, as in A0, a complete reduction of nitrates to 0 mg/kg was observed by the end of fermentation, with relatively low nitrite formation. These differences may point to a different type and concentration of pigments or other compounds, such as specific oligosaccharides, in carrots compared to beets. Such compositional differences variously affect the metabolic pathways of LAB, the activity of fungal enzymes, or promote the growth of microorganisms with potent nitrite reductase activity. The complete reduction of nitrates in A0 could have been enhanced by the higher proportion of mushrooms and, consequently, a larger quantity of their enzymes relative to the lower initial nitrate load. Nitrite formation, which is important for flavor development and stability, was highest in samples A4 and A2, correlating with their high initial nitrate content. The high nitrite concentrations in these samples could have more strongly stimulated the activity of flavor-producing bacteria, such as *Staphylococcus xylosus*, which use nitrites in their metabolism, thereby influencing the final organoleptic profile. Furthermore, the interaction between PSMPI, as a source of protein and peptides, and the components of the vegetable powders could have created unique niches and substrate conditions for microbial growth.

3.4. Microbiological investigation of *Naem Het* with natural nitrite sources and alternative proteins during storage

To ensure the food safety of *Naem Het* during storage, additional microbiological studies were conducted. The results of this microbiological investigation are presented in Table 4.

During the storage period of the fermented sausages for up to 14 days, dynamic changes in microbiological and physicochemical parameters were observed, reflecting both general trends of product stabilization and the specific influence of the added vegetable powders.

Table 4

Microbiological Investigation of *Naem Het* during Storage

Naem Het Samples	Microbiological parameters					
	Aerobic mesophilic bacteria, $\log_{10}$ CFU/g	Lactic acid bacteria, $\log_{10}$ CFU/g	Total coliforms, MPN/g	pH	Nitrate content, mg/kg	Nitrite content, mg/kg
7 days of storage						
A0	8.24 ± 0.12^{bc}	8.36 ± 0.08^{bc}	< 3	4.65 ± 0.02^{de}	0.00^{d}	0.00^{d}
A1	8.60 ± 0.17^{a}	8.70 ± 0.09^{a}	< 3	4.60 ± 0.01^{f}	0.00^{d}	1.22 ± 0.11^{c}
A2	8.02 ± 0.12^{cdef}	8.33 ± 0.10^{bcd}	< 3	4.62 ± 0.01^{ef}	8.00 ± 0.80^{a}	6.98 ± 0.51^{b}
A3	8.18 ± 0.09^{cd}	8.38 ± 0.10^{bc}	< 3	4.59 ± 0.01^{f}	0.00^{d}	0.00^{d}
A4	8.14 ± 0.10^{cde}	8.25 ± 0.07^{cd}	< 3	4.63 ± 0.02^{e}	8.15 ± 0.93^{a}	7.62 ± 0.48^{b}
14 days of storage						
A0	7.89 ± 0.09^{efg}	7.98 ± 0.09^{e}	< 3	4.77 ± 0.02^{a}	0.00^{d}	0.00^{d}
A1	8.48 ± 0.11^{ab}	8.48 ± 0.14^{b}	< 3	4.67 ± 0.02^{d}	0.00^{d}	1.04 ± 0.43^{c}
A2	7.74 ± 0.14^{g}	8.19 ± 0.09^{cde}	< 3	4.73 ± 0.01^{b}	3.18 ± 1.15^{c}	7.62 ± 0.39^{b}
A3	7.80 ± 0.16^{fg}	8.26 ± 0.08^{cd}	< 3	4.69 ± 0.01^{cd}	0.00^{d}	0.57 ± 0.22^{cd}
A4	7.93 ± 0.11^{defg}	8.13 ± 0.12^{de}	< 3	4.72 ± 0.02^{bc}	4.70 ± 0.85^{b}	9.35 ± 0.28^{a}

Notes: data are presented as mean ± standard deviation ($n = 5$). Values in the same column with different superscript letters are significantly different ($p < 0.05$)

After the first 7 days of storage, an expected decrease in the counts of AMB and LAB was noted in all samples compared to the end of active fermentation, which is typical for the transition of a microbial population into the stationary phase. Nevertheless, even at this stage, A1 was distinguished by better retention of LAB, which may be related to the prebiotic properties or the presence of protective compounds in beetroot. Coliforms remained consistently below the detection limit, and the pH, although it increased slightly, remained within a safely acidic range. Nitrate content continued to decrease, especially in A1, where they were completely reduced, and in A2 and A4. By the 14th day of storage, these trends had evolved: in samples with pigmented vegetable powders, A1 and A3, a slower subsequent decline in LAB counts and a slower increase in pH were observed compared to the control sample A0. This supports the hypothesis about the positive impact of components from these vegetables, such as antioxidant pigments or other bioactive substances, on the viability of beneficial microflora and the acidic stability of the product. The most intriguing finding was the dynamics of nitrites: after a general decrease by day 7, an unexpected increase occurred in A2, A4, and even partially in A3 between the 7th and 14th day. This could be related to the possible role of fungal enzymes in this process, which typically promote the reduction of nitrates/nitrites. However, a change in their activity or interaction with microbial metabolites during prolonged storage, which could have indirectly affected the nitrite balance, cannot be excluded. Therefore, a more plausible explanation is the prolonged metabolic activity of specific bacterial populations. These populations continue to slowly reduce the remaining nitrates (which were particularly abundant in A2 and A4) to nitrites. At this stage, the rate of nitrite formation may temporarily exceed the rate of its subsequent degradation, binding to myoglobin proteins, or conversion to nitric oxide. This is especially likely under conditions of gradual changes in pH and redox potential. This effect was absent in A0 and A1, where nitrates were depleted much earlier or their initial quantity was substantially lower.

The results of the storage study demonstrated that the introduction of vegetable powders significantly modifies the long-term stability and biochemical processes in fermented sausages. Specifically, the samples with beetroot (A1) and carrot (A3) were distinguished by better stability of AMB and LAB and a smaller increase in pH, likely due to the protective properties of their bioactive compounds. The dynamics of nitrates and nitrites were complex, as in most samples (A0, A1, A3), these compounds were depleted over time. However, in the variants with celeriac (A2) and radish (A4), a unique trend of increasing nitrite content in the later stages of storage was observed, indicating prolonged specific microbial activity and a different metabolism of nitrogenous compounds.

3.5. Sensory evaluation of *Naem Het* with natural nitrite sources and alternative proteins

The sensory evaluation of grilled *Naem Het* after 5 days of fermentation is presented in Fig. 1.

The sensory evaluation of the lightly grilled fermented sausages revealed a significant impact of the formulation modifications on the organoleptic characteristics. A1 and A3 received high overall scores. This occurred despite the experts' specific perception of their color. The lower color scores for A1 and A3 are explained by their distinct yellow pigments, inherited from the vegetable powders. Such pigments were considered a deviation from the traditional color of the dish. In contrast, A2 and A4 received high scores for color, similar to A0. This is because the celeriac and radish juice powders had a neutral color that did not distort the product's traditional appearance. The general improvement in texture across all modified samples (A1–A4) compared to A0 is clearly linked to the introduction of PSMPI. This, in turn, led to a higher protein content. The particularly high texture scores in A1 and A3 may further indicate a positive interaction between the plant fibers from these vegetables and the protein matrix. The pronounced and pleasant sour taste, highly rated in A1 and A3, is a direct consequence of the active metabolism of LAB. The lower pH values achieved in these samples also contributed to this. The higher scores for overall taste, pungency, and aftertaste in A1 and A3 were likely due to more than just intensive fermentation. A synergistic effect of the unique volatile and non-volatile compounds inherent in beetroot and carrot could have played an important role. These compounds might have served as precursors for new flavor and aroma compounds or directly enriched the flavor profile. As for A2 and A4, their good taste scores could have been partially shaped by a more pronounced "cured" or "umami" character. This could be related to the higher and more stable nitrite content, which is supported by the microbiological studies. At the same time, the pungency and complexity of the aftertaste in these samples were less pronounced than in A1 and A3. Thus, the organoleptic profile of the product is a complex result. It is formed by the interaction of the initial formulation, which affects texture through PSMPI and the specifics of the vegetable powders. Changes in nutritional value, particularly protein content, are also important. The characteristics of the microbiological processes play a key role: the activity of AMB and LAB, the pH level, and the dynamics of nitrites. All these factors collectively shape the final sensory perception by the consumer.

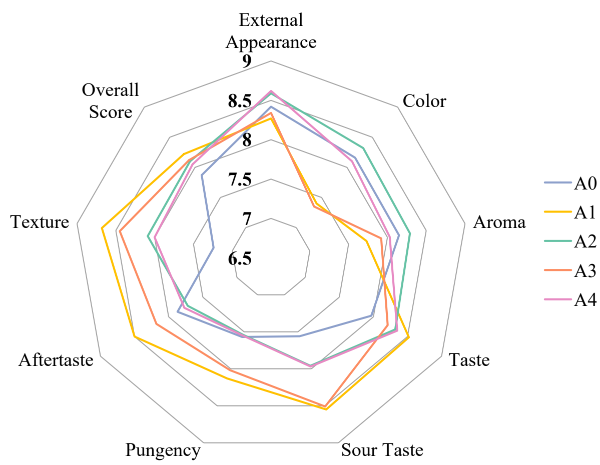

Fig. 1. Sensory evaluation of *Naem Het*

This research demonstrates the feasibility of creating innovative organic vegan fermented sausages with high nutritional value. The use of organic vegetable powders and pumpkin seed protein isolate (PSMPI) allows for the production of "clean label" products. This facilitates controlled fermentation, safety, and improved sensory characteristics, meeting the demand for healthy plant-based alternatives [20]. The results were obtained under laboratory conditions and require adaptation for industrial production, considering the specifics of raw materials, especially the quality control of wild-harvested mushrooms. The state of war in Ukraine critically restricts research: laboratories are being destroyed, scientists are forced to emigrate, and constant shelling poses direct threats. Consequently, experiments are often conducted under unpredictable conditions and in bomb shelters. Despite these extremely challenging current conditions, future research should focus on optimizing the technology with the help of AI and studying new plant-based ingredients [29]. It would also be pertinent to investigate the application of these vegetable powders in other fermented products and to compare their effectiveness with alternative sources of nitrates/nitrites for future foods. A deeper understanding of the microbial processes, especially the formation of nitrites by AMB and LAB, is important. Also relevant are studies on long-term stability, the complete bioactive profile, and expanded testing in the field of healthy nutrition.

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3.6. Research limitations and future research directions

The limitations of this research are determined by its design; specifically, the conclusions are directly applicable only to the specific ingredients used, namely pumpkin seed protein isolate and four types of vegetable powders. Furthermore, the research was conducted under laboratory conditions; therefore, scaling up the technology to an industrial level will require further optimization. The stability analysis was also limited to a 14-day period, which does not permit an assessment of the product's long-term quality. The depth of the microbiological analysis did not include strain identification, which complicated a full interpretation of the fermentation processes. Finally, the research design did not allow for the isolation of the protein component's specific effect on fermentation, although the observed process dynamics suggest its active participation not only in texture formation but also in microbial metabolism.

A promising direction for future work is the expansion of the raw material base by testing other plant-based proteins and vegetable powders. An in-depth investigation of the fermentation mechanisms is crucial, including analysis of the volatile compound profile and metagenomic analysis of the microbiota. In this context, a separate line of research is the investigation of the specific impact of the hydrated protein isolate on microbiological and biochemical processes, which would allow for the quantification of its contribution to fermentation kinetics. Future studies should adapt the technology to industrial conditions and examine the product's long-term stability using modern packaging solutions. An important subsequent step is also to conduct an expanded consumer evaluation to determine the market potential of the developed sausages.

4. Conclusions

This research demonstrates the successful adaptation of a formulation for fermented vegan sausages based on mushroom and predominantly organic plant-based components. It was established that the key characteristics of the product can be strategically modulated through the combined use of functional ingredients. Pumpkin seed protein isolate (PSMPI) significantly increased the protein content (from 8.50% in the control to 10.75% in sample A1) and improved textural properties, confirming its role as an effective nutritional and structural component. Simultaneously, the dried vegetable juice powders acted not merely as natural sources of nitrates (NO_3^-), but as a determining factor governing the biochemical and microbiological processes.

A clear dichotomy was identified: samples with pigment-rich powders from beetroot (A1) and carrot (A3) demonstrated the best microbiological stability (including effective pH control, which reached values of 4.49–4.51) and the highest sensory scores, which is explained by the antioxidant support of the fermentation processes. Conversely, in the samples with celeriac (A2) and radish (A4), significantly lower pigment content impaired the bacterial reduction of their extremely high initial NO_3^- content (270–290 mg/kg). This led to a unique dynamic of nitrite (NO_2^-) accumulation during storage, reaching a maximum value of 9.35 mg/kg in the radish sample (A4). Thus, the research demonstrates that the choice of vegetable powder is a powerful tool for the functional design of vegan fermented products. This allows for the predictable shaping of both the safety profile, related to nitrite dynamics, and the final organoleptic properties of the finished product.

Conflict of interest

The authors declare that they have no conflict of interest regarding this research, including any of a financial, personal, authorship, or other nature, that could have influenced the research and its results presented in this article.

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Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that artificial intelligence technologies were not used in the creation of the presented work.

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