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Upcycling Potato Juice Protein for Sustainable Plant-Based Gyros: A Multidimensional Quality Assessment

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Abstract

The growing demand for sustainable, nutritionally adequate plant-based foods has driven innovation in meat analogues. This study presents a novel approach to upcycling potato juice protein—a by-product of starch production—into plant-based gyros (PBG) enriched with iron and dietary fiber. Four formulations (PBG1-PBG4) were developed using a blend of potato, rice, wheat, and pea proteins, and fortified with either ferritin-rich sprout powder or ferrous sulfate. Comprehensive analyses were conducted to assess nutritional composition, mineral content, glycoalkaloid safety, antioxidant activity, texture, water mobility, sensory appeal, and microbiological stability. All variants met high-protein labeling criteria and exhibited favorable fiber and mineral profiles. In vitro digestion significantly enhanced antioxidant bioaccessibility, particularly phenolic acids. Sensory evaluations favored ferritin-enriched variants, which also demonstrated superior texture and consumer acceptance. Microbiological assessments confirmed safety for up to 10 days under refrigeration. These findings highlight the potential of potato juice protein as a sustainable, functional ingredient in next-generation plant-based meat analogues.

Keywords: antioxidant activity; iron fortification; plant-based meat; potato protein; sensory evaluation; sustainability

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1. Introduction

The global population is projected to surpass 9.7 billion by 2050 [1], which poses major challenges to food systems, especially in ensuring food security and nutritional adequacy. Meeting this demand requires more than caloric sufficiency—it necessitates nutritionally balanced diets that promote long-term health and well-being [2,3]. In recent years, a marked shift in consumer behavior has been observed, with an increasing number of individuals either reducing or eliminating meat from their diets. This shift is fueled by intersecting health, ethical, and environmental motivations [4–6]. As a result, the development of plant-based meat alternatives has become a central focus in the field of food science and technology.

Plant-based foods offer notable advantages over animal-derived products, especially regarding environmental sustainability. Meat production is associated with high greenhouse gas emissions, substantial land and water usage, and marked ecological footprints [7,8]. In contrast, the production of plant-based alternatives typically requires fewer natural resources and generates less environmental impact, making them an attractive solution in the context of sustainable food system transformation. Beyond ethical and environmental drivers, plant-based meat formulation enables the valorization of underutilized ingredients and food industry by-products, which aligns with circular economy principles. One such promising raw material is potato juice, a side stream generated during the industrial extraction of potato starch. Historically, potato juice has been treated as waste due to its high biochemical oxygen demand and disposal challenges [9]. But, recent research has highlighted its potential as a valuable source of bioactive compounds, particularly proteins with a favorable amino acid profile, including essential amino acids such as lysine and threonine, which are often limited in plant-based proteins [10,11]. Potato proteins, with a Digestible Indispensable Amino Acid Score (DIAAS) of 1.0, qualify as a complete protein source [12]. These proteins exhibit good nutritional quality as well as excellent functional properties, such as emulsifying and foaming capacity, which makes them suitable for use in a variety of food formulations [12,13]. Moreover, the recovery and utilization of potato juice proteins contribute to the valorization of agro-industrial by-products, thereby offering both economic and environmental benefits. Incorporating this protein-rich material into plant-based meat and dairy analogues aligns with sustainable development goals and supports the transition towards more efficient and circular food production systems [14–16].

Despite the growing popularity of plant-based meat analogues, one of the critical challenges in their development is achieving nutritional equivalence to conventional meat products. Many commercially available vegan products tend to be lower in essential nutrients such as high-quality protein, iron, zinc, and vitamin B_{12}, which are naturally abundant in animal-derived foods [17–19]. Furthermore, the processing methods and ingredient choices used in plant-based formulations can lead to an imbalance in macronutrient distribution or the presence of anti-nutritional factors that may affect nutrient bioavailability. Therefore, to ensure that plant-based alternatives support adequate nutrition, it is essential to carefully design their composition and fortify them with appropriate nutrients. Incorporating functional ingredients such as potato juice-based proteins, micronutrient-rich extracts, and bioavailable mineral sources can substantially enhance the nutritional value of vegan products and better align them with the dietary needs of consumers by reducing or eliminating meat from their diets.

Considering the outlined challenges, the objective of this study was to design a production technology for plant-based gyros (PBG) utilizing potato protein as a key ingredient, enriched with iron and carefully selected fats. The resulting PBGs were subsequently evaluated for their nutritional composition and bioactive properties prior to and following the in vitro digestion.

2. Materials and Methods

2.1. Plant-Based Gyros Preparation

Plant-based gyros prototypes were prepared according to the protocol described in Polish patent application P.446897 [20], which outlines the formulation and processing steps for plant-based gyros using potato protein. Briefly, a dry protein blend containing 70% potato protein, 20% rice protein (Beneo, Veendam, Belgium), and minor portions of wheat (Viresol, Visonta, Hungary) and pea (Brenntag, Kędzierzyn-Koźle, Poland) proteins (5% each) was dispersed in water at a 1:6 (*w*/*v*) ratio and homogenized for 5 min at 450 rpm with a RobotCook® (Robot-Coupe, Roubaix, France) gastronomic homogenizer to yield a viscous slurry. This slurry was transferred to 250 mL stainless-steel molds and baked in a convection oven (MIWE Michael Wenz GmbH, Arnstein, Germany) at 120 °C for 2 h. After cooling, the product was stored at 4 °C until use. A lipid phase consisting of rice-bran (Kasisuri, Bangkok, Thailand) and rapeseed (ZT Kruszwica, Kruszwica, Poland) oils (55:45, *v*/*v*) was formulated to secure an n6:n3 ratio of 5:1, and coconut oil was incorporated to reinforce textural integrity. The protein base was first combined with oils, after which dry ingredients were blended in (using RobotCook®, Robot-Coupe Inc., Ridgeland, MS, USA); water and vinegar were subsequently added in a thin stream under continuous agitation to obtain a homogeneous filling. Detailed composition is presented in Table 1. To ensure moisture equilibration and matrix stabilization, the mixture was vacuum-sealed in low-density polyethylene pouches (embossed vacuum pack bugs (75 µm, 60 µm PE, 15 µm PA, Hendi B.V., De Klomp, The Netherlands) and conditioned at 4 °C for 24 h. The matured mass was portioned into 75 g patties and thermally treated in a convection-steam oven (Vision Combi Oven, Retigo Ltd., Rožnov pod Radhoštěm, Czech Republic) at 160 °C for 30 min. Finally, the cooked product was cooled, comminuted into 1–5 cm^3 pieces, and packaged for further analyses.

Table 1. Ingredient composition of plant-based gyros prototypes (%).

Ingredient	PBG 1	PBG 2	PBG 3	PBG 4
Protein base	40.00	40.00	40.00	40.00
Coconut oil	5.00	5.00	5.00	5.00
Vegetable-oil blend	6.00	6.00	6.00	6.00
Potato starch	4.00	4.00	4.00	4.00
Corn starch	2.00	2.00	2.00	2.00
Yeast flakes + vit. B_{12}	4.00	4.00	4.00	4.00
Oat flakes	4.00	4.00	4.00	4.00
Methylcellulose	2.00	2.00	2.00	2.00
Carrageenan	2.00	2.00	2.00	2.00
Natural flavor	2.00	2.00	2.00	2.00
Dried beetroot juice	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50
Vinegar	0.50	0.50	0.50	0.50
Potato fiber	2.00	—	2.00	—
Oat fiber	—	2.00	—	2.00
Ferritin-rich sprout powder	1.50	1.50	—	—
Ferrous sulfate	—	—	0.007	0.007
Water *	23.75	23.75	25.243	25.243

* Water was added to bring the formulation up to 100%, and the differences result from the varying amounts of iron-source additives used.

The potato protein used in the present study was obtained with a proprietary membrane-filtration process applied to waste potato juice, as described in detail previously [11]

Ferritin-fortified lupine sprout powder was generated following a patented procedure described in details by Zielińska-Dawidziak, 2016 [21]. In brief, raw seeds underwent surface sterilization in 70% (*v*/*v*) ethanol for 15 min, after which they were thoroughly rinsed and submerged for 6 h in aqueous $FeSO_4$ solutions (0–25 mM). Germination proceeded for seven days in the same iron solutions under controlled temperature and humidity. On day 7 the sprouts were lightly irrigated and then dried with ambient air until their moisture content fell below 10%, after which they were milled to a fine powder.

2.2. Proximate Composition and In Vitro Digestibility

Nitrogen content was determined using the Kjeldahl method (ISO 1871 [22], using Kjeltec™ 8100, FOSS, Hillerød, Denmark) and converted to protein using a factor of 6.25, whereas crude lipids were extracted in a Soxhlet apparatus (Soxtec™ 8000, FOSS, Hillerød, Denmark) following AOAC 948.22 [23]. Soluble and insoluble dietary-fiber fractions were obtained by the enzymatic-gravimetric AACC 32-07 protocol using Fibertec™ 1023 (FOSS, Hillerød, Denmark), their sum constituting total fiber [24]. β-Glucan content was assessed according to AACC 32-23.01 approved method [25], colorimetric measurement was performed using Multiscan GO (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Moisture was measured gravimetrically according to AACC 44-19.01 [26] (universal ovenUF110, Memmert GmbH + Co.KG, Schwabach, Germany), and the residue remaining after dry-ashing at 550 °C yielded the ash content (ISO 763 [27], Nobertherm GmbH, Nabertherm, Germany). Available carbohydrate was calculated by difference:

$$\text{Carbohydrates (\%)} = 100 - (\text{P} + \text{F} + \text{Fi} + \text{Mi} + \text{M})$$

where: P—protein, F—fat, Fi—fiber, Mi—mineral, and M—moisture contents. The energetic value (kcal 100 g^{-1}) was estimated using the modified Atwater factors using:

$$\text{EV}\left(\frac{\text{kcal}}{100\ \text{g}}\right) = \frac{4 \times \text{P} + 4 \times \text{C} + 9 \times \text{F} + 2 \times \text{Fi}}{\text{M}}$$

where: C—carbohydrates, Fi—fiber, P—protein, F—fat, and M—moisture contents. Simulated gastrointestinal digestion followed the procedure of Wang et al. [28], and protein digestibility was expressed as the percentage difference between the protein introduced and that remaining after digestion.

Quantification of calcium, magnesium, potassium, sodium, copper, iron, manganese, zinc, and lead was performed in line with the previous protocol [29] with slight modifications. Briefly, accurately weighed portions of freeze-dried sample were transferred to chemically modified 30 mL Teflon vessels, after which 3 mL of concentrated HNO_3 and 1 mL of H_2O_2 were added. The sealed vessels were enclosed in steel jackets and digested in a closed high-pressure, high-temperature microwave system (10 min at 200 W). After cooling, digests were brought to 25 mL with ultrapure water and the elemental concentrations were determined by inductively coupled plasma–optical emission spectrometry (ICP-OES) using an IRIS HR spectrometer (Thermo Jarrell Ash, Franklin, MA, USA).

2.3. Potato Glycoalkaloids Content

Quantification of α-solanine and α-chaconine followed a protocol described in [30] with minor adaptations. Briefly, 1 g of freeze-dried sample was suspended in 15 mL of 5% (*v*/*v*) acetic acid, shaken for 15 min, and centrifuged at 10,000× *g* for 15 min at 4 °C. The clarified extract was decanted, and the residue underwent an identical second extraction; both supernatants were pooled. For clean-up, 3 mL of the combined extract was applied to an Oasis HLB SPE cartridge (30 mg, 1 cm^3; Waters, Milford, MA, USA) pre-conditioned sequentially with methanol, 10% methanol, and distilled water (2 mL each). After loading, the cartridge was washed with 3 mL of 10% methanol, and retained analytes were eluted with methanol containing 0.1% (*v*/*v*) formic acid. The eluate was filtered through a 0.22 µm PTFE membrane prior to instrumental analysis. Chromatographic separation was achieved on an UltiMate 3000 RSLC system (Thermo Scientific, Waltham, MA, USA) equipped with a Kinetex C18 column (1.7 µm, 100 × 2.1 mm; Phenomenex, Torrance, CA, USA) using a mobile phase of 0.1% formic acid (solvent A) and acetonitrile (solvent B). Detection and quantification were performed by UHPLC-MS/MS on an API 4000 QTRAP triple-quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) operated in positive-ion electrospray mode.

2.4. Impact of Simulated In Vitro Digestion on Antioxidants

2.4.1. Simulated In Vitro Digestion Procedure

The simulated gastrointestinal digestion followed, with minor adaptations, the procedure outlined earlier [31]. In brief, 10 g of the freeze-dried, milled sample were transferred to glass flasks, and the volume was brought to 100 mL with distilled water. The suspension was acidified to pH 2.0 using 4 M HCl, after which pepsin (1.92 mg/g, prepared in 0.1 M HCl) was introduced to reproduce gastric conditions; the mixture was kept at 37 °C for 2 h. Gastric digestion was quenched by raising the pH to 7.2–7.4 using 1 M $NaHCO_3$, and intestinal digestion commenced upon addition of a 0.1 M $NaHCO_3$ solution containing pancreatic extract (0.40 mg/g) and bile salts (2.40 mg/g). Incubation continued for a further 2.5 h at 37 °C in a shaking water bath. Digests were subsequently frozen at −80 °C and lyophilized for downstream analyses.

2.4.2. Antioxidants Extraction Procedure

Antioxidant constituents were isolated using an adapted vetted protocol [32]. Both freshly prepared samples and aliquots obtained after the in vitro digestion procedure described in Section 2.4.1 were examined. Precisely 1 g of freeze-dried material was suspended in 9 mL of 80% (*v*/*v*) methanol and gently agitated for 20 min, after which the mixture was clarified by centrifugation at 10,000× *g* for 15 min at 4 °C. The supernatant was then collected and filtered through a 0.22 μm PTFE syringe membrane to provide the extract used for subsequent analyses.

2.4.3. Total Phenolic Compounds and Antioxidant Activity

Total phenolics were quantified using a micro-scaled Folin-Ciocalteu assay [33]: 4 μL of extract, 16 μL of distilled water, and 100 μL of 0.1 N reagent were mixed, incubated at room temperature for 8 min, then 80 μL 0.75 g L^{-1} Na_2CO_3 were added and the mixture was incubated for 1.5 h at 20 °C in the dark. Absorbance read at 765 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific Inc., Waltham, USA) was converted to mg gallic acid equivalents per gram of dry matter. Antioxidant (radical-scavenging) capacity was measured using the $ABTS^{\bullet +}$ method [34]: 2 μL of the same extract reacted with 200 μL of pre-generated ABTS solution for 5 min at 30 °C under shaking, and the decrease in absorbance at 734 nm was expressed as mmol Trolox equivalents per gram of dry weight. Ferric-reducing antioxidant power followed the FRAP protocol [35], where 9 μL extract were combined with 270 μL freshly prepared TPTZ-Fe^{3+} reagent, incubated 6 min at 36 °C, and absorbance at 595 nm was converted to μmol Trolox equivalents per gram of dry weight.

2.4.4. Phenolic Acids Profiling

Quantitative profiling of chlorogenic, ferulic, gallic, and caffeic acids followed the vetted earlier procedure [36]. The phenolic extract prepared as outlined in Section 2.4.2 was subjected to LC-MS/MS analysis on the same instrument platform used for glycoalkaloid determination. Separation was carried out on a Luna C18 column (3 μm, 150 × 2.0 mm; Phenomenex, Torrance, CA, USA) employing a binary eluent of 5 mM ammonium acetate in water (solvent A) and methanol (solvent B).

2.5. Texture Analysis

To assess the texture characteristics, fifteen samples (cut to predetermined dimensions: 200 mm in length and 20 mm in diameter) were extracted from each batch. Each sample was positioned horizontally on the testing platform and subjected to a single shear using a Warner-Bratzler attachment equipped with a 73° V-shaped blade. The setup was mounted on a TA.XTplus Texture Analyzer (Stable Microsystems, Godalming, UK). A 50.0 N load cell was used, and the crosshead speed was set at 1.5 mm/s. Two primary parameters were recorded: shear force (N), which represented the peak force needed to cut through the sample, and total shear work (N × s), which indicated the energy expended to complete the shear.

2.6. LF NMR Relaxometry

Relaxation times, including T_1 (spin-lattice relaxation time) and T_2 (spin-spin relaxation time), were measured at a temperature of 20 °C. The measurements were conducted using a ^{1}H NMR PS15T pulse spectrometer (ELLAB, Poznań, Poland) operating at 15 MHz, equipped with an integrated temperature control system. Prior to the experiments, samples were placed in the spectrometer and allowed to equilibrate to 20 °C.

2.6.1. Measurement of Spin-Lattice Relaxation Time (T_1)

The inversion-recovery pulse sequence (π-t-π/2) was employed to measure T_1 relaxation times. The delay times (t) between pulses ranged from 100 to 1000 ms, with a repetition time of 20 s. For each measurement, 32 free induction decay (FID) signals were collected, with 119 points per FID signal.

Calculations of the spin-lattice relaxation times were performed using CracSpin software (Jagiellonian University, Kraków, Poland), applying the "spin grouping" method [37]. The Marquardt minimization method was utilized to fit the multi-exponential decays. The accuracy of the relaxation parameters was determined, and standard deviations were calculated. The temporal changes in the current amplitude of the FID signal at the applied pulse frequency were described by the equation:

$$M_z(t) = M_0\left(1 - 2e^{\frac{-t}{T_1}}\right)$$

where: $M_z(t)$ is the actual magnetization value, M_0 is the equilibrium magnetization value, t is the delay between pulses, and T_1 is the spin-lattice relaxation time.

2.6.2. Measurement of Spin-Spin Relaxation Time (T_2)

Spin-spin relaxation times (T_2) were measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (π/2-TE/2-(π)n) [38,39]. The delay time between π pulses (TE) was 2 ms, with a repetition time of 15 s. The number of spin echoes (n) was set to 100. Signal accumulations were used.

The calculation of spin-spin relaxation times was based on fitting the echo amplitudes to the equation:

$$M_{x,y}(TE) = M_0\sum_{i=1}^{n} p_i e^{\frac{-TE}{T_{2i}}}$$

where: $M_{x,y}(TE)$ is the echo amplitude, M_0 is the equilibrium amplitude, TE is the delay time between π pulses, and p_i is the fraction of protons relaxing with spin-spin relaxation time T_{2i}. The calculations were performed using dedicated software with a nonlinear least squares algorithm. Standard deviations were used to determine the accuracy of the relaxation parameters.

2.7. Consumer and Sensory Studies

Sensory and consumer acceptability evaluations were conducted on four developed products (PBG1–PBG4) and a commercial vegan gyros product available in a Polish retail chain (designated CPBG, Jeronimo Martins Polska S.A., Kostrzyn, Poland).

2.7.1. Consumer Study

To determine the appeal of plant-based gyros to the demographic most closely linked with vegan-food purchases—young adults—a sensory study was conducted with 100 untrained volunteers (19–40 years old; 67 women, 33 men) recruited from the student and staff community of the Poznań University of Life Sciences. After providing written informed consent, each participant evaluated samples that had been heated to approximately 60–70 °C to ensure serving temperatures reflected typical consumption conditions. Using a nine-point hedonic scale (1 = "dislike extremely" through 9 = "like extremely"), the participants rated taste, aroma, color, texture, appearance, and overall liking in a single controlled session.

2.7.2. Sensory Evaluation Protocol

The sensory profile of the experimental plant-based gyros was established through quantitative descriptive analysis (QDA) conducted in accordance with ISO 13299 [40]. Six trained panelists independently quantified the intensity of five descriptors—surface and cross-section appearance, color, odor, texture, and overall palatability—using anchored 10-point scales. Immediately before each session, portions were reheated to 60–70 °C, so that the sample temperature during evaluation faithfully reflected typical consumption conditions and minimized thermal variability across replicates.

2.8. Microbiological Stability and Shelf-Life Assessment

Microbiological stability and shelf-life were assessed under storage conditions designed to simulate refrigeration. Samples were stored at 3.0–8.0 °C in sealed, embossed vacuum pack bugs (75 µm, 60 µm PE, 15 µm PA, Hendi B.V., De Klomp, The Netherlands), protected from both light and atmospheric oxygen, for a total period of 15 days.

2.8.1. Detection of *Salmonella* spp.

Detection of *Salmonella* spp. was conducted in accordance with PN-EN ISO 6579-1 [41] in 25 g of each sample with application of Rappaport-Vassiliadis Soya (RVS) and Muller-Kauffmann Tetrathionate Novobiocin (MKTTn) broths as a pre-enrichment broths and Xylose Lysine Deoxycholate (XLD) and Chromagar Salmonella Plus agar as a selective plates.

2.8.2. Detection of Listeria Monocytogenes

The detection of *Listeria monocytogenes* was performed according to PN-EN ISO 11290-1 [42] in 25 g of each sample with application of ALOA and Oxford agar as a selective media.

2.8.3. Quantitative Microbiological Analyses

For enumeration analyses, 10 g of each sample was homogenized in 90 mL of peptone water. Serial 10-fold dilutions were prepared in the same diluent and used for planting. Enumeration of mesophilic aerobic microorganisms was conducted according to PN-EN ISO 4833-1 [43]. Enumeration of *Enterobacteriaceae* was carried out in accordance with PN-EN ISO 21528-2 [44]. Enumeration of *Clostridium perfringens* was performed according to PN-EN ISO 15213-2 [45]; colonies were verified by growth characteristics on blood agar and further tested for acid phosphatase activity. Enumeration of *Listeria monocytogenes* was performed in line with PN-EN ISO 11290-2 [46]. Quantification of coagulase-positive *Staphylococcus* spp. was performed according to PN-EN ISO 6888-1 [47]; confirmation was conducted by subculturing on RPF agar and assessing colony morphology. Enumeration of yeasts and molds was conducted following PN-EN ISO 21527-1 [48] using surface plating on Dichloran Rose Bengal Chloramphenicol (DRBC) agar.

2.9. Statistical Analysis

The experimental data, expressed as mean ± SD, underwent one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify statistically homogeneous groups at $\alpha = 0.05$ significance level.

3. Results and Discussion

3.1. General Nutritional Composition

The developed plant-based gyros (PBG1–PBG4) demonstrated a favorable nutritional profile, characterized by high protein content, moderate fat levels, and substantial dietary fiber. Protein content ranged from 20.85 g/100 g (PBG1) to 21.86 g/100 g (PBG3), which indicated that all formulations met the threshold for "high-protein" claims under EU regulation (Regulation (EC) No 1924/2006) [49]. The inclusion of concentrated plant proteins, particularly from legumes and potato protein isolate, contributed to this result. Fat content varied moderately, with values between 13.81 g/100 g (PBG3) and 14.98 g/100 g (PBG1). This range is typical for meat analogues designed to mimic traditional gyro products, allowing for desirable texture and mouthfeel without excessive lipid content [50]. The relatively low total carbohydrate content (6.94–9.47 g/100 g) reflects the formulation's emphasis on protein and fiber, with PBG3 presenting the highest value, likely due to its slightly elevated starch component. All variants were also rich in dietary fiber, with PBG2 (8.29 g/100 g) and PBG4 (8.26 g/100 g) demonstrating the highest levels. Detailed fiber analysis corroborated these findings, which showed a predominance of insoluble dietary fiber (IDF: 6.96–7.36 g/100 g), supplemented by small amounts of soluble fiber (SDF: 0.80–0.93 g/100 g). Notably, PBG2 and PBG4 also exhibited the highest β-glucan content (0.91 and 0.87 g/100 g, respectively). These components are known to support health, particularly by improving glycemic control and reducing cholesterol levels [51,52]. The energy values ranged from 267.76 to 286.80 kcal/100 g, which aligned with consumer expectations for satisfying yet balanced plant-based meals.

Protein digestibility is a critical determinant of nutritional quality in plant-based meat analogues, as it reflects amino acid bioavailability. In this study, in vitro analysis revealed significant differences in protein digestibility across the developed gyros variants (Table 2). PBG1 and PBG3 exhibited the highest digestibility values, whereas PBG2 and PBG4 showed significantly lower digestibility. These differences likely stem from variations in fiber composition among the formulations. The superior digestibility observed in PBG1 and PBG3 can be attributed to the use of potato fiber, which is rich in insoluble components that are less likely to form viscous gels or interact with proteins in a way that hinders enzymatic hydrolysis [53,54]. In contrast, the lower digestibility in PBG2 and PBG4 is likely a consequence of the inclusion of oat fiber, known for its high soluble fraction, particularly β-glucans [55]. Soluble fibers can increase the viscosity of the digesta and promote the formation of protein-polysaccharide complexes, both of which can limit the accessibility of proteolytic enzymes to their substrates [56,57]. These findings align with previous research indicating that soluble dietary fibers may negatively impact protein digestibility by creating physical barriers or restricting enzyme diffusion [16,58]. Our results underscore the importance of carefully selecting fiber types in the design of plant-based meat analogues to ensure high protein bioavailability without compromising technological properties. Further formulation strategies, such as matrix modification or enzymatic pre-treatment, may help optimize both the nutritional and functional qualities of these products. Future studies should expand the fiber matrix to include additional sources such as pea fiber, inulin, and cellulose derivatives. Moreover, gradient-based optimization of fiber inclusion levels could help identify ideal concentrations for balancing nutritional enhancement with sensory and textural performance.

Table 2. Nutritional composition of plant-based gyros.

Parameter	PBG1	PBG2	PBG3	PBG4
Protein content (g/100 g)	20.849 ± 0.233 [a]	21.573 ± 0.479 [a]	21.859 ± 0.243 [a]	21.031 ± 0.063 [a]
Fat content (g/100 g)	14.983 ± 0.645 [a]	14.343 ± 0.833 [a]	13.813 ± 0.723 [a]	14.172 ± 0.662 [a]
Fiber content (g/100 g)	7.911 ± 0.338 [b]	8.294 ± 0.388 [a]	7.762 ± 0.269 [b]	8.263 ± 0.365 [a]
Insoluble dietary fiber (g/100 g)	7.055 ± 0.308 [b]	7.363 ± 0.351 [a]	6.963 ± 0.245 [b]	7.349 ± 0.385 [a]
Soluble dietary fiber (g/100 g)	0.855 ± 0.038 [b]	0.931 ± 0.050 [a]	0.799 ± 0.023 [b]	0.914 ± 0.020 [a]
β-glucan content (g/100 g)	0.571 ± 0.049 [a]	0.906 ± 0.062 [b]	0.535 ± 0.054 [a]	0.872 ± 0.072 [b]
Carbohydrate content (g/100 g)	6.938 ± 0.643 [c]	9.037 ± 0.643 [b]	9.470 ± 1.150 [b]	7.459 ± 1.256 [c]
Ash content (g/100 g)	7.059 ± 0.156 [a]	6.824 ± 0.393 [b]	7.196 ± 0.290 [a]	6.705 ± 0.510 [b]
Energy value (kcal/100 g)	267.76 ± 4.04 [b]	286.80 ± 5.13 [a]	284.46 ± 2.96 [a]	270.89 ± 1.85 [b]
Protein digestibility (%)	96.02 ± 2.11 [a]	83.52 ± 1.64 [b]	95.22 ± 1.93 [a]	85.08 ± 1.98 [b]

Values marked with the same lowercase letter in the row do not differ significantly. Values expressed per 100 g fresh product mass, unless otherwise indicated.

The formulations also contained appreciable levels of essential minerals (Table 3). Calcium levels ranged from 1100 to 1200 μg/g dry matter, whereas magnesium content was highest in PBG2 (558 μg/g), likely reflecting the contribution of green leafy vegetable or legume-based components. Iron content was substantially higher in PBG1 and PBG2 (417–422 μg/g) compared to PBG3 and PBG4 (71.4–72.3 μg/g), which indicates that the ferritin-fortified lupine sprout powder is an excellent source of iron [59]. Sodium levels, although relatively high (14,500–15,600 μg/g dry matter), remain consistent with the flavor profile typically associated with gyro-style products. Potassium concentrations followed a decreasing trend from PBG1 (14,500 μg/g) to PBG3 (12,100 μg/g), which provided a favorable Na/K ratio in the PBG1-PBG2 variants. Trace minerals such as zinc (50.1–53.7 μg/g), copper (41.1–45.0 μg/g), and manganese (46.9–48.2 μg/g) were present at physiologically relevant levels, which indicated that these products may contribute to the recommended dietary intake of these micronutrients [60,61]. Lead (Pb) and cadmium (Cd) concentrations were within acceptable regulatory limits for plant-based food products [62]. But, a relatively elevated Pb content was observed across all samples (77.9–88.2 μg/g dry matter), which showed the importance of ingredient sourcing and quality control in minimizing contaminant levels. Cadmium levels ranged from 0.91 to 1.14 μg/g, which, although below the maximum levels set for plant products, should be monitored to ensure long-term product safety and compliance. The formulations contain physiologically relevant levels of minerals such as zinc, calcium, and copper. However, their bioavailability and stability during processing warrant further investigation. Future work will focus on assessing nutrient retention and absorption, particularly for critical micronutrients like vitamin B12 and zinc. Similarly, although ferritin-rich sprout powder demonstrated superior iron content, its bioavailability remains to be confirmed. Future studies will include animal models or human trials to assess iron absorption and its efficacy in addressing iron deficiency.

Table 3. Mineral profiles of analyzed gyros.

Parameter	PBG1	PBG2	PBG3	PBG4
Calcium, Ca (μg/g d.m.)	1120 ± 90 [a]	1110 ± 110 [a]	1200 ± 110 [a]	1100 ± 90 [a]
Magnesium, Mg (μg/g d.m.)	512 ± 55 [a]	558 ± 39 [a]	477 ± 31 [b]	481 ± 38 [b]
Sodium, Na (μg/g d.m.)	15,600 ± 1250 [a]	15,200 ± 1300 [a]	14,700 ± 1000 [a]	14,500 ± 1100 [a]
Potassium, K (μg/g d.m.)	14,500 ± 1300 [a]	14,400 ± 1200 [a]	12,100 ± 1000 [b]	12,700 ± 1100 [b]
Iron, Fe (μg/g d.m.)	417 ± 33 [a]	422 ± 29 [a]	71.4 ± 5.6 [b]	72.3 ± 6.1 [b]
Zinc, Zn (μg/g d.m.)	50.1 ± 3.2 [b]	51.3 ± 3.4 [ab]	53.7 ± 4.8 [a]	53.3 ± 4.7 [a]
Copper, Cu (μg/g d.m.)	44.7 ± 4.9 [a]	45.0 ± 3.8 [a]	41.1 ± 3.3 [b]	42.7 ± 3.8 [b]
Manganese, Mn (μg/g d.m.)	48.2 ± 2.7 [a]	46.9 ± 4.4 [a]	47.5 ± 4.3 [a]	47.2 ± 3.7 [a]
Lead, Pb (μg/g d.m.)	88.2 ± 8.1 [a]	86.7 ± 7.0 [a]	78.4 ± 7.3 [a]	77.9 ± 8.5 [a]
Cadmium, Cd (μg/g d.m.)	0.905 ± 0.088 [b]	1.141 ± 0.155 [a]	1.088 ± 0.184 [ab]	1.131 ± 0.123 [a]

Values marked with the same lowercase letter in the row do not differ significantly. Values expressed per 1 g dry matter unless otherwise indicated.

3.2. Glycoalkaloid Contents

The use of potato-derived protein isolate, obtained from waste potato juice, introduces a potential safety concern due to the natural occurrence of glycoalkaloids, particularly α-solanine and α-chaconine. These compounds are known for their toxicity at elevated levels, with documented effects including gastrointestinal distress, neurological symptoms, and in extreme cases, acute toxicity [63,64]. Therefore, monitoring their concentration in the final product is critical to ensure consumer safety.

The total glycoalkaloid (TGA) content in the analyzed vegan gyros variants ranged between 33.31 μg/g dry matter (PBG2) and 44.71 μg/g dry matter (PBG3), summing the levels of α-solanine and α-chaconine (Table 4). The highest levels were detected in PBG1 (32.41 μg/g α-solanine and 12.37 μg/g α-chaconine) and PBG3 (32.11 and 12.60 μg/g, respectively), whereas PBG2 and PBG4 demonstrated markedly lower concentrations of α-chaconine (5.26 and 5.42 μg/g, respectively), which suggested possible differences in the batch of protein isolate or additional TGA content in potato fiber used for the production of PBG1 and PBG3. Although present, the glycoalkaloid concentrations remained below established safety thresholds. According to the European Food Safety Authority (EFSA), the total glycoalkaloid content in food should not exceed 100 μg/g dry weight [65], and ideally, for processed foods, levels below 20 μg/g fresh weight (approximately 50–60 μg/g dry matter depending on moisture) are considered safe for chronic consumption. None of the formulations approached this upper limit, although PBG1 and PBG3 were closer to the critical range, and as such, warrant continued monitoring during scale-up or commercial production. It is important to emphasize that α-chaconine is typically more toxic than α-solanine, with synergistic effects when both are present [32,66]. Therefore, the significantly lower α-chaconine levels in PBG2 and PBG4 are particularly beneficial in terms of toxicological risk reduction. These variants may thus be more suitable from a regulatory and consumer safety perspective.

Table 4. Results of glycoalkaloid contents in analyzes gyros.

Parameter	PBG1	PBG2	PBG3	PBG4
α-Solanine content (μg/g d.m.)	32.405 ± 0.619 [a]	28.047 ± 1.186 [b]	32.110 ± 1.028 [a]	27.971 ± 1.327 [b]
α-Chaconine content (μg/g d.m.)	12.365 ± 1.895 [a]	5.264 ± 0.375 [b]	12.601 ± 1.301 [a]	5.418 ± 0.478 [b]

Values marked with the same lowercase letter in the row do not differ significantly. Values expressed per 1 g dry matter unless otherwise indicated.

3.3. Antioxidant Activity and Phenolic Acid Bioaccessibility

To evaluate the bioaccessibility and potential health-promoting properties of the developed vegan gyros formulations, in vitro gastrointestinal digestion was performed. Antioxidant activity and levels of selected phenolic acids were analyzed before and after digestion to assess the release and transformation of antioxidant compounds under simulated physiological conditions. Initial total phenolic content (TPC) values ranged from 1.60 to 1.86 mg GAE/g (gallic acid equivalent per gram) dry matter (Table 5), which aligned with values reported for other plant-based meat analogues containing legume proteins and fiber-rich carriers [16]. Notably, after digestion, TPC values increased significantly by approximately 2- to 2.5-fold, which indicated an improved extractability of polyphenolic compounds from the food matrix. The gastric and intestinal phases likely disrupted protein-phenolic and fiber-phenolic complexes, thus enhancing phenolic release [67,68].

Table 5. Content of antioxidants and overall antioxidant potential measured pre- and post-digestion.

Parameter	PBG1	PBG2	PBG3	PBG4
Prior to the in vitro digestion				
TPC (mg/g d.m.)	1.763 ± 0.077 ^{a}	1.863 ± 0.065 ^{a}	1.601 ± 0.079 ^{b}	1.623 ± 0.101 ^{b}
$TEAC_{ABTS}$ (μmol/g d.m.)	9.915 ± 1.927 ^{a}	6.983 ± 1.173 ^{ab}	8.163 ± 1.054 ^{a}	6.067 ± 0.634 ^{b}
$TEAC_{FRAP}$ (μmol/g d.m.)	1.522 ± 0.147 ^{a}	1.497 ± 0.032 ^{b}	1.443 ± 0.099 ^{b}	1.606 ± 0.192 ^{a}
Gallic acid (μg/g d.m.)	0.564 ± 0.018 ^{a}	0.388 ± 0.159 ^{b}	0.554 ± 0.032 ^{a}	0.567 ± 0.035 ^{a}
Caffeic acid (μg/g d.m.)	0.162 ± 0.005 ^{b}	0.188 ± 0.015 ^{a}	0.124 ± 0.01 ^{c}	0.199 ± 0.015 ^{a}
Ferulic acid (μg/g d.m.)	0.827 ± 0.016 ^{a}	0.652 ± 0.013 ^{c}	0.759 ± 0.02 ^{b}	0.723 ± 0.092 ^{bc}
Chlorogenic acid (μg/g d.m.)	0.375 ± 0.011 ^{c}	0.381 ± 0.063 ^{b}	0.331 ± 0.02 ^{d}	0.499 ± 0.025 ^{a}
After the in vitro digestion				
TPC (mg/g d.m.)	4.276 ± 0.222 ^{a}	3.994 ± 0.220 ^{b}	3.876 ± 0.197 ^{b}	4.127 ± 0.391 ^{a}
$TEAC_{ABTS}$ (μmol/g d.m.)	55.900 ± 7.543 ^{b}	52.952 ± 9.377 ^{b}	71.138 ± 13.716 ^{a}	37.248 ± 7.393 ^{c}
$TEAC_{FRAP}$ (μmol/g d.m.)	2.671 ± 0.334 ^{a}	2.728 ± 0.336 ^{a}	2.316 ± 0.195 ^{a}	2.507 ± 0.474 ^{a}
Gallic acid (μg/g d.m.)	1.145 ± 0.045 ^{b}	1.652 ± 0.012 ^{a}	1.644 ± 0.091 ^{a}	1.662 ± 0.044 ^{a}
Caffeic acid (μg/g d.m.)	0.150 ± 0.001 ^{b}	0.155 ± 0.003 ^{b}	0.161 ± 0.011 ^{a}	0.166 ± 0.002 ^{a}
Ferulic acid (μg/g d.m.)	0.859 ± 0.023 ^{a}	0.682 ± 0.026 ^{d}	0.767 ± 0.020 ^{b}	0.707 ± 0.001 ^{c}
Chlorogenic acid (μg/g d.m.)	0.980 ± 0.039 ^{b}	1.144 ± 0.018 ^{a}	1.102 ± 0.105 ^{a}	1.072 ± 0.011 ^{ab}

Values marked with the same lowercase letter in the row do not differ significantly. Values expressed per 1 g dry matter unless otherwise indicated.

The antioxidant activity measured by ABTS assay increased post-digestion, with PBG3 exhibiting the highest final activity (71.14 μmol TE/g dry matter), which suggested that its specific matrix composition favored the liberation of bioactive compounds. This observation aligns with prior studies demonstrating enhanced polyphenol bioaccessibility following digestion [69,70]. FRAP values also increased across all variants, although the changes were comparably more moderate, thus reflecting the specificity of the assay toward electron transfer mechanisms. The discrepancy between ABTS and FRAP suggests that digestion favors the release of radical scavengers rather than reducing agents alone [71,72]. Before digestion, ferulic acid was the most abundant free phenolic acid (0.65–0.83 μg/g dry matter), which is expected given its prevalence in cereal and fiber-rich components. But, following digestion, chlorogenic and gallic acid levels increased substantially, with chlorogenic acid levels rising by more than 2.5-fold in all variants, particularly in PBG1 and PBG2. This suggests the breakdown of chlorogenic acid precursors or the release of bound forms during enzymatic hydrolysis [73,74]. The post-digestive increase in gallic acid, particularly in PBG2-PBG4 (up to ~1.66 μg/g), indicates hydrolysis of galloylated tannins or protein-polyphenol complexes. These results reinforce the understanding that phenolic acids exist in bound forms within plant matrices and become bioaccessible upon enzymatic digestion [75–77]. Notably, ferulic and caffeic acid concentrations remained relatively stable before and after digestion. This could be attributed to their partial degradation under alkaline intestinal pH or to limited release from insoluble structures [78]. Nevertheless, the net antioxidant capacity increased significantly, which indicated that other phenolic or non-phenolic antioxidants (e.g., Maillard reaction products) may have contributed to the post-digestive antioxidant potential. Although in vitro digestion revealed enhanced antioxidant release, future research should include in vivo comparative studies to evaluate physiological effects such as anti-inflammatory or anti-fatigue responses. These investigations will help establish the health relevance of the observed antioxidant activity.

3.4. Consumer Acceptance and Sensory Attractiveness

The consumer and sensory evaluation of the developed gyros formulations demonstrated clear differences between products enriched with ferritin-rich sprout powder and those fortified with inorganic iron salts. Figures 1 and 2 illustrate that formulations enriched with sprout powder consistently outperformed those fortified with inorganic iron salts across all sensory attributes. In particular, products containing sprout powder achieved higher scores for flavor, aroma, appearance, and texture. Consumers described these variants as having a more harmonious and appealing taste profile, with the sprout powder functioning as a natural flavor enhancer that subtly enriched the seasoning notes of the PBGs. The sprout powder enhanced flavor complexity and balance, thus mitigating the off-notes often associated with iron fortification. In stark contrast, formulations fortified with inorganic iron salts, such as ferrous sulfate, were consistently rated lower in overall acceptability. Participants frequently reported the presence of a metallic or astringent aftertaste in these samples, which negatively influenced their sensory appeal. Such metallic off-flavors are a known limitation of inorganic iron fortification in plant-based products. Visual appeal and texture were also favorably influenced by the incorporation of sprout powder. Products enriched with sprout powder were described as having a more uniform color, an attractive appearance, and a cohesive, pleasant texture. Sprout powder integrated seamlessly into the matrix, which improved structural cohesion and contributed to a more pleasant mouthfeel. Conversely, products with added iron salts were often noted to have minor color irregularities and a slightly grainier texture, which further decreased their sensory quality.

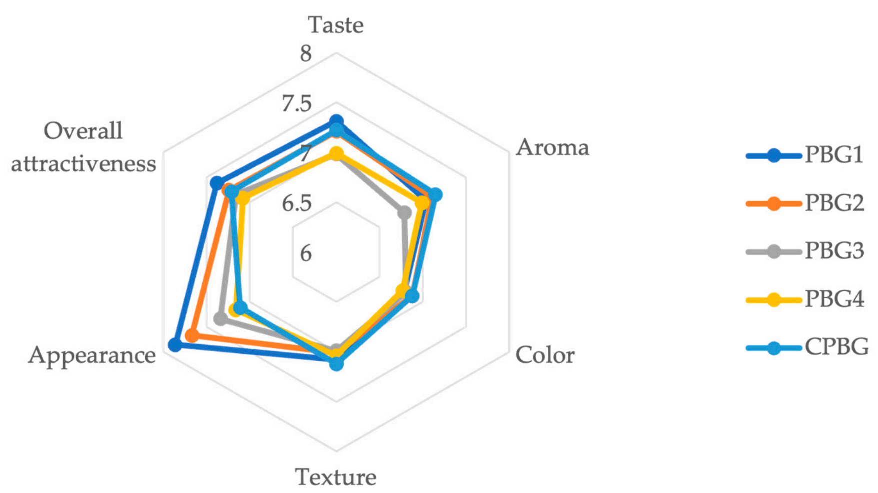

Figure 1. Mean consumer acceptability scores for plant-based gyros formulations enriched with ferritin-rich sprout powder or fortified with inorganic iron salts (PBG1-PBG4) and commercial plant-based gyros (CPBG).

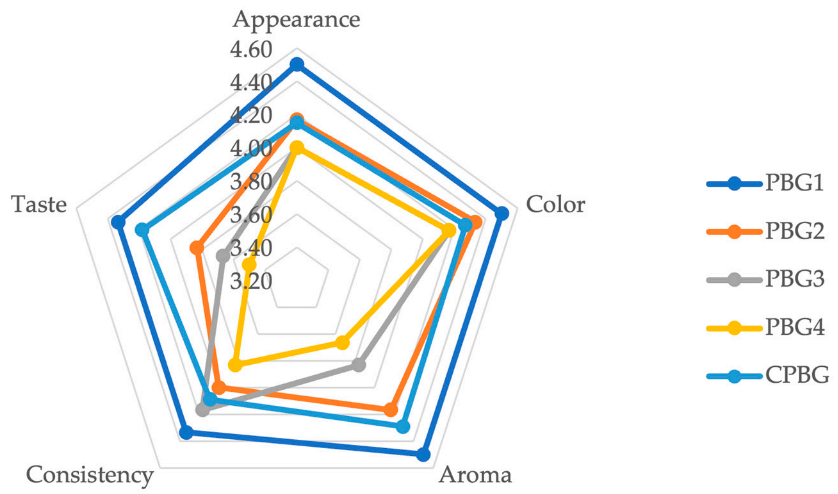

Figure 2. Detailed sensory profiling of plant-based gyros formulations: comparison of products containing ferritin-rich sprout powder and those fortified with inorganic iron salts (PBG1-PBG4) and commercial plant-based gyros (CPBG).

To contextualize consumer acceptance, we included a commercially available plant-based gyros (CPBG) as a benchmarking control. In the consumer test, the commercial product received the lowest rating for overall appearance, despite being judged to have the most appealing color. Across the remaining attributes, consumers rated PBG1 as the most attractive product, with scores exceeding those of CPBG. A similar pattern was observed in the sensory evaluation, where PBG1 achieved the highest overall score. Importantly, these findings highlight the potential of ferritin-rich sprout powder not only as a source of bioavailable iron, but also as a multifunctional ingredient that improves the sensory characteristics of plant-based meat analogues. The sprout powder acts similarly to a seasoning component by adding complexity and depth to the flavor profile without the need for artificial additives [79]. The patented production method for these ferritin-enriched sprouts thus represents a promising innovation for the design of novel plant-based products and offers a dual benefit of enhanced nutritional value and improved consumer appeal. This approach could play a pivotal role in future food development strategies aimed at addressing iron deficiency while meeting growing consumer demand for plant-based foods that are both health-promoting and sensorially attractive.

3.5. Textural Properties

Texture plays a pivotal role in consumer acceptance of plant-based meat analogues, as it influences the perceived quality and meat-like authenticity [80,81]. In the present study, two fundamental textural parameters were evaluated: firmness, expressed as the maximum shear force (N), and shear work (N × s), which represented the energy required to cut through the sample and is associated with cohesiveness and structural integrity [82]. Among the tested formulations, PBG4 demonstrated the highest firmness (3.83 ± 0.61 N) and shear work (19.64 ± 2.81 N × s), which indicated a more compact and cohesive structure relative to the other variants (Table 6). In contrast, PBG2 exhibited the lowest firmness (3.11 ± 0.54 N) and the lowest shear resistance. These textural differences are attributable to variations in matrix composition, particularly fiber type and carbohydrate content. The main compositional differences arose from the type of dietary fiber employed: PBG1 and PBG3 incorporated potato fiber, whereas PBG2 and PBG4 contained oat fiber, characterized by relatively higher β-glucan content and superior water-binding capacity. Notably, oat fiber typically enhances moisture retention and elastic cohesiveness, whereas the markedly higher firmness of PBG4 compared to PBG2 suggests that additional matrix interactions played a role. The PBG2 contained a higher carbohydrate content (9.04 g/100 g) than PBG4 (7.46 g/100 g), which could have exerted a plasticizing effect, reducing matrix rigidity and mechanical resistance in PBG2. Conversely, the elevated β-glucan content in PBG4 (0.872 g/100 g) likely contributed positively to gelation and viscoelasticity, which aligned with the observed increase in shear work. Furthermore, variations in fat and sodium content may have influenced the structural properties. The lower fat content in PBG3 and PBG4 likely facilitated denser matrix formation during thermal processing. In contrast, the higher sodium levels in PBG1 and PBG2 could have altered protein-polysaccharide interactions by weakening matrix strength. PBG3, with intermediate firmness and shear work values, contained both ferrous sulfate and a lower fiber level compared to PBG4, which potentially affected water retention, protein cross-linking, and overall textural integrity. These findings underscore the critical role of fiber type and minor ingredients in modulating textural performance. Oat fiber, rich in soluble fractions such as β-glucans, appears to enhance matrix viscosity and gelation, which supported higher shear resistance when balanced with water content [83,84]. In contrast, potato fiber, with a greater proportion of insoluble components, may contribute to less cohesive textures, as reflected by the lower shear work in PBG1 and PBG3. The interactions between protein, starch, fiber, and fat—modulated by water activity and emulsifier profile—emerge as key factors defining mechanical performance.

Table 6. Results of texture analysis.

Parameter	PBG1	PBG2	PBG3	PBG4
Firmness (N)	3.210 ± 0.319 [b]	3.107 ± 0.336 [b]	3.337 ± 0.238 [b]	3.833 ± 0.207 [a]
Share work (Ns)	14.72 ± 1.94 [c]	15.84 ± 3.11 [c]	17.35 ± 1.86 [b]	19.64 ± 2.81 [a]

Values marked with the same lowercase letter in the row do not differ significantly.

3.6. Water Behavior

Low-field nuclear magnetic resonance (LF NMR) relaxometry was employed to characterize the water mobility and physical state of water fractions in both raw PBG batters and the final cooked products. Relaxation times T_1 (spin-lattice) and T_2 (spin-spin), including T_{21} and T_{22} components, were used to characterize water mobility and distribution [85]. In the uncooked batters (Table 7), T_1 values ranged from 118.07 ms in PBG2 to 139.92 ms in PBG4, which indicated variations in proton-lattice energy exchange rates that reflect differences in protein-water and fiber-water interactions. The longest T_1 values in PBG3 and PBG4 suggest the presence of more mobile water populations, likely related to higher hydration and looser binding within the protein-fiber network. The higher β-glucan content in PBG4 may also contribute to increased water entrapment in a viscous gel phase [83], by slowing spin-lattice relaxation. Regarding spin-spin relaxation, T_{21} values (tightly bound water) ranged from approximately 29.9 ms in PBG2 to 35.8 ms in PBG3, whereas T_{22} values (loosely bound or free water) ranged from about 84.7 ms in PBG1 to 91.1 ms in PBG3, with higher T_{22} values in PBG3 and PBG4 implying greater free water availability or weaker matrix entrapment, which correlated with higher hydration and more porous structure in the raw state. Thermal processing led to a notable reduction in both T_1 and T_2 values across all variants and indicated protein denaturation and matrix densification. T_1 values decreased by 10–20 ms, with the lowest in PBG2 (106.09 ms), which indicated strong protein-water interactions and tighter proton-lattice coupling, potentially influenced by oat fiber. T_{21} values also decreased by 8–10 ms, which suggested reduced mobility of bound water fractions [85] as protein aggregation and starch retrogradation progressed. PBG1 and PBG2 exhibited the shortest T_{21} times (22.4 and 21.7 ms), which indicated greater water immobilization consistent with their slightly higher insoluble fiber density and starch-fiber synergy. T_{22} values decreased but remained higher in PBG3 and PBG4 (around 69–70 ms), which suggested the retention of more loosely bound water, likely due to their higher water content and soluble fiber (SDF, β-glucans). Correlating these findings with texture and composition, PBG4, which had the highest firmness (3.83 N, Table 6) and shear work (19.64 N × s, Table 6), also exhibited the highest post-cooking T_1 and T_{22} values, which suggested partial water retention in a structured yet flexible matrix enabled by high β-glucan content and balanced fat and protein levels. In contrast, PBG2, with the lowest firmness and shortest relaxation times, reflected tighter water binding but lower structural resistance, perhaps due to a denser yet brittle matrix lacking plasticity. PBG3, though softer, showed the highest pre-cooking water mobility, which highlighted the potential for reformulation with gelling aids or alternative fibers to improve structural stability upon cooking. These results confirm that water mobility is intricately linked to matrix composition and processing, which in turn influences final texture and stability [86,87]. LF NMR thus provided sensitive indicators of microstructural transitions during processing and revealed how ingredient choices modulate water dynamics, ultimately to shape the textural profile of plant-based meat analogues.

Table 7. LF NMR relaxometry results of batters and final products.

Relaxation Time	PBG1	PBG2	PBG3	PBG4
Batters				
T_1 (ms)	121.81 ± 0.44 [b]	118.07 ± 0.51 [b]	137.55 ± 0.58 [a]	139.92 ± 0.47 [a]
T_{21} (ms)	31.25 ± 0.91 [ab]	29.94 ± 1.02 [b]	35.77 ± 1.09 [a]	33.93 ± 0.93 [a]
T_{22} (ms)	84.66 ± 4.21 [b]	85.31 ± 4.30 [b]	91.06 ± 5.78 [a]	90.94 ± 5.09 [a]
Final products				
T_1 (ms)	110.22 ± 0.51 [b]	106.09 ± 0.43 [c]	111.03 ± 0.46 [b]	118.00 ± 0.52 [a]
T_{21} (ms)	22.39 ± 0.79 [b]	21.73 ± 0.66 [b]	23.37 ± 0.83 [a]	24.06 ± 0.98 [a]
T_{22} (ms)	64.31 ± 5.77 [c]	67.92 ± 4.58 [b]	68.92 ± 3.05 [b]	70.04 ± 3.66 [a]

Values marked with the same lowercase letter in the row do not differ significantly.

3.7. Microbiological Stability

To evaluate the microbiological stability of the developed PBG formulations, samples were stored under refrigerated conditions and analyzed on days 1, 8, and 15 of storage (Table 8). The assessment included enumeration of total mesophilic aerobic microorganisms, yeasts and molds, Enterobacteriaceae, as well as screening for key foodborne pathogens, specifically *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *Clostridium perfringens*. Initial mesophilic counts were low across all variants (2.47–2.87 log CFU/g), which reflected effective thermal processing and hygienic handling. By day 8, mesophilic counts increased modestly (2.99–3.47 log CFU/g) but remained well within acceptable levels for chilled ready-to-eat (RTE) products. By day 15, microbial counts increased significantly in PBG3 and PBG4, exceeding the 7 log CFU/g threshold associated with spoilage. In contrast, PBG1 (6.36 log CFU/g) and PBG2 (6.66 log CFU/g) remained below this limit, which suggested relatively greater microbial stability that could be attributed to differences in matrix composition or lower moisture availability in these variants. Precarious increase in mesophilic counts in all formulations at the 15th day has been recognized as a point in which formulations became microbiologically unstable and further measurements have been suspended.

Table 8. Microbiological stability of plant-based gyros formulations (PBG1-PBG4) during refrigerated storage over 15 days: counts of mesophilic aerobic microorganisms, yeasts and molds, Enterobacteriaceae, *Staphylococcus aureus*, *Clostridium perfringens*, and *Listeria monocytogenes* (log CFU/g).

Sample	Day	Mesophilic Aerobic Microorganisms	Yeast and Mould	Enterobacteriaceae	*S. aureus*	*C. perfringens*	*L. monocytogenes*
PBG1	1	2.864	1.100	<0.100	<0.100	<0.100	<0.100
	8	3.175	2.595	<0.100	<0.100	<0.100	<0.100
	15	6.359	3.006	1.65	<0.100	<0.100	<0.100
PBG2	1	2.473	1.460	<0.100	<0.100	<0.100	<0.100
	8	3.474	2.555	<0.100	<0.100	<0.100	<0.100
	15	6.663	5.145	<0.100	<0.100	<0.100	<0.100
PBG3	1	2.652	1.454	<0.100	<0.100	<0.100	<0.100
	8	2.998	2.471	<0.100	<0.100	<0.100	<0.100
	15	8.967	3.983	<0.100	<0.100	<0.100	<0.100
PBG4	1	2.868	0.774	<0.100	<0.100	<0.100	<0.100
	8	3.155	2.988	<0.100	<0.100	<0.100	<0.100
	15	7.059	5.907	<0.100	<0.100	<0.100	<0.100

Yeasts and molds exhibited a comparable growth pattern over time. Initial levels were below 1.5 log CFU/g in all formulations. By day 15, PBG2 (5.15 log CFU/g) and PBG4 (5.91 log CFU/g) exhibited higher fungal counts, which may be associated with the inclusion of oat fiber in these variants. The water-binding capacity and nutrient composition of oat fiber could have supported fungal proliferation under refrigerated condition [88,89]. In comparison, PBG1 (3.01 log CFU/g) and PBG3 (3.98 log CFU/g) displayed lower yeast and mold growth, potentially due to tighter matrix structures and reduced water mobility, as indicated by complementary LF NMR analysis. Enterobacteriaceae were detected only in PBG1 at day 15 (1.66 log CFU/g), whereas *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus,* and *Clostridium perfringens* were absent from all samples at all storage time points. Despite signs of spoilage after 10 days, all formulations remained microbiologically safe throughout the 15-day storage period. All formulations can be considered microbiologically stable for at least 8 days under refrigerated conditions. Given the observed increases in microbial counts beyond this period—particularly in PBG3 and PBG4—a conservative shelf-life of approximately 10 days is recommended for these variants unless additional preservation measures are adopted such as preserving ingredients, MAP, etc. The enhanced stability of PBG1 and PBG2 may be linked to compositional factors limiting microbial proliferation, such as lower levels of free water or differences in matrix structure. Overall, the results demonstrate that the developed PBG formulations are safe, minimally processed alternatives with potential for short-term commercial distribution under refrigerated storage.

4. Conclusions

This study demonstrates the feasibility of transforming potato juice protein—a previously underutilized by-product—into a nutritionally robust and sensorially appealing plant-based gyros. The incorporation of ferritin-rich sprout powder enhanced iron bioavailability as well as improved flavor and texture, thereby addressing common limitations of plant-based meat analogues. The formulations exhibited high protein content, favorable fiber profiles, and significant antioxidant potential, particularly after simulated digestion. Microbiological analyses confirmed product safety for up to 10 days under refrigerated storage.

Future research should explore shelf-life extension strategies, such as natural preservatives or modified atmosphere packaging. Additionally, scaling up production and conducting in vivo bioavailability studies will be critical for commercial translation. The valorization of agro-industrial side streams such as potato juice aligns with circular economy principles and offers a promising pathway toward more sustainable food systems. To improve commercial applicability, future work will explore preservation strategies such as natural antimicrobial agents, modified atmosphere packaging (MAP), and high-pressure processing. These approaches may extend shelf-life while maintaining product safety and quality.

5. Patents

The results presented in this article were used to prepare a patent application to The Patent Office of the Republic of Poland (patent application No. P.446897, dated 29 November 2023).

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Institutional Review Board Statement: The sensory survey protocol received approval from the Rector's Committee for the Ethics of Scientific Research Involving Humans at Poznań University of Life Sciences (Resolution No. 1/2023; 7 March 2023).

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References

1. United Nations. *World Population Prospects: The Highlights*; United Nations: New York, NY, USA, 2019.
2. Hussain, M.A.; Li, L.; Kalu, A.; Wu, X.; Naumovski, N. Sustainable Food Security and Nutritional Challenges. *Sustainability* **2025**, *17*, 874. https://doi.org/10.3390/su17030874.
3. Shrestha, S.; Mahat, J. Sustainable Food Security: How To Feed An Increasing Population? A Review. *INWASCON Technol. Mag.* **2022**, *4*, 15–18. https://doi.org/10.26480/itechmag.04.2022.15.18.
4. Kirbiš, A.; Korže, V.; Lubej, M. Predictors of Meat Reduction: The Case of Slovenia. *Foods* **2024**, *13*, 2346. https://doi.org/10.3390/foods13152346.
5. Zimmerman, A. Willingness to reduce meat consumption among U.S. flexitarians: Sampling, segmentation, and judgmental latitudes. *Food Qual. Prefer.* **2024**, *118*, 105194. https://doi.org/10.1016/j.foodqual.2024.105194.

6. Hansen, A.; Wethal, U.; Efstathiou, S.; Volden, J. Towards plantification: Contesting, negotiating and re-placing meaty routines. *Consum. Soc.* **2023**, *2*, 165–181. https://doi.org/10.1332/WPKF9257.
7. Tipan-Torres, C. Evaluación del impacto ambiental de los sistemas intensivos de producción animal según la literatura reciente. *Multidiscip. Collab. J.* **2024**, *2*, 40–54. https://doi.org/10.70881/mcj/v2/n4/5.
8. Watwani, D.; Gonal, V.; Wadke, P.; Chavan, P.; Sahu, S. Prediction of Carbon Footprint Due to Meat Industry. In Proceedings of the 2023 International Conference on IoT, Communication and Automation Technology (ICICAT), IEEE, Gorakhpur, India, 23–24 June 2023; pp. 1–5.
9. Nowak, J.; Lasik, M.; Miskiewicz, T.; Czarnecki, Z. Biodegradation of high temperature wastewater from potato starch industry. In *Proceedings of the Waste Management and the Environment*; Lmorza, D., Brebbia, C.A., Sales, D., Popov, V., Eds.; WIT PRESS Southampton: Boston, MA, USA, 2002; pp. 655–663.
10. Waglay, A.; Karboune, S. Potato Proteins. In *Advances in Potato Chemistry and Technology*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 75–104.
11. Kowalczewski, P.Ł.; Olejnik, A.; Białas, W.; Rybicka, I.; Zielińska-Dawidziak, M.; Siger, A.; Kubiak, P.; Lewandowicz, G. The Nutritional Value and Biological Activity of Concentrated Protein Fraction of Potato Juice. *Nutrients* **2019**, *11*, 1523. https://doi.org/10.3390/nu11071523.
12. Herreman, L.C.M.; de Vos, A.M.; Cosijn, M.M.; Tjalma, L.F.; Spelbrink, R.E.J.; van der Voort Maarschalk, K.; Laus, M.C. Potato: A Sustainable Source of Functional and Nutritional Proteins. In *Sustainable Protein Sources*; Elsevier: Amsterdam, The Netherlands, 2024; pp. 471–491.
13. Jeżowski, P.; Polcyn, K.; Tomkowiak, A.; Rybicka, I.; Radzikowska, D. Technological and antioxidant properties of proteins obtained from waste potato juice. *Open Life Sci.* **2020**, *15*, 379–388. https://doi.org/10.1515/biol-2020-0046.
14. Rybicka, I.; Bohdan, K.; Kowalczewski, P.Ł. Meat alternatives—Market and cunsumption. In *Sustainable Food. Production and Consumption Perspectives*; Pawlak-Lemańska, K., Borusiak, B., Sikorska, E., Eds.; Wydawnictwo Uniwersytetu Ekonomicznego w Poznaniu: Poznań, Poland, 2024; pp. 118–131.
15. Kowalczewski, P.Ł.; Smarzyński, K.; Biegalski, J.; Muzolf-Panek, M.; Cais-Sokolińska, D.; Ruszkowska, M.; Lewandowicz, J.; Miedzianka, J.; Wróbel, M.M.; Kačániová, M.; et al. Insight into the potato protein-based vegan cheese: A comprehensive study on physicochemical, mechanical and molecular properties. *Food Sci. Technol. Int.* **2025**. https://journals.sagepub.com/doi/10.1177/10820132251315810.
16. Kowalczewski, P.Ł.; Wróbel, M.M.; Smarzyński, K.; Zembrzuska, J.; Ślachciński, M.; Jeżowski, P.; Tomczak, A.; Kulczyński, B.; Zielińska-Dawidziak, M.; Sałek, K.; et al. Potato Protein-Based Vegan Burgers Enriched with Different Sources of Iron and Fiber: Nutrition, Sensory Characteristics, and Antioxidants before and after In Vitro Digestion. *Foods* **2024**, *13*, 3060. https://doi.org/10.3390/foods13193060.
17. Zeuschner, C.L.; Hokin, B.D.; Marsh, K.A.; Saunders, A. V.; Reid, M.A.; Ramsay, M.R. Vitamin B12 and vegetarian diets. *Med. J. Aust.* **2013**, *199*, S27–S32. https://doi.org/10.5694/mja11.11509.
18. Koeder, C.; Perez-Cueto, F.J.A. Vegan nutrition: A preliminary guide for health professionals. *Crit. Rev. Food Sci. Nutr.* **2024**, *64*, 670–707. https://doi.org/10.1080/10408398.2022.2107997.
19. Fields, H.; Ruddy, B.; Wallace, M.R.; Shah, A.; Millstine, D.; Marks, L. How to Monitor and Advise Vegans to Ensure Adequate Nutrient Intake. *J. Osteopath. Med.* **2016**, *116*, 96–99. https://doi.org/10.7556/jaoa.2016.022.
20. Kowalczewski, P.; Smarzyński, K.; Kubiak, P.; Lesiecki, M.; Jeżowski, P.; Tomczak, A.; Tomkowiak, A.; Grygier, A.; Kmiecik, D. Method of Producing a Plant-Based Analogue of Gyros. Polish Patent Aplication No. P.446897, 29 November 2023.
21. Zielińska-Dawidziak, M.; Staniek, H.; Król, E.; Piasecka-Kwiatkowska, D.; Twardowski, T. Legume seeds and cereal grains? Capacity to accumulate iron while sprouting in order to obtain food fortificant. *Acta Sci. Pol. Technol. Aliment.* **2016**, *15*, 333–338. https://doi.org/10.17306/J.AFS.2016.3.32.
22. *ISO 1871:2009*; Food and Feed Products—General Guidelines for the Determination of Nitrogen by the Kjeldahl Method. ISO: Geneva, Switzerland, 2009.
23. AOAC. *AOAC Official Methods of Analysis*, 21st ed.; AOAC International: Rockville, MD, USA, 2019.
24. AACC. AACCI 32-07.01. Soluble, Insoluble, and Total Dietary Fiber in Foods and Food Products. In *AACC International Approved Methods*; AACC International: Rockville, MD, USA, 2009.
25. AACC. Technical beta-Glucan Content of Barley and Oats—Rapid Enzymatic Procedure. In *AACC International Approved Methods*; AACC International: Rockville, MD, USA, 2009.
26. AACC. AACC 44-19.01. Moisture—Air-Oven Method, Drying at 135 Degrees. In *AACC International Approved Methods*; AACC International: Rockville, MD, USA, 2009.

27. *ISO 763:2003*; Fruit and Vegetable Products—Determination of Ash Insoluble in Hydrochloric Acid. ISO: Geneva, Switzerland, 2003.
28. Wang, X.-S.; Tang, C.-H.; Yang, X.-Q.; Gao, W.-R. Characterization, amino acid composition and in vitro digestibility of hemp (*Cannabis sativa* L.) proteins. *Food Chem.* **2008**, *107*, 11–18. https://doi.org/10.1016/j.foodchem.2007.06.064.
29. Matusiewicz, H.; Ślachciński, M. A Comparison of ETV and LA for the Determination of Trace Elements in Solid Samples by MIP OES. *Ecol. Chem. Eng. S* **2019**, *26*, 429–441. https://doi.org/10.1515/eces-2019-0032.
30. Kowalczewski, P.Ł.; Zembrzuska, J.; Drożdżyńska, A.; Smarzyński, K.; Radzikowska, D.; Kieliszek, M.; Jeżowski, P.; Sawinska, Z. Influence of potato variety on polyphenol profile composition and glycoalcaloid contents of potato juice. *Open Chem.* **2021**, *19*, 1225–1232. https://doi.org/10.1515/chem-2021-0109.
31. Olejnik, A.; Rychlik, J.; Kidoń, M.; Czapski, J.; Kowalska, K.; Juzwa, W.; Olkowicz, M.; Dembczyński, R.; Moyer, M.P. Antioxidant effects of gastrointestinal digested purple carrot extract on the human cells of colonic mucosa. *Food Chem.* **2016**, *190*, 1069–1077. https://doi.org/10.1016/j.foodchem.2015.06.080.
32. Kowalczewski, P.Ł.; Olejnik, A.; Wieczorek, M.N.; Zembrzuska, J.; Kowalska, K.; Lewandowicz, J.; Lewandowicz, G. Bioactive Substances of Potato Juice Reveal Synergy in Cytotoxic Activity against Cancer Cells of Digestive System Studied In Vitro. *Nutrients* **2023**, *15*, 114. https://doi.org/10.3390/nu15010114.
33. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1999; Volume 299, pp. 152–178.
34. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
35. Benzie, I.F.F.; Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70–76. https://doi.org/10.1006/abio.1996.0292.
36. Cybulska, I.; Zembrzuska, J.; Brudecki, G.; Hedegaard Thomsen, M. Optimizing methods to characterize caffeic, ferulic, and chlorogenic acids in *Salicornia sinus-persica* and *Salicornia bigelovii* extracts by tandem mass spectrometry (LC-MS/MS). *BioResources* **2021**, *16*, 5508–5523. https://doi.org/10.15376/biores.16.3.5508-5523.
37. Węglarz, W.P.; Harańczyk, H. Two-dimensional analysis of the nuclear relaxation function in the time domain: The program CracSpin. *J. Phys. D. Appl. Phys.* **2000**, *33*, 1909–1920. https://doi.org/10.1088/0022-3727/33/15/322.
38. Carr, H.Y.; Purcell, E.M. Effects of Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments. *Phys. Rev.* **1954**, *94*, 630–638. https://doi.org/10.1103/PhysRev.94.630.
39. Meiboom, S.; Gill, D. Modified Spin-Echo Method for Measuring Nuclear Relaxation Times. *Rev. Sci. Instrum.* **1958**, *29*, 688–691. https://doi.org/10.1063/1.1716296.
40. International Organization for Standardization. *ISO 13299:2016*; Sensory Analysis—Methodology—General Guidance for Establishing a Sensory Profile. ISO: Geneva, Switzerland, 2016.
41. *PN-EN ISO 6579-1:2017-04;* Microbiology of the Food Chain—Horizontal Method for the Detection, Enumeration and Serotyping of *Salmonella*—Part 1: Detection of *Salmonella* spp. (ISO 6579-1:2017). Polish Committee for Standardization: Warsaw, Poland, 2017.
42. *PN-EN ISO 11290-1:2017-07*; Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* and of *Listeria* spp.—Part 1: Detection Method (ISO 11290-1:2017). Polish Committee for Standardization: Warsaw, Poland, 2017.
43. *PN-EN ISO 4833-1:2013-12*; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms—Part 1: Colony Count at 30 degrees C by the Pour Plate Technique (ISO 4833-1:2013). Polish Committee for Standardization: Warsaw, Poland, 2013.
44. *PN-EN ISO 21528-2:2017-08*; Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of Enterobacteriaceae—Part 2: Colony-Count Technique (ISO 21528-2:2017). Polish Committee for Standardization: Warsaw, Poland, 2017.
45. *PN-EN ISO 15213-2:2024-05;* Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of *Clostridium* spp.—Part 2: Enumeration of *Clostridium perfringens* by Colony-Count Technique (ISO 15213-2:2023). Polish Committee for Standardization: Warsaw, Poland, 2023.
46. *PN-EN ISO 11290-2:2017-07;* Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* and of *Listeria* spp.—Part 2: Enumeration Method (ISO 11290-2:2017). Polish Committee for Standardization: Warsaw, Poland, 2017.
47. *PN-EN ISO 6888-1:2022-03*; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (*Staphylococcus aureus* and other species)—Part 1: Method Using Baird-Parker Agar Medium (ISO 6888-1:2021). Polish Committee for Standardization: Warsaw, Poland, 2022.

48. *PN-ISO 21527-1:2009*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Molds Part 1: Colony Count Technique in Products with Water Activity Greater Than 0,95 (ISO 21527-1:2008). Polish Committee for Standardization: Warsaw, Poland, 2009.
49. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on Nutrition and Health Claims Made on Foods. Available online: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32006R1924 (accessed on 12 July 2024).
50. Munialo, C.D.; Vriesekoop, F. Plant-based foods as meat and fat substitutes. *Food Sci. Nutr.* **2023**, *11*, 4898–4911. https://doi.org/10.1002/fsn3.3421.
51. Han, X.; Luo, R.; Ye, N.; Hu, Y.; Fu, C.; Gao, R.; Fu, S.; Gao, F. Research progress on natural β-glucan in intestinal diseases. *Int. J. Biol. Macromol.* **2022**, *219*, 1244–1260. https://doi.org/10.1016/j.ijbiomac.2022.08.173.
52. McCarthy, C.; Papada, E.; Kalea, A.Z. The effects of cereal β-glucans on cardiovascular risk factors and the role of the gut microbiome. *Crit. Rev. Food Sci. Nutr.* **2025**, *65*, 2489–2505. https://doi.org/10.1080/10408398.2024.2345159.
53. McGill, C.R.; Kurilich, A.C.; Davignon, J. The role of potatoes and potato components in cardiometabolic health: A review. *Ann. Med.* **2013**, *45*, 467–473. https://doi.org/10.3109/07853890.2013.813633.
54. Furrer, A.N.; Chegeni, M.; Ferruzzi, M.G. Impact of potato processing on nutrients, phytochemicals, and human health. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 146–168. https://doi.org/10.1080/10408398.2016.1139542.
55. Xu, R. Oat fibre: Overview on their main biological properties. *Eur. Food Res. Technol.* **2012**, *234*, 563–569. https://doi.org/10.1007/s00217-012-1666-2.
56. Cameron-Smith, D.; Collier, G.R.; O'dea, K. Effect of soluble dietary fibre on the viscosity of gastrointestinal contents and the acute glycaemic response in the rat. *Br. J. Nutr.* **1994**, *71*, 563–571. https://doi.org/10.1079/BJN19940163.
57. Dikeman, C.L.; Murphy, M.R.; Fahey, G.C. Dietary Fibers Affect Viscosity of Solutions and Simulated Human Gastric and Small Intestinal Digesta. *J. Nutr.* **2006**, *136*, 913–919. https://doi.org/10.1093/jn/136.4.913.
58. Grundy, M.M.-L.; Edwards, C.H.; Mackie, A.R.; Gidley, M.J.; Butterworth, P.J.; Ellis, P.R. Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. *Br. J. Nutr.* **2016**, *116*, 816–833. https://doi.org/10.1017/S0007114516002610.
59. Zielińska-Dawidziak, M.; Twardowski, T. Preparation with Higher Plant Ferritin Content and Other Forms of Iron, Process for Preparation and the Use Thereof. Polish Patent No. PL218747, 24 June 2009.
60. Li, J.; Cao, D.; Huang, Y.; Chen, B.; Chen, Z.; Wang, R.; Dong, Q.; Wei, Q.; Liu, L. Zinc Intakes and Health Outcomes: An Umbrella Review. *Front. Nutr.* **2022**, *9*, 798078. https://doi.org/10.3389/fnut.2022.798078.
61. Charkiewicz, A.E. Is Copper Still Safe for Us? What Do We Know and What Are the Latest Literature Statements? *Curr. Issues Mol. Biol.* **2024**, *46*, 8441–8463. https://doi.org/10.3390/cimb46080498.
62. Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. Available online: https://eur-lex.europa.eu/legal-content/SK/TXT/PDF/?uri=CELEX:32006R1881 (accessed on 4 May 2025).
63. Friedman, M.; McDonald, G.M.; Filadelfi-Keszi, M. Potato Glycoalkaloids: Chemistry, Analysis, Safety, and Plant Physiology. *CRC. Crit. Rev. Plant Sci.* **1997**, *16*, 55–132. https://doi.org/10.1080/07352689709701946.
64. Korpan, Y.I.; Nazarenko, E.A.; Skryshevskaya, I.V.; Martelet, C.; Jaffrezic-Renault, N.; El'skaya, A.V. Potato glycoalkaloids: True safety or false sense of security? *Trends Biotechnol.* **2004**, *22*, 147–151. https://doi.org/10.1016/j.tibtech.2004.01.009.
65. Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; del Mazo, J.; Hogstrand, C.; Hoogenboom, L.R.; Leblanc, J.; Nebbia, C.S.; Nielsen, E.; et al. Risk assessment of glycoalkaloids in feed and food, in particular in potatoes and potato-derived products. *EFSA J.* **2020**, *18*, e06222. https://doi.org/10.2903/j.efsa.2020.6222.
66. Rayburn, J.R.; Friedman, M.; Bantle, J.A. Synergistic interaction of glycoalkaloids α-chaconine and α-solanine on developmental toxicity in xenopus embryos. *Food Chem. Toxicol.* **1995**, *33*, 1013–1019. https://doi.org/10.1016/0278-6915(95)00081-X.
67. Marinea, M.; Ellis, A.; Golding, M.; Loveday, S.M. Delivering Phenolic Acids in Soy Protein Gels: Noncovalent Interactions Control Gastrointestinal Bioaccessibility. *Food Biophys.* **2023**, *18*, 218–227. https://doi.org/10.1007/s11483-022-09763-6.
68. Huang, G.; Wang, Z.; Wu, G.; Cao, X.; Zhang, R.; Dong, L.; Huang, F.; Zhang, M.; Su, D. In vitro simulated digestion and colonic fermentation of lychee pulp phenolics and their impact on metabolic pathways based on fecal metabolomics of mice. *Food Funct.* **2021**, *12*, 203–214. https://doi.org/10.1039/D0FO02319A.
69. Aalim, H.; Hashim, S.B.H.; Zhou, C.; Zou, X.; Luo, Z. Matrix characteristics modulate black rice phenolic compounds bioaccessibility and antioxidant activity during simulated gastrointestinal digestion. *Food Biosci.* **2024**, *58*, 103628. https://doi.org/10.1016/j.fbio.2024.103628.

70. Domingues, Y.O.; Lemes, G.A.; de Oliveira, F.L.; de Souza, T.R.; Silva, B.; Bento, J.A.C.; Caldeira Morzelle, M. In vitro simulated gastrointestinal digestion and bioaccessibility of phenolic compounds and antioxidants of soursop (*Annona muricata* L.) peel and pulp. *LWT* **2024**, *208*, 116694. https://doi.org/10.1016/j.lwt.2024.116694.
71. Nadjamuddin, M.; Auliah, N.; Mulyadin; Tajuddin, Z.; Andriawan, R. Test the Antioxidant Effectiveness of Oyster Mushroom (Pleurotus ostreatus) Ethyl Acetate Fraction Cream Formula using FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2′-azino-bis (3-3) ethylbenzothiazoline-6-sulphonic acid) Methods). *J. Penelit. Pendidik. IPA* **2023**, *9*, 10985–10990. https://doi.org/10.29303/jppipa.v9i12.6118.
72. Nilsson, J.; Pillai, D.; Önning, G.; Persson, C.; Nilsson, Å.; Åkesson, B. Comparison of the 2,2′-azinobis-3-ethylbenzotiazo-line-6-sulfonic acid (ABTS) and ferric reducing anti-oxidant power (FRAP) methods to asses the total antioxidant capacity in extracts of fruit and vegetables. *Mol. Nutr. Food Res.* **2005**, *49*, 239–246. https://doi.org/10.1002/mnfr.200400083.
73. Miao, M.; Xiang, L. Pharmacological action and potential targets of chlorogenic acid. *Adv. Pharmacol.* **2020**, *87*, 71–88.
74. Clifford, M.N.; Kerimi, A.; Williamson, G. Bioavailability and metabolism of chlorogenic acids (acyl-quinic acids) in humans. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 1299–1352. https://doi.org/10.1111/1541-4337.12518.
75. Okuda, T.; Ito, H. Tannins of Constant Structure in Medicinal and Food Plants—Hydrolyzable Tannins and Polyphenols Related to Tannins. *Molecules* **2011**, *16*, 2191–2217. https://doi.org/10.3390/molecules16032191.
76. Le Bourvellec, C.; Renard, C.M.G.C. Interactions between Polyphenols and Macromolecules: Quantification Methods and Mechanisms. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 213–248. https://doi.org/10.1080/10408398.2010.499808.
77. Shahidi, F.; Peng, H. Bioaccessibility and bioavailability of phenolic compounds. *J. Food Bioact.* **2018**, *4*, 11–68. https://doi.org/10.31665/JFB.2018.4162.
78. Chen, Y.; Teng, W.; Wang, J.; Wang, Y.; Zhang, Y.; Cao, J. The intestinal delivery systems of ferulic acid: Absorption, metabolism, influencing factors, and potential applications. *Food Front.* **2024**, *5*, 1126–1144. https://doi.org/10.1002/fft2.366.
79. Lee, C.-W.; Kim, T.-K.; Hwang, K.-E.; Kim, H.-W.; Kim, Y.-B.; Kim, C.-J.; Choi, Y.-S. Combined Effects of Wheat Sprout and Isolated Soy Protein on Quality Properties of Breakfast Sausage. *Korean J. Food Sci. Anim. Resour.* **2017**, *37*, 52–61. https://doi.org/10.5851/kosfa.2017.37.1.52.
80. Jang, J.; Lee, D.-W. Advancements in plant based meat analogs enhancing sensory and nutritional attributes. *npj Sci. Food* **2024**, *8*, 50. https://doi.org/10.1038/s41538-024-00292-9.
81. Godschalk-Broers, L.; Sala, G.; Scholten, E. Meat Analogues: Relating Structure to Texture and Sensory Perception. *Foods* **2022**, *11*, 2227. https://doi.org/10.3390/foods11152227.
82. Szczesniak, A.S. Texture is a sensory property. *Food Qual. Prefer.* **2002**, *13*, 215–225. https://doi.org/10.1016/S0950-3293(01)00039-8.
83. Geng, X.; Zhao, N.; Song, X.; Wu, J.; Zhu, Q.; Wu, T.; Chen, H.; Zhang, M. Fabrication and Characterization of Konjac Glucomannan/Oat β-Glucan Composite Hydrogel: Microstructure, Physicochemical Properties and Gelation Mechanism Studies. *Molecules* **2022**, *27*, 8494. https://doi.org/10.3390/molecules27238494.
84. Inglett, G.E.; Chen, D.; Xu, J.; Lee, S. Pasting and Rheological Properties of β-Glucan-Enriched Hydrocolloids from Oat Bran Concentrate*. *J. Food Process. Preserv.* **2013**, *37*, 792–798. https://doi.org/10.1111/j.1745-4549.2012.00690.x.
85. Brosio, E.; Gianferri, R.R. Low-resolution NMR—An analytical tool in foods characterization and traceability. In *Basic NMR in Foods Characterization*; Brosio, E., Ed.; Research Signpost: Kerala, India, 2009; pp. 9–37; ISBN 978-81-308-0303-6.
86. Fundo, J.F.; Silva, C.L.M. Microstructure, composition and their relationship with molecular mobility, food quality and stability. In *Food Microstructure and Its Relationship with Quality and Stability*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 29–41.
87. Cornillon, P.; Salim, L.C. Characterization of water mobility and distribution in low- and intermediate-moisture food systems. *Magn. Reson. Imaging* **2000**, *18*, 335–341. https://doi.org/10.1016/S0730-725X(99)00139-3.
88. Rousta, N.; Larsson, K.; Fristedt, R.; Undeland, I.; Agnihotri, S.; Taherzadeh, M.J. Production of fungal biomass from oat flour for the use as a nutritious food source. *NFS J.* **2022**, *29*, 8–15. https://doi.org/10.1016/j.nfs.2022.09.001.
89. Thamilnesan, M.; Cheng, L.-H. Advancements in oat processing technologies and their impact on nutritional and functional properties: A review. *Nutr. Food Sci.* **2025**, *55*, 783–800. https://doi.org/10.1108/NFS-08-2024-0281.