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Effects of Spinach Addition on the Nutritional Value, Functional Properties, Microstructure and Shelf Life of Lamb Meat Dumplings

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Abstract

The incorporation of leafy vegetables into meat products offers a promising strategy for enhancing nutritional value and shelf-life while reducing reliance on synthetic additives. This study evaluated the substitution of lamb (Edilbaev breed) with spinach (0%, 10%, 20%, and 30%) in meat dumplings to assess effects on composition, functionality, microbial stability, lipid oxidation, and sensory quality. Spinach addition enriched the products with minerals, vitamins, and dietary fiber while moderating fat and protein content. Functional properties such as water- and fat-binding capacity were improved, contributing to lower cooking losses, and microbiological tests confirmed slower proliferation of spoilage organisms during chilled storage. Moreover, spinach components contributed to improved oxidative stability, as evidenced by lower thiobarbituric acid values and reduced acid numbers, indicating slower lipid oxidation and hydrolysis. Sensory evaluation revealed that substitution up to 20% maintained favorable appearance, texture, and taste, while higher levels diminished acceptability. Overall, incorporating spinach at a 20% substitution level provides an optimal balance of nutritional enhancement, functional performance, microbial and oxidative stability, and sensory acceptance, making it a practical approach for developing healthier lamb-based dumplings with strong potential for consumer acceptance and market application.

Keywords: lamb; spinach; minced meat; nutritional quality; functional properties; sensory evaluation; microbiological safety

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1. Introduction

Optimal nutrition is fundamental to human health, work capacity, and adaptation to environmental stressors. In the Republic of Kazakhstan, traditional dietary patterns have long centred on meat dishes prepared from horse, mutton, and beef, reflecting the country's pastoral heritage and the ecological suitability of steppe regions for livestock production [1]. While global average meat consumption varies, and general protein requirements are estimated around 55 g per day for adult males and 45 g for adult females, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) primarily

emphasize the importance of balanced diets, often recommending moderation in red and processed meat intake, and promoting diversified protein sources for sustainable and healthy eating [2]. Within Kazakhstan, however, mutton surpasses other categories in production, processing volume, and per capita consumption, followed by beef and horse meat. This prominence is underpinned by the historic importance of sheep husbandry, which supplies not only meat but also wool, hides, and lanolin, thereby contributing substantially to the national economy and rural livelihoods [3].

Lamb is recognized for its favorable nutrient profile. Relative to pork it contains approximately 30% more iron, as well as appreciable levels of phosphorus, iodine, sodium, magnesium, potassium, calcium, and vitamins E, B_1, B_{12}, and PP [4]. A comparatively low cholesterol content characterises the lipid fraction, and lecithin present in lamb fat contributes to cardiovascular health by lowering low-density lipoprotein (LDL, 'bad' cholesterol) and supporting high-density lipoprotein (HDL, 'good' cholesterol), thereby contributing to the prevention and management of atherosclerotic cardiovascular disease [5]. Moreover, lamb provides complete proteins rich in essential amino acids and lipids containing polyunsaturated fatty acids [6]. Nevertheless, carcass composition varies widely among breeds; meat-specialised genotypes yield muscle tissue of superior tenderness and marbling, whereas dual-purpose meat–milk breeds tend to display higher proportions of bone and connective tissue. Meat composition, including marbling, has a significant influence on consumer decisions at the point of meat purchase [7]. Genetic factors, therefore, influence pH, autolytic changes, and morphological characteristics that ultimately determine culinary quality.

In parallel with safeguarding national food traditions, Kazakhstan faces the need to diversify and modernise its meat industry in line with United Nations Sustainable Development Goals, which emphasise food security, sustainable agriculture, and the reduction in non-communicable diseases. Consumers increasingly demand products that combine high sensory appeal with improved nutritional and functional attributes. One promising route is the partial replacement of animal raw materials with plant ingredients, thereby decreasing energy density and saturated-fat content while introducing dietary fibre, bioactive phytochemicals, and additional micronutrients [8]. Such hybrid formulations can also optimise resource use by mitigating pressure on animal production systems. Although this study focuses on Kazakhstan, the challenges it addresses are shared globally. These include balancing traditional meat consumption with health concerns, enriching animal products with plant-derived bioactive compounds, and reducing reliance on synthetic additives.

Among potential plant enrichers, spinach (*Spinacia oleracea* L.) warrants particular attention. This leafy vegetable is calorically dilute yet dense in bioflavonoids, carotenoids (β-carotene and lutein), folate, vitamins C, E, and K, and minerals including potassium, calcium, magnesium, and iron [9]. Numerous studies report that many of these constituents remain appreciably stable under culinary heat treatments [10,11]. Spinach additionally supplies insoluble and soluble fibres, flavonoids, and chlorophyll, all of which possess antioxidant and, to some extent, antimicrobial activity. The integration of spinach into meat matrices could therefore enrich products with nutrients often under-represented in ruminant flesh, notably provitamin A, ascorbate, α-tocopherol, and folates, while the fibrous cell-wall network may enhance water- and fat-binding, improve cooking yield, and modulate texture [12,13].

In particular, spinach is rich in ascorbic acid, carotenoids, and tocopherols, which act as natural antioxidants capable of scavenging free radicals and inhibiting lipid peroxidation. These bioactive compounds are expected to attenuate the formation of secondary oxidation products, thereby lowering thiobarbituric acid reactive substances (TBARS) and acid values in enriched meat formulations [14]. In addition, spinach contains relatively high levels of

naturally occurring nitrates, which can be enzymatically reduced to nitrites. This process may further contribute to prolonging shelf-life by exerting inhibitory effects on spoilage and pathogenic microorganisms [15].

Recent studies highlight the potential of spinach and other leafy greens as functional ingredients in meat and meat-alternative products. Vegetable powders such as spinach, celery, and beetroot can partially replace synthetic nitrites, improving color stability, antioxidant capacity, and safety of fermented sausages [16]. Spinach and moringa powders enhanced the sensory quality and cooking yield of chicken meatballs [17], while spinach extract extended the shelf-life of chicken sausages under refrigeration [18]. Incorporation of spinach powder improved antioxidant properties and overall quality of pork sausages [19]. Similarly, spinach-derived chlorophyll enhanced color, texture, and antioxidant activity in plant-based meat patties [20].

The adoption of plant fortifiers is consistent with global and national strategies aimed at expanding the supply of safe, nutritious, and locally sourced foods. Kazakhstan's agroclimatic conditions support spinach cultivation, and domestic processing would foster value-added production chains. Despite these advantages, the incorporation of spinach into lamb mince has not been comprehensively investigated with respect to its effects on proximate composition, mineral and vitamin profiles, techno-functional behaviour, microstructure, microbiological stability, and sensory quality. Filling this knowledge gap is essential for the rational design of combined meat–plant semi-finished products that meet both regulatory standards and consumer expectations.

The hypothesis of this study was to improve the nutritional and functional properties of minced meat formulations by partially replacing lamb meat with spinach, while maintaining microbiological safety and consumer acceptability.

The aim of this study is to investigate how varying levels of spinach incorporation affect the nutritional composition, functional properties, microbiological stability, and sensory quality of lamb meat dumplings.

2. Materials and Methods

2.1. Samples

Lamb meat used in this study was obtained from sheep raised on the Mukinov collective farm, located in the Abai region of the Republic of Kazakhstan. Lamb meat was obtained from four- to five-month-old Edilbaev sheep, a fat-tailed breed native to Kazakhstan, with an average live weight of 40–42 kg. Following slaughter, selected carcass portions were transported to the laboratory in insulated freezer containers to maintain appropriate temperature conditions. Meat samples were collected from the loin and pelvic regions of the carcass. Before use, all visible connective tissue, tendons, cartilage, and excess fat were carefully trimmed and removed to standardize raw material quality.

2.2. Preparation of Spinach

Fresh spinach (*Spinacia oleracea* L.) was purchased from local markets in the city of Semey. Immediately after purchase, the spinach was placed in portable insulated containers and transported to the laboratory under refrigerated conditions at a temperature of +4 to +6 °C to preserve its freshness and minimize microbial proliferation. Upon arrival, the spinach was subjected to visual inspection for the removal of wilted or damaged leaves. All further handling and processing were carried out under hygienic and aseptic conditions.

Before use, the spinach was thoroughly washed to remove surface contaminants and reduce microbial load. The leaves were immersed in a large volume of cold potable water and gently agitated to dislodge soil and debris. This washing step was repeated twice, each time with fresh water. To further reduce the potential presence of pathogenic

microorganisms, the spinach was soaked for 2 min in a 1% (*v*/*v*) acetic acid solution (food-grade vinegar), prepared by adding 10 mL of vinegar to 990 mL of water. Following this, the leaves were rinsed thoroughly with potable water to remove residual acid.

For additional microbiological safety, the spinach leaves were blanched by immersing them in boiling water for 1–2 min, immediately followed by cooling in ice water to halt thermal processing. The blanched leaves were drained and dried using sterile paper towels prior to incorporation into the meat formulations.

2.3. Production Technology of Meat Dumplings

A total of 16 kg of trimmed lamb meat was used for the experimental trials. The study design comprised four treatment groups corresponding to different spinach inclusion levels (0%, 5%, 10%, and 15% *w*/*w* of minced meat). For each treatment, three independent biological replicates were prepared, where each replicate consisted of a separately processed batch of minced lamb with spinach addition. Within each biological replicate, all analytical determinations were performed in triplicate as technical replicates to ensure measurement accuracy and reproducibility.

The production of lamb dumplings involved several distinct stages: preparation of meat and vegetable raw materials, dough mixing, dumpling formation, and subsequent freezing. Chilled lamb, which served as the primary raw material, underwent boning and trimming to remove cartilage, fascia, and connective tissues. The meat was then minced using a meat grinder with a die plate hole diameter of 3–5 mm. To enhance succulence and flavor, the minced meat was combined with finely chopped onions, table salt, and spices (black pepper, coriander), along with a small quantity of chilled potable water. The mixture was thoroughly blended to a homogeneous consistency, ensuring optimal water-binding capacity.

Wrapper Preparation

For the wrapper (casing), premium wheat flour was sieved to aerate it and remove any foreign particles. Dough mixing was performed in a laboratory dough mixer using wheat flour, water (at 25–30 °C), and table salt. Mixing continued until a homogeneous, pliable dough with a moisture content of 39–42% was obtained. Following mixing, the dough was rested for 20–30 min at 18–22 °C to reduce elasticity and improve its formability.

Dumpling Manufacturing and Freezing

Dumplings were formed manually. The prepared dough was rolled into a sheet with a thickness of 1.5–2.0 mm. Circular pieces, 45–50 mm in diameter, were cut from the sheet. Each piece was then filled with 8–10 g of the prepared meat mixture, and the edges were firmly crimped to seal the dumpling. The resulting semi-finished products were placed on metal trays, which were lightly dusted with flour, and subjected to shock freezing at –25 °C until the core temperature of the dumplings reached –10 °C or lower. The frozen dumplings were then packaged in polyethylene bags and stored at –18 °C until further use. The meat dumplings are made according to the recipe specified in Table 1. For the study, different types of minced meat without wrappers were used.

Table 1. Recipe of meat dumplings, %.

Ingredient	Variant 1—Control	Variant 2—10%	Variant 3—20%	Variant 4—30%
Lamb	90	80	70	60
Spinach	0	10	20	30
Onion	10	10	10	10
Total	100	100	100	100

Table 1. *Cont.*

Ingredient	Variant 1—Control	Variant 2—10%	Variant 3—20%	Variant 4—30%
Spices				
Salt	1.5	1.5	1.5	1.5
Pepper	0.5	0.5	0.5	0.5
Wrapper (Dough)				
Flour	63.5	63.5	63.5	63.5
Egg	9	9	9	9
Water	27	27	27	27
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100

2.4. Determination of Chemical Composition

Protein content was determined using the Kjeldahl method, as specified in GOST 25011 [21]. Homogenized samples (1–2 g) were digested with concentrated sulfuric acid and catalysts, then distilled after alkalization with sodium hydroxide. Liberated ammonia was collected in boric acid and titrated with standardized sulfuric acid. The protein content was calculated from the measured nitrogen using a conversion factor and expressed as a percentage of sample mass.

Fat content was determined using the Soxhlet extraction method [22]. Homogenized samples (2–5 g) were dried to constant weight, then extracted with petroleum ether for 4–6 h. After solvent removal and further drying, the increase in flask mass was used to calculate fat content as a percentage of the sample's dry weight.

Water content was determined gravimetrically by drying homogenized samples (5–10 g, weighed on analytical scales (RADWAG Company, Radom, Poland) with a readability of 0.01 mg) mixed with pre-dried sand in heat-resistant dishes at 103 ± 2 °C until constant weight. The dish was cooled and weighed between cycles. Moisture content was calculated as the loss in mass and reported as a percentage of the original sample weight.

Ash content was determined by gravimetric incineration. Homogenized samples (2–5 g) were weighed into pre-cleaned, pre-weighed porcelain crucibles and incinerated in a muffle furnace at 550 °C until a constant grayish-white ash was obtained. The crucibles were cooled, reweighed, and ash content was calculated as a percentage of the original sample mass.

The carbohydrate content was determined by difference using the equation:

$$Carbohydrates,\ \% = 100 - (Moisture\ (\%) + Protein\ (\%) + Fat\ (\%) + Ash\ (\%)$$

2.5. Determination of pH

The active acidity (pH) of the samples was determined by the potentiometric method using a calibrated pH meter (Model 340 or equivalent) equipped with a combined glass pH electrode and reference electrode. Representative samples of the food product were thoroughly homogenized and a water extract was prepared by mixing 10 g of crushed sample with 100 mL of distilled water (1:10 ratio). The mixture was stirred to ensure complete dispersion and then allowed to infuse for 30 min at a controlled temperature of 20 °C. During measurement, the pH meter was calibrated using standard buffer solutions (pH 4.0, 7.0, and 10.0) according to the manufacturer's instructions. The electrodes were immersed in the extract and the pH value was recorded once the reading stabilized. Electrodes were rinsed with distilled water between each measurement to avoid cross-contamination [23].

2.6. Determination of Water Activity

Water activity (aw) in samples was determined using an Aqualab 4TE water activity meter (Addium Inc., Pullman, Washington, DC, USA). Meat samples were homogenized by grinding at least three times through a food processor (<3 mm plate opening) to ensure uniform moisture distribution. Approximately 7–10 g of the homogenized sample was placed into disposable sample cups, with care taken to avoid residue on the rim, before being sealed in the instrument's chamber. Measurements were recorded after the system reached equilibrium, typically within 5 min, and internal temperature control of the Aqualab 4TE maintained a consistent temperature (e.g., 25 °C) during analysis.

2.7. Determination of Water-Binding Capacity

Water-binding capacity was performed according to Okuskhanova et al. (2017) [24].

2.8. Determination of Water-Holding Capacity

Water-holding capacity (WHC) of the minced meat was determined using a gyrometer method. A 4–6 g sample of minced meat was accurately weighed, evenly spread on the inner surface of the gyrometer, and tightly sealed. The sealed gyrometer was placed in a water bath, narrow side down, and heated to boiling for 15 min. After heating, the mass of released moisture was determined by reading the gyrometer scale, and the water release capacity (WRC, %) was calculated based on the number of scale divisions and sample mass. The total moisture content (W, %) was determined separately using the oven drying method. WHC was then calculated as the difference between total moisture content and WRC, and expressed as a percentage [25].

The water-holding capacity of the meat (*WHC*, %) was calculated according to Formula (1):

$$WHC = W - WRC, \quad (1)$$

The water release capacity (*WRC*, %) was calculated according to Formula (2):

$$WRC = a\ n\ m^{-1}\ 100, \quad (2)$$

where W is the total mass fraction of moisture in the sample, %;

a is the gyrometer graduation rate, $a = 0.01$ cm^3;

n is the number of graduations;

m is the mass of the sample, g.

2.9. Determination of Fat-Holding Capacity

For the determination of fat-holding capacity (FHC), the water-binding capacity (WBC) is first calculated, followed by weighing the meat remaining in the fat meter with an accuracy of ±0.0001 g. The meat is then placed in a weighing bottle and dried to constant mass at 150 °C for 1.5 h. After drying, a sample weighing (2.0000 ± 0.0002) g is transferred to a porcelain mortar, where 2.5 g (1.6 cm^3) of fine calcined sand and 6 g (4.3 cm^3) of α-monobromonaphthalene are added. The contents are thoroughly ground for 4 min and filtered through a folded paper filter [25].

Three to four drops of the resulting filtrate are evenly applied with a glass rod to the lower prism of a refractometer. The prisms are closed and secured with a screw. A beam of light is directed onto the prism using a mirror, and the telescope is adjusted so that the intersection of the crosshairs (aliada) is clearly visible. The boundary between the illuminated and dark sections is aligned with the intersection point of the crosshairs, and the refractive index is recorded. The refractive index of pure monobromonaphthalene is determined in parallel.

Measurements are repeated several times, and the average values are used in calculations. The fat-holding capacity of meat (FHC, %) is calculated using the following Formula (3):

$$FHC = (g_1/g_2) \times 100 \quad (3)$$

where g_1 is the mass fraction of fat in the sample after heat treatment (%);

g_2 is the same value before heat treatment (%).

The fat content in the sample (g, %) is calculated according to the Formula (4):

$$g = (10^4 \times \alpha \times (n_1 - n_2) \times m_1)/m_2 \quad (4)$$

where α is a coefficient characterizing the fat content in the solvent that changes the refractive index by 0.0001%;

n_1 is the refractive index of the pure solvent;

n_2 is the refractive index of the test solution;

m_1 is the mass of 4.3 cm^3 α-monobromonaphthalene (g);

m_2 is the mass of the sample (g).

The coefficient α is established experimentally by comparing the results of fat content determination by the Soxhlet method and the refractometric method using the following Formulas (5) and (6):

$$\alpha = c_1/(10^4 \times \Delta n) \quad (5)$$

$$c_1 = (c \times 100)/m_0 \quad (6)$$

where c_1 is the mass fraction of fat in the filtrate (%);

Δn is the difference between the refractive indices of the pure solvent and the filtrate;

c is the fat content in the sample determined by the Soxhlet method (g);

m_0 is the mass of the solvent sample (g).

2.10. Determination of Cooking Loss

The cooking loss of forcemeat was determined by measuring the weight of sample before and after cooking at temperature 75 °C for 40 min. The loss was calculated as per Formula (7).

$$CL = \frac{m_1 - m_2}{m_1} \quad (7)$$

where m_1—mass of meat product sample before cooking, g;

m_2—mass of meat product sample after cooking, g.

2.11. Microstructure Analysis

Microstructural analysis was carried out using a low vacuum scanning electron microscope (JSM-6390LV JEOL, Tokyo, Japan). Meat samples were cut to fit the sample holders, mounted on SEM stubs, and analyzed at 15 kV. Images were captured at ×200 magnification to observe structural features for comparison between control and experimental groups.

2.12. Determination of Vitamins

The vitamin composition of the samples was determined by high-performance liquid chromatography (HPLC) using a Dionex UltiMate™ 3000 system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a UV-Vis photodiode array detector. Samples were homogenized and subjected to vitamin-specific extraction: water-soluble vitamins were extracted with potassium phosphate buffer (Sigma-Aldrich, St. Louis, MO, USA), while fat-soluble vitamins were extracted with organic solvents (HPLC grade, Merck, Darmstadt, Germany). Extracts were filtered through 0.22–0.45 μm syringe filters (Millipore, Billerica, MA, USA) prior to injection. Chromatographic separation was performed on a Supelco SUPELCOSIL LC-PAH column (5 μm, 4.6 × 150 mm; Sigma-Aldrich, St. Louis, MO,

USA). The mobile phase consisted of methanol-water (92:8, v/v) for fat-soluble vitamins and potassium phosphate buffer for water-soluble vitamins, using an isocratic program. Calibration was carried out with certified reference standards and CRM (Sigma-Aldrich, USA), and quantification employed the external standard method.

In this study, thiamine (B1), riboflavin (B2), niacin (B3), folate (B9), vitamin A, and vitamin E were determined. The method demonstrated detection limits in the range of 0.5–1.0 µg/kg, with linear concentration ranges of 0.5 µg/kg to 100 µg/kg depending on the vitamin. Measurement uncertainty ranged from 5 to 8%, ensuring reliable quantification. Results were expressed as mg/100 g or µg/g of product, corrected for dilution and extraction factors [26].

2.13. Determination of Mineral Elements

The mineral content of the samples was determined using inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent Technologies, Santa Clara, CA, USA) after microwave-assisted ashing, following the AOAC 984.27-1986 method [27]. Precisely 5 g of each homogenized sample was weighed on an analytical balance and placed into clean, acid-washed crucibles suitable for microwave ashing. Samples were gradually heated in a muffle furnace (SNOL, UAB "Umega", Lithuania) to 600 °C over 12 h and held at this temperature until complete ashing was achieved. After cooling, the resulting ash was transferred to a beaker or volumetric flask and digested with 10 mL of hydrochloric acid (1:1 v/v with distilled water). The mixture was thoroughly mixed, allowed to stand, and then filtered through qualitative filter paper into a clean volumetric flask. The filter and original vessel were rinsed with additional acid solution, and the filtrate was diluted to the required final volume.

The ICP-OES instrument was calibrated using certified multi-element standard solutions (ICP Multi-element Standard IV, Merck, Germany) containing known concentrations of minerals such as Ca, K, Mg, Na, Fe, Zn, Cu, and Mn. Calibration standards were prepared at concentration ranges of 0.01–10 mg/L for trace elements (Fe, Zn, Cu, Mn) and 1–100 mg/L for macroelements (Ca, K, Mg, Na), ensuring coverage of the anticipated sample mineral concentrations. Validation of the analytical method was conducted, demonstrating linearity with correlation coefficients (R^2) greater than 0.999 for all calibration curves. Limits of detection (LOD) were determined, ranging from 0.005 mg/L for trace elements to 0.1 mg/L for macroelements. Blank measurements were also performed to ensure no contamination occurred during analysis. Prepared sample solutions were then aspirated into the ICP-OES, and the emission intensities for each mineral element were recorded. Mineral concentrations were calculated by comparing emission intensities with the established calibration curves, and final results were expressed as mg/100 g or µg/g, accounting for all dilution factors and the initial sample weight.

2.14. Determination of Total Viable Count

Total viable count (TVC) was determined by aseptically homogenizing 10 g of each sample in 90 mL sterile saline, followed by preparation of serial tenfold dilutions. Aliquots (0.2 mL) of the appropriate dilutions were inoculated onto Petritest™ substrates and incubated at 36 ± 1 °C for 18 h. After incubation, colonies were counted and TVC was calculated as colony-forming units (CFU) per gram of sample, using the relevant dilution factors. All analyses were conducted in triplicate for accuracy [28].

2.15. Determination of the Acid Number

The acid number of meat products was determined titrimetrically according to GOST R 55480 [29]. A homogenized meat product sample (5–10 g) was accurately weighed into a conical flask and mixed thoroughly with 50 mL of a neutralized ethanol-diethyl ether

solvent (1:1 v/v) for 5 min. The mixture was then heated in a water bath at 50–60 °C for 10 min to extract free fatty acids. After cooling to room temperature, 2–3 drops of phenolphthalein indicator were added, and the solution was titrated with 0.1 M sodium hydroxide (NaOH) until a stable pink color persisted for at least 10–15 s. The consumed volume of NaOH was recorded and used for calculation.

The acid value (mg KOH/g) was calculated using Formula (8):

$$X = \frac{V \cdot K \cdot 5.61}{m} \quad (8)$$

V—volume of 0.1 mol/dm^3 potassium hydroxide solution consumed for titration, cm^3;

K—correction factor to the nominal concentration of solutions;

m—mass of fat in the analyzed sample, g;

5.61—mass of potassium hydroxide corresponding to 1 cm^3 0.1 mol/dm^3 of potassium hydroxide solution, mg.

2.16. Determination of the Thiobarbituric Acid Reactive Substances

Determination of the thiobarbituric acid reactive substances (TBARS) in meat products was performed using the photometric method according to GOST R 55810 [30]. This method relies on the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA), a product formed by oxidation of unsaturated fatty acids in meat, producing a colored complex measurable at an absorbance of (535 ± 10) nm. The measurement range for TBARS is from 0.039 to 2.000 mg MDA per kg of product. The procedure includes sample homogenization, extraction of MDA by distillation after hydrochloric acid treatment, reaction of the distillate with TBA solution in a boiling water bath for 35 min, cooling, and subsequent photometric analysis. The TBARS value (X, mg MDA/kg product) is calculated using Formula (9):

$$X = A \times 7.8 \quad (9)$$

where A is the absorbance of the solution,

7.8 is a constant coefficient representing the proportional relationship between absorbance and MDA concentration in the solution.

2.17. Determination of Organoleptic Properties of Lamb Meat Dumplings

The organoleptic evaluation of lamb meat dumplings was conducted to assess their sensory attributes, including appearance, color, aroma, taste, consistency, and juiciness. A sensory panel comprising 11 trained evaluators (six female, five male, aged 23–48 years) from the Laboratory of Food Analysis at Shakarim University performed the analysis. Inclusion criteria required that participants were healthy adults without known food allergies, non-smokers, and regular consumers of meat products. Each evaluator received scoring sheets and drinking water for palate cleansing between samples. Each dumpling sample was evaluated three times. Before evaluation, dumplings were cooked uniformly, ensuring consistency.

Evaluation involved four sequential stages: First, visual inspection assessed the dumplings' overall appearance, surface condition, and color. Second, olfactory assessment determined the aroma directly from the product surface. Third, tactile assessment was carried out by gently pressing dumplings with fingers and further examining texture during chewing. Lastly, gustatory evaluation examined the flavor profile and juiciness during tasting. Evaluators scored each attribute using a 5-point scale (1—very poor, 2—poor, 3—satisfactory, 4—good, 5—excellent). Samples were presented anonymously with randomized coding to prevent bias. Collected data were subsequently analyzed statistically to determine sensory quality.

The study protocol and sensory testing procedures were reviewed and approved by the Local Ethical Committee of Shakarim University (protocol #359, 18 March 2025) in accordance with the Declaration of Helsinki guidelines for research involving human participants.

2.18. Statistical Analysis

The experiment was conducted using a completely randomized design (CRD) with four treatments (0%, 10%, 20%, and 30% spinach inclusion) and measurements taken over five days of refrigerated storage. For each treatment and storage time point, three independent (biological) samples were prepared, and each biological replicate was analyzed in triplicate (technical replicates), resulting in nine measurements (3×3) per parameter. Data are expressed as mean ± standard deviation (SD) of these nine values. Statistical analysis was performed using one-way analysis of variance (ANOVA) to determine significant differences among treatments, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p \leq 0.05$. Analyses were conducted using the Data Analysis add-in for Microsoft Excel and Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Study the Chemical Composition of Edilbaev Lamb

Proximate analysis of the trimmed loin and pelvic muscles gave the following composition: moisture 67.8 ± 0.4%, protein 15.6 ± 0.2%, lipid 16.3 ± 0.3%, and ash 0.80 ± 0.01%. These values are within the typical range reported for fat-tailed breeds under comparable feeding regimes [31]. Macro- and micro-element profiling showed sodium 80 mg 100 g^{-1}, potassium 270 mg 100 g^{-1}, calcium 9 mg 100 g^{-1}, magnesium 20 mg 100 g^{-1}, phosphorus 168 mg 100 g^{-1}, and iodine 2 mg 100 g^{-1}. Vitamin analysis revealed thiamine (B_1) 0.08 mg 100 g^{-1}, riboflavin (B_2) 0.14 mg 100 g^{-1}, and niacin (B_3) 3.8 mg 100 g^{-1} (Table 2).

Table 2. Chemical, mineral and vitamin composition of lamb meat (n = 10), mg/100 g (mean ± SD).

Indicator	Content
Moisture	67.8 ± 0.62
Protein	15.6 ± 0.17
Fat	16.3 ± 0.23
Ash	0.80 ± 0.02
Sodium (Na)	81.85 ± 1.07
Potassium (K)	273.77 ± 4.61
Calcium (Ca)	8.76 ± 0.12
Magnesium (Mg)	19.67 ± 0.32
Phosphorus (P)	169.86 ± 2.26
Iodine (I)	2.10 ± 0.03
Thiamine	0.08 ± 0.00
Riboflavin	0.14 ± 0.01
Niacin	3.8 ± 0.05

Technologically, lamb meat provides a favourable pH (5.6–5.7 post-rigor) and moderate intramuscular fat, giving consistent emulsion stability in comminuted products. Their moderate myoglobin content yields an appealing light-red hue that remains stable after mincing, thereby reducing reliance on curing agents [32]. The mineral profile supports ionic strength and buffering capacity, factors that influence water-binding and gelation during

cooking. Vitamins B_1, B_2 and B_3, although present at modest levels, act as precursors of metabolic co-enzymes and participate in lipid oxidation control, indirectly improving shelf-life [33,34]. Collectively, these compositional and physicochemical attributes confirm that Edilbaev lamb offers an excellent matrix for developing value-added meat products. Its balanced nutrient profile and favourable processing behaviour establish a robust baseline against which the functional effects of spinach inclusion can be evaluated.

3.2. Studying the Chemical Composition of Meat Dumplings

Replacing part of the lamb in the minced meat with spinach resulted in statistically significant changes in its chemical composition. Spinach contains about 91% water, low amounts of protein (2–3%), very little fat (≤0.4%), and approximately 2% fibre and minerals. As the proportion of spinach increased from 10% to 30%, the product's composition shifted accordingly.

Moisture content increased from 67.14% in the control to 74.36% in the 30% variant. Variant 2 (10% spinach) slightly increased to 68.80%, while Variant 3 (20%) reached 71.58%. The difference in moisture content between the control and Variant 4 (30%) was 7.2%, which is explained by the higher water content of spinach compared to lamb (Table 3).

Table 3. Chemical composition of meat dumplings, % (mean ± SD).

Component	V1—Control	V2—10%	V3—20%	V4—30%	Regression Equation	R^2	*p*-Value
Moisture (%)	67.14 ± 0.74 ^{a}	68.80 ± 0.83 ^{a}	71.58 ± 1.22 ^{b}	74.36 ± 1.08 ^{c}	y = 66.804 + 0.244x	0.988	0.006
Protein (%)	14.54 ± 0.19 ^{d}	13.23 ± 0.19 ^{c}	11.92 ± 0.12 ^{b}	10.61 ± 0.14 ^{a}	y = 14.540 − 0.131x	1.000	<0.001
Fat (%)	17.32 ± 0.24 ^{d}	15.47 ± 0.17 ^{c}	13.59 ± 0.25 ^{b}	11.69 ± 0.10 ^{a}	y = 17.333 − 0.188x	1.000	<0.001
Ash (%)	0.93 ± 0.01 ^{a}	1.03 ± 0.01 ^{b}	1.15 ± 0.01 ^{c}	1.26 ± 0.01 ^{d}	y = 0.926 + 0.011x	0.999	<0.001
Carbohydrate (%)	nd	1.45 ± 0.01 ^{b}	1.78 ± 0.02 ^{c}	2.11 ± 0.03 ^{a}	y = 0.0332x + 1.1176	0.998	<0.001

$^{a–d}$ Different lowercase letters indicate statistically significant differences within the columns ($p < 0.05$). nd—not detected.

Protein content decreased from 14.54% in the control to 10.61% in Variant 4 ($p < 0.05$). In Variants 2 and 3, the protein was 13.23% and 11.92%, respectively. The reduction ($p < 0.05$) is due to replacing meat proteins with those from spinach, which are present in lower concentrations. The decrease reflects the replacement of muscle proteins with leaf proteins of lower concentration and a different amino-acid pattern. Although total protein declined, spinach contributes lysine-poor but leucine-rich plant proteins and chlorophyll–protein complexes, potentially improving the overall antioxidant profile [35].

Fat content dropped sharply, from 17.32% in the control to 11.69% in the 30% variant ($p < 0.05$). Intermediate values were 15.49% (10% spinach) and 13.59% (20% spinach). As lamb fat was reduced, the overall energy value of the product also decreased. Ash content increased from 0.93% in the control to 1.26% in the 30% variant ($p < 0.05$), confirming that spinach enriched the minced meat with mineral micronutrients, notably potassium, magnesium, iron, and calcium. Compared to the control, the ash content in Variant 3 was 1.13%, and in Variant 2 it was 1.03%. These minerals also raise ionic strength, which can affect myofibrillar protein solubility and water-binding [36].

Carbohydrate content reached 2.11% in the 30% spinach variant due to the dietary fibre in spinach. This increase was gradual: 1.45% in Variant 2 and 1.78% in Variant 3 ($p < 0.05$). Fibre may also contribute to the higher water retention observed in spinach-containing variants. The primary source of these components is the dietary fiber found in spinach, which includes cellulose, hemicellulose, and pectic substances. Besides contributing to the

slight ash increment through associated minerals, this insoluble fibre may act as a natural texturiser by retaining water within its matrix, explaining why moisture increased more than predicted from simple mass balance alone [37].

Replacing lamb with spinach reduced the fat and protein content of the product while increasing moisture, minerals, and fibre. The 20% and 30% variants showed the most pronounced changes, suggesting that spinach addition modifies the nutritional profile and functional properties of minced meat. These findings confirm the potential for using spinach as a partial meat replacer in semi-finished products and justify further study on texture, shelf life, and consumer acceptance.

3.3. Determination of pH and Water Activity

Integration of the pH and a_w data confirms that spinach inclusion modulates the chemical micro-environment of the raw matrix only marginally but in a direction consistent with its intrinsic composition. Fresh lamb muscle exhibits a post-rigor pH close to 5.8–6.0; the control mince (6.37) was slightly higher, reflecting low glycogen reserves typical of mature lambs and the alkaline influence of onion sap (Figure 1). Introducing 10% spinach increased the pH to 6.61. This effect is due to spinach's natural buffering compounds, such as potassium, magnesium, calcium, and bicarbonate salts, which help neutralize the acids in meat. When the substitution level increased to 20% and 30%, the pH dropped to 6.51 and 6.46. This decrease is explained by the higher amount of spinach organic acids, such as oxalic, citric, and malic acids, which become more influential as spinach exceeds 20% of the mix. Although none of the shifts reached statistical significance, the trend suggests a plateau of pH adjustment between 20% and 30% substitution, where leaf acids and mineral bases counterbalance each other. From a technological viewpoint, values between 6.4 and 6.6 remain within the optimal zone for myofibrillar protein functionality; therefore water-binding and textural integrity should not be compromised, while the slight acidification relative to the 10% variant may marginally retard microbial outgrowth [38].

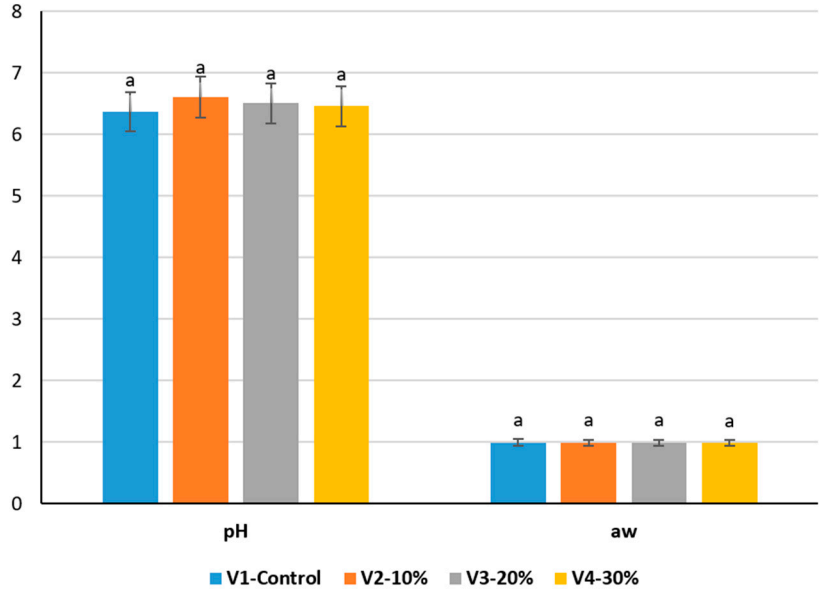


Figure 1. Change in pH and aw in different variants of meat dumplings (Different letters above the bars indicate significant differences between samples, $p < 0.05$).

Water activity declined monotonically from 0.9989 to 0.9905 as spinach replaced meat (Figure 1). The difference is analytically small, yet mechanistically informative. Spinach fibre bound a portion of the added moisture into cell-wall capillaries, converting free water into matrix-bound water without altering total moisture content. Although an aw of

0.9905 remains well above the minimum required for growth of most spoilage bacteria, even modest reductions in available water can slow microbial proliferation rates and nutrient diffusion. Moreover, lower available water may temper lipid oxidation by restricting diffusive oxygen flow within the matrix, complementing the antioxidant polyphenols already supplied by the leaf [39]. When combined with spinach-derived antimicrobial factors such as nitrates, nitrites, and polyphenols, this modest reduction in aw may further support slower microbial growth during refrigerated storage, helping extend shelf life.

3.4. Studying the Functional Properties of Meat Dumplings

The functional properties of the minced meat changed along with its composition, but the interaction between meat proteins and spinach fibre was not simply linear. Water-binding capacity, which reflects the ability of the crude matrix to immobilize added or endogenous water without mechanical stress [40], fell from 51.3% in the control to 45.7% at 10% substitution, confirming that initial dilution of salt-soluble lamb proteins removed the principal hydration sites responsible for electrostatic binding [41]. When the spinach level rose to 20% and 30%, water binding climbed sharply to 65.6% and 85.5%, respectively (Figure 2). At these higher inclusions, the hydrophilic pectins, hemicelluloses and cellulose microfibrils provided abundant capillary and hydrogen-bonding sites, more than compensating for lost protein [42].

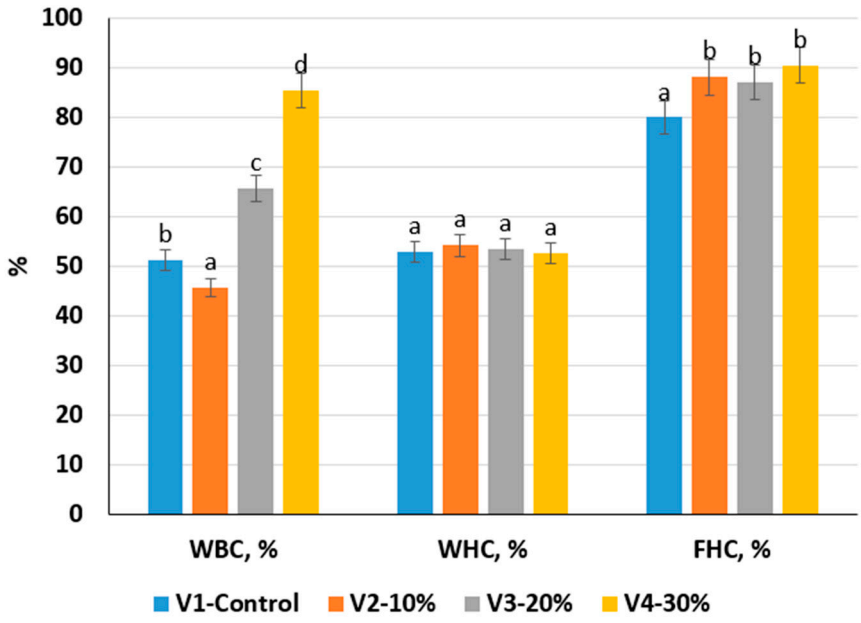


Figure 2. Change in WBC, WHC, FHC in different variants of meat dumplings (Different letters above the bars indicate significant differences between samples, $p < 0.05$).

Water-holding capacity remained statistically unchanged ($p > 0.05$) across all variants. This stability is consistent with the modest pH differences: values between 6.4 and 6.6 lie far enough from the myofibrillar isoelectric point to preserve protein–water interactions, and the extra fibre appears to bind water tightly but not release it under the relatively mild test force [43]. Consequently, cooking loss is unlikely to increase, preserving yield even as total moisture rises.

Fat-holding capacity improved progressively from 80% to 90.5%. This can be explained that spinach fibres possess amphiphilic domains capable of physically entrapping liquid fat within their porous network [44]. Also, chlorophyll and membrane phospholipids originating from leaf chloroplasts act as natural emulsifiers, stabilising fat globules against coalescence [45]. This translates into reduced fat exudation during pan-frying or oven-

baking, mitigating surface greasing and improving sensory perception despite the overall reduction in lipid content.

Taken together, the techno-functional data strengthen the argument that 20–30% spinach substitution delivers a nutritionally lighter product without compromising, and in some respects enhancing, processing performance. Elevated water- and fat-binding predict better juiciness and shape retention in meat dumplings.

3.5. Studying the Cooking Loss of Meat Dumplings

The results of the cooking loss analysis demonstrate a statistically significant reduction in mass loss during thermal treatment with increasing levels of spinach inclusion. In the control sample (Variant 1), cooking loss was 24.61%. In comparison, the incorporation of spinach at 10%, 20%, and 30% reduced cooking loss to 21.47%, 20.56%, and 19.64%, respectively ($p < 0.05$ for all compared to the control). These reductions represent absolute decreases of 3.14, 4.05, and 4.97 percentage points, corresponding to relative improvements of 12.8%, 16.5%, and 20.2% compared with the control. No statistically significant difference was observed between the 20% and 30% variants, indicating that further increasing the spinach content above 20% does not lead to additional reduction in cooking loss (Figure 3).

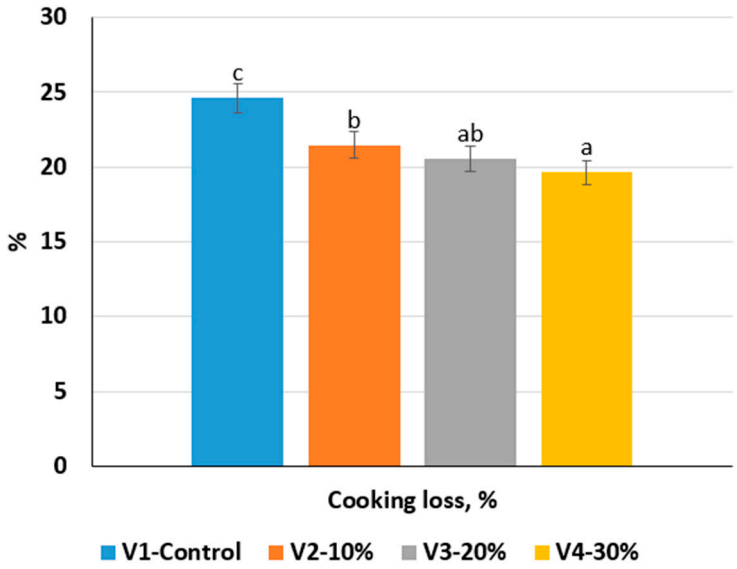


Figure 3. Change in cooking loss in different variants of meat dumplings (Different letters above the bars indicate significant differences between samples, $p < 0.05$).

The observed decrease in cooking loss with spinach addition is primarily attributed to the functional role of spinach-derived dietary fibre and plant cellular structures in retaining moisture and lipids during heating. Spinach contains high levels of insoluble polysaccharides, such as cellulose, hemicellulose, and soluble pectic substances, which contribute to the formation of a hydrated matrix capable of physically entrapping water and fat within the minced meat system. During thermal treatment, this fibre-rich matrix reduces the mobility of water molecules and limits their evaporation or exudation, thereby improving water retention [46]. In addition to water-binding, spinach components influence the emulsion stability of the system. The presence of plant fibers and residual membrane phospholipids from spinach cells can enhance fat retention by creating a denser, more stable protein–fiber–lipid network [47]. This structural reinforcement helps reduce fat separation during heating, which contributes to the overall lower cooking loss observed in experimental variants.

These findings directly support the research objective of assessing the technological benefits of spinach incorporation in minced meat systems. The reduction in cooking loss with spinach addition suggests improved yield and product quality. Moreover, the effect appears to be dose-dependent up to 20%, beyond which no additional significant benefit is observed. This aligns with the previously discussed microstructural and functional results, reinforcing the conclusion that 20% spinach addition represents a technologically and nutritionally favourable level that enhances water and fat retention without compromising structural integrity.

3.6. Studying the Microstructure of the Meat Dumplings

Figure 4 shows the microstructure of the control minced meat sample (Variant 1) at ×200 magnification. The image reveals a compact and relatively dense structure, dominated by overlapping muscle fiber bundles that are partially disrupted by grinding. These bundles are oriented in different directions and are embedded in a fine protein matrix. The fibers appear flattened with indistinct striations, which is typical after mincing and sample preparation for SEM. There are minimal spaces between fibers, indicating tight packing and low porosity. The surface is generally smooth and continuous, with no clear signs of moisture loss or phase separation. No fragments of plant material are present, confirming the absence of spinach in this variant. This homogeneous and cohesive protein matrix with intact connective tissue supports the moderate water-binding and water-holding capacities measured in the control sample and serves as a baseline for comparing the structural changes introduced by spinach addition.

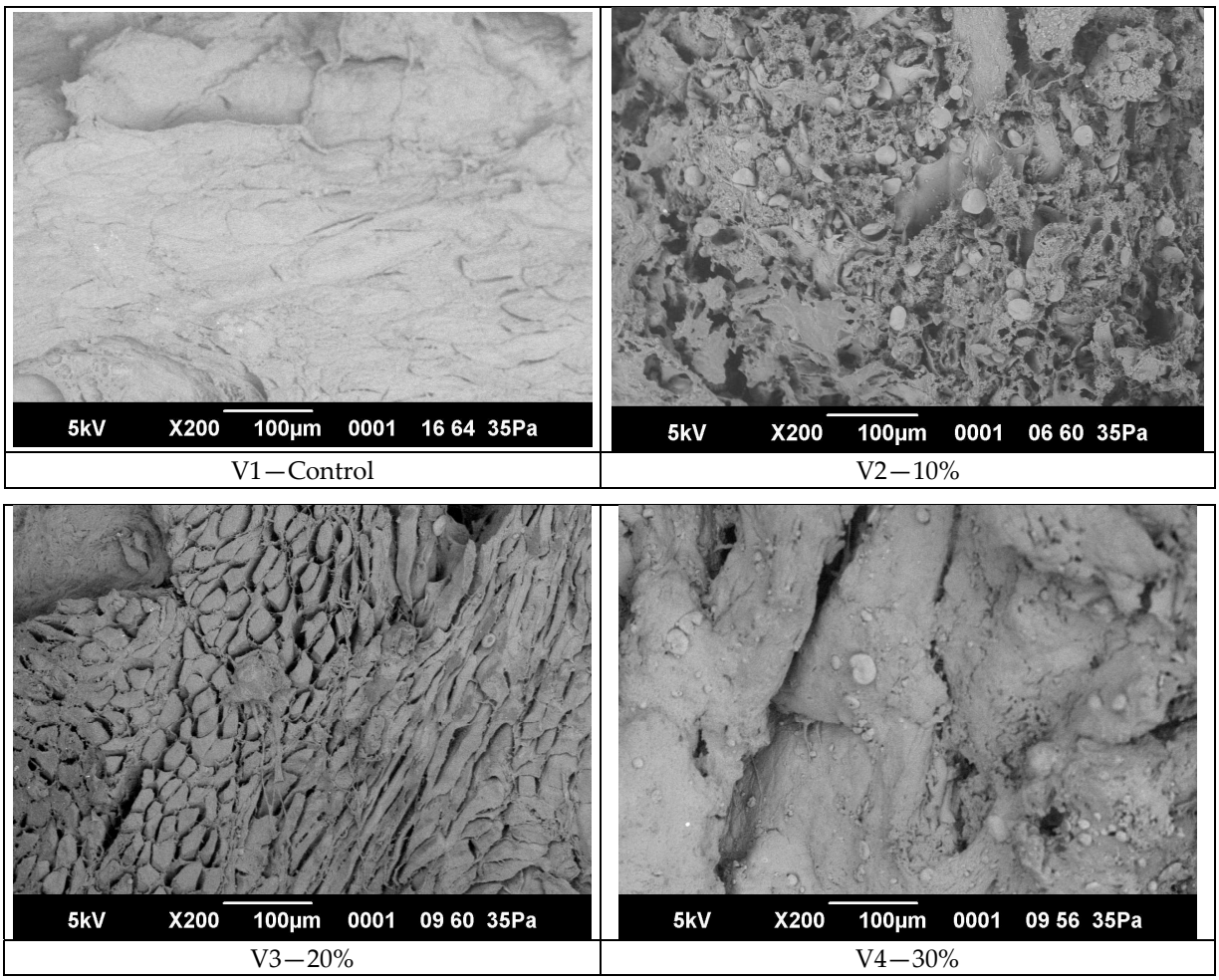


Figure 4. Microstructure of different variants of meat dumplings.

Compared to the control sample, the microstructure of Variant 2 demonstrates clear differences in organization, porosity, and phase distribution, indicating the influence of spinach inclusion on the physical architecture of the product. The overall structure is less compact and more heterogeneous than the control. Numerous irregular voids and pores are visible across the field, indicating partial phase separation and an increase in the interstitial space within the protein matrix. This more open structure is consistent with the higher moisture content and increased carbohydrate (fibre) content. Spherical and oval particulates, likely spinach-derived plant cell fragments or leaf parenchyma, are distributed throughout the matrix. These components differ in texture and electron density from the surrounding meat fibres, suggesting the integration of plant material that retains partial structural identity post-grinding. The surface texture is rougher and more granulated, and the continuity of fibrous structures is lower. The addition of 10% spinach introduces noticeable microstructural changes to the minced meat matrix: reduced compactness, increased porosity, and the presence of plant cell debris. These changes are consistent with the measured improvements in functional properties and support the role of spinach as a textural and compositional modifier in semi-finished meat products.

The SEM image of Variant 3 (minced meat with 20% spinach addition) reveals a distinct and more structured microarchitecture compared to both the control and 10% spinach variant. The structural transformation suggests that at this level of plant inclusion, a more organized composite matrix forms between meat proteins and spinach-derived material. The image shows clear zones of regular cellular alignment, likely corresponding to intact or semi-intact plant tissue from spinach leaves, particularly the parenchyma with their characteristic polygonal or rounded outlines. These plant structures are more numerous and densely packed than in Variant 2. Adjacent to these regions are elongated fibrous meat fragments, which appear more aligned than in previous variants, possibly due to better integration with plant fibre. Notably, the void fraction is lower than in Variant 2, with fewer and smaller pores, suggesting that the spinach fibre network may now play a stabilising role in structuring the matrix. This correlates with the highest water-binding capacity (WBC) observed in this variant, as plant cell walls and fibre retain moisture through capillary action and physical entrapment. The fat-holding capacity also remains high, likely due to the dense network inhibiting fat migration during processing. The interface between plant and meat structures appears more cohesive than in Variant 2, indicating improved compatibility and possibly synergistic gel formation.

The SEM image of Variant 4 (minced meat with 30% spinach addition) shows a microstructure that differs markedly from the control and lower-level spinach variants. The matrix appears highly disrupted and disordered, with fewer distinct fibrous structures and an increase in amorphous regions, indicating a breakdown of muscle fibre continuity. The surface is irregular and fragmented, with numerous spherical or granular inclusions, which are likely to be spinach-derived cellular remnants, such as ruptured parenchyma cells or aggregated cytoplasmic material. These inclusions are more frequent and randomly distributed than in Variants 2 or 3, suggesting oversaturation of plant material in the protein matrix. Compared to Variant 3, the connectivity between muscle components appears weaker, and the overall matrix is less cohesive. This structure corresponds to the lower sensory scores for appearance, consistency, and flavour observed in this variant. The absence of a clear network may also explain the decrease in water-holding capacity and the less favourable mouthfeel due to reduced structural integrity. The observed microvoids and irregular surface features indicate poor integration of the components. While spinach fibres can improve texture at moderate levels, at 30% inclusion, the high content likely exceeded the capacity of the meat matrix to incorporate the plant matter efficiently. This may lead to phase separation, reduced gel formation, and a more fragile, less elastic texture.

3.7. Studying the Vitamin Composition of Meat Dumplings

The vitamin profile of the spinach–lamb composites shows a coherent shift toward leaf-derived micronutrients while modestly diluting meat-specific niacin. Thiamine and riboflavin rose incrementally with spinach inclusion, reaching 83 µg B1 and 158 µg B2 per 100 g at 30% substitution. Although the absolute gains are small, they counteract the slight thermal destruction expected during cooking and underline spinach's capacity to enrich B-vitamins that are otherwise sub-optimal in lamb. Folate content increased from 24.6 to 28.5 µg per 100 g with the addition of spinach (Table 4). This 15% increase is important because red meat is usually low in folate, even though it is rich in protein. Vitamin B3 (niacin), conversely, declined by about 11% as meat was displaced, confirming that lamb remains the primary contributor of this vitamin in the blend. Despite this reduction, the 30% variant still supplies more than 30% of the adult male daily niacin requirement per 100 g portion, so biochemical sufficiency is preserved.

Table 4. Vitamin composition of meat dumplings, µg/100 g (mean ± SD).

Sample	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B9	Vitamin A	Vitamin E
V1-Control	77 ± 1 ^{a}	128 ± 2 ^{a}	3440 ± 59 ^{a}	24.57 ± 0.40 ^{a}	0	560 ± 11 ^{a}
V2-10%	79 ± 1 ^{a}	139 ± 2 ^{b}	3320 ± 50 ^{a}	26.10 ± 0.48 ^{b}	75 ± 1 ^{a}	750 ± 15 ^{b}
V3-20%	81 ± 1 ^{ab}	144 ± 3 ^{c}	3215 ± 47 ^{b}	27.31 ± 0.46 ^{c}	330 ± 4 ^{b}	1170 ± 19 ^{c}
V4-30%	83 ± 1 ^{b}	158 ± 3 ^{d}	3050 ± 60 ^{c}	28.48 ± 0.42 ^{d}	410 ± 7 ^{c}	1180 ± 17 ^{c}

$^{a–d}$ Different lowercase letters indicate statistically significant differences within the columns ($p < 0.05$).

The most notable changes concern the fat-soluble antioxidants absent in the control. Spinach contributed provitamin A carotenoids, elevating retinol activity equivalents from non-detectable to 410 µg β-carotene per 100 g at the highest substitution. Tocopherol concentration doubled, rising from 560 to 1180 µg per 100 g. This increase in vitamin E is important because it helps protect fats in the product from oxidation [48]. As a result, the higher fat-holding capacity in Variants 3 and 4 may help extend shelf life, even though the overall fat content is lower.

The vitamin results show that adding 20% spinach offers the best balance: niacin levels stay high, B vitamins and folate increase, and new antioxidants like carotenoids and vitamin E are introduced without reducing sensory quality. These changes may also work together to improve health, as more folate supports blood formation alongside increased iron, and higher vitamin E can help protect against oxidation caused by the added iron [49].

3.8. Study the Mineral Composition of Meat Dumplings

Progressive enrichment of the mince with spinach produced a clear, dose-dependent rise in calcium, magnesium and iron, indicating that the leafy matrix served as an effective mineral fortifier rather than merely diluting meat nutrients. Calcium increased from 5.86 mg/100 g in the control to 21.07 mg/100 g in the 30% variant. Magnesium rose from 14.03 mg/100 g to 27.48 mg/100 g, and iron increased from 0.94 mg/100 g to 2.68 mg/100 g over the same range (Figure 5).

The mineral pattern also helps explain several physicochemical observations reported earlier. Calcium contributes to the slight pH buffering seen at moderate inclusion and partly offsets the acidifying influence of spinach organic acids [50]. Iron in spinach, mainly in non-heme form, is the main factor giving a green-brown color to the product interior, which became noticeable and undesirable at 30% substitution. While iron can promote lipid oxidation, this risk is likely reduced in samples due to the lower fat content and the presence of spinach polyphenols and chlorophylls, which have antioxidant properties [51].

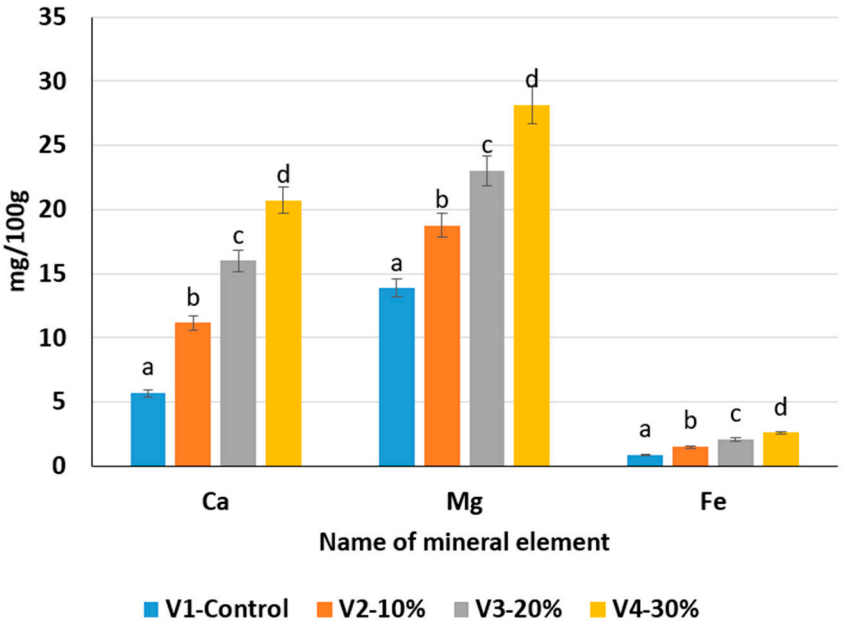


Figure 5. Mineral composition of meat dumpling samples (Different letters above the bars indicate significant differences between samples, $p < 0.05$).

3.9. Studying the Total Viable Count of Meat Dumplings

The viable-count curves demonstrate that substituting lamb with spinach suppressed both the initial microbial load and its subsequent proliferation during chilled storage (+2 °C), and the magnitude of the effect increased monotonically with the spinach fraction. At day 0 the control mince contained 2.5×10^3 CFU g^{-1}, whereas Variant 4 began at 1.2×10^3 CFU g^{-1}, a 52% reduction (Table 5). Over five days, the total viable count in the control sample increased to 2.56×10^6 CFU/g, which is above the maximum limit specified by TR CU 034/2013 [52]. In contrast, all samples with added spinach had lower microbial counts and stayed below this regulatory threshold. Spinach acts as a natural barrier that significantly slows the growth of mesophilic aerobic microorganisms.

Table 5. Total viable count of meat dumplings, Log CFU g^{-1}.

Storage Time at +2 °C, day	V1—Control	V2—10%	V3—20%	V4—30%
0	3.40 ^{Abc}	3.26 ^{Ab}	3.15 ^{Aab}	3.08 ^{Aa}
1	4.00 ^{Bb}	3.86 ^{Bab}	3.75 ^{Bab}	3.68 ^{Ba}
2	4.60 ^{Cb}	4.46 ^{Cab}	4.35 ^{Cab}	4.28 ^{Ca}
3	5.20 ^{Db}	5.06 ^{Dab}	4.95 ^{Dab}	4.89 ^{Da}
4	5.81 ^{Eb}	5.66 ^{Eab}	5.55 ^{Eab}	5.49 ^{Ea}
5	6.41 ^{Fb}	6.26 ^{Fab}	6.16 ^{Fa}	5.90 ^{Fa}

$^{A–F}$ Different uppercase letters indicate statistically significant differences within the columns ($p < 0.05$). $^{a–c}$ Different lowercase letters indicate statistically significant differences within the rows ($p < 0.05$).

The inhibitory effect is due to several components of spinach. Fresh spinach leaves contain 2500–3000 mg/kg nitrate. During storage and exposure to light, both plant and microbial enzymes convert some of this nitrate into nitrite and nitric oxide, which are known to inhibit the growth of many Gram-positive and Gram-negative bacteria [53]. The linear relation between inclusion level and final CFU is consistent with a dose-dependent increase in the in situ concentration of these reactive nitrogen species.

Spinach also alters the physical habitat of the microbial cells. Its insoluble cell-wall polysaccharides immobilise free water, as evidenced by the lower a_w values, reducing solute diffusion and nutrient availability in the micro-pores where bacteria reside [54]. At the same time, the modest rise in calcium and magnesium increases ionic strength, exerting a mild osmotic challenge; although insufficient alone to inhibit growth, this stress acts synergistically with the chemical hurdles described above. Notably, the pH remained within the optimum range for myofibrillar functionality (≈6.4–6.6) and thus did not confound the antimicrobial interpretation: the suppression is attributable to spinach constituents rather than acidification.

From a product-development standpoint, the microbiological advantage reinforces the formulation window previously defined by sensory constraints. A 20% spinach substitution kept the five-day count at 1.43×10^6 CFU g^{-1}, well inside the regulatory limit, while preserving overall palatability. Although 30% provided an extra log unit of safety, it simultaneously introduced color and flavor defects that were unacceptable to typical consumers. It should be noted that only total viable counts were assessed in this study; species-level identification of microbial communities was not performed. Future research will address this gap by characterizing the dominant bacterial groups affected by spinach inclusion, thereby strengthening the safety interpretation of the observed antimicrobial effects. The present findings therefore recommend a 15–20% inclusion level as the optimal compromise, delivering statistically significant shelf-life extension without sensory penalty.

3.10. Changes in TBARS and Acid Number During Storage of Meat Dumplings

The progression of lipid oxidation and hydrolysis in meat dumplings was assessed using thiobarbituric acid reactive substances (TBARS) and acid number measurements during five days of refrigerated storage. Both indicators revealed that spinach addition significantly improved lipid stability compared to the control.

Initially, the control sample exhibited a TBARS level of 0.17 mg MDA/kg and an acid number of 0.80 mg KOH/g. In contrast, spinach-enriched variants (10%, 20%, 30%) demonstrated lower initial TBARS values (0.15, 0.15, and 0.13 mg MDA/kg, respectively) and reduced acid numbers (0.70, 0.70, and 0.60 mg KOH/g, respectively), highlighting an immediate antioxidative and protective effect of spinach (Figures 6 and 7).

Throughout storage, both TBARS and acid numbers progressively increased across all samples, reflecting continuous lipid oxidation and hydrolysis. However, spinach incorporation consistently resulted in lower levels of both parameters compared to the control. By day five, TBARS reached 0.53 mg MDA/kg in the control but significantly lower values of 0.45, 0.40, and 0.35 mg MDA/kg in the 10%, 20%, and 30% spinach samples, respectively ($p < 0.05$). Concurrently, the acid number rose to 2.00 mg KOH/g in the control but was notably lower in spinach-containing variants: 1.70 mg KOH/g (10%), 1.38 mg KOH/g (20%), and 1.19 mg KOH/g (30%) ($p < 0.05$).

The observed protective effects can be attributed to spinach's phytochemical composition, rich in polyphenols, carotenoids, vitamins, and chlorophyll derivatives. These bioactive compounds effectively scavenge free radicals, inhibit lipid peroxidation pathways, and suppress enzymatic lipid hydrolysis [55]. While Variants 2 and 3 showed statistically similar acid numbers, the 30% spinach inclusion consistently offered superior lipid stabilization. From both technological and nutritional perspectives, the lower TBARS and acid numbers achieved with spinach addition indicate enhanced product stability, reduced risk of rancidity, and potentially extended shelf life. Overall, spinach at inclusion levels of 20–30% emerged as an effective natural functional additive, significantly mitigating lipid deterioration in lamb-based dumplings during refrigerated storage.

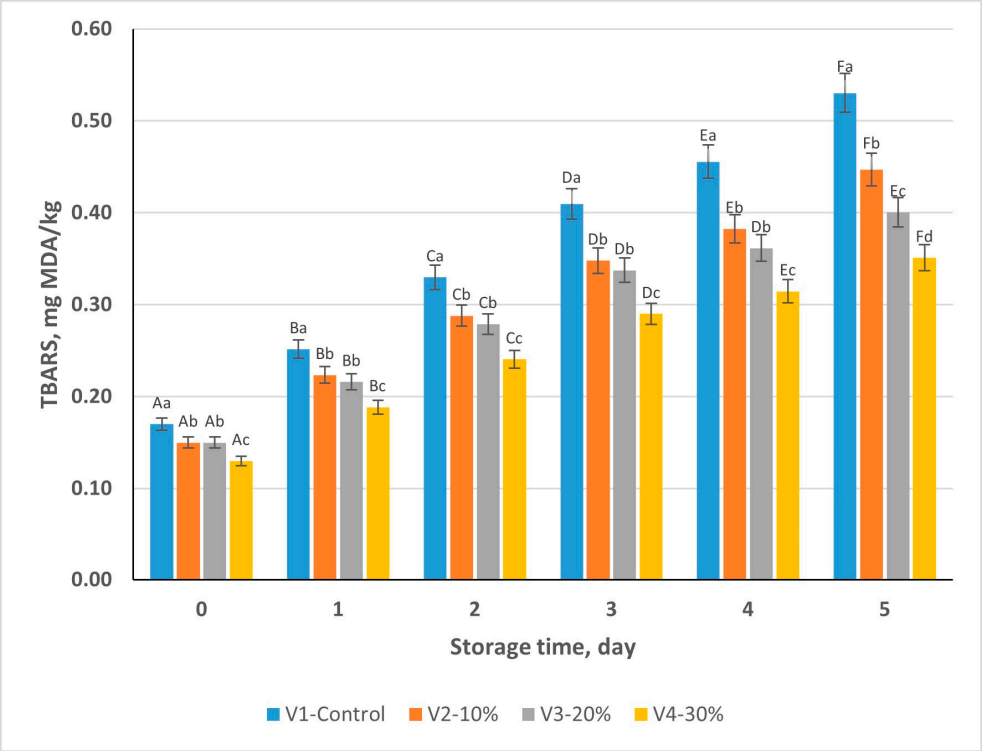


Figure 6. Effect of spinach addition on TBARS values of meat dumpling during storage. Different lowercase letters (a–d) indicate statistically significant differences between variants and within the same time ($p < 0.05$). Different uppercase letters (A–F) indicate a significant difference within the same variant but at different times of storage ($p < 0.05$).

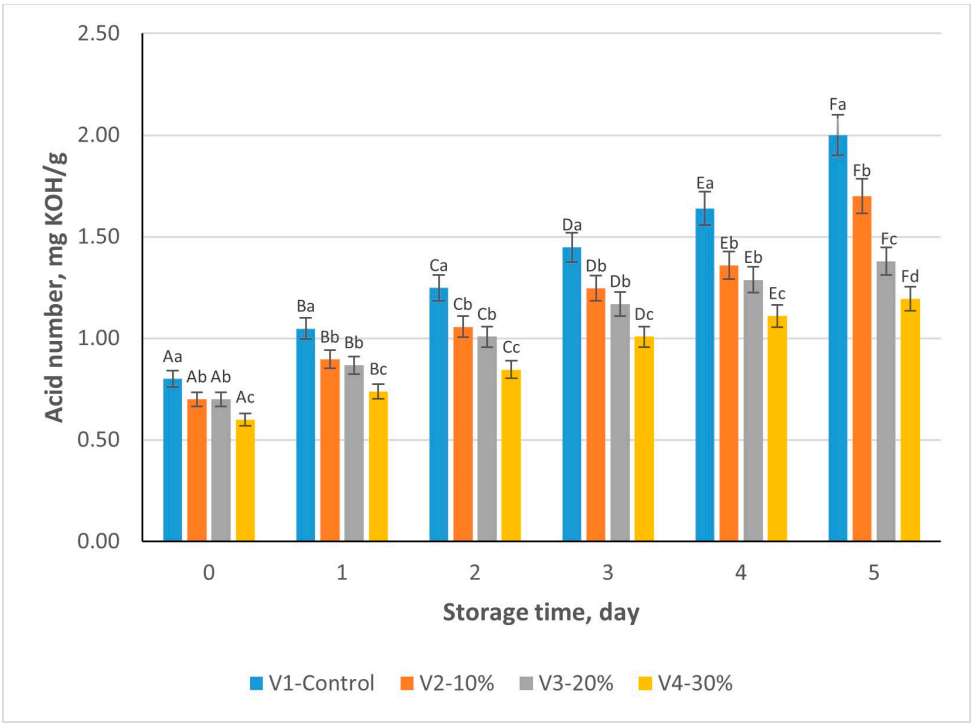


Figure 7. Effect of spinach addition on acid number values of meat dumpling during storage. Different lowercase letters (a–d) indicate statistically significant differences between variants and within the same time of storage ($p < 0.05$). Different uppercase letters (A–F) indicate a significant difference within the same variant but at different times of storage ($p < 0.05$).

3.11. Studying the Sensory Profile of the Meat Dumplings

The sensory data support the earlier compositional and functional results, showing that moderate amounts of spinach do not reduce, and may even slightly improve, overall consumer acceptance. However, adding too much spinach causes noticeable negative changes in product quality. In the control mince the panelists awarded high scores across all descriptors, confirming the baseline palatability of boiled lamb. Replacing 10% of the meat with spinach raised the overall rating from 22.8 to 23.1 despite the statistical equivalence of individual attributes; the marginal gain can be ascribed to a slightly brighter interior surface and marginally firmer bite. At 20% substitution the total score rose again to 23.2, driven chiefly by the highest consistency score 4.7 (Figure 8). This aligns with the dramatic increase in water-binding capacity recorded for Variant 3: spinach cell-wall polysaccharides created a cohesive particulate network that stabilised the structure after cooking, translating into a mouthfeel judged marginally superior to the control.

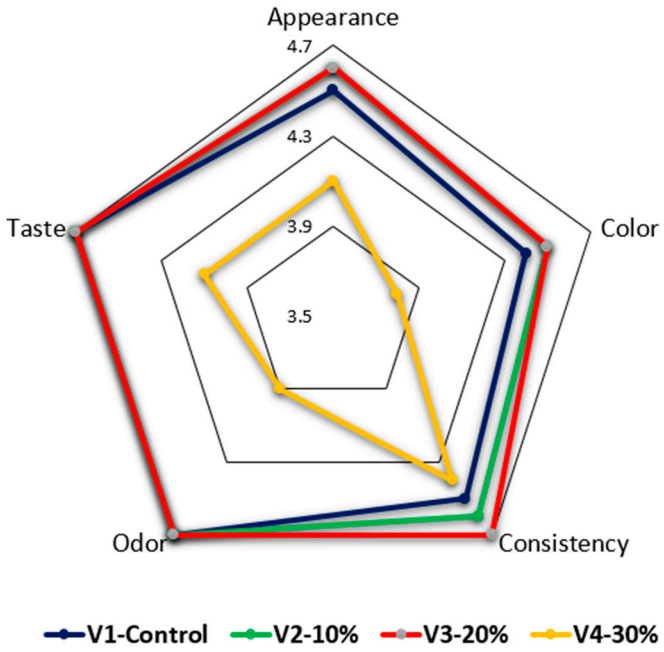


Figure 8. Sensory score of meat dumplings.

The sensory penalty emerged only at 30% substitution. Panel comments revealed a pronounced spinach after-taste, a greenish internal hue and a less appealing aroma; quantitatively, scores for color and odor fell to 3.8 and 3.9, respectively, dragging the composite rating down to 20.3 ($p < 0.05$). The loss of visual appeal is mechanistically linked to the elevated chlorophyll concentration and the concomitant fat reduction that ordinarily imparts a light, pinkish opacity to lamb.

From a product development perspective, the results suggest that using up to approximately 20% spinach in meatballs is appropriate to meet conventional consumer expectations for lamb (Supplementary File). Below this level, spinach fibres enhance juiciness and bite without introducing conspicuous vegetal notes, and the modest colour shift reads as appetising freshness rather than deviance. The organoleptic results show that adding 20% spinach improves nutritive value without reducing consumer acceptance. However, increasing spinach to 30% leads to poorer taste and appearance, making the product less appealing and marketable.

Although sensory evaluation by a trained panel (n = 11) provided reliable and controlled assessments, the small panel size limits the generalizability of the results. Trained judges may not fully reflect consumer preferences, and the five-point scale used offers lim-

ited sensitivity. Therefore, future work should include larger consumer studies to validate these findings under real market conditions.

3.12. Comparative Analysis of Spinach Impact on Quality and Technological Characteristics of Meat Products

The present findings regarding spinach incorporation into lamb meat dumplings align closely with several studies in the literature, confirming the beneficial impact of spinach on meat product quality. Levkovskaya and Muzykina (2022) reported that adding spinach at levels between 10 and 20% significantly improved nutritional and functional properties in meat cutlets, enhancing vitamin and mineral content and water retention while concurrently reducing fat and energy density [56]. Their study also highlighted the sensory drawbacks associated with spinach inclusion above 20%, specifically undesirable changes in color and taste. The study by Gorlov et al. (2019) on rabbit meatballs confirmed that spinach addition improved protein content, amino acid profiles, texture, and moisture retention [57]. Our research supports these conclusions, with spinach addition significantly enriching the nutritional profile and functional attributes of lamb dumplings at moderate levels, though sensory acceptability declined beyond 20% spinach inclusion. Similarly, Khachatryan and Gerashchenko (2021) demonstrated that spinach in minced meat products improved water- and fat-holding capacities, increased moisture content, and reduced cooking loss [58], which parallels our results showing improved functional properties and lower cooking losses in spinach-enriched dumplings. Their optimal spinach concentration of 15% for maintaining sensory quality aligns with our findings, suggesting that moderate spinach inclusion balances enhanced nutritional and technological characteristics without compromising taste or texture. Pathade et al. (2022) similarly demonstrated that moderate spinach addition in chicken meatballs increased cooking yields and improved sensory attributes, with sensory quality declining at higher spinach levels [17].

From a microbiological perspective, studies [14,59] provided strong evidence supporting the antimicrobial and antioxidant properties of spinach extracts in meat products. Fermented spinach extract significantly reduced microbial growth and lipid oxidation in pork loin [14], while spinach extract inclusion effectively suppressed pathogens such as Clostridium perfringens and improved oxidative stability in Spanish chorizo [59]. This study similarly demonstrated enhanced microbiological stability and oxidative stability in spinach-enriched lamb dumplings, highlighting spinach's potential as a natural preservative and antioxidant additive in meat products. It should be noted that the hygienic pre-treatment of raw materials, including chilling, trimming, washing, acetic acid immersion, and blanching, likely contributed to reducing the initial microbial load. These measures, together with the intrinsic antioxidant and antimicrobial properties of spinach, acted synergistically to support the enhanced microbiological stability observed during storage.

According to Lee et al. (2019), incorporating spinach into pork emulsion sausages enhances antioxidant properties, with notable improvements in both antioxidant activity and sensory qualities observed at lower levels of spinach [19]. However, sensory quality diminished with excessive spinach inclusion due to undesirable color and off-flavor development. Kantale et al. (2022) [18] also reported that adding spinach leaf extract to chicken sausages improved both oxidative and microbiological stability. They found that sensory qualities such as flavor and texture were better maintained during storage in samples with spinach [18]. Pennisi et al. (2020) showed that spinach powder effectively maintained microbiological safety and structural properties in fermented sausage formulations, although higher spinach levels negatively impacted sensory color acceptance [16]. These findings mirror our results in lamb dumplings, indicating spinach's capability to extend shelf-life and maintain quality attributes during storage.

The results of our study, in conjunction with the comparative research, highlight spinach's importance as a functional ingredient that can significantly improve the nutritional quality, safety against microbial contamination, oxidative resilience, and sensory characteristics of meat-based products when added in optimal amounts.

4. Conclusions

The study demonstrated that partial substitution of lamb with spinach (up to 20%) effectively enhanced the nutritional, functional, and microbiological properties of minced lamb dumplings. Spinach addition significantly increased calcium, magnesium, iron, and folate content while reducing fat and overall energy density. Functionally, spinach improved water- and fat-binding capacities and decreased cooking losses, beneficial for product quality and consumer acceptability. Importantly, spinach-enriched samples showed significantly lower levels of lipid oxidation (TBARS) and reduced acid numbers during refrigerated storage, indicating better oxidative and hydrolytic stability compared to the control. Sensory analysis confirmed consumer acceptance up to 20% spinach addition, whereas higher amounts negatively affected taste and appearance. Spinach thus represents a promising natural ingredient to improve nutritional and technological attributes of lamb meat products. Future research should therefore test spinach fortification in other meat systems, evaluate long-term storage and investigate consumer acceptance. Based on current results, an applied recommendation is to incorporate up to 20% spinach into lamb dumplings to improve nutrition, shelf-life, and sensory quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app15179556/s1, Figure S1: Vacuum-packed lamb meat dumplings with spinach.

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