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Development of Meat-Substituted Beef Burgers Using Single-Cell Protein and Legume Powders: Physicochemical, Nutritional and Microbiological Assessment

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Abstract

This study evaluated the effect of partial substitution of beef with red kidney beans (*Phaseolus vulgaris*) and brown lentils (*Lens culinaris*) and fortification with *Spirulina platensis* microalgae biomass or single-cell protein (1%) on the nutritional, technological, microbiological, and sensory quality of beef burgers during 90 days of frozen storage at $-18°C$. The formulations showed significant ($p < 0.05$) improvements in total protein, essential amino acids, mineral content, and antioxidant activity (DPPH assay) compared to the control (T_1). Fortification with *S.platensis* combined with legumes significantly inhibited lipid oxidation, maintaining TBARS values below the oxidation threshold (0.981 mg MDA/kg in T_7 versus 2.018 mg MDA/kg in T_1 at day 90. Cooking yield and water-holding capacity (WHC) were significantly improved in fortified formulations ($p < 0.05$). Microbiologically, red kidney bean and *Spirulina* combinations ($T_5 - T_7$) effectively reduced total bacterial counts, completely inhibited yeast and mold growth throughout storage, and suppressed psychrotrophic bacterial growth. Sensory evaluation revealed that formulations containing red kidney beans with 1% *Spirulina* (T_6 and T_7) maintained the highest overall acceptability scores. In conclusion, the incorporation of single-cell protein (*S. platensis*) alongside legume extenders provides a highly nutritious, shelf-stable, and organoleptically acceptable alternative in processed meat products.

Keywords: Beef burgers, single-cell protein, *Spirulina platensis*, red kidney beans, brown lentils, frozen storage, antioxidant activity, shelf-life quality.

1. Introduction

Red meat products, particularly processed beef burgers, are widely consumed worldwide due to their high nutritional value, protein density, and convenience for consumers **[1]**. However, growing global

food security challenges, rising production costs of animal proteins, and increasing consumer demand for healthier, functional meat products have accelerated the search for sustainable, non-conventional protein sources **[2]**.

Plant-based extenders, such as legumes (e.g., red kidney beans, Phaseolus vulgaris, and brown lentils, Lens culinaris), have gained significant interest in meat technology. Legumes are rich sources of dietary fiber, minerals, and bioactive phytochemicals, while possessing desirable functional attributes such as high water-binding and fat-emulsifying capacities **[3]**. Nevertheless, plant proteins often lack sufficient sulfur-containing essential amino acids (such as methionine and cysteine) to fully match animal protein nutritional profiles **[4].**

To overcome these nutritional limitations, single-cell protein (SCP) sources particularly microalgae like *Spirulina platensis* present a promising complementary solution **[5]**. *Spirulina platensis* is renowned for its high protein content (up to 60–70% dry weight), balanced amino acid spectrum, and rich concentration of bioactive constituents, including phycocyanin, carotenoids, and phenolic compounds, which exhibit potent antioxidant and antimicrobial properties **[6]**. Combining legume proteins with microalgal biomass offers a synergistic strategy to enhance the biological value and stability of meat formulations while optimizing processing dynamics.

Therefore, this study aims to evaluate the incorporation of single-cell protein (*Spirulina platensis*) as a non-conventional protein source in combination with red kidney beans and brown lentils in beef burger formulations. Specifically, it investigates the physicochemical, technological, oxidative, microbiological, and sensory dynamics of fortified beef burgers over a 90-day frozen storage period.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Raw Materials

Spirulina (*Arthrospira platensis*) powder was obtained from the National Research Centre, Giza, Egypt. Fresh beef cuts, fatty tissues, legume seeds (Brown lentil and Red kidney bean), and other essential formulation ingredients were purchased from local markets in Ismailia Governorate, Egypt.

2.1.2. Chemicals and Reagents

All chemicals and reagents used throughout the chemical analyses were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Methods

2.2.1. Preparation of Legume Powders

Legume seeds (lentil and red kidney bean) were prepared following the procedure of **[7]**. Cleaned raw seeds were soaked in water for 12 h, dried in a hot-air oven at 40–50 °C for 12 h, ground into fine flour, and stored at room temperature until use.

2.2.2. Processing and Formulation of Beef Burgers

Beef burgers were prepared according to **[8]** with minor modifications. Lean beef cuts and fat tissues were frozen at -18 °C for 24 h prior to processing.

The control burger (T_1) was prepared using the standard baseline formulation. For the experimental treatments (T_2–T_7), lean beef was partially substituted with legume powders (lentil or red kidney bean) at replacement levels of 20%, 30%, and 40%, in combination with a fixed 1% fortification of *Spirulina platensis* powder across all formulations. Non-meat ingredients were kept constant for all treatments.

2.2.3. Analytical Methods for Beef Burger Samples

2.2.3.1. Physicochemical Analysis

Determinations of moisture, ash, crude protein ($N \times 6.25$), and crude fat contents were performed following the standard procedures of **[9]**. Moisture was measured by oven drying (102 °C for 18 h), ash by muffle furnace incineration (580 °C for 8 h), protein via the Kjeldahl method, and fat by Soxhlet extraction with petroleum ether.

2.2.3.1.1. Amino Acid Profile

The amino acid composition was analyzed as described by [10] using a Beckman 120 °C amino acid analyzer

amino acid analyzer fitted with a DC-28 resin column (0.9×30 cm) and an Infotronics CRS 110A integrator.

2.2.3.1.2. Thiobarbituric Acid Reactive Substances (TBARS)

TBARS values were spectrophotometrically estimated at 538 nm after steam distillation, based on the protocol outlined by **[11]**. Results were expressed as mg malonaldehyde/kg sample using a conversion factor of 7.8.

2.2.3.1.3. Antioxidant Activity (DPPH Assay)

Free radical scavenging activity was evaluated following the method of **[12]** . Absorbance was recorded at 515 nm after a 30-min incubation period, and activity was calculated using the following equation:

DPPH Scavenging Activity (%) = $\left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$ Equation (1)

Where $A_{control}$ is the absorbance of the DPPH solution without the extract/sample, and A_{sample} is the absorbance of the sample reaction mixture.

2.2.3.1.4. pH Determination

The pH was measured according to **[13]**. Sample homogenates (5 g in 45 mL distilled water) were prepared using a stomacher for 1 min, and the supernatant pH was recorded in triplicate at room temperature using a digital pH meter.

2.2.3.1.5. Cooking Properties

Cooking loss and cooking yield were determined by measuring weight differences before and after cooking, following the approach of **[14]**.

2.2.3.1.6. Water Holding Capacity (WHC)

WHC was assessed using the filter paper press technique (0.5 g sample pressed under a 1-kg weight for 20 min) as adapted from **[15]**.

2.2.3.2. Microbiological Analysis

Serial decimal dilutions (10^{-1} to 10^{-7}) were prepared in 1% peptone water for the following microbial assays:

- Total Bacterial Count: Enumerated on Nutrient Agar following incubation at 35 °C for 48 h **[16]**.
- Yeasts and Molds: Assayed on Plate Count Agar supplemented with 10 μg/mL chloramphenicol after incubation at 37 °C for 48 h **[17]**.
- Psychrotrophic Bacterial Count: Evaluated on Plate Count Agar incubated at 7 °C for 7 days **[18]**.

All counts were reported as $\log_{10}$ CFU/g.

2.2.3.3. Sensory Evaluation

Organoleptic attributes of warm cooked burger samples were evaluated by a trained 15-member panel for appearance, color, tenderness, juiciness, flavor, and overall acceptability using a 7-point Hedonic scale ($7 =$ extremely like, $1 =$ extremely dislike), based on **[19]**.

2.2.3.4. Statistical analysis

Analysis of data was carried out using SPSS statistical software version 21 (SPSS Inc., Chicago, IL). To identify any significant differences between the oil samples or treatments of fortified products, analysis of variance (ANOVA) followed by **Duncan's** multiple range procedure was used at a level of $p \leq 0.05$. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Chemical Analysis

3.1.1. Moisture content (%)

The moisture content of the different beef burger samples during frozen storage is

presented in **Table (1)**. Moisture content showed a gradual decline throughout the storage period (0–90 days), although the extent of reduction varied according to the formulation. This reduction may be attributed to moisture migration, ice crystal formation during frozen storage, and dehydration of the product. T_7 showed the greatest reduction, decreasing from 53.480% at day 0 to 50.182% after 90 days, whereas T_1 exhibited the highest moisture retention, declining only from 61.327% at day 0 to 61.170% at day 90, reflecting superior water-holding capacity. This finding suggests that increasing the level of red kidney bean substitution reduced the water-holding capacity of the burger matrix, resulting in greater moisture loss during storage. On the other hand, there were no statisticall significant changes ($p > 0.05$) in the moisture content of treatments T_2, T_3, T_4, and T_6 during storage. The overall decrease in moisture content during the storage of burger samples is consistent with the findings of **[20]**, who found a gradual decrease in the moisture content of beef burger patties during frozen storage. They attributed this reduction to moisture migration and sublimation from the product surface, together with structural changes in muscle proteins caused by freezing and prolonged storage. The present results show that both storage time and formulation significantly affect the moisture content of beef burgers. The combination of legume and *Spirulina* altered the water-holding characteristics of the burger matrix, with formulations containing higher proportions of red kidney bean showing more moisture loss than the control and brown lentil-based samples during frozen storage.

Table (1): Changes in Moisture content (%) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	**30**	**60**	**90**	
T_1	$61.327^{a} \pm 0.015$	$61.230^{a} \pm 0.030$	$61.186^{a} \pm 0.005$	$61.170^{a} \pm 0.044$	<0.001
T_2	$60.833^{a} \pm 0.164$	$60.741^{a} \pm 3.008$	$60.350^{a} \pm 0.062$	$59.558^{b} \pm 1.375$	0.778
T_3	$60.127^{a} \pm 0.031$	$59.833^{a} \pm 0.764$	$56.473^{a} \pm 9.765$	$58.563^{b} \pm 0.170$	0.794
T_4	$59.220^{a} \pm 0.035$	$59.267^{a} \pm 0.600$	$58.727^{a} \pm 0.057$	$58.703^{b} \pm 0.006$	0.091
T_5	$58.743^{a} \pm 0.265$	$56.133^{b} \pm 0.015$	$55.931^{a} \pm 0.047$	$55.310^{c} \pm 0.010$	0.000
T_6	$55.927^{b} \pm 3.698$	$54.193^{bc} \pm 0.006$	$53.910^{a} \pm 0.090$	$52.747^{d} \pm 0.049$	0.284
T_7	$53.480^{b} \pm 0.020$	$53.107^{c} \pm 0.092$	$52.297^{a} \pm 0.205$	$50.182^{e} \pm 0.020$	<0.001
***p* value**	<0.001	<0.001	<0.001	<0.001	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.

Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.1.2. Fat content (%)

Changes in fat content of burger samples during storage from day 0 to day 90 are presented in **Table (2)**. The fat content reduced gradually throughout the storage period (0–90 days), This decline may be attributed to lipid This decline may be attributed to lipid oxidation, free fatty acid release, and fat exudation resulting from structural changes in the meat matrix during frozen storage. Within-treatment analysis revealed statistically significant decreases ($p \leq 0.05$) in fat content for T_1, T_3, T_4, and T_5 ($p = 0.000$), indicating reduction throughout storage. Among these treatments, the control sample T_1 consistently exhibited the highest fat content, decreasing from 19.323% at day 0 to 17.189% after 90 days. Similarly, T_5 showed a notable reduction from 18.157% to 15.837%, while T_3 and T_4 decreased from 14.307% to 13.063% and 12.874% to 11.593%, respectively.

In contrast, T_2, T_6, and T_7 showed no significant differences in fat content during storage ($p > 0.05$), suggesting that these formulations maintained stable lipid levels throughout the frozen storage period. This may be due to the incorporation of brown lentil or red kidney bean, together with *Spirulina*, which contributed to improved retention of lipids within the burger matrix and reduced fat migration or exudation during storage.

Comparison among treatments during storage time shows highly significant differences ($p < 0.001$), indicating that formulation had a marked effect on fat content during storage. The control sample (T_1) was the highest fat content, whereas T_4 (40% brown lentil + 1% *Spirulina*) showed the lowest fat value at all storage times, declining from 12.874% at day 0 to 11.593% after 90 days. Similarly, formulations containing higher content of brown lentil or red kidney bean generally exhibited lower fat contents than the control, reflecting the replacement of meat with plant-based ingredients that naturally contain less fat.

These results are consistent with **[21]**, who reported significantly higher fat contents in conventional beef burgers than in reformulated products containing plant-based ingredients. Similarly, **[22]** reported that replacement of meat with legume ingredients effectively reduced the fat content of meat products while improving their nutritional profile.

Overall, the results demonstrate that both storage time and formulation significantly affected the fat content of beef

burgers. Partial replacement of beef with brown lentil or red kidney bean accompanied with *Spirulina* effectively reduced the fat content while maintaining comparatively stable lipid levels during frozen storage, thus improving the nutritional value of the developed products.

Table (2): Changes in Fat content (%) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	30	60	90	
T_1	$19.323^{a} \pm 0.006$	$18.971^{a} \pm 0.017$	$18.130^{a} \pm 0.010$	$17.189^{a} \pm 0.009$	<0.001
T_2	$15.818^{bc} \pm 2.977$	$15.464^{c} \pm 0.152$	$15.315^{d} \pm 0.005$	$14.887^{bc} \pm 1.644$	0.924
T_3	$14.307^{cd} \pm 0.012$	$13.960^{d} \pm 0.165$	$13.440^{f} \pm 0.000$	$13.063^{d} \pm 0.055$	<0.001
T_4	$12.874^{d} \pm 0.038$	$12.420^{e} \pm 0.246$	$12.007^{g} \pm 0.067$	$11.593^{e} \pm 0.025$	<0.001
T_5	$18.157^{ab} \pm 0.575$	$17.577^{b} \pm 0.349$	$16.847^{b} \pm 0.045$	$15.837^{b} \pm 0.035$	<0.001
T_6	$16.262^{bc} \pm 0.004$	$16.013^{c} \pm 0.878$	$15.821^{c} \pm 0.019$	$15.703^{b} \pm 0.006$	0.470
T_7	$14.947^{cd} \pm 0.558$	$14.473^{d} \pm 0.046$	$14.433^{e} \pm 0.058$	$14.206^{c} \pm 0.005$	0.063
***p* value**	<0.001	<0.001	<0.001	<0.001	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.

Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.1.3. Protein content (%)

The protein content of the beef burger samples during frozen storage is presented in **Table (3)**. The results showed that both formulation and storage duration significantly influenced the protein content of burger samples. Comparison among treatments at each storage time showed highly significant differences ($p < 0.001$) during the storage period (0, 30, 60, and 90 days), indicating that the formulation had a pronounced effect on protein content. The control treatment T_1 consistently demonstrated the lowest protein content; however, protein concentration increased gradually with the combination of legume and *Spirulina*. The highest protein values were consistently recorded in T_7 (40% red kidney bean + 1% *Spirulina*), reaching 26.060% at day 0. and remaining high (24.255%) after 90 days. Similarly, treatments containing higher substitution levels (T_4, T_6, and T_7) exhibited significantly greater protein content than the control throughout storage. This increase can be due to the naturally high protein content of legumes and *Spirulina*, which enhanced the nutritional quality of the reformulated burgers.

These findings agree with those reported by **[22, 23]**, who observed that partial replacement of meat with protein-rich plant ingredients significantly increased the protein content of meat products in proportion to the substitution level. The protein value reduced significantly ($p \leq 0.05$) during frozen storage in treatments T_1, T_2, T_3, T_4, T_5, and T_7. The decrease was most clear in the control sample (T_1), where protein content declined from 14.823% at day 0 to 12.930% after 90 days. Similar but less pronounced decreases were detected in the reformulated treatments. In contrast, T_6 did not demonstrate a statistically significant change during storage ($p = 0.328$), revealing greater stability of its protein content during frozen storage. The slight reduction in protein content during storage may be attributed to protein denaturation, oxidative modifications, and the loss of water-soluble nitrogenous compounds through drip loss during thawing. Frozen storage can also promote structural changes in muscle proteins, reducing protein extractability and contributing to the evident decline in measured protein value. However, the reformulated burgers retained significantly higher protein levels than the control throughout storage, suggesting that the incorporation of brown lentil or red kidney bean flour together with *Spirulina* improved the nutritional value while keeping acceptable protein stability.

Overall, the present results exhibit that the combination of plant-based components significantly improved the protein content of beef burgers, while frozen storage affected only slight reductions in most formulations. Among all treatments, T_7 consistently maintained the highest protein content, demonstrating that the highest level of red kidney bean replacement combined with *Spirulina* produced the greatest enhancement in the nutritional value of the burger formulation.

Table (3): Changes in Protein content (%) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	30	60	90	
T_1	$14.823^{g} \pm 0.000$	$14.673^{g} \pm 0.170$	$14.033^{f} \pm 0.058$	$12.930^{c} \pm 0.027$	<0.001
T_2	$18.340^{f} \pm 0.035$	$18.110^{f} \pm 0.000$	$17.567^{e} \pm 0.404$	$17.783^{b} \pm 0.257$	0.018
T_3	$19.936^{d} \pm 0.012$	$19.850^{d} \pm 0.009$	$18.830^{d} \pm 0.000$	$18.118^{b} \pm 0.027$	<0.001
T_4	$20.370^{c} \pm 0.000$	$20.323^{c} \pm 0.020$	$20.110^{c} \pm 0.000$	$19.865^{b} \pm 0.056$	<0.001
T_5	$19.535^{e} \pm 0.525$	$19.210^{e} \pm 0.115$	$18.787^{d} \pm 0.101$	$17.913^{b} \pm 0.081$	0.001
T_6	$21.875^{b} \pm 0.000$	$21.070^{b} \pm 0.093$	$20.493^{b} \pm 0.000$	$20.050^{b} \pm 2.357$	0.328

T_7	26.060[a] ± 0.035	25.073[a] ± 0.042	24.207[a] ± 0.012	24.255[a] ± 0.264	<0.001
***p* value**	<0.001	<0.001	<0.001	<0.001	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.
Data are presented as Mean ± SD. Values within the same row/column with different superscript letters ([a,b,c,d]) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.1.4. Amino acid content (%)

Protein quality is assessed not only by total protein content but also by amino acid composition and digestibility. **Table (5)** shows the amino acid composition (%) of burger samples supplemented with brown lentil, red kidney bean, and *Spirulina*; **Table (4)** exhibits the amino acid profiles of the raw ingredients (*Spirulina* platensis, brown lentil, and red kidney bean). Of the raw materials analyzed, *S. platensis* showed the highest total amino acid content (39.29%), significantly surpassing that of brown lentils (24.88%) and red kidney beans (25.54%). This aligns with the established high protein content of *Spirulina*, which typically ranges from 60–70% of its dry weight. Across all three materials, glutamic acid (4.76–6.59%) and aspartic acid (3.13–4.21%) were the most abundant amino acids, while methionine (0.23–0.83%) and cystine (0.21–0.73%) were the least prevalent. The lower levels of sulfur-containing amino acids in the legumes are consistent with prior research identifying methionine and cysteine as the primary limiting amino acids in pulse proteins **[24, 25]**. However, *Spirulina*'s higher concentrations of these specific amino acids compared to brown lentils and red kidney beans indicate that it could be used to enhance the amino acid profile of legume-based food formulations.

The amino acid profile of the burger formulations **Table (5)** revealed that glutamic acid (9.89–13.80%), aspartic acid (6.81–9.60%), and leucine (7.52–8.56%) were the most abundant amino acids in all treatments. Conversely, tyrosine (1.41–2.18%), methionine (1.97–2.77%), and cystine (2.12–3.27%) were present at lower concentrations. These findings align with previous reports on legume-enriched meat products, where glutamic acid, aspartic acid, and leucine typically serve as the primary amino acids influencing both nutritional value and sensory properties.

Although legumes are commonly deficient in sulfur-containing amino acids, the combination of 1% *Spirulina* appeared to partially compensate for this limitation. Methionine and cystine contents remained stable among the supplemented treatments,

ranging from 1.97 to 2.11% and 2.12 to 2.45%, respectively. These results indicate that Spirulina supplementation improved the amino acid balance of the formulations. without influencing observed decreases in sulfur-containing amino acids.

Generally, the incorporation of brown lentil, red kidney bean, and *Spirulina* improved the nutritional value of beef burgers by enhancing their amino acid profile while supporting a balanced distribution of essential and non-essential amino acids. The treatments containing higher levels of red kidney bean generally showed superior lysine and arginine contents, whereas brown lentil formulations were characterized by greater concentrations of glutamic and aspartic acids. These findings indicate that combining legume proteins with S. platensis effectively increases the nutritional value of beef burgers and provides a more balanced amino acid composition compared to the control samples.

Table (4): Amino acid profile (%) of *Spirulina*, Brown lentil, and Red kidney bean.

Amino acid	*spirulina*	Brown lentil	Red kidney bean
Essential amino acids (%)			
Lysine (LYS)	2.014	1.74	1.74
Leucine (LEU)	3.38	1.94	2.07
Phenylalanine (PHE)	2.04	1.32	1.51
Methionine (MET)	0.83	0.23	0.37
Threonine (THR)	1.97	0.97	1.13
Valine (VAL)	2.46	1.16	1.19
Histidine (HIS)	0.86	0.69	0.71
Isoleucine (ISO)	2.36	1.14	1.13
Cystine (CYS)	0.73	0.21	0.30
Tyrosine (TYR)	0.21	0.67	0.69
Non-essential amino acids (%)			
Aspartic (ASP)	4.21	3.13	3.44
Serine (SER)	1.75	1.35	1.59
Glutamic (GLU)	6.59	4.78	4.76
Glycine (GLY)	2.05	1.02	0.98
Alanine (ALA)	2.89	1.08	1.05
Arginine (ARG)	3.23	2.36	1.77
Proline (PRO)	1.72	1.09	1.11
Total			
	39.29	**24.88**	**25.54**

Table (5) :Amino acid composition (%) of burger samples supplemented with brown lentil, red kidney bean, and *Spirulina*.

Amino acid	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Essential amino acids (%)							
Lysine (LYS)	4.11	4.31	4.3	4.33	5	5.05	5.09
Leucine (LEU)	7.52	8.1	8.24	8.11	8.40	8.55	8.56
Phenylalanine (PHE)	5.72	4.3	4.26	4.27	5.45	5.43	5.1
Methionine (MET)	2.77	2.11	2.1	2.1	2.1	1.98	1.97
Threonine (THR)	4.51	3.9	4.12	4	4.09	4.1	4.15
Valine (VAL)	5.98	4.91	4.87	4.9	5.78	5.72	5.7
Histidine (HIS)	3.43	2.81	2.88	2.87	3.11	3.15	3.22
Isoleucine (ISO)	4.87	5.2	5.5	5.25	4.63	4.66	4.70
Cystine (CYS)	3.27	2.2	2.12	2.15	2.41	2.4	2.45
Tyrosine (TYR)	1.41	1.46	1.567	1.48	2.15	2.18	2.14
Non-essential amino acids (%)							
Aspartic (ASP)	6.81	9.45	9.6	9.47	8.40	8.44	8.49
Serine (SER)	2.34	5.48	5.6	5.5	3.41	3.44	3.45
Glutamic (GLU)	9.89	13.2	13.8	13.55	11.95	12.1	12.23
Glycine (GLY)	4.60	5.1	5.17	5.12	4.42	4.56	4.7
Alanine (ALA)	4.5	4.33	4.1	4.21	4.49	4.44	4.4
Arginine (ARG)	5.38	3.8	3.6	3.78	6.7	6.78	6.9
Proline (PRO)	3.12	5	5.41	5.3	3.64	3.66	3.78

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.

3.1.5. Ash content (%)

Table (6) shows the statistical study of ash content during frozen storage (zero time to 90 days) for both the control and enriched raw beef burger treatments (T_1 to T_7). At the beginning of storage, the control treatment T_1 showed the lowest ash content (2.451%); however, ash content increased gradually with the combination of brown lentil, red kidney bean, and *Spirulina*. Samples T_2, T_3, and T_4 recorded ash values of 2.947%, 3.103%, and 3.354%, respectively. A more significant increase in ash content was observed in the formulations of red kidney beans, demonstrating levels of 6.826% (T_5), 9.298% (T_6), and 11.113% (T_7), with the latter indicating the highest mineral content observed during the entire experiment.

The higher ash values observed in the fortified formulations may be due to the naturally high mineral content of brown lentil, red kidney bean, and specifically *Spirulina platensis*, which is recognized as a rich source of essential minerals. The same results were reported by **[26]**, who exhibited that the combination of plant-based functional ingredients significantly raised the ash content of meat products because of their higher mineral composition.

Frozen storage affected ash content differently; significant differences over time ($p < 0.05$) were observed in samples T_1, T_2, T_4, T_5, and T_6. On the other hand, no significant differences were noticed in T_3 ($p = 0.332$) or T_7 ($p = 0.399$), demonstrating better stability in mineral concentration during storage. These variations are related to the gradual reduction in moisture content during frozen storage, which increases non-volatile elements, including minerals, and exhibiting increase in mineral content. This result agrees with the findings of **[27]**, who conceded that moisture loss during frozen storage increases ash percentage of meat products due to concentration effects.

Finally, using brown lentil, red kidney bean, and *Spirulina* significantly improved the mineral content of beef burgers, while frozen storage had only a lower influence on ash values compared with the pronounced effect of formulation.

Table (6): Changes in Ash content (%) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	**30**	**60**	**90**	
T_1	$2.451^{d} \pm 0.020$	$2.357^{d} \pm 0.006$	$2.281^{g} \pm 0.020$	$2.240^{d} \pm 0.026$	<0.001
T_2	$2.947^{d} \pm 0.006$	$2.840^{d} \pm 0.010$	$2.693^{f} \pm 0.123$	$2.457^{d} \pm 0.025$	<0.001
T_3	$3.103^{d} \pm 0.100$	$3.079^{d} \pm 0.193$	$2.973^{e} \pm 0.006$	$2.960^{d} \pm 0.026$	0.332
T_4	$3.354^{d} \pm 0.021$	$3.347^{d} \pm 0.012$	$3.310^{d} \pm 0.010$	$3.310^{d} \pm 0.010$	0.008
T_5	$6.826^{c} \pm 0.031$	$6.943^{c} \pm 0.040$	$7.053^{c} \pm 0.046$	$7.127^{c} \pm 0.031$	<0.001
T_6	$9.298^{b} \pm 0.197$	$9.367^{b} \pm 0.029$	$10.030^{b} \pm 0.052$	$10.153^{b} \pm 0.015$	<0.001
T_7	$11.113^{a} \pm 2.213$	$12.100^{a} \pm 1.652$	$13.213^{a} \pm 0.220$	$13.167^{a} \pm 1.756$	0.399
***p* value**	<0.001	<0.001	<0.001	<0.001	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.

Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.1.6. TBARS (Thiobarbituric Acid Reactive Substances)

Lipid oxidation is one of the main factors affecting the quality and shelf life of meat products during storage. Thiobarbituric Acid Reactive Substances (TBARS) are used as an indicator of secondary lipid oxidation products, particularly malondialdehyde (MDA), and provide a reliable measure of oxidative deterioration. The changes in TBARS estimates of beef burger samples during frozen storage are reported in **Table (7).** Inter-group comparisons showed highly significant differences ($p < 0.001$) among all

treatments during storage, indicating that formulation clearly affected lipid oxidative stability.

The control sample T_1 showed the highest TBARS values during storage, increasing significantly from 0.753 mg MDA/kg at day 0 to 2.018 mg MDA/kg after 90 days of frozen storage. On the other hand, all fortified samples indicated significantly lower TBARS values **(**$p \leq 0.05$**)** than the control during the storage period.

In the brown lentil formulations enriched with 1% *Spirulina*, the TBARS values observed at day 0 showed a steady decline from 0.460 mg/kg (T_2) to 0.393 mg/kg (T_3) and decreased to 0.360 mg/kg (T_4). The red kidney bean formulation demonstrated even lower initial oxidation values, with T_5, T_6, and T_7 recording measurements of 0.338, 0.244, and 0.189 mg/kg, respectively. These data suggest that increasing the percentage of meat replaced by legume, particularly red kidney bean, along with *Spirulina* supplementation, improved the oxidative stability of the beef burgers. Improving oxidative stability of fortified formulations is attributable to the plenty of naturally occurring antioxidant compounds found in legumes and *Spirulina platensis*, including phenolic compounds, flavonoids, carotenoids, phycocyanin, and other bioactive components capable of scavenging free radicals and interrupting lipid oxidation reactions. Similar observations were reported by **[28]**, who demonstrated that plant-based protein extenders enhance lipid stability in meat products, and by **[29]**, who reported the antioxidant activity of *Spirulina* to its rich content of natural antioxidant compounds.

TBARS values increased significantly during frozen storage **(**$p \leq 0.004$**),** showing the developing formation of secondary lipid oxidation products during time. However, the significance of this increase differed between treatments. After 90 days, the control sample reached 2.018 mg/kg, while sample T_7 showed 0.981 mg/kg**,** showing a 51% reduction in lipid oxidation compared to the control. This demonstrates the effectiveness of the combined red kidney bean–*Spirulina* formulation in retarding oxidative deterioration during frozen storage.

Increase in TBARS values during storage is consistent with the results of **[30]** who reported gradual lipid oxidation in frozen beef burgers regardless of formulation. Likewise, **[31]** monitored that TBARS values increased continuously throughout frozen storage due to the gradual oxidation of unsaturated fatty acids.

The incorporation of brown lentil, red kidney bean, and *Spirulina* substantially

enhanced the oxidative stability of beef burgers. Among all formulations, sample T_7 (40% red kidney bean + 1% *Spirulina*) showed the highest antioxidant protection, keeping the lowest TBARS values during the 90-day frozen storage period, so indicating superior resistance to lipid oxidation compared with the control sample.

Table (7): Changes in TBARS (mg/kg) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	30	60	90	
T_1	$0.753^{a} \pm 0.029$	$1.056^{a} \pm 0.005$	$1.466^{a} \pm 0.021$	$2.018^{a} \pm 0.070$	<0.001
T_2	$0.460^{b} \pm 0.020$	$0.747^{b} \pm 0.025$	$0.967^{b} \pm 0.006$	$1.010^{b} \pm 0.026$	<0.001
T_3	$0.393^{c} \pm 0.059$	$0.687^{c} \pm 0.015$	$0.930^{b} \pm 0.020$	$1.499^{b} \pm 0.498$	0.004
T_4	$0.360^{c} \pm 0.010$	$0.553^{d} \pm 0.012$	$0.893^{b} \pm 0.084$	$1.370^{b} \pm 0.000$	<0.001
T_5	$0.338^{c} \pm 0.036$	$0.662^{c} \pm 0.040$	$0.905^{b} \pm 0.089$	$1.160^{b} \pm 0.083$	<0.001
T_6	$0.244^{d} \pm 0.039$	$0.576^{d} \pm 0.040$	$0.706^{c} \pm 0.038$	$1.026^{b} \pm 0.047$	<0.001
T_7	$0.189^{d} \pm 0.011$	$0.472^{e} \pm 0.025$	$0.686^{c} \pm 0.008$	$0.981^{b} \pm 0.011$	<0.001
p value	<0.001	<0.001	<0.001	<0.001	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.

Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.1.7. DPPH radical scavenging activity

Table (8) shows the DPPH radical scavenging activity of the control and fortified beef burger formulations during frozen storage for 90 days. DPPH activity is generally documented as an indicator of the antioxidant capacity of food systems, showing the ability of bioactive compounds to neutralize free radicals.

The inter-group comparison showed very significant differences ($p < 0.001$) among all treatments at each storage time (0, 30, 60, and 90 days), showing that the combination of brown lentil, red kidney bean, and *Spirulina* noticeably improved the antioxidant ability of beef burgers. At zero time, the control treatment T_1 exhibited the lowest DPPH scavenging activity (13.50%), while the fortified treatments showed significantly higher contents. Brown lentil-enhanced burgers (T_2 – T_4) showed a progressive increase in antioxidant activity (34.23 – 46.45%) as the substitution level increased. Similarly, red kidney bean formulations (T_5 – T_7) exhibited even greater scavenging activity, reaching the highest value in T_7 (65.09%).

The improvement in antioxidant activity may be due to the high intensity of phenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid, and phycocyanin naturally found in legumes and *Spirulina platensis*. These bioactive compounds effectively provide hydrogen atoms or electrons to neutralize free radicals, so interrupting oxidative chain reactions within the meat matrix. The same data were reported by **[32]**, who established that adding microalgae with legume ingredients significantly improves the antioxidant properties of meat products.

The intra-group comparison also showed statistically significant reductions ($p \leq 0.026$) in DPPH activity during frozen storage for all treatments. Even though antioxidant capacity gradually declined with increasing storage time, all fortified samples retained significantly higher DPPH values than the control. At the end of storage (90 days), the control sample reduced to only 7.21%, whereas T_7 kept the highest antioxidant activity (60.80%). This finding showed that the combination of red kidney bean and *Spirulina* efficiently maintains antioxidant capacity during frozen storage and delays oxidative deterioration. Similar notes were described by **[33]**, who reported that natural plant-derived antioxidants maintain effective during frozen storage and significantly enhance the oxidative stability of meat products.

Generally, the results demonstrate that replacement part of the meat with legume enhanced with 1% *Spirulina platensis*, specially red kidney bean at higher replacement levels, substantially improves the antioxidant potential of beef burgers whereas maintaining considerable free-radical scavenging activity during frozen storage.

Table (8): Changes in DPPH content (%) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	30	60	90	
T_1	$13.500^{g} \pm 0.800$	$11.797^{g} \pm 0.439$	$9.183^{g} \pm 0.076$	$7.207^{f} \pm 0.092$	<0.001
T_2	$34.233^{f} \pm 0.777$	$32.403^{f} \pm 0.300$	$30.593^{f} \pm 0.160$	$28.827^{e} \pm 0.110$	<0.001
T_3	$40.500^{e} \pm 0.200$	$38.820^{e} \pm 0.288$	$37.490^{e} \pm 0.205$	$36.167^{d} \pm 0.115$	<0.001
T_4	$46.453^{d} \pm 0.999$	$45.167^{d} \pm 0.115$	$43.530^{d} \pm 0.478$	$42.247^{c} \pm 2.531$	0.026
T_5	$51.443^{c} \pm 0.513$	$49.487^{c} \pm 0.081$	$47.983^{c} \pm 0.081$	$43.863^{c} \pm 0.208$	<0.001
T_6	$57.927^{b} \pm 1.016$	$56.047^{b} \pm 0.075$	$54.670^{b} \pm 0.572$	$46.233^{b} \pm 0.749$	<0.001
T_7	$65.087^{a} \pm 0.674$	$63.800^{a} \pm 0.448$	$62.523^{a} \pm 1.194$	$60.797^{a} \pm 0.204$	0.001
p value	<0.001	<0.001	<0.001	<0.001	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.
Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.2. Physical and Technological Properties

3.2.1 pH values

Table (9) displays the statistical analysis of pH values during frozen storage (zero time to 90 day) for both the control and supplemented raw beef burger treatments (T_1 to T_7). The inter-group comparison shows extremely significant changes across all individual intervals ($p < 0.001$ for zero-time, 30 day, 60 day, and 90 day) in the pH levels across the various treatment groups at time periods. At the start of storage, the pH values of the supplemented burger samples were generally slightly higher than those of the control burger T_1. As the addition levels of the legume along with the fixed 1% *Spirulina* concentration, the pH values within the fortified samples tended to rise gradually. It was shown that substituting increasing amounts of brown lentil powder for meat in treatments T_2, T_3, and T_4 raised the pH values of the beef burgers relative to the control sample T_1.

Along with the enriched samples, T_3, T_4, T_5, and T_7 showed the highest pH values at the end of storage (6.78 – 6.84), whereas T_2 and T_6 kept relatively lower values (6.58). Despite these increases, all samples stay on within the normal pH range generally reported for frozen beef products. The gradual breakdown of proteins by endogenous enzymes or microbial activity, which results in the accumulation of volatile basic nitrogen compounds (such as free amino acids and amines) that raise the alkalinity of the meat matrix, is primarily responsible for the continuous rise in pH values for the fortified samples during the storage period. The findings published by **[34]** strongly align with the well-documented phenomena of a steady increase in pH during frozen storage.

Finally, the data suggest that partial replacement of meat with brown lentil or red kidney bean improved with 1% *Spirulina platensis* significantly influenced the pH values of beef burgers. Although pH increased during frozen storage, the fortified samples retained appropriate physicochemical stability, indicating that these functional components can be combined without negatively affecting product quality.

Table (9): Changes in pH value of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	**30**	**60**	**90**	
T_1	$6.1500^{c} \pm 0.044$	$6.1800^{e} \pm 0.000$	$6.6100^{a} \pm 0.010$	$6.270^{c} \pm 0.010$	<0.001
T_2	$6.2800^{a} \pm 0.020$	$6.3267^{c} \pm 0.015$	$6.5700^{ab} \pm 0.000$	$6.580^{b} \pm 0.026$	<0.001
T_3	$6.2600^{a} \pm 0.000$	$6.3133^{c} \pm 0.015$	$6.5667^{ab} \pm 0.012$	$6.840^{a} \pm 0.053$	<0.001
T_4	$6.2600^{a} \pm 0.000$	$6.3133^{c} \pm 0.015$	$6.5667^{ab} \pm 0.012$	$6.840^{a} \pm 0.053$	<0.001
T_5	$6.2100^{b} \pm 0.010$	$6.3700^{b} \pm 0.000$	$6.6600^{a} \pm 0.010$	$6.783^{a} \pm 0.021$	<0.001
T_6	$6.1800^{bc} \pm 0.000$	$6.5133^{a} \pm 0.015$	$6.4933^{b} \pm 0.110$	$6.580^{b} \pm 0.000$	<0.001
T_7	$6.1700^{bc} \pm 0.017$	$6.2700^{d} \pm 0.000$	$6.6600^{a} \pm 0.010$	$6.833^{a} \pm 0.031$	<0.001
***p* value**	<0.001	<0.001	<0.001	<0.001	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.
Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.2.2. Cooking loss and cooking yield (%)

Tables (10) show the physical quality parameters of beef burgers during frozen storage, specifically cooking loss (%) and cooking yield (%). Inter-group comparisons showed highly significant differences ($p < 0.001$) in cooking loss and cooking yield among treatments at all storage time points (T_1 through T_7) at particular time points.

The control samples T_1 had the lowest cooking yield (63.447%) and the highest cooking loss (36.553%) during the first storage interval (zero time). On the other hand, cooking loss was dramatically decreased ($p \leq 0.05$) when brown lentil powder (T_2, T_3, and T_4) and red kidney bean powder (RKBP) mixed with 1% *Spirulina* (T_5, T_6, and T_7) were used in place of some meat protein. The greatest improvement was seen in T_4 (highest lentil replacement), which had the highest cooking output (84.147%) and the lowest initial cooking loss (15.853%), closely followed by the other enriched formulations.

Chemically, The plant and algal proteins added by these extenders function effectively as water binders, whereas fat, being hydrophobic, readily escapes during thermal processing. By limiting excessive moisture and nutrient exudation during cooking, this structural alteration increases the meat matrix's water-holding capacity (WHC) and flexibility, which directly improves the texture and juiciness of the final product **[35].**

Furthermore, the hydrocolloidal dietary fibers included in brown lentil and legume seeds are mostly responsible for this exceptional capacity to reduce cooking loss. To successfully limit thermal losses, these functional fibers create a robust, three-dimensional matrix that can physically trap and store both water and the fat added to the formulations. The use of dietary fibers to preserve fat and moisture in beef burger matrices provides compelling evidence for this process **[36].** Because of the remarkable water-binding and emulsifying qualities of the microalgae, prior research on the microalgal biomass effect confirms that burgers made with 1% *Spirulina* show improved structural stability and lower cooking loss profiles during storage when compared to control samples. In treatments T_5, T_6, and T_7, the synergistic interaction between *Spirulina* proteins and legume macromolecules effectively created a thick, cross-linked network that limited the initial cooking loss to 31.557%, 29.530%, and 27.600%, respectively.

On the other hand, the intra-group comparison monitors the evolution of cooking yield and cooking loss values within each treatment. Every treatment group (T_1 through T_7) in this dataset shows statistically significant changes over the time periods (p = 0.000), indicating that the intra-group variation is consistently extremely significant.

Protein denaturation and ice crystal formation, which structurally alter the meat matrix over time, caused an overall progressive rise in cooking loss and a concomitant decline in cooking yield across most groups as frozen storage progressed from zero time to 90 days. Nevertheless, this degradation was remarkably controlled by the fortified groups. In contrast to the severe moisture and fat losses seen in the control group, the cooking loss of the 1% *Spirulina*-fortified blends remained more functionally controlled. All substituted treatments especially the brown lentil formulations (T_2 to T_4), retained significantly lower cooking losses (32.497%, 31.447%, and 30.500%, respectively) and superior cooking yields at the final storage point (90 days) when compared to the control baselines, demonstrating that these natural protein replacers successfully optimize the functional stability of processed beef products during extended commercial freezing.

Regarding the sharp increase in cooking loss observed in T_5 between day 60 (40.920%) and day 90 (49.927%), this distinct behavior can be attributed to structural degradation within the protein-polysaccharide matrix caused by prolonged ice recrystallization

during extended frozen storage. While T_6 (30% Red Kidney Bean) and T_7 (40% Red Kidney Bean) incorporated higher proportions of dietary fiber and starch which effectively reinforced the matrix by absorbing released exudates and maintaining water/fat-binding capacity T_5 (20% Red Kidney Bean) possessed lower non-meat functional solids. Consequently, prolonged freezing compromised the meat-legume protein network in T_5, leading to a marked reduction in its water holding capacity (WHC) and a significantly higher drip and fat loss during thermal processing at day 90. Overall, these findings demonstrate that natural protein replacers successfully optimize the functional stability of processed beef products during extended commercial freezing.

Table (10): Changes in Cooking loss (%) and Cooking yield (%) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	30	60	90	
Cooking loss (%)					
T_1	$36.553^{a} \pm 0.038$	$48.330^{a} \pm 0.021$	$41.250^{a} \pm 0.018$	$40.919^{b} \pm 0.688$	<0.001
T_2	$20.243^{e} \pm 0.012$	$29.333^{e} \pm 0.006$	$31.206^{e} \pm 0.052$	$32.497^{c} \pm 3.908$	<0.001
T_3	$18.143^{f} \pm 0.035$	$26.363^{f} \pm 0.773$	$30.250^{f} \pm 0.035$	$31.447^{c} \pm 2.086$	<0.001
T_4	$15.853^{g} \pm 0.012$	$21.773^{g} \pm 0.693$	$28.333^{g} \pm 0.025$	$30.500^{c} \pm 0.100$	<0.001
T_5	$31.557^{b} \pm 1.045$	$37.450^{b} \pm 0.507$	$40.920^{b} \pm 0.030$	$49.927^{a} \pm 0.930$	<0.001
T_6	$29.530^{c} \pm 0.030$	$35.690^{c} \pm 0.101$	$38.887^{c} \pm 0.103$	$47.087^{a} \pm 0.023$	<0.001
T_7	$27.600^{d} \pm 0.100$	$30.117^{d} \pm 0.015$	$38.193^{d} \pm 0.012$	$41.713^{b} \pm 2.994$	0.001
***p* value**	<0.001**	<0.001**	<0.001**	<0.001**	
Cooking yield (%)					
T_1	$63.447^{f} \pm 0.038$	$51.670^{g} \pm 0.020$	$58.750^{e} \pm 0.017$	$59.080^{b} \pm 0.689$	<0.001
T_2	$79.757^{c} \pm 0.012$	$70.667^{c} \pm 0.006$	$68.780^{c} \pm 0.035$	$66.777^{a} \pm 3.376$	<0.001
T_3	$81.863^{b} \pm 0.025$	$73.633^{b} \pm 0.777$	$69.747^{b} \pm 0.040$	$68.557^{a} \pm 2.086$	<0.001
T_4	$84.147^{a} \pm 0.012$	$78.220^{a} \pm 0.697$	$71.670^{a} \pm 0.030$	$69.497^{a} \pm 0.095$	<0.001
T_5	$68.443^{e} \pm 1.045$	$62.350^{f} \pm 0.328$	$59.087^{e} \pm 0.032$	$50.033^{c} \pm 0.981$	<0.001
T_6	$71.547^{d} \pm 1.865$	$64.300^{e} \pm 0.118$	$61.103^{d} \pm 0.091$	$52.913^{c} \pm 0.023$	<0.001
T_7	$72.382^{d} \pm 0.078$	$69.883^{d} \pm 0.015$	$61.477^{d} \pm 0.577$	$58.287^{b} \pm 2.994$	0.001
***p* value**	<0.001	<0.001	<0.001	<0.001	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.
Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.2.3. Water holding capacity (WHC) (%)

Table (11) shows the changes in water-holding capacity (WHC) of beef patties formulated with varying amounts of plant and algal proteins during frozen storage.

The inter-group comparison assesses the variations in water holding capacity (WHC)

between the various treatment groups (T_1 through T_7) at time points, showing extremely significant differences at all individual intervals ($p < 0.001$ for zero-time, 30 day, 60 day, and 90 day). When compared to the control sample T_1 (5.733), the results showed that the addition of brown lentil powder (T_2 to T_4) resulted in a highly significant ($p \leq 0.05$) rise in WHC values (7.297, 8.373, and 8.707, respectively) during the initial storage period (zero time). The WHC of the burgers increased at a consistent rate as the brown lentil substitution ratio increased. The remarkable capacity of brown lentil components to absorb and hold a lot of water is the main cause of this improvement. Proteins can effectively interact with water and oil in food systems because they have both hydrophilic and hydrophobic mechanisms **[37]**. The high protein content of these extenders improves the burger's structural matrix since water-protein interactions are generally responsible for the functional characteristics of proteins.

Conversely, formulations including 1% *Spirulina* and red kidney bean powder (T_5 to T_7) showed lower starting WHC values at "zero time" in comparison to the control, but as storage went on, they showed a strikingly quick and significant increase. T_7 had the greatest overall WHC value (18.130) by the conclusion of the storage period (90 day), followed by T_6 (13.523) and T_5 (9.170).

On the other hand, the intra-group comparison monitors the evolution of WHC values within each distinct treatment. Every treatment group (T_1 through T_7) in this dataset shows statistically significant changes over the time periods ($p \leq 0.024$), indicating that the intra-group variation is consistently extremely significant.

All treatments, including the control, showed a steady rise in WHC levels throughout the course of the 90-day frozen storage period (from zero time to 90 days).

This increasing trend during freezing can be explained by gradual changes in protein structure and the degree of denaturation that occurs during processing and frozen storage. These changes significantly alter the interactions between water and proteins and restrict the mobility of free water within the formulation matrix. Over time, the enriched formulas, especially the synergistic combinations of *Spirulina* and legumes, showed a superior ability to bind water dynamically, demonstrating their effectiveness in enhancing the structural integrity of beef burgers

Table (11): Changes in water-holding capacity (WHC) (%) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	30	60	90	
T_1	$5.733^{c} \pm 0.666$	$6.120^{d} \pm 0.010$	$6.820^{g} \pm 0.044$	$7.297^{d} \pm 0.006$	0.002
T_2	$7.297^{b} \pm 0.006$	$7.563^{c} \pm 0.025$	$7.913^{e} \pm 0.023$	$8.050^{d} \pm 0.050$	<0.001
T_3	$8.373^{a} \pm 0.497$	$8.950^{b} \pm 0.853$	$9.653^{c} \pm 0.021$	$9.817^{c} \pm 0.015$	0.024
T_4	$8.707^{a} \pm 0.055$	$9.100^{b} \pm 0.000$	$9.240^{d} \pm 0.061$	$9.333^{c} \pm 0.015$	<0.001
T_5	$3.570^{e} \pm 1.017$	$5.193^{e} \pm 0.205$	$7.133^{f} \pm 0.021$	$9.170^{c} \pm 0.868$	<0.001
T_6	$4.347^{de} \pm 0.015$	$6.767^{d} \pm 0.802$	$11.367^{b} \pm 0.153$	$13.523^{b} \pm 0.112$	<0.001
T_7	$5.057^{cd} \pm 0.035$	$10.770^{a} \pm 0.020$	$16.107^{a} \pm 0.110$	$18.130^{a} \pm 1.097$	0.001
***p* value**	<0.001	<0.001	<0.001	<0.001**	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.

Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.3. Microbiological Analysis

The changes in total bacterial count (TBC), mold and yeast counts, and psychrotrophic bacterial counts of raw beef burgers during 90 days of frozen storage ($-18 \pm 1°C$) are presented in **Table 12.**

At day 0, the control (T_1) recorded the highest initial TBC (5.155 log CFU/g) and psychrotrophic count (4.250 log CFU/g), exceeding the standard limit (5.0 log CFU/g) **[38]**. Partial meat replacement with legume powders and 1% *Spirulina platensis* significantly ($p \leq 0.05$) decreased initial microbial loads, with T_7 exhibiting the lowest initial TBC (2.150 log CFU/g).

During 90 days of frozen storage, T_7 maintained the highest microbial stability, retaining the lowest TBC (2.000 log CFU/g) and psychrotrophic count (2.690 log CFU/g) at day 90. Furthermore, while the control developed fungal growth up to 3.550 log CFU/g at day 90, red kidney bean formulations with 1% *Spirulina* ($T_5 - T_7$) achieved 100% fungal inhibition (ND) throughout storage. This antimicrobial effectiveness is attributed to the synergistic action of legume polyphenols and *Spirulina* bioactive compounds, combined with ice crystal formation during freezing **[26, 39].**

Table (12): Changes in Total Bacterial Count, Mold & Yeast, and Psychrotrophic Bacterial Counts (log CFU/g) of beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days).

Treatment	Storage period (days)				*P* value
	0	30	60	90	
Total Bacterial Count (log CFU/g)					
T_1	$5.155^{a} \pm 0.005$	$5.505^{a} \pm 0.095$	$6.810^{a} \pm 0.020$	$5.945^{a} \pm 0.005$	<0.001
T_2	$5.135^{a} \pm 0.005$	$5.440^{a} \pm 0.050$	$5.705^{b} \pm 0.005$	$5.928^{a} \pm 0.030$	<0.001
T_3	$5.063^{a} \pm 0.015$	$5.200^{a} \pm 0.000$	$5.625^{b} \pm 0.155$	$5.895^{a} \pm 0.005$	<0.001
T_4	$4.983^{a} \pm 0.015$	$5.190^{a} \pm 0.110$	$5.390^{c} \pm 0.010$	$4.933^{b} \pm 0.015$	<0.001
T_5	$3.625^{b} \pm 0.005$	$3.739^{b} \pm 0.341$	$2.923^{d} \pm 0.025$	$2.250^{c} \pm 0.250$	<0.001
T_6	$2.250^{c} \pm 0.250$	$2.750^{c} \pm 0.250$	$2.300^{e} \pm 0.000$	$2.150^{cd} \pm 0.150$	0.005
T_7	$2.150^{c} \pm 0.150$	$2.550^{c} \pm 0.050$	$2.300^{e} \pm 0.300$	$2.000^{c} \pm 0.000$	0.089
***p* value**	<0.001	<0.001	<0.001	<0.001	
Yeast and Mold (log CFU/g)					
T_1	$0.000^{b} \pm 0.000$	$3.950^{a} \pm 0.050$	$4.813^{b} \pm 0.063$	$3.550^{c} \pm 0.050$	<0.001
T_2	$3.515^{c} \pm 0.025$	$4.023^{a} \pm 0.166$	$5.710^{a} \pm 0.000$	$5.445^{a} \pm 0.045$	<0.001
T_3	$3.890^{a} \pm 0.010$	$3.710^{b} \pm 0.010$	$3.989^{c} \pm 0.211$	$3.825^{b} \pm 0.025$	0.064
T_4	$3.826^{b} \pm 0.004$	$3.716^{b} \pm 0.000$	$3.818^{d} \pm 0.012$	$3.855^{b} \pm 0.015$	0.000
T_5	$0.000^{b} \pm 0.000$	$0.000^{c} \pm 0.000$	$0.000^{e} \pm 0.000$	$0.000^{d} \pm 0.000$	ND
T_6	$0.000^{b} \pm 0.000$	$0.000^{c} \pm 0.000$	$0.000^{e} \pm 0.000$	$0.000^{d} \pm 0.000$	ND
T_7	$0.000^{b} \pm 0.000$	$0.000^{c} \pm 0.000$	$0.000^{e} \pm 0.000$	$0.000^{d} \pm 0.000$	ND
***p* value**	<0.001	<0.001	<0.001	<0.001	
Psychrotrophic bacteria (log CFU/g)					
T_1	$4.250^{a} \pm 0.050$	$3.717^{a} \pm 1.158$	$5.150^{a} \pm 0.150$	$4.710^{a} \pm 0.010$	0.077
T_2	$3.350^{b} \pm 0.050$	$2.900^{a} \pm 0.100$	$3.603^{b} \pm 0.105$	$3.750^{b} \pm 0.050$	<0.001
T_3	$3.128^{c} \pm 0.016$	$3.165^{a} \pm 0.065$	$3.310^{c} \pm 0.010$	$3.420^{bc} \pm 0.182$	0.021
T_4	$2.790^{e} \pm 0.010$	$3.110^{a} \pm 0.036$	$3.130^{d} \pm 0.020$	$3.130^{bc} \pm 0.050$	<0.001
T_5	$3.095^{c} \pm 0.015$	$3.114^{a} \pm 0.000$	$2.925^{e} \pm 0.025$	$2.800^{c} \pm 0.000$	<0.001
T_6	$2.090^{d} \pm 0.010$	$3.165^{a} \pm 0.015$	$3.150^{d} \pm 0.000$	$3.100^{bc} \pm 0.800$	0.028
T_7	$2.890^{f} \pm 0.110$	$2.950^{a} \pm 0.000$	$2.790^{f} \pm 0.010$	$2.690^{c} \pm 0.000$	0.002
***p* value**	<0.001	<0.001	<0.001	<0.001**	

Treatments: T_1: Control sample, T_2: 20% Brown Lentil + 1% *Spirulina*, T_3: 30% Brown Lentil + 1% *Spirulina*, T_4: 40% Brown Lentil + 1% *Spirulina*, T_5: 20% Red Kidney Bean + 1% *Spirulina*, T_6: 30% Red Kidney Bean + 1% *Spirulina*, T_7: 40% Red Kidney Bean + 1% *Spirulina*.

Data are presented as Mean ± SD. Values within the same row/column with different superscript letters (^{a,b,c,d}) are statistically significantly different ($p < 0.05$). ** Highly significant at $p < 0.001$.

3.4. Sensory evaluation

The most important factors affecting consumer preference and palatability of processed beef products are sensory characteristics, including color, odor, texture, taste, and overall acceptability **[40]**. **Figures (1)** describe the sensory evaluation scores of cooked beef burgers during frozen storage.

Inter-group comparisons showed highly significant differences ($p < 0.001$) among all samples across all storage time points and sensory parameters. in the sensory

scores (color, odor, texture, taste, and overall acceptability) These results showed that the partial replacement of meat with legume powders improved with *Spirulina* significantly affected the sensory properties of the burgers. Adding plant and microalgae proteins had a major impact on sensory scores at the first storage point day 0). Formulations with 1% *Spirulina* and red kidney bean powder (RKBP) (T_5 to T_7) maintained high, desired color scores; T_7 reached 6.500. The natural components of germinated red beans are mostly responsible for this color improvement or stability **[41]**. On the other hand, high brown lentil substitutes (T_3 and T_4) had lower starting color ratings (3.250 and 4.125, respectively). According to the results of **[42]** Adding brown lentil in the proper percentages T_2 successfully optimized sensory acceptability in terms of texture and flavor when compared to the control baseline T_1. Because of their high fiber content and remarkable water-holding capacity, these functional extenders enhance texture and juiciness structurally. On the other hand, the intra-group comparison monitors the evolution of sensory scores within each treatment:

relating color, samples having red kidney bean powder (T_5–T_7) showed greatest scores during storage. At day 0, T_7 got the highest color score (6.50), followed by T_6 (6.00) and T_5 (5.88). However, higher replacement amounts of brown lentil (T_3 and T_4) showed significantly lower color scores (4.13 and 3.25, respectively). The enhanced color stability opined in red kidney bean samples due to the natural pigments and phenolic composites found in germinated red kidney beans, which improve preserve color through storage time **[41]**.

- Also, odor scores were significantly changed by the treatment. Samples having red kidney bean powder had higher odor scores compared to brown lentil samples during storage. The improved odor stability may be explained by the antioxidant action. of *Spirulina* and legume phytochemicals, which retard lipid oxidation and the progress of unwanted volatile compounds responsible for off-odors throughout storage **[41]**.
- Texture scores changed significantly between samples. Medium substitute levels, specifically T_2, kept texture comparable to control, while higher levels of brown lentil replacement moderately reduced panelist scores. Group T_4 is the only exception,

exhibiting no statistically significant change over time ($p = 0.821$).

- Taste scores demonstrated moderately smaller variations among samples compared to the other sensory properties. Extremely enriched formulations provided acceptable taste during storage, demonstrating that the combination with 1% *Spirulina* did not negatively affect flavor preference. Notably, red kidney bean samples showed good flavor stability throughout frozen storage
- Overall Acceptability: For most treatments, combination effect of all sensory properties this intra-group variance is statistically significant because groups T_1, T_2, T_3, T_6, and T_7 all show significant variations over time ($p < 0.05$). On the other hand, groups T_4 and T_5 do not exhibit statistically significant changes over time ($p = 0.391$ and $p = 0.266$, respectively). The samples containing red kidney bean powder with *Spirulina* (remarkably T_6 and T_7) reported the highest overall acceptability scores during storage, however burgers had high concentrations of brown lentil (T_3 and T_4) were less acceptable. These findings indicate that red kidney bean powder offered extra positive balance between nutritional enhancement and sensory quality.

The control group's sensory scores gradually declined as frozen storage advanced to "day 90" as a result of normal storage changes. But up to 90 days, the supplemented showed superior overall acceptance and good sensory stability, especially those that combined beans and *Spirulina* proteins not only improves the nutritional value but at the same time improves the sensory attributes and consumer preference of beef burgers during storage time. This suggests that these components effectively prevent items from losing quality when frozen.

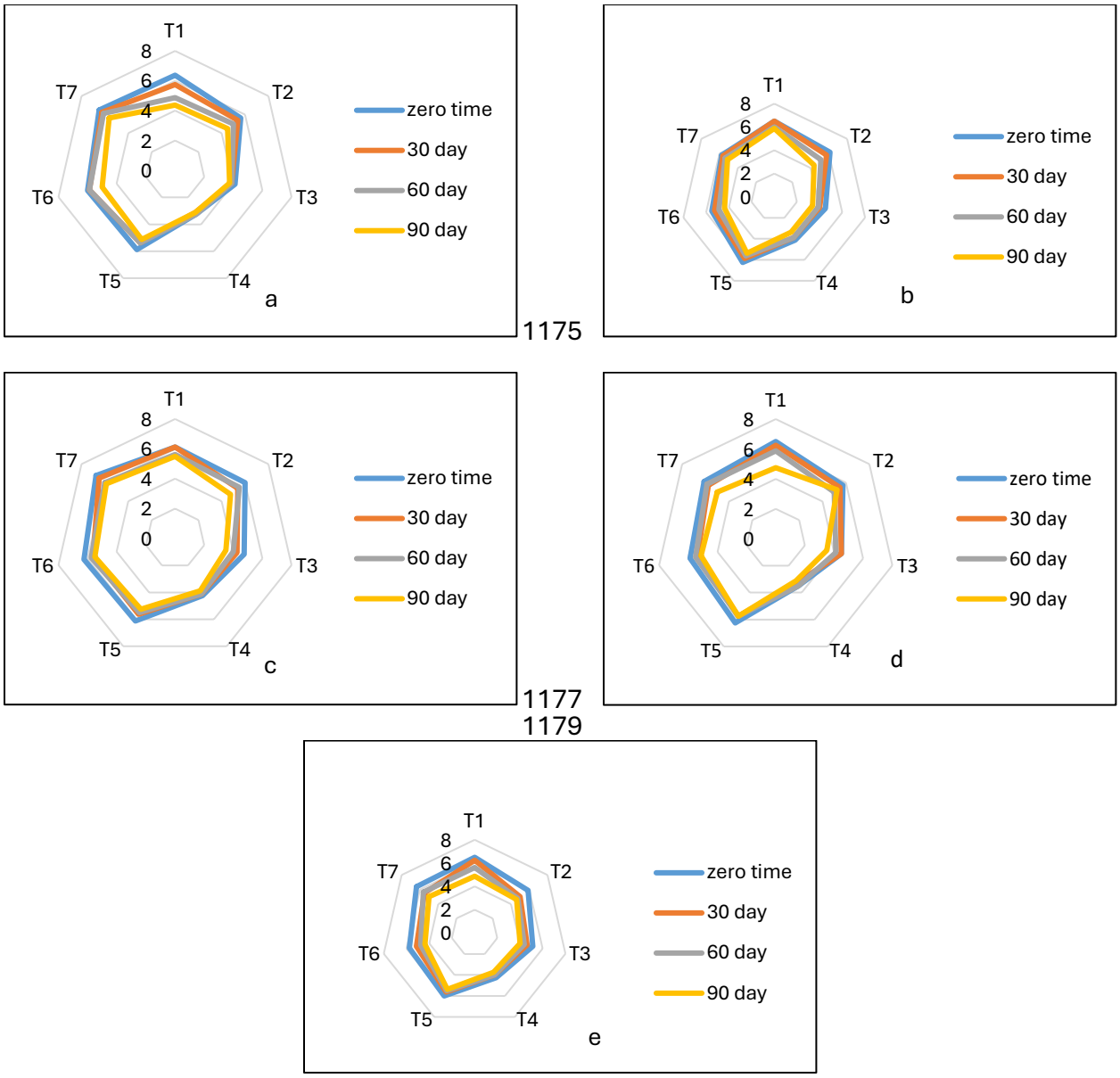


Figure (1): Changes in sensory quality scores of cooked beef burger samples subjected to different treatments during storage periods (0, 30, 60, and 90 days): (a) Color, (b) Odor, (c) Texture, (d) Taste, and (e) Overall Acceptability.

Values are presented as mean ± SD. Sensory evaluation was conducted by 15 trained panelists using a 7-point Hedonic scale (7 = like extremely, 1 = dislike extremely).

4. Conclusion

The application of *Spirulina platensis* as a single-cell protein supplement, combined with red kidney beans or brown lentils as meat extenders, successfully improves the nutritional and functional profile of beef burgers without compromising consumer acceptability.

Key findings from this research demonstrate that:

- **Nutritional Enrichment:** Legume and microalgae fortification significantly enhanced protein quality, mineral content, and essential amino acid profiles.
- **Oxidative and Microbial Stability:** Bioactive compounds in *Spirulina* effectively delayed lipid oxidation (lowering TBARS levels by ~51%) and demonstrated strong antimicrobial activity, completely preventing yeast and mold development over 90 days of frozen storage.
- **Technological and Sensory Superiority:** Cooking yield and water retention were improved across fortified treatments. Formulations incorporating red kidney beans supplemented with 1% *Spirulina* (T_6 and T_7) achieved the best balance of structural integrity, flavor, color stability, and overall consumer acceptability.

Economic analysis

From an economic perspective, the partial substitution of beef with red kidney beans and brown lentils presents a highly cost-effective strategy for commercial meat processing. Legumes provide a significantly lower raw-material cost per kilogram compared to lean beef, directly reducing the overall formulation cost while maintaining or enhancing protein functionality. Furthermore, the inclusion of legume extenders improves cooking yield and water-holding capacity, effectively mitigating thermal processing losses and maximizing finished product weight. Although fortification with Spirulina platensis biomass introduces an added ingredient cost, its low inclusion rate (1%) is economically counterbalanced by the substantial reduction in meat raw-material expenditure and the extended shelf life achieved through lipid oxidation inhibition. Consequently, this hybrid formulation offers meat manufacturers a scalable, high-margin, and cost-efficient alternative that aligns with commercial feasibility and consumer demand for affordable, value-added meat products.

Recommendation:

Incorporating 1% *Spirulina platensis* alongside legume extenders is highly recommended for commercial meat processing to produce healthier, cost-effective, and shelf-stable functional meat products.

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