International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(Research Article)

Check for updates

THE EFFECT OF FILLERS ON BEEF BURGERS AND A STUDY OF THEIR SENSORY AND BIOLOGICAL CHARACTERISTICS

Sarah J. Ghazi *

Department of Food Sciences, College of Agriculture, University of Kufa, Iraq.
* Corresponding Author

ORCID Details
Sarah J. Ghazi: https://orcid.org/0009-0001-0549-6127

International Journal of Science and Research Archive, 2026, 20(02), 575–586

Publication history: Received on 17 July 2026; revised on 23 August 2026; accepted on 25 August 2026

Article DOI: https://doi.org/10.30574/ijsra.2026.20.2.1671

ABSTRACT

The deliberate inclusion of plant-based fillers to meet worldwide demand for cheaper and healthier processed meat products is difficult to quantify. Filler type, inclusion amount, and refrigerated storage affect sensory acceptability and microbiological safety. This study examined the impact of soybean flour, breadcrumbs, and rusk at 10%, 20%, and 30% levels on the sensory and microbiological quality of beef burger patties stored at 4±1°C for 1, 4, 6, and 9 days. The study evaluated color, shape uniformity, texture, flavor/aroma, and chewability, while microbiological analyzes examined total aerobic bacterial counts and *Escherichia coli (E.coli*). A 10% filler addition improved sensory scores compared to the control, while 20-30% concentrations decreased texture, shape integrity, and chewability ($P < 0.05$) due to excessive dilution of the meat protein matrix. Moreover, 10% soybean flour scored highest in color (23.07) and taste (23.0). Also, soybean filler showed the strongest inhibition of spoilage bacteria, with total aerobic and *E. coli* counts significantly reduced and psychrophilic bacterial proliferation prevented compared to the control at day 9 ($P < 0.01$). Breadcrumbs and rusk rely on physical water-binding in the absence of phytochemical protection. Soybean flour, especially at 10% inclusion, offers an appropriate techno-functional balance to retain sensory quality, while greater quantities (30%) can improve refrigerated shelf life through natural antibacterial activities. This study supports the development of hybrid beef burgers in accordance with global sustainability, production cost reduction, and red meat health hazards, benefiting the meat processing business.

Keywords: Beef Burger, Soybean, Breadcrumbs, Rusk, Sensory Evaluation, Antimicrobial, Hybrid Meat Products, Food Safety.

1 INTRODUCTION

The nutritive value of meat is renowned, and it can be attributed, partly, to the balance of the amino acid components and the high protein content, as well as the high content of B-complex vitamins (particularly niacin and riboflavin) and various minerals, which are absorbed easily through the digestive system, such as iron, phosphorus, calcium, etc. [1]. Several recent studies have confirmed the nutritive value of meat and found that non-meat ingredients could be employed to modify and enhance the functionality and nutritional properties of processed meat products [2].

Among these products, the position of the beef burgers is preeminent, acceptable to consumers, and a high-volume market of at the international level. This has required a lot of emphasis on cost reduction and quality improvement, with a particular emphasis on the use of filler materials known to improve tenderness, juiciness, and textural integrity but which have also been reported to decrease the production costs [3,4]. Nevertheless, the use of raw and processed meat has been positively correlated with non-communicable diseases like hypertension, hypercholesterolemia, and cardiovascular diseases worldwide. Epidemiological studies have frequently proven that vegetarian groups have a much lower incidence of these diseases than do frequent meat consumers [5,6].

Employing plant-based fillers in meat products has become a dual-use approach to decrease the health risks and improve the techno-economic viability of these products [7,8]. Moreover, these fillers have attracted significant interest as meat extenders, with research showing they can alter textural characteristics, enhance nutritional content, and lower production costs [9]. Among these fillers is soy protein, which is one of the most widely studied protein extenders because of its desirable functional properties, such as water-holding capacity and emulsification [10,11].

Fresh minced beef is a highly perishable substrate and is easily affected by microbial growth from a safety point of view. The grinding process can evenly spread the contaminating pathogenic species on the surface of the product, such as *Salmonella spp*., enterotoxigenic *Staphylococcus* spp., *shiga-toxin-producing Escherichiacoli,* and *Clostridium* and *Listeria* species, increasing the risk of foodborne illnesses [12]. Refrigerated storage is still the most actual bacterial control measure; nevertheless, some species, such as psychrotrophic bacteria, can grow at chilling temperatures, releasing extracellular enzymes that break down fats and proteins, resulting in deterioration of products' quality over time [13]. Simultaneously, coliforms, as well as yeasts and molds, are considered microbiological quality indicators, and their proliferation is highly dependent on the product formulation and time of storage [14].

While individual fillers have been previously investigated in meat products, there is a lack of studies that have examined the interactions between type of Filler, level of inclusion, and extended chilled storage time on the various microbiological indicators of meat spoilage. In particular, the effect of protein-rich fillers (*soybean*) versus carbohydrate-rich fillers (breadcrumbs and rusk) on the competitive ecology of spoilage microbiota has not been systematically explained. Besides, the role these fillers play in the function of the product has been well established, but their effect on microbial safety over a determined chilling period (up to 9 days) and at the presented concentrations (10-30%) has not been quantified. Therefore, our study aims to evaluate the effects of various filler types (soybean, bread crumbs, and rusk) with 10, 20, and 30% inclusion levels on the sensory and microbiological quality of beef burgers refrigerated for 1, 4, 6, and 9 days.

2 MATERIALS AND METHODS

2.1 Raw material and formation

Table 1: Formulation of beef burger patties with different filler types and concentrations.

Ingredients (g/100 g)	Control	Filler's inclusion levels		
Lean beef	78.0	68.0	58.0	48.0
Beef fat	20.0	20.0	20.0	20.0
Fillers1	0.0	10.0	20.0	30.0
Salt and spices[2]	2.0	2.0	2.0	2.0

[1] Filler's type: breadcrumbs, soybeans, and rusks.; [2] Sodium chloride (1.5%) and spice mix (0.5%): black pepper, paprika, and garlic powder.

Fresh beef (thigh muscle, *Longissimus femoris*) and subcutaneous fat were obtained from the local retail markets in the Al-Najaf Al-Ashraf Governorate, Iraq. All raw meat was chilled (4±1°C) and processed on the day of its arrival in the laboratory. The meat was used within 48 hours post-slaughter to standardize the initial microbial load and ensure consistent freshness across all replicates. Each experimental batch of one kilogram contained a mixture of 750 g lean beef and 250 g beef fat (fat content, 25% w/w). All treatments were supplemented with 2% (w/w) sodium chloride (NaCl) and a spice mixture (black pepper, paprika, and garlic powder). Soybean flour, rusk (traditionally toasted wheat crumb), and wheat breadcrumbs (prepared by fine grinding of dried bread) were added as filler material at 3 levels, including 10%, 20%, and 30% (w/w per 100 g meat). Additionally, the control treatment did not receive any filler (0%). Table1 displays the detailed formulation.

2.2 Burger patty preparation

The lean beef and fat were ground individually in a sterile electric meat grinder with a 3 mm orifice plate. All other equipment, including mixing bowls, spatulas, and weighing utensils, was thoroughly washed with hot water, sterilized with 70% ethanol, and air-dried before use to prevent cross-contamination between treatments. The constituents of the ground mixture were then well mixed in the prescribed proportions to form a homogeneous base mixture. Each filler material was added to the meat-fat blend separately at the concentrations (10%, 20%, or 30% w/w), and the meat-fat blend without any fillers was employed as a control group. The mixtures were manually homogenized to achieve even distribution of ingredients for about five minutes. The batter was formed into patties of even weight (100 g each), which were about 10 cm in diameter and 1.2 cm in thickness, and separated by parchment sheets. The raw patties were then stored in a refrigerator at 4±1°C for 1, 4, 6, and 9 days before sensory and microbiological analyses. The experiment was conducted in three independent replicates on different days; for each replicate, three patties were prepared [15,16].

2.3 Sensory evaluation

A trained sensory panel consisting of 12 members from the faculty and the postgraduate students of the Department of Food Science was employed for the sensory evaluation of cooked burger patties. The patties were cooked on a preheated electric grill at 180°C for 4 minutes on each side until the internal temperature reached 72°C. The assessment was carried out using the standardized assessment sheet developed from Meilgaard et al. [17], which included the five key quality attributes involving color, shape uniformity, texture, odor and taste, and chewability. Table 2 (a) exhibits detailed scoring criteria and descriptive anchors, along with the maximum score allocated for each attribute. Whereas Table 2 (b) shows the sensory evaluation form (score sheet) used by the panelists to record their judgments. The sensory evaluation was conducted directly after patty preparation (day 0), before the refrigerated storage period. The overall sensory score for each sample was determined by summing the individual attribute scores.

Table 2: (a). Sensory evaluation scoring criteria for beef burger patties.

Quality Attributes	Score Range	Descriptive Scale
Color	25 (Excellent) 20 (Very good) 15 (Average) 8 (Acceptable) 5 (Rejected)	Maximum score assigned for desirable browned color
Shape Uniformity	20 (Excellent) 16 (Very good) 12 (Average) 10 (Acceptable) 4 (Rejected)	Maximum score assigned for regular shape and edge integrity
Texture	20 (Excellent) 16 (Very good) 12 (Average) 10 (Acceptable) 4 (Rejected)	Maximum score assigned for firm, non-gummy consistency
Odor and Taste	25 (Excellent) 20 (Very good) 15 (Average) 10 (Acceptable) 5 (Rejected)	Maximum score assigned for characteristic meat-like flavor and aroma
Chewability	10 (Excellent) 8 (Very good) 6 (Average) 4 (Rejected)	Maximum score assigned for ease of cutting and mastication

Table 2: (b). Laboratory sensory analysis of the total quality score based on weighted attribute criteria.

Laboratory remarks/Evaluation signature and notes	Color (25)	Shape uniformity (20)	Texture (20)	Odor and taste (25)	Chewability (10)	Total score (100)

2.4 Microbial analysis

Raw burger patties were analyzed microbiologically at each designated time of storage (days 1, 4, 6, and 9). Standard microbiological media, such as Nutrient Agar (NA), Potato Dextrose Agar (PDA), and 0.1% peptone water, were obtained from HiMedia (China) and then prepared as specified by the manufacturer. All media were autoclaved at 121°C for 15 min at 1 atm before use. All subsequent incubations were performed under aerobic conditions, as the study focused on aerobic and facultative aerobic microorganisms.

2.4.1 Sample preparation

A sample of 10 g was aseptically removed from each patty and added to 90 mL of sterile 0.1% (w/v) peptone water to make the first 10^{-1} dilution. This stock solution was then well homogenized with a vortex mixer for 2 minutes, and then ten-fold serial dilutions were made for enumeration.

2.4.2 Total Viable Count (Aerobic Plate Count)

The spread plate method of Acharya [18] was employed to count the total viable aerobic mesophilic bacteria. A suitable dilution of 0.1 mL was aseptically streaked on the surface of pre-solidified nutrient agar plates. The inoculated plates were incubated under aerobic conditions for 24-48 hours at 35°C. After incubation, the colonies were enumerated with a digital colony counter, and the number of colonies was multiplied by the reciprocal of the dilution factor. The $\log_{10}$ of the number of colony-forming units per gram (CFU/g) was reported.

2.4.3 Psychrotrophic Bacterial Count

Total psychrotrophic bacterial counts were estimated according to the standard method described by the American Public Health Association (APHA) [19] for enumeration of psychrotrophic microorganisms in foods. Nutrient agar plates were inoculated as labeled above and then incubated under aerobic conditions at 5±1°C for ten days. Then, colonies were counted and stated as $\log_{10}$ CFU/g.

2.4.4 Yeast and Mold Count

The quantification of Yeast and mold was conducted with the pour plate technique following the standard microbiological method for yeast and mold enumeration in foods[19]. A 1 mL aliquot from the suitable dilutions was aseptically placed into sterile Petri dishes, thereafter, supplemented with around 15 mL of molten PDA chilled to 45±1°C. Upon solidification, the plates were kept under aerobic conditions at a temperature of 25 °C for five days. Yeasts and molds were recognized by their macroscopic and microscopic morphological characters, with results reported as $\log_{10}$ CFU/g.

2.4.5 Escherichia coli count

The *E. coli* counts were determined using the selective differential medium MacConkey agar (HiMedia, China). Enumeration was performed by the spread plate method as mentioned in section 2.4.2. In brief, 0.1 mL aliquots of the serial dilutions (section 2.4.1) were spread aseptically onto the surface of pre-solidified MacConkey agar plates. The inoculum was spread uniformly with the use of a sterile bent glass rod. The plates samples were incubated aerobically at 37 °C for one day. Typical presumptive *E. coli* colonies were pink to dark red colonies after incubation and differentiated from other enterobacteriaceae based on their morphology. Typical colonies were counted using a digital colony counter and multiplied by the reciprocal of the dilution factor, and results were expressed as $\log_{10}$ CFU/g [20].

2.5 Statistical Analysis

Data were analyzed using SAS software version 9.4 [21]. We investigated the frequency distribution of sensory estimation scores for beef patties treated with different fillers using the chi-square test [22].The general linear model (GLM) was employed to check the data of total aerobic bacteria, *E. coli*, Yeast and molds, and psychrophilic bacterial counts employing the least squares method. Average differences were checked using the post-hoc Duncan [23]. The model used is as follows:

$$Y_{ij}= \mu + T_i+ \varepsilon_{ij}$$

Yij= the observed value of the concerned treatment, μ= the observed mean for the concerned treatment, Ti= treatments impacts, and eij= the error related to individual observations.

3 RESULTS

3.1 Sensory Evaluation

The sensory properties of beef burger patties formulated using breadcrumbs, soybeans, or rusks at 10, 20, and 30% inclusion levels were evaluated. Table 3 presents the scores for color, uniformity of shape, texture, flavor and aroma, and chewability.

Table 3: Sensory Evaluation Scores for Meat Formulations with Various Additives.

Items	Color	Shape Uniformity	Texture	Flavor & Aroma	Chewability
Control	19.6	16.4	18	18	8
Breadcrumbs 10%	20.4	18	18.6	21	9.5
Breadcrumbs 20%	21.6	17.9	17.5	20.8	8.4
Breadcrumbs 30%	21	16.8	17.6	19.3	8
Soybeans 10%	23.07	18.4	18.2	23	8.8
Soybeans 20%	22.4	17.08	17.5	21	9
Soybeans 30%	21.7	17.9	17.5	20.4	8
Rusks 10%	22.5	18.8	18.6	20.5	8.9
Rusks 20%	21.3	17.5	17.4	20.3	8.3
Rusks 30%	21.3	17.9	16.6	20	8.4
Chi-Square	0.4409	0.01550	0.0369	0.7235	0.0498
P-Value	N. S	*	*	N. S	*

N.S: non-significant; *(Statistically Significant): Indicates significant differences between treatments at P<0.05.

3.1.1 Color

Color scores ranged from 19.6 for control to 23.07 for 10% soybean. Generally, all the filler treatments were higher than the control, with the 10% soybean treatment having the highest value. However, statistical analysis showed no significant differences among treatments for this attribute (chi-square = 0.4409, $P > 0.05$).

3.1.2 Shape uniformity

Shape uniformity scores ranged from 16.4 (control) to 18.8 (10% rusk). The addition of Filler improved the shape integrity of all the burgers over the control. In all types, an overall trend of decreasing score with increasing filler concentration was observed. The chi-square value of 0.0155 indicated a significant difference among various treatments ($P < 0.05$).

3.1.3 Texture

The texture score was 16.6 (30% rusk) to 18.6 (10% breadcrumbs and 10% rusk). At 10% inclusion, all fillers had the same or slightly better texture than the control (18.0). However, higher concentrations (20% and 30%) tended to generate lower scores. Statistical analysis showed a significant effect of treatments on texture quality (Chi-square = 0.0369, $P < 0.05$).

3.1.4 Flavor and aroma

Flavor and aroma scores ranged from 18.0 (control) to 23.0 (10% soybeans). Fillers at a level of 10% were higher than the control group, with the soybean 10% treatment exhibiting the most pronounced improvement. Increasing filler concentration generally lowered scores. There were no significant differences among treatments (Chi-square = 0.7235, $P > 0.05$).

3.1.5 Chewability

Chewability scores ranged from 8.0 (control, 30% breadcrumbs and 30% soybeans) to 9.5 (10% breadcrumbs). All the fillers at a 10% level of inclusion improved the chewability over the control, but at higher concentrations (30%) the scores were reduced. The Chi-square value of 0.0498 showed significant differences among treatments ($P < 0.05$).

3.2 Microbiological quality

3.2.1 Total Aerobic Bacterial Counts

All the treatments showed a gradual increase in total aerobic bacterial counts (TABC) during the storage period as shown in Table 4. In all cases, the control samples had the highest counts, with values ranging from 1.90×10^5 CFU/g (day 1) to 6.30×10^5 CFU/g (day 9). On the contrary, the treatment of 30% soybean displayed the lowest counts of

TABC, which ranged from 1.00 to 3.70 × 10^5 CFU/g. A clear dose-dependent impact was detected, with higher filler addition levels mostly causing lower TABC values. Statistical analysis showed highly significant differences (P < 0.01) between all treatments at each storage interval.

Table 4: Total aerobic bacterial counts (CFU/g10^5) in beef patties treated with various levels of filler powders during various storage periods.

Treatments	Days			
	1	4	6	9
Control	1.90^{a}	3.10^{a}	4.70^{a}	6.30^{a}
Breadcrumbs 10%	1.60^{c}	2.80^{b}	4.50^{b}	5.20^{c}
Breadcrumbs 20%	1.40^{d}	2.60^{c}	4.10^{c}	5.00^{cd}
Breadcrumbs 30%	1.30^{de}	2.20^{de}	3.90^{d}	4.80^{e}
Soybeans 10%	1.40^{d}	2.30^{d}	3.50^{f}	4.60^{f}
Soybeans 20%	1.20^{e}	2.10^{ef}	3.00^{g}	4.10^{g}
Soybeans 30%	1.00^{f}	1.80^{g}	2.60^{h}	3.70^{h}
Rusks 10%	1.70^{b}	2.60^{c}	3.90^{d}	$5.10b^{c}$
Rusks 20%	1.40^{d}	2.10^{ef}	3.70^{e}	4.90^{de}
Rusks 30%	1.30^{de}	2.00^{f}	3.50^{f}	4.60^{de}
SEM	0.029	0.031	0.019	0.032
P-Value	**	**	**	**

a,b,c…etc. Means, within column, within treatments, with different superscripts are significantly different (**=p < 0.01).

3.2.2 E. coli count

Table 5 shows that the *E. coli* counts increased in all samples from 1 day to 9 days. The control samples had the highest counts (0.90 × 10^2 to 4.10 × 10^2 CFU/g), whereas the 30% soybean treatment had the lowest (0.50 × 102 to 2.10 × 10^2 CFU/g). A concentration-dependent reduction of *E. coli* counts was observed for all fillers. Whereas soybean treatments showed a better inhibitory effect as compared to breadcrumbs and rusks at equal concentrations. Differences among treatments were highly significant (P < 0.01) at all storage intervals.

Table 5: *E. coli* counts (CFU/g10^2) in beef patties treated with various levels of filler powders during various storage periods.

Treatments	Days			
	1	4	6	9
Control	0.90^{a}	1.50^{ab}	2.70^{a}	4.10^{a}
Breadcrumbs 10%	0.90^{a}	1.60^{a}	2.40^{b}	3.40^{b}
Breadcrumbs 20%	0.80^{ab}	1.40^{bc}	2.20^{c}	3.10^{c}
Breadcrumbs 30%	0.60^{cd}	1.20^{de}	1.80^{f}	2.90^{d}
Soybeans 10%	0.80^{ab}	1.30^{cd}	2.00^{de}	2.80^{d}
Soybeans 20%	0.60^{cd}	1.10^{e}	1.80^{f}	2.50^{e}
Soybeans 30%	0.50^{d}	0.90^{f}	1.40^{g}	2.10^{f}
Rusks 10%	0.80^{ab}	1.50^{ab}	2.10^{cd}	3.20^{c}
Rusks 20%	0.70^{bc}	1.30^{cd}	1.90^{ef}	2.90^{d}
Rusks 30%	0.60^{cd}	1.10^{e}	1.80^{f}	2.60^{e}
SEM	0.014	0.031	0.039	0.028

P-Value	**	**	**	**

a,b,c…etc. Means, within column, within treatments, with different superscripts are significantly different (**=p < 0.01).

3.2.3 Yeast and molds

Yeast and mold populations increased progressively in all treatments throughout storage (Table 6). Maximum counts were observed for control samples (0.50×10^2 to 2.80×10^2 CFU/g), and minimum counts were observed for 30% soybean treatment (0.20×10^2 to 1.50×10^2 CFU/g). All types of Filler showed a dose-dependent reduction, with the strongest effect being in the soybean-treated samples. Statistical analysis revealed significant differences among treatments for all storage periods ($P < 0.01$).

Table 6: Yeast and Molds Count (CFU/g10²) in beef patties treated with various levels of filler powders during various storage periods.

Treatments	Days			
	1	4	6	9
Control	0.50^{ab}	1.10^{a}	1.90^{a}	2.80^{a}
Breadcrumbs 10%	0.60^{a}	1.00^{ab}	1.80^{a}	2.50^{b}
Breadcrumbs 20%	0.40^{bc}	0.80^{cd}	1.50^{b}	2.30^{c}
Breadcrumbs 30%	0.30^{cd}	0.60^{ef}	1.30^{c}	1.90^{e}
Soybeans 10%	0.40^{bc}	0.80^{cd}	1.30^{c}	1.80^{ef}
Soybeans 20%	0.40^{bc}	0.60^{ef}	1.10^{d}	1.70^{f}
Soybeans 30%	0.20^{d}	0.50^{f}	0.90^{e}	1.50^{g}
Rusks 10%	0.50^{ab}	0.90^{bc}	1.40^{bc}	2.50^{b}
Rusks 20%	0.40^{bc}	0.80^{cd}	1.30^{c}	2.30^{c}
Rusks 30%	0.30^{cd}	0.70^{de}	1.30^{c}	2.10^{d}
SEM	0.011	0.021	0.030	0.026
P-Value	**	**	**	**

a,b,c…etc. Means, within column, within treatments, with different superscripts are significantly different (**=p < 0.01).

3.2.4 Psychrophilic Bacterial count

The psychrophilic bacterial populations showed considerable growth during the storage period (Table 7). The control samples had the highest counts (1.00×10^3 to 4.95×10^3 CFU/g), and the lowest was in the 30% soybean treatment (0.51×10^3 to 2.13×10^3 CFU/g). A similar concentration-dependent decrease was found for all filler types. The inhibitory effects of soybean-based formulations were more effective against psychrophilic bacteria than breadcrumbs and rusks. Statistical analysis showed very significant differences among treatments at all storage intervals ($P < 0.01$).

Table 7: Psychrophilic bacterial counts (CFU/g10³) in beef patties treated with various levels of filler powders during various storage periods.

Treatments	Days			
	1	4	6	9
Control	1.00^{a}	1.88^{a}	3.12^{a}	4.95^{a}
Breadcrumbs 10%	0.88^{b}	1.81^{a}	2.88^{b}	3.98^{b}
Breadcrumbs 20%	0.81^{bc}	1.56^{b}	2.52^{c}	3.82^{c}
Breadcrumbs 30%	0.75^{cde}	1.36^{c}	2.26^{d}	3.22^{d}
Soybeans 10%	0.81^{bc}	1.38^{c}	2.42^{c}	3.25^{d}
Soybeans 20%	0.65^{ef}	1.21^{d}	2.23^{d}	2.92^{e}

Soybeans 30%	0.51^{g}	0.97^{e}	1.62^{f}	2.13^{f}
Rusks 10%	0.78^{bcd}	1.82^{a}	2.84^{b}	3.75^{c}
Rusks 20%	0.68^{def}	1.58^{b}	2.28^{d}	3.24^{d}
Rusks 30%	0.59^{fg}	1.21^{d}	1.98^{e}	2.87^{e}
SEM	0.021	0.009	0.013	0.022
P-Value	**	**	**	**

a,b,c..etc.. Means, within column, within treatments, with different superscripts are significantly different (**=p < 0.01).

4 DISCUSSION

The present study systematically evaluated the effects of three types of fillers, soybean flour, breadcrumbs, and rusk, at inclusion levels of 10%, 20%, and 30% on the sensory and microbiological quality of beef burger patties during refrigerated storage (4±1°C) for up to 9 days. We show that the type and concentration of Filler differentially and often dose-dependently affect sensory attributes and microbial stability. Notably, the carbohydrate-based fillers (breadcrumbs and rusk) were consistently outperformed by the soybean flour in suppressing spoilage microorganisms. Moderate addition (10%) of any filler generally preserved or improved sensory scores. The outcomes of these studies have significance to the meat industry for cost reduction, shelf life extension, and the growing consumer demand for healthier plant-supplemented meat products. The results corroborate the ongoing literature on hybrid meat products that link the properties of animal and plant proteins for sustainability, health, and economic requirements while preserving consumers' acceptance [2,8].

As seen in our study, all fillers at 10% addition enhanced most attributes compared to the control group, whereas higher levels (20–30%) yielded the lower scores for shape uniformity, texture, and chewability, attributes for which statistical significance was noted ($P < 0.05$). This biphasic response highlights the need to optimize the fillers' levels to take advantage of technological benefits without compromising sensory acceptance. This dose-dependent sensory response is consistent with findings in other hybrid meat systems, where increasing plant protein inclusion beyond optimal levels often leads to structural flaws and reduced consumer appeal [3, 4].

The soybean flour with 10% soybean flour of the fillers tested had the highest color score (23.07) and flavor/aroma score (23.0). Kumar et al. [7] and Ergezer et al. [16] indicated that the increased color could be related to the greater availability of free amino groups in soy protein, which undergo caramelization with sugars to yield the desired browned appearance in the product during cooking; moreover, the burger surface color and the overall appearance are enhanced by using soy-based extenders at moderate levels. Furthermore, Huang et al. [24] noted that the inclusion of 10% soybean in meat products significantly improves the flavor, which is in line with the fact that soybeans are rich in several amino acids, such as glutamic and aspartic acids, which are known as flavor contributors. Furthermore, the functional roles of charged amino acids in soy proteins improve the qualities of the meat through non-covalent bonds [11,25]. The current study revealed that there was no significant difference among the different treatments ($P > 0.05$), which means that plant-based fillers can be added without affecting the sensory properties. These results align with Zhang et al. [26], who reported that careful optimization of the formulation of hybrid meat products can yield sensory acceptance equal to that of conventional meat products.

Both rusk and breadcrumbs had similar performance to the soybean, and the lowest scores for shape uniformity and texture were at 10% inclusion for all the fillers. This increased shape integrity is in keeping with the water-binding and fat emulsifying capacities of these fillers. In particular, the high water absorbency of the rusk forms a gel-like network that limits the shrinkage of patties during cooking and increases the cohesion between the patties [3]. Rusk binds around three parts of water, which is very important for the firmness and consistency of bite and to help prevent shrinkage during all the cooking processes [27,28]. But at 30% inclusion, there was a significant loss in texture scores, particularly in the case of the ruse (16.6). This may be linked to over-dilution of the meat protein matrix, which creates a softer, crumbly matrix. Similar findings were noted for wheat filler-extended beef patties by [4]. In addition, the high carbohydrate content decreases the gel integrity of myosin and actin [9,10].

Consumer satisfaction was related to chewability, which also had a similar dose-dependency. It was found that the most points were obtained with 10% of breadcrumbs added to the diet and that any filler added at the 30% level resulted in chewability being at control levels or less. The chi-square value obtained (0.0498) suggests that there is a significant effect of filler level on mastication properties. Some meat alternatives made from plants have been found to be softer than beef patties [8]. So, from a sensory point of view, 10% inclusion seems optimum for all filler types, with additional advantages of soybean in color and flavor. Recent studies on hybrid meatballs with texturized soy protein have similarly reported acceptability scores compared to conventional meatballs when constructions were enhanced, confirming the high importance of filler types and levels in reaching customer acceptance [6,16].

Both type and filler level had a big impact ($P < 0.01$) on the TABC, *E. coli*, yeasts and molds, and psychrophilic bacteria during the 9 days of storage. All soybean-treated patties, especially the 30% inclusion, had the lowest counts for all indicators with the exception of control samples, which consistently showed the highest counts. One of the most important and promising results of the antimicrobial ability of soybean flour is that it can be used for the shelf-life extension of food. These findings align with and considerably extend recent studies into the antimicrobial potential of plant-based components within meat matrices [2,29].

The observed antimicrobial effects of soybean filler in our study are likely multifactorial. While previous studies have attributed antimicrobial features to bioactive peptides and isoflavones, e.g., genistein and daidzein, found in soy [11, 25], it is important to note that our experimental design did not include chemical analysis of these specific components in the tested samples. Therefore, the inhibitory effects observed here cannot be exclusively attributed to these phytochemicals. In addition, the strong water-holding capacity and high protein content of soybeans may have contributed to a reduction in water activity in the patty matrix, which is a well-known physical factor that limits the availability of free water for microbial metabolism and growth, particularly for psychrophilic bacteria and yeasts [30,31]. Furthermore, the dilution of the meat itself at higher filler concentrations reduces the nutrient density available for spoilage organisms. Thus, while a potential bioactive role of soy components cannot be ruled out, the current data suggest that physicochemical factors, primarily water activity reduction and substrate dilution, are the most plausible primary mechanisms for the observed dose-dependent microbial inhibition. In a contrasting study recently published, Moazzam et al. [32] found that beef patties with 25% rehydrated soy protein concentrate (SPC) had significantly higher ($p < 0.05$) total viable counts than control patties after 7 days of refrigerated storage (2±2°C). This apparent discrepancy with our results can be plausibly attributed to differences in the form of soy used (whole flour versus purified protein concentrate), the hydration state of the ingredient, and the specific storage temperature regimes. Soybean flour has a greater range of intact phytochemicals, oligosaccharides, and fibers, which may influence the competitive microbial ecology differently than fractionated soy protein concentrates. Moreover, Dogan et al. [29] showcased that soy-based meat matrices follow different trajectories of spoilage microbiota succession relative to pea-based counterparts, further supporting the notion that the protein matrix fundamentally influences spoilage trajectories and acidity profiles during chilled storage. Recent studies have confirmed that isoflavones and peptides from soybeans effectively kill microbes and may be more effective than synthetic additives currently used to prevent bacterial proliferation [12,25]. The intrinsic antioxidant and antimicrobial properties of soy components have also been demonstrated in various meat product applications[2].

Breadcrumbs and rusk, carbohydrate-rich, had a lesser amount of pronounced antimicrobial activity; this may be linked to reduced free water and water activity, and this may be the reason why they lack the intrinsic antimicrobial phytochemical features of soy [33,34]. Our study indicated that breadcrumbs at 30% also had microbial suppression compared to the untreated group, confirming the importance of physical factors like bound water and water activity as mentioned by [30]. This physical mechanism is strongly confirmed by Ershidat et al. [35], who narrated that raising the substitution levels of dehydrated wheat germ flour in beef burgers causes a gradual, dose-dependent decrease in bacterial load, principally linked to diminished water availability rather than bioactive inhibition. The different antimicrobial impacts noted between soy-based and carbohydrate-based fillers are aligned with other hybrid meat products where the microbial proliferation depends on the ingredients' composition [9, 14].

The noted rise in each of the microbial populations throughout the storage period indicates that beef burgers are very perishable even in the refrigerator. The psychrophilic bacteria demonstrated substantial growth, which suggests that refrigeration is not sufficient to prevent spoilage. This is in the same line with the findings of Saha et al. [13], who reported that psychrotrophic bacteria have extracellular proteases and lipases, which have the ability to break down meat components, generating off-odors and texture degradation over time. The benefits of soybean overcarbohydrate fillers are that it has been exhibited to be more actual in reducing psychrotrophic counts, which can extend the sensory and microbial shelf life of any refrigerated meat product [31,36]. In addition, the current study builds on recent research that has shown that the protein source impacts spoilage pathways, as soy-based meat products have microbial profiles that are diverse from those of other products as a result of the product ingredients and the spoilage bacteria [1, 5]. Lombardi et al. [37] further reinforced this challenge by stating that, even though the usage of fat replacers and plant-based extenders could guarantee the nutritional and sensory quality, they are not inherently sufficient to meet microbiological quality requirements. Moreover, they noted that the safety and extended shelf-life of low-fat or hybrid burgers can only be achieved by the strategic combination of appropriate plant-based fillers with well-selected natural antimicrobial agents, a strategy obviously supported by the superior performance of soybean in our study.

The antimicrobial activity of soybean flour is especially remarkable because of the increasing awareness of the inefficiency of synthetic antimicrobial agents and the appearance of bacterial strains resistant to them [12]. However, this study's dose-dependent antibacterial advantages must be assessed against greater addition levels' sensory drawbacks. Inclusion soybean at a level of 30% resulted in a significant microbial suppression, reducing total aerobic counts by 40-50% in comparison to the untreated group; however, it also affected texture, shape integrity, and chewability ($P < 0.05$). Hybrid meat systems show that excessive plant protein inclusion dilutes the meat protein matrix, causing structural flaws and lower consumer acceptance [3, 4]. From a commercial and purchaser acceptance standpoint, it can be concluded that the 30% inclusion is not the best formulation. In contrast, it could only be recommended in conditions where the prolonged shelf life is the top priority or in conjunction with technological interventions such as binders or hydrocolloids to reduce the impact of the significant quantity of meat dilution on the textural damage of the products. The use of soy-based isoflavones and peptides in food preservation is a potential substitute, which is biodegradable, environmentally friendly, and not harmful [11,38]. In addition, pea protein-based patties can be texturized to be used in hybrid patties, and research has revealed that the growth of Yeast and mold may be higher in hybrid patties, which can shorten the shelf life [3]. Our results show, however, that soybean flour is a very effective inhibitor of the growth of Yeast and mold, thus presenting greater antimicrobial activity than other plant-based fillers.

5 CONCLUSION

To sum up, the results of this study showed that the type and level of the Filler could have a significant effect on the sensory and microbiological characteristics of beef burger patties during storage in refrigerator conditions. Soybean flour is more effective at reducing total aerobic bacteria, *E. coli*, Yeast and mold, and psychrophilic bacteria counts by up to 40-50% than the control, especially at a 30% inclusion level. However, these microbiological features were accompanied by a significant sensory penalty, as 30% inclusion adversely affected texture, shape integrity, and chewability ($p < 0.05$). In contrast, the 10% inclusion level of all fillers, particularly soybean, provided the optimal balance, as it kept or even enhanced sensory attributes, including color and flavor for soybean, while still offering considerable microbial protection compared to the control group. Consequently, for the development of hybrid beef burgers that meet consumer expectations, a 10% soybean inclusion is strongly recommended as the ideal formulation to achieve cost savings, sensory acceptance, and an acceptable refrigeration shelf life. The 30% inclusion level is only advisable when extended preservation is prioritized over sensory quality or when combined with additional structural agents. These findings support the global trend of incorporating plant-based ingredients into meat products while offering a scientifically grounded strategy that harmonizes health, economic, and sensory objectives.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

The author would like to express their sincere gratitude to the Ministry of Higher Education and Scientific Research, Iraq, and Kufa University for their valuable support of scientific research and for providing the laboratory facilities necessary to complete this work.

Funding Source

No funding was received for conducting this study.

Disclosure of conflict of interest

The author declares no conflict of interest.

REFERENCES

[1] Fang, R., & Zhu, Z. (2025). Advances in reducing salt content in processed meats with basic amino acids.Foods,14(6), 940. https://doi.org/10.3390/foods14060940

[2] Ciobanu, M. M., Manoliu, D. R., Ciobotaru, M. C., Flocea, E. I., & Boișteanu, P. C. (2025). Dietary fibres in processed meat: A review on nutritional enhancement, technological effects, sensory implications and consumer perception.Foods,14(9), 1459. https://doi.org/10.3390/foods14091459

[3] Oliveira, T. S., Almeida, R. C. D. C., Silva, V. D. L., Ribeiro, C. V. D. M., Bezerra, L. R., & Ferreira Ribeiro, C. D. (2025). Enhancing beef hamburger quality: A comprehensive review of quality parameters, preservatives, and nanoencapsulation technologies of essential and edible oils.Foods,14(2), 147. https://doi.org/10.3390/foods14020147

[4] Yamini, S., Paswan, V. K., Hmar, B. Z., Verma, D. K., & Baruah, S. (2025). Quality, nutrition, and functionality of meat products. Trends in Animal-Based Foods, 149-179. https://doi.org/10.1007/978-3-032-00575-5_8

[5] Ridwan, H., Ahmed, S., Singh, R. B., Chakravorty, S., Medvedev, O., Singh, R. G., & Lisdwiyani, B. K. (2026). Sustainable diets and functional foods for the prevention of cardio-metabolic diseases and sustainable development goals of the UNO. An international consensus of scientific statement of the international college of nutrition and 28th world congress on clinical nutrition, Bogor, Indonesia.BMC Cardiovascular Disorders, 26(1), 569. https://doi.org/10.1186/s12872-026-05557-7

[6] Elsherbeni, A. I., & Abd El-Hack, M. E. (2026). Functional Foods for Chronic Disease Prevention. In Functional Foods from Animal Sources: Properties, Human Health Benefits, and Applications (pp. 239-274). Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-032-15798-0_9

[7] Kumar, P., Chatli, M. K., Mehta, N., Singh, P., Malav, O. P., & Verma, A. K. (2017). Meat analogues: Health promising sustainable meat substitutes.Critical reviews in food science and nutrition, 57(5), 923-932. https://doi.org/10.1080/10408398.2014.939739

[8] Ledesma-Amaro, R., & Bamezai, S. (2025).Sustainable use and conservation of fermentation-associated microorganisms within the agrifood system. Food & Agriculture Org.

[9] Rocchetti, G., Leni, G., Rebecchi, A., Dordoni, R., Giuberti, G., & Lucini, L. (2024). The distinctive effect of different insect powders as meat extenders in beef burgers subjected to cooking and in vitro gastrointestinal digestion. Food Chemistry,442, 138422. https://doi.org/10.1016/j.foodchem.2024.138422

[10] Al-Mazrouei, M. A., Al-Kharousi, Z. S., Al-Kharousi, J. M., & Al-Barashdi, H. M. (2024). Microbiological evaluation of local and imported raw beef meat at retail sites in Oman with emphasis on spoilage and pathogenic psychrotrophi bacteria.Microorganisms,12(12), 2545. https://doi.org/10.3390/microorganisms12122545

[11] Rashwan, A. K., Osman, A. I., Abdelshafy, A. M., Mo, J., & Chen, W. (2025). Plant-based proteins: advanced extraction technologies, interactions, physicochemical and functional properties, food and related applications, and health benefits.Critical Reviews in Food Science and Nutrition,65(4), 667-694. https://doi.org/10.1080/10408398.2023.2279696

[12] Sokra, I., Meta, H., & Somaly, S. (2026). Microbial spoilage and pathogen control strategies in meat and meat products.Journal of Agriculture and Technology,2(1), 228-243.

[13] Saha, S., Majumder, R., Rout, P., & Hossain, S. (2024). Unveiling the significance of psychrotrophic bacteria in milk and milk product spoilage–A review.The Microbe,2, 100034. https://doi.org/10.1016/j.microb.2024.100034

[14] Silva, I. F., de Rezende-Lago, N. C. M., de Marchi, P. G. F., Messias, C. T., & Silva, L. A. (2023). Microbiological quality of food.Seven Ed, 1501-1518. https://doi.org/10.56238/colleinternhealthscienv1-120

[15] Ahmed, B. H., & Mohammed, N. A. M. (2024). Effects of Adding Clove (Syzygium aromaticum L.) Seed Extract on Oxidative Stability, Microbial Activity, and Sensory Attributes in Beef Patties During Refrigerated Storage.Kurdistan Journal of Applied Research,9(2), 131-140. https://doi.org/10.24017/science.2024.2.10

[16] Ergezer, H., Akcan, T., & Serdaroğlu, M. (2014). The effects of potato puree and bread crumbs on some quality characteristics of low fat meatballs. Korean Journal for Food Science of Animal Resources, 34(5), 561.https://doi.org/10.5851/kosfa.2014.34.5.561

[17] Meilgaard, M. C., Civille, G. V., & Carr, B. T. (1999). Sensory evaluation techniques. 3 rd edCRC Press.Boca Raton, FL, USA, 416-424. https://doi.org/10.1201/9781003040729

[18] Acharya, K., & Khatua, S. (Eds.). (2024). Ganoderma: Cultivation, Chemistry, and Medicinal Applications, Volume 2. CRC Press. https://doi.org/10.1201/9781003490258

[19] American Public Health Association (APHA). (2015).Compendium of methods for the microbiological examination of foods (5th ed.). American Public Health Association.

[20] Ahmad Zahra, M., Niaj Murshidi, G., Das Moon, U., Sultana, S., & Haque, F. K. M. (2025). Seasonal analysis of pathogenic Escherichia coli contamination in Vegetables, washing Water, and vendor hygiene: virulence group classification and antibiotic resistance.Food Science & Nutrition,13(1), e4723. https://doi.org/10.1002/fsn3.4723

[21] SAS (2014). SAS User's Guide: Statistics. Version 9.4 SAS Inst. Inc., Cary, NC., USA.

[22] Snedecor, G. W. and Cochran, W.G. (1993). Statistical Methods ISBN: 0-8138-1561-4.

[23] Duncan, D.B. (1955). The Multiple Ranges and multiple FTests. Biometrics, 11,1-42. https://doi.org/10.2307/3001478

[24] Huang, X., Wang, P., Xue, W., Cheng, J., Yang, F., Yu, D., & Shi, Y. (2023). Preparation of meaty flavor additive from soybean meal through the Maillard reaction.Food chemistry: X,19, 100780.

[25] Elsheikh, M., Osman, A., Edris, S., Dawam, W., Sitohy, M., & Sabeq, I. (2025). Soybean Glycinin's Antibacterial Properties Provide a Feasible Natural Alternative for Improving the Overall Quality and Shelf-Life of Beef Steaks and Combating Foodborne Pathogens.Food and Bioprocess Technology,18(2), 1777-1792. https://doi.org/10.1007/s11947-024-03557-z

[26] Zhang, R., Farouk, M. M., Realini, C. E., & Thum, C. (2025). Hybridization in meat-based dual protein foods: Mechanisms, challenges, and consumer insights.Comprehensive Reviews in Food Science and Food Safety,24(4), e70216.

[27] Dinçer, E. A., Büyükkurt, Ö. K., Candal, C., Bilgiç, B. F., & Erbaş, M. (2018). Investigation of the usability of retrograded flour in meatball production as a structure enhancer.Korean Journal for Food Science of Animal Resources,38(1), 78-87.

[28] Ravani, A., & Sharma, H. P. (2022). Meat based functional foods.Functional foods, 235-287. https://doi.org/10.1002/9781119776345.ch7

[29] Dogan, M., Mann, D. A., & Deng, X. (2025). Characterization of the spoilage microbial communities in plant-based meat alternatives during refrigerated storage.Microbiology Spectrum,13(10), e01650-25.

[30] Cauvain, S. P. (2016). Bread and other bakery products. In The stability and shelf life of food (pp. 431-459). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100435-7.00015-0

[31] Gani, A., & Benjakul, S. (2020). Effect of antioxidants in combination of VCO nanoemulsion on gel properties and storage stability of refrigerated sardine surimi gel.International Journal of Food Science & Technology,55(6), 2451-2461. https://doi.org/10.1111/ijfs.14496

[32] Moazzam, M., Ali, S., Khan, M. A., Waseem, M., Javed, M. R., Rajput, N., & Osei Tutu, C. (2026). Impact of soy protein concentrate and storage on the safety, quality and shelf stability of beef patties.Food Science & Nutrition,14(1), e71327.https://doi.org/10.1002/fsn3.71327.

[33] Jay, J. M., Loessner, M. J., & Golden, D. A. (2005).Modern food microbiology. Boston, MA: Springer US.

[34] Kotsubanska, O., Halenko, O., & Kovaliova, S. (2019). Section 1 Food and Chemical Technology. In International scientific conference of young scientists and students" Youth Scientific Achievements to the 21st (Vol. 16, p. 4).

[35] Ershidat, O. T. M., Atta, M. B., Elsebaie, E. M., & Badr, M. R. (2025). Effect of wheat germ as a partial meat substitute on the nutritional and qualitative properties of beef burgers. Food Science and Technology International. Advance online publication. https://doi.org/10.1177/10820132251353396

[36] Jideani, V. A. (2011). Functional properties of soybean food ingredients in food systems. Soybean-Biochemistry, chemistry and physiology, 345-366.

[37] Lombardi, S. J., Pannella, G., Coppola, F., Vergalito, F., Maiuro, L., Succi, M., & Coppola, R. (2024). Plant-based ingredients utilized as fat replacers and natural antimicrobial agents in beef burgers.Foods,13(20), 3229. https://doi.org/10.3390/foods13203229

[38] De Rossi, L., Rocchetti, G., Lucini, L., & Rebecchi, A. (2025). Antimicrobial potential of polyphenols: Mechanisms of action and microbial responses-A narrative review. Antioxidants, 14(2), 200. https://doi.org/10.3390/antiox14020200