

EFFECT OF ADDITION CHICKPEAS AND MASH SEED'S LEGUMES POWDER ON THE REFRIGERATED STORAGE STABILITY AND QUALITY CHARACTERISTICS OF BEEF SAUSAGES

Moawya I. Y. Abdalla¹, Mohammed Z. Eskander², Ahmed R. M. Abd³, Tilal M. A. Mudawi⁴, Saad I. Yousif⁵, Zeinab I. Ali^{*6}

Address(es):

¹ Dept. of Food Science & Technology, Faculty of Agric. Sci., University of Gezira, WadMedani, Sudan.

² Food Sciences Department, Faculty of Agriculture, University of Basrah, Iraq.

³ Dept. Food Inspection, Anbar Municipalities Directorate, Anbar, Iraq.

⁴ Goat Research Center, Faculty of Agric. Sci., University of Gezira, WadMedani, Sudan.

⁵ Dept. Food science Department, Collage of Agriculture, University of Anbar, Anbar, Iraq.

⁶ Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Egypt.

*Corresponding author: Zeinab_ali@cu.edu.eg dr.zeinab2010@gmail.com

<https://doi.org/10.55251/jmbfs.11331>

ARTICLE INFO

Received 13. 5. 2024

Revised 18. 3. 2025

Accepted 27. 3. 2025

Published 1. 6. 2025

Regular article

OPEN ACCESS

ABSTRACT

Sausage should be stored under hygienic conditions and protected from microbial contamination. The aims of this study were to evaluate the influence of substituting a part of the prime mixture with different proportions of ground chickpea and mash seeds on the refrigerated storage time and quality characteristics of the processed beef sausages. Beef sausages were processed by adding 5%, 10%, 15% and 20% of chickpeas (K) and Mash (M). The sausages were kept under refrigeration (4 - 8 °C) for 1, 3, 6 and 9 days. The results point-out that significant differences ($P < 0.01$) between means were observed among the storage periods, with the highest pH value recorded at the ninth day of storage with an average of 6.182. On the other side, peroxide value of treatments control (TC) was the highest (2.071), while the lowest average (1.240) was recorded in T1K¹⁵ and the interaction demonstrated that T2M¹⁰ combined with one days of storage recorded the lowest peroxide value (1.098). No contamination with *Salmonella* was observed during all storage periods. T1K¹⁵ recorded the highest water-holding capacity (41.24). Overall, the addition of ground legume seeds prevented shrinkage and weight loss in sausages after thawing. The sensory evaluation results showed no significant differences ($P < 0.01$) between the mean values of the treatments in terms of flavor, texture, and overall acceptability. Therefore, the study recommended the use of legume seeds, up to 20%, in sausage manufacturing, as it helped extend the shelf life, improved the quality attributes, and overall acceptance of the product.

Keywords: Sausage, chickpea, mash seeds, shelf life, sensory

INTRODUCTION

Fresh sausages are highly perishable products since it is manufactured from fresh ground meat, that's make it a good medium for microbial growth, for both spoilage and pathogenic organisms. Moreover, Sausage has a high fat content favourable for lipid oxidation, is stored in oxygen semi-permeable packaging and is kept at refrigeration temperatures. These products, therefore, need to be preserved to maintain their quality (Sultana *et al.*, 2014). One of the main causes of meat and meat product spoilage is excessive oxidation, which breaks down the fats and proteins of the meat. Oxidation leads to the breakdown of oxidized cholesterol products, which have negative effects on consumers' health, as well as the degradation of unsaturated fatty acids (Dzudie *et al.*, 2004, and Ali *et al.*, 2024). Bacterial contamination can result in significant economic losses, as it leads to meat spoilage and quality deterioration. It can also cause food poisoning when consuming meat contaminated with harmful bacteria. The potential health effects of food poisoning range from mild symptoms such as nausea and diarrhea to severe symptoms like vomiting, fever, and pain. It can lead to serious complications such as kidney failure and even death in some cases (Noor, 2014). To avoid these risks, meat and meat products should be stored under hygienic conditions and protected from microbial contamination (Andres *et al.*, 2014).

Recently, there have been advances in production and manufacturing methods to meet the demand for meat and meat products. Several studies have been conducted to find natural ways to control contamination in meat and develop safe and natural preservatives. Some plants rich in antioxidants have been used as natural preservatives to extend the shelf life of meat without compromising consumer health. These natural preservatives are preferred by consumers because they are considered safe, healthy, and free from synthetic chemicals (Meyer *et al.*, 2002). One of the key objectives pursued by nutrition experts is to obtain a high-quality and cost-effective food product. Therefore, combining plant protein and animal protein can improve the performance of the food product and help achieve these goals. The main objective of the current study is to investigate the effect of different concentrations of crushed legume seeds on the quality of the sausage and to what extent it can affect its sensory attributes, safety and shelf life.

MATERIALS AND METHODS

Site of the study

The experiment was conducted in the Department of Food Science, College of Agriculture, Al-Anbar University, Iraq. The research and processing of sausages took place from October 10th, 2022, to December 20th, 2022.

Manufacturing of Beef sausage

Beef meat was purchased from meat shops in Ramadi city, Anbar province, Iraq. The meat was ground twice to achieve homogeneity in the mixture, and approximately a chemical analysis of moisture, proteins, fats and ash were conducted on the meat according to the method described by A.O.A.C. (2005). Meat and sheep fat (tallow) were stored at a temperature of 2°C for 24 hours. The fats were placed in special plastic bags to prevent air entry and stored in the refrigerator for later use. Then, the meat and sheep fat were cut into small cubes with dimensions of 2-3 cm³ before starting the sausage manufacturing process to facilitate the grinding process (Al-Faidi, 1996). Subsequently, the sausage blend was prepared according to (Aswad, 2000). This blend consists of the ingredients listed in table (1). The operations of substitution were carried out using powdered legume seeds of chickpeas (K) and mash (M) beans at ratios of 5%, 10%, 15% and 20% of the total meat and fat mixture used. These substitutions were performed with three replicates for each treatment, in addition to a control treatment. Natural casings were used for sausage packaging. Fresh small intestines were obtained directly after sheep slaughter. They were cleaned according to method mentioned by Al-Faidi, (1996).

All treatments were preserved in a refrigerator at a temperature of (4°C). After that, physical, chemical, and microbiological tests were performed at time point of 1, 3, 6 and 9 days of storage.

Table 1 Sausage mixer ingredient used in the study

Material	Meat	Fat	Ice	Salt	Black Pepper	spices	Sugar	Starch	Skimmed milk	onion	Garlic
Percentage	79.9	8.15	1.5	0.8	0.25	1.75	0.5	2.5	2.4	1.5	0.75

Chemical analysis

Approximate chemical composition analysis of samples was done for determining moisture, protein, fat and ash according to the method described by **A.O.A.C. (2005)**.

Oxidation tests

Estimation Free Fatty Acids (FFAs)

Neutral alcohol solution was prepared by adding 50 ml of ethyl alcohol (99.9%) to 5 drops of phenolphthalein indicator solution. A few drops of sodium hydroxide solution (N 0.1) were added to this mixture to obtain a neutral solution. Then, 10 ml of the previously prepared chloroform filtrate was mixed with 10 ml of the neutral alcohol solution. The mixture was titrated using sodium hydroxide solution (N 0.1) until the appearance of a pink color, indicating the endpoint. According to method described by **Edirisingha et al., (2021)** the percentage of FFAs based on oleic acid was calculated using the following equation:

$$FFA\% = \frac{\text{volume of NaOH} \times \text{NaOH con.} \times 0.0282}{\text{Sample Wt. (g)}} \times 100$$

Peroxide value estimation

Ten ml of the previously prepared chloroform filtrate was placed in a 100 ml volumetric flask. Fifteen ml of glacial acetic acid and 0.5 ml of saturated potassium iodide solution were added to the flask. The mixture was left for one minute. Twelve ml of distilled water was added to the flask. The mixture was titrated using sodium thiosulfate solution (N 0.01) and starch indicator, until it reached the endpoint, indicated by the appearance of a white color. Then peroxide value was calculated according to **Low et al., (2015)** by following equation:

$$PV = \frac{\text{volume of Na}_2\text{S}_2\text{O}_3 \text{ ml} \times \text{Na}_2\text{S}_2\text{O}_3 \text{ con.}}{\text{sample Wt. (g)}} \times 1000$$

Estimation of pH

The pH value was determined according to the method described by **(Capita et al., 2006)**. Ten grams of the sausage sample were weighed and 90 mL of deionized water were added to it. The mixture was then homogenized using an electric homogenizer for 30 seconds to obtain a homogeneous mixture. The pH value was directly measured using a pH meter.

Thiobarbituric acid (TBA) value estimation

The method by **Edirisingha et al., (2021)** was used to measure the amount of Malondialdehyde, which represents one of the products of fat oxidation in various tissues, by estimating the Thiobarbituric Acid (TBA) value. Ten grams of the meat sample were grinded with 25 ml of a solution containing 20% trichloroacetic acid (TCA) dissolved in 2 Molar phosphoric acid for 2 minutes. The resulting mixture was transferred to a 50 ml volumetric flask and fill it up to the mark with distilled water and shake gently. Then 25 ml was take it from the flask and subjected to centrifugation at 3000 revolutions per minute for 30 minutes. The mixture was filtered through a Whatman filter paper (No. 1), then transfer 5 ml of the filtrate to a test tube, adding 5 ml of TBA reagent solution (0.005 Molar) dissolved in distilled water to the test tube. the control solution (Blank) was prepared by mixing 5 ml of distilled water with 5 ml of TBA reagent solution. The absorbance (A) of the resulting color at a wave length of 530 nanometers using a spectrophotometer was measured. The calculation is done by using the following equation: TBA value (mg MDA/kg meat) = A × 5.2 (dilution factor).

Microbiological analysis

Estimation of Coliform Bacteria Count (CBC)

MacConkey agar medium was used to determine the CBC in the sample using the Pour-Plate method described by **Simon et al., (2021)**. The plates were then allowed to harden and incubated at 37 degrees Celsius for 24 hours. Afterward, the colony count was estimated using a microscope to determine the CBC in the sample.

Detection of Salmonella bacteria

The test was performed according to the method described by **Heide (2021)** using SS agar medium and incubated at 37°C for 24 hours. This test was conducted on raw meat samples and during the preparation of the sausage mixture for the meat

model, as well as after cooking and refrigerated storage periods 1, 3, 6 and 9 days of storage.

Physical tests

Thawing loss

The thawing loss percentage of sausage patties was determined using the method described by **Hemn (2021)**. The process involved weighing the solid sausage patties before placing them on a wire rack in the refrigerator for 24 hours until complete thawing. Afterward, the dripping water was removed, and the patties were reweighed to calculate the thawing loss percentage using the following equation:

$$\text{Thawing loss\%} = \frac{\text{freezing sample wt.} - \text{sample wt. removed water}}{\text{freezing sample wt.}} \times 100$$

Cooking loss

The cooking loss is measured according to the method described by **Meiselman (2019)** to measure the weight loss during the cooking process of foods such as sausages. It involves measuring the difference between the initial sample weight (before cooking) and the final sample weight (after cooking), and calculating the percentage of cooking loss using the following equation:

$$\text{Cooking loss\%} = \frac{\text{sample wt. before cooking (g)} - \text{sample wt after cooking (g)}}{\text{sample wt. before cooking (g)}} \times 100$$

Diameter change after cooking

The percentage of diameter change after cooking for the sausage discs was calculated using the method described by **Meiselman (2019)**. The diameter of three discs from each treatment was measured using a Vernier caliper to obtain three readings for each disc before and after cooking. The percentage of diameter change after cooking was calculated using the following equation:

$$\text{Diameter change \%} = \frac{\text{Diameter before cooking (cm)} - \text{Diameter after cooking (cm)}}{\text{Diameter before cooking (cm)}} \times 100$$

Thickness change after cooking

The percentage of thickness change after cooking for the sausage discs was calculated using the method described by **Meiselman (2019)**. The thickness of three discs from each treatment was measured using a Vernier caliper, with three readings taken for each disc before and after cooking. The percentage of thickness change after cooking was calculated using the following equation:

$$\text{Thickness change \%} = \frac{\text{Thickness before cooking (cm)} - \text{Thickness after cooking (cm)}}{\text{Thickness before cooking (cm)}} \times 100$$

Sensory evaluation

A sensory evaluation study was conducted for the produced sausage by using Hedonic Scale method of five points (like, like very much, neither like or dislike, dislike, and dislike very much), by ten experienced individuals -(4 males and 6 females)-specialized in food science at the College of Agriculture, University of Anbar. The sausages were evaluated for attributes such as color, flavor, juiciness, tenderness, and overall acceptability. The sensory evaluation was conducted after cooking by experts in the field of food. Based on the sensory evaluation, the level of acceptability and palatability of the product by consumers was determined, according to previous studies conducted by **Meiselman (2019)** and **Adriana et al. (2022)**.

Statistical analysis and mathematical model:

The data was analyzed using a factorial experiment (4×9) with a completely randomized design (CRD) to study the effects of the treatment and storage duration on different attributes. Means were compared using the Duncan's multiple range test (**Gerstman, 2014**). The SAS program (2012) was used for statistical analysis of all data.

RESULTS AND DISCUSSION

Chemical analysis

Major components in sausage production

The result illustrates the chemical composition of the beef used in sausage production. It can be observed that the meat had a high moisture content of 74.33%. Other components such as protein, ash, and fat had average values of 21.23%, 1.31%, and 3.13%, respectively. Table (2) showed that chemical composition of beef ranged within normal composition of beef, the minor different can be attributed to factors such as the nature, breed, and age of the animal, as well as the diet and environmental conditions, which can influence the meat composition. The variation in chemical composition among the legumes used can be attributed to the genetic nature of the cultivars employed in the study.

The statistical analysis results demonstrate significant differences (P<0.01) in the moisture, protein, fat, carbohydrate and ash content of the produced sausages compare to control treatment (TC). Skylas et al., 2019; Pelagalli et al., 2020 reported that the chemical composition of broad bean seeds depends significantly on genetic composition, farming practices, environmental conditions, and agricultural management expertise. The variation in composition contents may be attributed to the effect of additives from crushed legume seeds, which were used to replace part of the meat. Furthermore, the high protein content in mash seeds, which slightly differed from chickpeas in this aspect, resulting in an improvement and enhancement of this characteristic in the produced sausages. These results are consistent with the findings of Gasim et al. (2015); Khan et al., (2015); Chelladurai, and Erkinbaev, (2020) and Monnet, et al., (2019). Whom reported that broad bean seeds contain crude protein range from 21 - 34%. The total carbohydrate content in broad bean seeds ranges from 457 to 701 g/kg of dry matter, including sugars, starch, and fibers. The carbohydrate content in lentil seeds

ranges from 62% to 69% and includes starch (35-53%) with a low glycemic index (GI) of 21-22.

Table 2 Average values of Chemical Composition of beef meat, check peas and mash

Components %	Beef Meat	Check Peas	Mash
Moisture	74.33 ±.29	11.00 ±.21	10.40 ±.15
Protein	21.23 ±.004	23.40 ±.006	24.10 ±.005
Fat	3.13 ±.004	2.00 ±.007	1.30 ±.006
Carbohydrate	-	60.20 ±1.10	60.80 ±.25
Ash	1.31 ±.01	3.40 ±.15	3.50 ±.02

Estimation of hydrogen ion concentration (pH)

As seen in the analysis of variance table (3), the interaction effect between the treatment means and the refrigeration storage period on the pH value of chilled meat sausages is evident. Significant differences (P<0.01) were observed among the storage periods, with the highest pH value recorded at 9 days of storage with an average of 6.182. Furthermore, significant differences (P<0.01) were observed among the treatments, with treatment T2M²⁰ recording the highest average of 6.216 for this characteristic. Regarding the interaction between treatments and refrigeration periods, treatment T2M²⁰ exhibited the highest average for the characteristic at 9 days of storage, reaching 6.286. This result is close to study conducted by Maqsood, et al., (2015) and Al-Alwani, (2017) their conclusions were, a degradation of meat proteins as the storage period progresses, resulting in an increase in nitrogen compounds, which leads to an increase in pH value.

Table 3 The pH value of processed sausages

Treatments	Storage time day/s				Treatment means
	1	3	6	9	
pH					
TC	6.165	6.205	6.225	6.125	6.180 ^{dfg}
	T1K ⁵	6.135	6.055	6.196	6.120 ^b
Chickpea	T1K ¹⁰	6.085	6.125	6.165	6.130 ^{bc}
	T1K ¹⁵	6.216	6.236	6.056	6.184 ^{fg}
	T1K ²⁰	6.236	6.196	6.196	6.213 ^h
	T2M ⁵	6.065	6.115	6.205	6.095
Mash	T2M ¹⁰	6.175	6.105	6.145	6.155 ^{cde}
	T2M ¹⁵	6.185	6.085	6.155	6.168 ^{def}
	T2M ²⁰	6.176	6.186	6.176	6.216 ^{gh}
	Day mean	6.158 ^b	6.145 ^a	6.169 ^{bc}	6.182 ^c

*Means with different letters are significantly different in probability (P ≤ 0.01).

Peroxide value (PV) test

From the analysis of variance table in table (4), the interaction effect between the treatments and refrigeration storage period on the PV of chilled meat sausages is evident. Significant differences (P<0.01) were observed among the storage periods, with the highest PV recorded at 9 days of storage, with an average of 1.646 meq/kg. The lowest average for this characteristic was recorded after one day of storage, with a mean of 1.117 meq/kg. Regarding the study treatments, TC exhibited the highest average for this characteristic at 2.071 meq/kg, while the lowest average was recorded in the T2K¹⁵ at 1.240 meq/kg. As for the interaction between storage days and additive concentrations, significant differences were observed. The highest average for this characteristic was recorded in the TC after

9 days of storage, reaching 3.181 meq/kg, while the lowest average was observed in T2M¹⁰ after one day of storage. Similar results were obtained by Al-Alwani, (2017), who indicated that peroxide and TBARS values of sausage beef with 20 ppm of MPEO were significantly lower than samples with 40 and 60 ppm of MPEO and control, at the end of storage period. This may be attributed to the natural additives in the treatments, which have been reported to inhibit fat oxidation and reduce PV, (O'Brien, 2008). It has been noted that the PV decreased in meat samples with the addition of legume powder compared to the control treatment during refrigeration storage. The natural additives have a clear inhibitory effect on fat oxidation in fresh meat, and this may be due to their influence on suppressing fat oxidation and reducing the PV, (Kim et al., 2016).

Table 4 Peroxide values (PV) of processed beef sausage

Treatments	Storage time day/s				Treatment means
	1	3	6	9	
PV (meq/kg)					
TC	1.162	1.501	2.441	3.181	2.071 ^f
	T1K ⁵	1.17	1.181	1.454	1.325 ^{bcd}
Chickpea	T1K ¹⁰	1.10	1.167	1.396	1.284 ^b
	T1K ¹⁵	1.116	1.123	1.304	1.414
	T1K ²⁰	1.11	1.337	1.347	1.667
	T2M ⁵	1.111	1.287	1.307	1.448
Mash	T2M ¹⁰	1.098	1.276	1.306	1.536
	T2M ¹⁵	1.111	1.294	1.327	1.449
	T2M ²⁰	1.116	1.298	1.306	1.436
	Day mean	1.1173 ^a	1.2888 ^b	1.4532 ^c	1.6456 ^d

*Means with different letters are significantly different in probability (P ≤ 0.01).

Determination of free fatty acids (FFAs)

As in the analysis of variance in table (5), the interaction effect between the treatments and refrigeration storage period on the free fatty acid ratio of chilled beef sausages is evident. Significant differences ($P < 0.01$) were observed among the storage periods, with the highest free fatty acid ratio recorded at 9 days of storage with an average of 0.4583%. The lowest average for this characteristic was recorded after one day of storage, with a mean of 0.2822%. Regarding the study treatments, the TC exhibited the highest average for this characteristic at 0.4540%, while the T1K⁵ recorded the lowest average at 0.3255%, similar findings were reported by Al-Dhaheri (2012) and Al-Alwani (2017). All treatments containing natural additives showed a higher reduction in the free fatty acid ratio compared to

the control treatment. The natural additives contain multiple phenolic compounds, which have stronger antioxidant activity than synthetic antioxidants. These compounds help break the reaction chain by donating a hydrogen atom to the fatty acid and free radicals (Jordan et al., 2014; Maqsood et al., 2015; Alawani, 2017). The study disagrees with finding by Krzysztof et al., (2021) who studied the fatty acid profile and oxidative changes (TBARS) in vacuum-packed (VP) or modified-atmosphere-packed (MAP) finely-comminuted poultry sausages with liquid fish oil and microencapsulated fish oil (MC) additives. They reported that an analysis of omega-3 fatty acids (EPA and DHA) showed that their content in the samples with the fish oil additive decreased from the initial value of 0.22 g·100 g⁻¹ of the product to 0.18 g·100 g⁻¹ (MAP) and 0.17 g·100 g⁻¹ (VP), respectively.

Table 5 Free fatty acids (FFA) ratio in processed sausage

Treatments	Storage time day/s				Treatment means	
	1	3	6	9		
	FFA %					
TC	0.3250	0.3950	0.4750	0.6200	0.4540 ^b	
Chickpea	T1K ⁵	0.2555	0.3155	0.3555	0.3755	0.3255 ^a
	T1K ¹⁰	0.2855	0.3355	0.3755	0.4155	0.3530 ^c
	T1K ¹⁵	0.2850	0.3150	0.3650	0.3850	0.3375 ^b
	T1K ²⁰	0.3155	0.3955	0.4555	0.5855	0.4380 ^g
Mash	T2M ⁵	0.2655	0.3355	0.3455	0.4155	0.3405 ^b
	T2M ¹⁰	0.2760	0.3560	0.3660	0.4060	0.3510 ^c
	T2M ¹⁵	0.2560	0.3560	0.3760	0.4260	0.3535 ^c
	T2M ²⁰	0.2760	0.3360	0.4360	0.4960	0.3860 ^d
Day mean	0.2822 ^a	0.3489 ^b	0.3944 ^c	0.4583 ^d	0.3710	

*Means with different letters are significantly different in probability ($P \leq 0.01$).

Thiobarbituric acid (TBA) test

Table (6) showed that significant differences ($P < 0.01$) were observed among the storage periods, with the highest TBA value recorded at 9 days of storage, averaging 0.0803 malondialdehyde (mg MDA)/kg. The lowest average for this characteristic was recorded after one day of storage, with a mean of 0.0267 MDA/kg.

Regarding the study treatments, the TC exhibited the highest average for this characteristic at 0.074 mg MDA/kg, while the T2M⁵ recorded the lowest average at 0.047 mg MDA/kg. As for the interaction between storage days and additive concentrations, significant differences were observed. The highest average for this characteristic was recorded in the TC after 9 days of storage, reaching 0.093 mg MDA/kg. The lowest average was observed at T2M⁵ after one day of storage, with a value of 0.0215 mg MDA/kg /kg. Similar results were obtained by Al-Alwani,

(2017), who indicated that peroxide and TBARS values of sausage beef with 20 ppm of MPEO were significantly lower than samples with 40 and 60 ppm of MPEO and control, at the end of storage period. The results mentioned above and the superiority of the sausages produced with additives over the TC can be attributed to the presence of crushed legume seeds, which contain various compounds that delay the process of fat oxidation. Numerous studies have confirmed that compounds derived from plant sources play a significant role in preventing oxidation due to their antioxidant properties (Kim et al., 2013; Maqsood et al., 2015). The increase in the TBA value for the TC in fresh minced beef stored under refrigeration may be due to the accumulation of MDA as the storage period progresses. Malondialdehyde is one of the secondary products of fat oxidation in meat and meat products due to the breakdown of peroxides (Alawani, 2017).

Table 6 Thio-barbituric acids (TBA) in processed sausage

Treatments	Storage time day/s				Treatment means	
	1	3	6	9		
	TBA (mg MDA/kg)					
TC	0.0280	0.0870	0.0860	0.0930	0.0735 ^d	
Chickpea	T1K ⁵	0.0260	0.0460	0.0850	0.0660	0.0558 ^b
	T1K ¹⁰	0.0330	0.0490	0.0810	0.0630	0.0565 ^b
	T1K ¹⁵	0.0330	0.0540	0.0630	0.0760	0.0565 ^b
	T1K ²⁰	0.0260	0.0720	0.0490	0.0880	0.0588 ^b
Mash	T2M ⁵	0.0215	0.0475	0.0495	0.0695	0.0470 ^a
	T2M ¹⁰	0.0270	0.0780	0.0450	0.0880	0.0595 ^b
	T2M ¹⁵	0.0216	0.0816	0.0466	0.0910	0.0602 ^b
	T2M ²⁰	0.0246	0.0736	0.0886	0.0886	0.0689 ^c
Day mean	0.0267 ^a	0.0654 ^b	0.0660 ^b	0.0803 ^c	0.059	

*Means with different letters are significantly different in probability ($P \leq 0.01$).

Microbiological analysis

The microscopic images demonstrate the results of the total bacterial count in fresh and refrigerated beef sausages for the periods of 1, 3, 6 and 9 days. It is observed that the growth of *Escherichia coli* bacteria cultured in MacConkey medium occurred after a storage period of 3 days in the control treatment. No contamination was observed in the rest of the treatments until the sixth day of storage. This indicates the positive effect of the additives on the manufactured sausages, as they extended the refrigerated storage period, and the ratio of the additive corresponded proportionally with the storage periods. Similarly, the results of bacterial culture for *Salmonella* on S.S agar medium, no contamination observed. This indicates

that the meat used in the study was free from contamination, and the produced sausages were not exposed to any type of contamination during the manufacturing process. The finding was in line with Atef et al. (2015) and Ibrahim, (2008) who were not detected *E. coli* and salmonella bacteria, but disagree with Elhashmi et al., (2021) who detected *E. coli* and salmonella in beef sausage. The study disagrees with finding by Fahim et al., (2021), who reported that the percentages of Salmonellae in the examined samples of *kofta*, liver, *shawerma* and sausage products were 32%, 60%, 8% and 40%, respectively. The obtained results indicated that the incidence of *E. coli* was 20%, 8% and 32% and 40% in the examined *kofta*, *shawerma*, sausage and liver samples, respectively.

Table 7 Detection of bacterial growth for *Escherichia coli* and *Salmonella*

Treatments	Storage time day/s			
	1	3	6	9
	Growth of <i>E. coli</i> / <i>Salmonella</i>			
TC	-/-	+/-	+/-	+/-
Chickpea	T1K ⁵	-/-	+/-	+/-
	T1K ¹⁰	-/-	-/-	-/-
	T1K ¹⁵	-/-	-/-	-/-
	T1K ²⁰	-/-	-/-	-/-
Mash	T2M ⁵	-/-	-/-	-/-
	T2M ¹⁰	-/-	-/-	+/-
	T2M ¹⁵	-/-	-/-	+/-
	T2M ²⁰	-/-	-/-	-/-

*Positive growth = (+) and negative growth = (-).

Table 8 The Water Holding Capacity of processed sausages

Treatments	Storage time day/s				Treatment means
	1	3	6	9	
	WHC%				
TC	26.26	27.2	43.12	29.58	31.54 ^a
Chickpea	T1K ⁵	39.99	30.5	42.14	34.68 ^b
	T1K ¹⁰	40.11	32.85	33.89	35.15 ^{bc}
	T1K ¹⁵	43.64	43.14	39.08	41.24 ^e
	T1K ²⁰	34.84	52.78	20.52	36.65 ^f
Mash	T2M ⁵	35.89	40.9	31.59	35.30 ^e
	T2M ¹⁰	43.29	34.97	32.9	35.50 ^e
	T2M ¹⁵	35.83	43.6	26.6	36.77 ^f
	T2M ²⁰	39.28	44.68	19.79	36.09 ^e
Day mean	37.68 ^c	38.96 ^d	32.18 ^a	34.69 ^b	35.88

*Means with different letters are significantly different in probability (P ≤ 0.01).

Physicochemical tests.

Water holding capacity (WHC)

The analysis of variance in table (8) reveals significant differences (P<0.01) in the WHC of the meat among different storage periods. The storage treatment after 3 days of refrigeration recorded the highest mean value of 38.96, while the storage treatment after 6 days of refrigeration had the lowest mean value of 32.18. Furthermore, table (8) shows that treatment T1K¹⁵ had the highest mean value (41.24%), while control treatment had the lowest mean value (31.54%) for WHC among all the treatments. The findings were greater than **Elhashmi et al., (2021)**, who found that the water holding capacity of sausage processed from ostrich and beef were 2.24, 3.35 respectively, but lower than **Ibrahim, (2008)** who stated out that the water holding capacity of beef sausage was (59.55%). This result is in agreement with the results of previous reports by **Eilers et al., (2021)**, showing that the extent of the pH decrease is a key factor influencing meat quality. Moreover, less cooking loss was observed in the high pH group than the intermediate and low pH beef (P < 0.05) at all ageing periods. All groups had no difference in percentage of cooking loss throughout 9 days of postmortem ageing (P ≥ 0.05). This can be attributed to the presence of active compounds in these treatments, which have the ability to protect cell membranes from damage, thereby preserving proteins and preventing the loss of water. This leads to the water remaining bound to the protein through protein-water bonds (**Viuda-Martos et al., 2015**).

The percentage of drip loss after thawing

Figure (1) show the drip loss percentage among the study treatments. There were no significant differences (P>0.05) observed among the different treatments, with the highest value recorded for treatment T1K¹⁵ (24.07) and the lowest value recorded for the T2M¹⁵ (13.57%). These findings in the beef burger were less than that found by **Ibrahim (2008)** who observed that drip loss of beef burger was (68.26%) however, these findings is greater than that found by **Elhashmi et al., (2021)**, were (3.52 %). They observed that the treatments with higher additive concentrations exhibited an inverse relationship with drip loss after thawing, indicating a positive effect of the high concentrations of legume seeds on reducing the drip loss. This may be due to protein degradation caused by enzymes, which could lead to subtle changes in cell membrane permeability or protein structure, resulting in a decreased ability of the meat to retain water, particularly water bound to proteins, and consequently an increase in its proportion (**Alawani, 2017**).

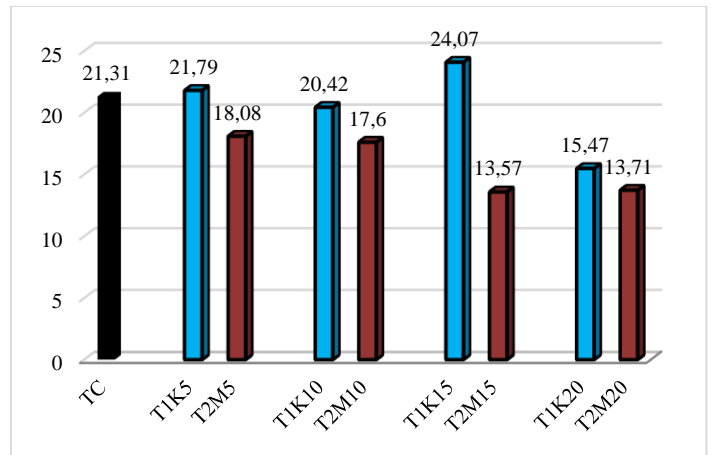


Figure 1 The percentage of drip loss after thawing sausages

The analysis of variance showed significant differences (p<0.05) among the treatments in weight loss after cooking, figure (2). This was particularly evident in treatment TC, T1K⁵, and T2M¹⁰, which recorded the highest mean values of 48.68, 41.65, and 40.78%, respectively. Conversely, treatment T1M²⁰ recorded the lowest level of this attribute at 37.38%. This could be attributed to the melting of fats within the sausages, which was more pronounced in treatments with lower additive concentrations (below 10%). However, higher concentrations of additives preserved the weight compared to the lower concentrations. These findings were greater than that obtained by **Elhashmi et al., (2021)**, who found that the cooking loss was (38.41%) and **Ibrahim, (2008)** reported the cooking loss was (42.34%) was lower than our study. This may be due to a significant decrease in the weight loss during cooking and shrinkage in the sausage fingers treated with plant extracts compared to the control treatment. This is possibly a result of the antioxidant compounds' ability to increase the meat tissue's water-holding capacity and reduce water loss during storage and cooking (**Alzari, 2016**). Furthermore, the analysis of variance indicated significant differences (p>0.05) in the diameter changes of sliced sausages after cooking, figure (3). The highest and lowest mean values of diameter loss were recorded by treatments T2M⁵ (23.85%) and T2M¹⁵ (12.64%), respectively. These findings were greater than that obtained by **Elhashmi et al., (2021)**, who found that the diameter changes were (10.59%) and **Ibrahim, (2008)** reported the diameter changes was (26.19%) was lower than our study. From these results, it can be observed that different concentrations of chick-peas had a negative effect on the diameter changes after cooking, while the addition of low concentration (5%) of mung beans had a positive effect on maintaining the diameter stability of the sausages after cooking. Similarly, the analysis of variance results indicates significant differences (P<0.05) in figure (3) the thickness changes of sliced sausages after cooking. Treatment

T2M¹⁵ recorded the highest mean value of 22.40% for thickness changes, while treatments T1K⁵ and T1K¹⁰ did not show any significant thickness changes during the cooking process. Overall, the additive treatments did not significantly differ from each other in terms of thickness changes, which is considered a positive indication for the additives. The finding was in line with Adeniyi et al., (2018), who found that the thickness changes was (21.91%) and Ibrahim, (2008) reported it was (17.24%) was lower than our study.

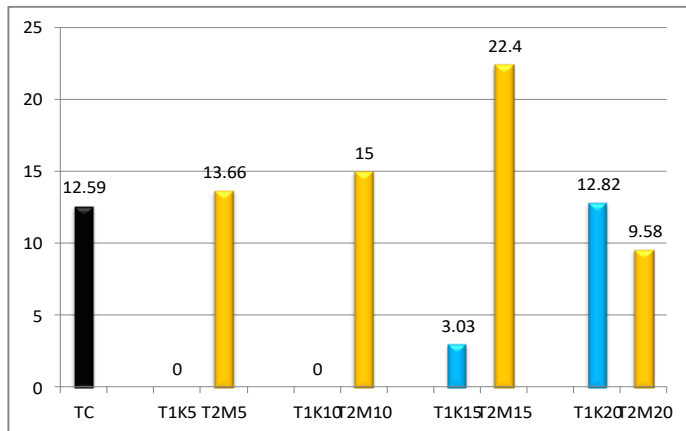


Figure 2 The Percentage of changes in sausage thickness after cooking

Sensory evaluation

The results from the analysis of variance in Fig. (4) indicate that there were no significant differences (P>0.01) in flavor, texture, and overall acceptability between different treatments. The flavor attribute had the highest average in treatment T2M5, with a mean of 7.267, while the lowest average for this attribute was recorded in treatment T1K10, with a mean of 6.133. The texture attribute had the highest average in TC and T1K5 treatments, with a mean of 6.733, while the lowest average was recorded in treatment T2M10, with a mean of 6.067. Similar results were reported by Adeniyi et al., (2018), who reported that soy burger is similar to chicken burger in color attributes. It is more preferred than chicken burger in taste, texture and overall acceptability.

The lack of significant impact from the added crushed legume seeds can be seen as a positive indicator for consumers, indicating that the sausages were not negatively affected by the plant additives. Additionally, the addition of legume seed powder may have improved juiciness, flavor, and freshness while acting as an antioxidant, thus preserving the meat flavor, preventing off-flavors, and maintaining meat moisture, which positively influenced overall acceptability (Cai et al., 2015; Alawani, 2017).

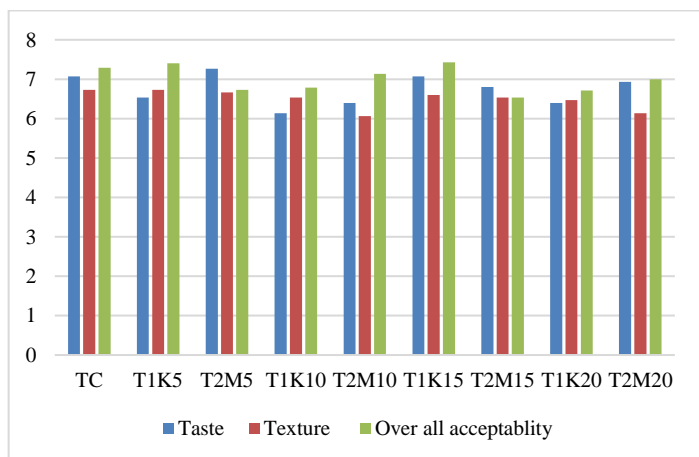


Figure 3 Results of sensory evaluation of treats

CONCLUSION

The addition of crushed legume seeds ranges from 5 – 20% had a positive effect on extending the storage periods, reducing contamination, increased the protein and ash content, thus enhancing the nutritional value of the sausages. Increasing the concentration of crushed legume seed substitution helped reduce the peroxide value, free fatty acid percentage, as these crushed seeds contain compounds that inhibit fat oxidation. The additive legumes had a positive effect on the loss during thawing, weight loss and thickness, diameter loss after cooking, improved juiciness, flavor, and freshness while acting as an antioxidant, thus preserving the meat flavor, preventing off-flavors, and maintaining meat moisture, which positively influenced overall acceptability.

REFERENCES

Adriana, G. P., Laure, S., & Julien, D. (2022). Hedonic response sensitivity to variations in the evaluation task and culinary preparation in a natural consumption context. *Frontiers in Nutrition*, 9, 1008577. <https://doi.org/10.3389/fnut.2022.1008577>

Adeniyi, P. O., Obatolu, V. A., & Kehinde, H. A. (2018). Comparative evaluation of the nutritional, physical, and sensory properties of beef, chicken, and soy burgers. *Journal of Agriculture and Food Sciences Research*, 5(2), 57–63. <https://doi.org/10.20448/journal.512.2018.52.57.63>

Association of Official Analytical Chemists (AOAC). (2005). *Official methods of analysis: Microbiological food testing* (Chapter 17). USA.

Al-Alwani, D. H. (2017). The effect of adding carnosic acid and rosemary on fresh and cooked ground beef during refrigerated storage for different periods [Master's thesis]. College of Agriculture, Al-Qasim Green University, Iraq.

Al-Dhaheeri, S. K. M. (2012). The effect of adding marjoram (*Origanum majorana* L.) and its extracts on some quality characteristics of frozen ground beef [Master's thesis]. College of Agriculture, University of Baghdad.

Al-Faidi, I. A. (1996). Technical and technological study for the development of fermented Iraqi sausages [PhD thesis]. College of Agriculture, University of Baghdad.

Ali, Z. I., Shawki, M. A., Abdel-Latif, E. F., & Saudi, A. M. (2024). Assessment of fatty acid content and lipid nutritional quality indicators for some cheese kinds using gas chromatography–mass spectrometry. *Journal of Advanced Veterinary Research*, 14(7), 1258–1263.

Andres, S., Huerga, L., Mateo, J., Tejido, M. L., Bodas, R., & Moran, L. (2014). The effect of quercetin dietary supplementation on meat oxidation processes and texture of fattening lambs. *Meat Science*, 96, 806–811. <https://doi.org/10.1016/j.meatsci.2013.09.020>

Atef, A. A., Nahed, M. A., Amr, A., & May, M. A. (2015). The effect of arak stems extracts on chemical characteristics, bacterial activity, and sensory evaluation of beef sausage products. *International Journal of Advances in Agricultural and Environmental Engineering*, 2, 39–43. <https://doi.org/10.15242/ijaaec.c0815031>

Cai, R., McCurdy, A., & Baik, B. K. (2015). Textural property of 6 legume curds in relation to their protein constituents. *Journal of Food Science*, 67, 1725–1730. <https://doi.org/10.1111/j.1365-2621.2002.tb08713.x>

Capita, R., Liorente-Marigomez, S., Prieto, M., & Carlos, A.-C. (2006). Microbiological profiles, pH, and titratable acidity of Chorizo and Salchichón (two Spanish sausages) manufactured with ostrich, deer, or pork meat. *Journal of Food Protection*, 69(5), 1183–1189. <https://doi.org/10.4315/0362-028x-69.5.1183>

Chelladurai, V., & Erkinbaev, C. (2020). Lentils. In *Pulses: Processing and product development* (pp. 129–143).

Domínguez, R., Barba, F. J., Gómez, B., Putnik, P., Kovačević, D. B., Pateiro, M., Santos, E. M., & Lorenzo, J. M. (2018). Active packaging films with natural antioxidants to be used in meat industry: A review. *Food Research International*, 113, 93–101. <https://doi.org/10.1016/j.foodres.2018.06.073>

Dzudie, T., Kouebou, C. P., Essia-Ngang, J. J., & Mboufong, C. M. F. (2004). Lipid sources and essential oils effect on quality and stability of beef patties. *Journal of Food Engineering*, 65, 67–72. <https://doi.org/10.1016/j.jfoodeng.2003.12.004>

Edirisingha, D. N. S. A., Kichang, N., & Dong, U. A. (2021). Analytical methods for lipid oxidation and antioxidant capacity in food systems. *Antioxidants*, 10(10), 1587. <https://doi.org/10.3390/antiox10101587>

Eilers, J. D., Tatum, J. D., Morgan, J. B., & Smith, G. C. (2021). Modification of early-postmortem muscle pH and use of postmortem aging to improve beef tenderness. *Journal of Animal Science*, 74(4), 790–798.

Elhashmi, Y. H., Falih, S. Y., & Abdalmageed, M. E. I. (2021). Characteristics of ostrich meat in manufacturing sausage in comparison with beef. *Online Journal of Animal and Feed Research*, 11(1), 23–27. <https://doi.org/10.51227/ojaftr.2021.5>

Food and Agriculture Organization (FAO). (2011). *FAOSTAT*. <http://faostat.fao.org/site/291/default.aspx>

Fahim, A. E. S., Mohammed, F., Hosam, A. A., & Mostafa, E. M. (2017). Incidence of E. coli and Salmonellae in ready-to-eat fast foods. *Benha Veterinary Medical Journal*, 32(1), 18–22.

Gasim, S., Hamad, S. A., Abdelmula, A., & Mohamed Ahmed, I. A. (2015). Yield and quality attributes of faba bean inbred lines grown under marginal environmental conditions of Sudan. *Food Science & Nutrition*, 3(6), 539–547. <https://doi.org/10.1002/fsn3.245>

Gerstman, B. B. (2014). *Basic biostatistics*. Jones & Bartlett Learning.

Heide, S. (2021). *Salmonella: Methods and protocols* (3rd ed., Vol. 2182). Springer. ISBN 978-1-0716-0790-9.

Hemn, G. Z. (2021). Impact of different thawing methods on physico-chemical characteristics, electrophoretic profile, and sensory evaluation of frozen beef Longissimus dorsi muscle. *Journal of Animal and Poultry Production*, 12(1), 7–14.

Ibrahim, S. (2008). Utilization of wheat germ flour in the processing of sausage [Master's thesis]. Khartoum University, Sudan.

Jordan, M. J., Castillo, J., Banon, S., Martínez-Conesa, C., & Sotomayor, J. A. (2014). Relevance of the carnosic acid/carnosol ratio for the level of rosemary diterpene transfer and for improving lamb meat antioxidant status. *Food Chemistry*, 151, 212–218. <https://doi.org/10.1016/j.foodchem.2013.11.068>

- Khan, M. A., Ammar, M. H., Migdadi, H. M., El-Harty, E. H., Osman, M. A., Farooq, M., & Alghamdi, S. S. (2015). Comparative nutritional profiles of various faba bean and chickpea genotypes. *International Journal of Agriculture and Biology*, 17(3), 449–457. <https://doi.org/10.17957/ijab/17.3.14.990>
- Kim, H. S., & Chin, K. B. (2016). Evaluation of different drying temperatures on physico-chemical and antioxidant properties of water-soluble tomato powders and on their use in pork patties. *Journal of the Science of Food and Agriculture*, 96, 742–750. <https://doi.org/10.1002/jsfa.7141>
- Kim, S.-J., Cho, A. R., & Han, J. (2013). Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control*, 29, 112–120. <https://doi.org/10.1016/j.foodcont.2012.05.060>
- Krzysztof, K., Jerzy, S., & Piotr, K. (2021). An analysis of oxidative changes and the fatty acid profile in stored poultry sausages with liquid and microencapsulated fish oil additives. *Molecules*, 26(14), 4293. <https://doi.org/10.3390/molecules26144293>
- Maqsood, S., Abushelaibi, A., Manheem, K., Al Rashedi, A., & Kadim, I. T. (2015). Lipid oxidation, protein degradation, microbial and sensorial quality of camel meat as influenced by phenolic compounds. *LWT-Food Science and Technology*, 63, 953–959. <https://doi.org/10.1016/j.lwt.2015.03.106>
- Meiselman, H. L. (2019). *The effects of environment on product design and evaluation* (1st ed.). Woodhead Publishing.
- Meyer, A. S., Suhr, K. I., Nielsen, P., & Kolm, F. (2002). Minimal processing technologies in the food industry. In *Natural food preservation* (Chapter 6). Woodhead Publishing. <https://doi.org/10.1201/9781439823132.ch6>
- Monnet, A. F., Laleg, K., Michon, C., & Micard, V. (2019). Legume enriched cereal products: A generic approach derived from material science to predict their structuring by the process and their final properties. *Trends in Food Science & Technology*, 86, 131–143. <https://doi.org/10.1016/j.tifs.2019.02.027>
- Noor, R. (2014). Microbiological analysis of common preservatives used in food items and demonstration of their in vitro anti-bacterial activity. *Asian Pacific Journal of Tropical Diseases*, 4, 452–456. [https://doi.org/10.1016/s2222-1808\(14\)60605-8](https://doi.org/10.1016/s2222-1808(14)60605-8)
- O'Brien, R. D. (2008). *Fats and oils: Formulating and processing for applications* (3rd ed.). CRC Press. <https://doi.org/10.1201/9781420061673.ch2>
- Pelagalli, A., Musco, N., Trotta, N., Cutrignelli, M. I., Di Francia, A., Infascelli, F., & Calabrò, S. (2020). Chemical characterisation and in vitro gas production kinetics of eight faba bean varieties. *Animals*, 10(3), 398. <https://doi.org/10.3390/ani10030398>
- Simon, A. A., Benjamin, M. S., & Patrick, O. L. (2021). Comparative assessment of the relationship between coliform bacteria and water geochemistry in surface and ground water systems. *PLoS ONE*, 16(9), e0257715. <https://doi.org/10.1371/journal.pone.0257715>
- Skylas, D. J., Paull, J. G., Hughes, D. G., Gogel, B., Long, H., Williams, B., & Quail, K. J. (2019). Nutritional and anti-nutritional seed-quality traits of faba bean (*Vicia faba*) grown in South Australia. *Crop and Pasture Science*, 70(5), 463–472. <https://doi.org/10.1071/cp19017>
- Sultana, T., Rana, J., Chakraborty, S. R., Das, K. K., Rahman, T., & Viuda-Martos, M. (2015). Assessment of chemical, physicochemical, techno-functional, and antioxidant properties of fig (*Ficus carica* L.) powder co-products. *Industrial Crops and Products*, 69, 472–479. <https://doi.org/10.1016/j.indcrop.2015.03.005>
- Yolmeh, M., Najafi, M. B. H., & Farhoosh, R. (2014). Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). *Food Chemistry*, 155, 319–324. <https://doi.org/10.1016/j.foodchem.2014.01.059>
- Zulfa, Z., Chia, C. T., & Rukayadi, Y. (2016). In vitro antimicrobial activity of *Cymbopogon citratus* (lemongrass) extracts against selected foodborne pathogens. *International Food Research Journal*, 23(3), 1262. <https://doi.org/10.3390/foods5030052>