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Effect of Chickpeas and Lentil Seed Powder on the Refrigerated Storage Period and Quality Characteristics of Beef Sausages

Moawyal. Y. Abdalla1 Ahmed R. M. Abd2, Tilal M. A. Mudawi3 , Saad I. Yousif 4*

1. Department of Food Science and Technology, Faculty of Agricultural Sciences, University of Gezira, WadMedani, Sudan.
2. Department of Food Inspection, Anbar Municipalities Directorate, Anbar, Iraq.
3. Goat Research Center, Faculty of Agricultural Sciences., University of Gezira, Sudan.
4. Dept.of Food science, Collage of Agriculture, University of Anbar, Anbar, Iraq.

*Corresponding author email: moawya2009@yahoo.co.uk

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ABSTRACT

Meat and its products such as sausages are susceptible to spoilage during storage due to microbial contamination and fat oxidation, which generally leads to deterioration in quality, consequently reducing their shelf life. The objectives of this study were to study the effect of replacing a portion of the main mixture with different ratios of ground legume seeds on the refrigerated storage period and to evaluate the chemical, physical, and sensory characteristics of the produced beef sausages. Beef sausages were manufactured by replacing a portion of the main mixture with 5%, 10%, 15% and 20% of ground legume seeds, chickpeas (P) and lentils (L) with control treatment (TC). The sausages were stored under refrigeration (4 - 8 °C) for 1, 3, 6 and 9 days. The results indicated that the chemical analysis of the manufactured sausages showed the highest and lowest moisture, ash, protein, and fat percentages for treatments TC, $T1P^{20}$, $T1P^{20}$,$T2L^{5}$, $T2L^{10}$, TC, TC and $T2L^{20}$ with values of 62.59, 51.77, 2.56, 1.86, 23.44, 20.02, 13.91 and 11.03, respectively. The differences between the mean pH values of the treatments were significant ($P \leq 0.01$) after refrigerated storage. The storage for a period of 3 days recorded the highest average (6.169), while treatment $T2L^{20}$ recorded the highest average (6.275). Regarding the interaction between treatments and refrigerated storage periods, treatment $T2L^{20}$ combined with 6 days of storage showed the highest pH value of 6.31. The peroxide value analysis showed significant differences ($P < 0.01$) between the mean values of the treatments. TC recorded the highest value (2.071), while the lowest average (1.24) was recorded in $T1P^{15}$. The results of the interaction demonstrated that treatment $T1P^{15}$ combined with 9 days of storage recorded the lowest peroxide value (1.41). The analysis of free fatty acids (FFA) also showed significant differences ($P \leq 0.01$) between the mean values of the treatments. TC outperformed all other treatments with an average of 0.455. The interaction between treatments and storage periods was significant ($P < 0.01$), and the lowest FFA value was recorded with the interaction of $T1P^{5}$ and one day of storage, with an average of 0.255. The bacterial tests indicated a positive effect of replacement on storage periods, as the refrigerated storage periods correlated positively with the concentrations of legume seed replacement. No contamination with *Salmonella* bacteria was observed during all storage periods. $T1P^{15}$ 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 ($P \leq 0.01$) 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 refrigerated storage life, and improved the quality attributes, and overall acceptance of the product.

INTRODUCTION

Meat is considered one of the most important sources of nutrition for humans, as it contains a high nutritional value that includes a wide range of essential nutrients. It primarily contains a high proportion of protein, which is an important element needed by the body for tissue building and repair. Protein consists of several essential amino acids that the body requires. Meat also contains various other nutrients, such as fats, minerals, and vitamins, which play a vital role in maintaining overall body health. Therefore, consuming meat is necessary for obtaining a balanced and healthy diet (Dominguez *et al*., 2018). Meat products have high nutritional values due to their physiological and chemical characteristics. However, they prone to spoilage during storage due to microbial contamination and fat oxidation, which generally results in changes in color, taste, and texture, and reduces its shelf life (Yolmeh *et al*., 2014).

Sausages are processed meat products that usually consist of beef often combined with spices, preservatives, salt, and water. Manufacturers may add additional ingredients such as starch, gluten, salt, and preservatives to improve texture and taste. The composition of sausages varies from one company to another, but generally, lower-value meats and tissues are used to reduce costs. The meats used in sausage making are typically finely minced and mixed with spices and other ingredients before being stuffed into thick artificial casings (Dominguez *et al*., 2018). Commonly added ingredients to sausages include salt, sugar, paprika, garlic, onion, sodium nitrite, and water. Additional components like gluten, starch, preservatives, and nitrate salts may be added as needed (F.A.O., 2011).

Recent studies have focused on the use of natural additives, such as legumes, as a safe source of plant-based protein instead of synthetic chemicals added to meat and meat products. These synthetic additives have raised concerns among consumers due to their harmful effects on human health and their association with cancer (Meyer *et al*., 2002). In recent times, consumers' interest in food safety has increased due to the excessive use of preservatives and chemicals in food production, which negatively affects consumer health. Therefore, the idea of using natural enhancers and additives emerged to improve the nutritional value of products, enhance their preservation, and promote taste, serving as a safe alternative to preservatives (Zulfa *et al*., 2016). 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 aim of the study was to investigate the effect of different concentrations of crushed legume seeds on the quality of the sausage and its refrigerated storage periods.

MATERIALS AND METHODS

Site of the study

The experiments were 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.

Method of manufacturing

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 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 cm^3 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 (P) and lentils (L) 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 by conducted in a refrigerator at a temperature of (4-8ºC). After that, physical, chemical, and microbiological tests were performed after 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 tests

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)

According to method described by Srinivasan *et al.*, (1998) the percentage of FFAs based on oleic acid was calculated using the following equation:

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

Peroxide value estimation

The peroxide value was calculated according to Srinivasan *et al.*, (1998) by following equation:

$$PV = \frac{\text{volume of } Na_2\,S_2\,O_3\ ml\ \times\ Na_2\,S_2\,O_3\ \text{con.}}{\text{Sample Wt. (g)}} \times 1000$$

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.

Bacterial Tests

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 APHA (1978). The plates were then allowed to harden and incubated at 37°C for 24 hours. Afterward, the colony count was estimated using a microscope to determine the CBC in the sample.

Testing for the presence of *Salmonella* bacteria

The test was performed according to the method described by Al-Dulaimi (1988) 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 Nam *et al*. (2000). 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:

$$thawing\ loss\% = \frac{Freezing\ Sample\ Wt. - Samle\ Wt. removed\ water}{Freezing\ Sample\ Wt.} \times 100$$

Sensory evaluation

A sensory evaluation study was conducted for the produced sausage by ten experienced individuals 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 Cross *et al*. (1978) and Igene *et al*. (1979).

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

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). 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 lentil 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).

Table (2): Average values of Chemical Composition of beef meat, check peas and lentils.

Components %	Beef Meat	Check Peas	Lentils
Moisture	74.33	11.00	12.40
Protein	21.23	23.40	25.10
Fat	3.13	2.00	0.70
Carbohydrate	-	60.20	59.70
Ash	1.31	3.40	2.10

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 3 days of storage with an average of 6.169. Furthermore, significant differences (P≤0.01) were observed among the treatments, with treatment $T2L^{20}$ recording the highest average of 6.275 for this characteristic. Regarding the interaction between treatments and refrigeration periods, treatment $T2L^{20}$ exhibited the highest average for the characteristic at 6 days of storage, reaching 6.31. This may be attributed to the degradation of meat proteins as the storage period progresses, resulting in an increase in nitrogen compounds, which leads to an increase in pH value (Al-Alwani, 2017).

Peroxide value (PV) tests

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 1.725 meq/kg. The lowest average for this characteristic was recorded after one day of storage, with a mean of 1.120 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 T2P^{15} 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 control treatment after 9 days of storage, reaching 3.181 meq/kg, while the lowest average was observed in T2L^{15} after one day of storage. 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).

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 ($P \leq 0.01$) were observed among the storage periods, with the highest free fatty acid ratio recorded at 9 days of storage with an average of 0.5002 %. The lowest average for this characteristic was recorded after one day of storage, with a mean of 0.2898 %. Regarding the study treatments, the TC exhibited the highest average for this characteristic at 0.4550 %, while the T1P^{5} 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).

Table (3): The pH value of processed sausages

Treatment		Storage time (day/s)				Treatment mean
		1	3	6	9	
		pH				
TC		6.165	6.205	6.225	6.125	**6.180^{efg}**
Chickpea	**T1P^{5}**	6.135	6.055	6.196	6.095	**6.121^{b}**
	T1P^{10}	6.085	6.125	6.165	6.145	**6.130^{bc}**
	T1P ^{15}	6.216	6.236	6.056	6.226	**6.184^{fg}**
	T1P^{20}	6.236	6.196	6.196	6.225	**6.213^{h}**
Lentil	**T2L^{5}**	6.015	6.055	6.175	6.115	**6.090^{a}**
	T2L^{10}	6.105	6.035	6.095	6.085	**6.081^{a}**
	T2L^{15}	6.205	6.105	6.105	6.195	**6.153^{cd}**
	T2L^{20}	6.29	6.23	6.31	6.27	**6.275^{i}**
Day mean		**6.165^{b}**	**6.169^{a}**	**6.138^{bc}**	**6.161^{c}**	**6.158**

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

Table (4): peroxide values (PV) of processed sausage

Treatment		Storage time (day/s) 1	3	6	9	Treatment mean
		PV (meq/kg)				
TC		1.162	1.501	2.441	3.181	**2.071^{f}**
Chickpea	**T1P^{5}**	1.17	1.181	1.454	1.493	**1.325^{bcd}**
	T1P^{10}	1.10	1.167	1.396	1.474	**1.284^{b}**
	T1P^{15}	1.116	1.123	1.304	1.414	**1.240^{a}**
	T1P^{20}	1.11	1.337	1.347	1.667	**1.365^{de}**
Lentil	**T2L^{5}**	1.113	1.351	1.353	1.638	**1.364^{de}**
	T2L^{10}	1.108	1.35	1.528	1.612	**1.400^{e}**
	T2L^{15}	1.097	1.296	1.415	1.558	**1.342^{cd}**
	T2L^{20}	1.112	1.293	1.407	1.486	**1.325^{bcd}**
Day mean		**1.1209^{a}**	**1.2888^{b}**	**1.5161^{c}**	**1.7248^{d}**	**1.413**

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

Table (5): free fatty acids (FFA) ratio in processed sausage.

Treatment		Storage time (day/s) 1	3	6	9	Treatment mean
		FFA %				
TC		0.325	0.395	0.475	0.62	**0.4550^{h}**
Chickpea	T1P^{5}	0.2555	0.3155	0.3555	0.3755	**0.3255^{a}**
	T1 P^{10}	0.2855	0.3355	0.3755	0.4155	**0.3530^{c}**
	T1P^{15}	0.285	0.315	0.365	0.385	**0.3375^{b}**
	T1P^{20}	0.3155	0.3955	0.4555	0.5855	**0.4380^{g}**
Lentil	T2L^{5}	0.2755	0.3855	0.3955	0.5355	**0.3980^{e}**
	T2L^{10}	0.295	0.375	0.415	0.565	**0.4125^{f}**
	T2L^{15}	0.295	0.325	0.435	0.535	**0.3975^{e}**
	T2L^{20}	0.2755	0.3455	0.4755	0.4855	**0.3955^{e}**
Day mean		**0.2898^{a}**	**0.3542^{b}**	**0.4164^{c}**	**0.5002^{d}**	**0.3901**

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

Bacteriological tests

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 ninth 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.

Physicochemical tests of refrigerated beef sausages

Water holding capacity (WHC)

The analysis of variance in table (6) reveals significant differences ($p \leq 0.01$) in the WHC of the meat among different storage periods. The storage treatment after 3 days of refrigeration recorded the highest mean value of 35.31, while the storage treatment after 9 days of refrigeration had the lowest mean value of 31.09. Furthermore, table (6) shows that treatment $T1P^{15}$ had the highest mean value (41.24%), while treatment $T2L^{10}$ had the lowest mean value (29.52%) for WHC among all the treatments. 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 observed among the different treatments, with the highest value recorded for treatment $T1P^{15}$ (24.07) and the lowest value recorded for the $T2L^{5}$ (12.69%). It can be 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).

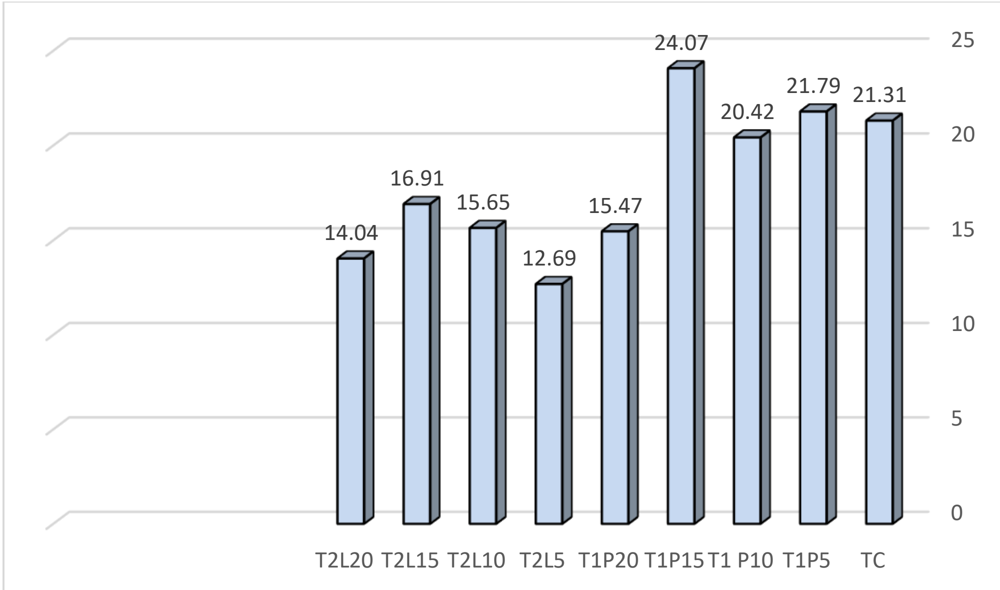

Figure (1) : The Percentage of losses after defrosting of processed sausages

Table (6): The ability of processed sausages to hold water.

Treatment		Storage time (day/s)				Treatment mean
		1	3	6	9	
		WHC%				
TC		26.26	27.2	43.12	29.58	**31.54^{b}**
Chickpea	**$T1P^{5}$**	39.99	30.5	42.14	26.07	**34.68^{c}**
	$T1P^{10}$	40.11	32.85	33.89	33.76	**35.15^{c}**
	$T1P^{15}$	43.64	43.14	39.08	39.1	**41.24^{e}**
	$T1P^{20}$	34.84	52.78	20.52	38.44	**36.64^{d}**
Lentil	**$T2L^{5}$**	30.5	31.81	31.13	33.06	**31.63^{b}**
	$T2L^{10}$	29.41	35.59	28.69	24.4	**29.52^{a}**
	$T2L^{15}$	34.46	37.38	22.1	26.7	**30.16^{ab}**
	$T2L^{20}$	29.14	26.52	34.11	28.73	**29.63^{a}**
Day mean		**34.26^{d}**	**35.31^{c}**	**32.75^{b}**	**31.09^{a}**	**33.35**

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

The results from the analysis of variance in Fig. (2) indicate that there were no significant differences in flavor, texture, and overall acceptability between different treatments. The flavor attribute had the highest average in treatment $T1P^{15}$, with a mean of 7.067, while the lowest average for this attribute was recorded in treatment $T1P^{10}$, with a mean of 6.133. The texture attribute had the highest average in treatment $T2L^{10}$, with a mean of 6.933, while the lowest average was recorded in treatment T2L[5], with a mean of 5.800. 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).

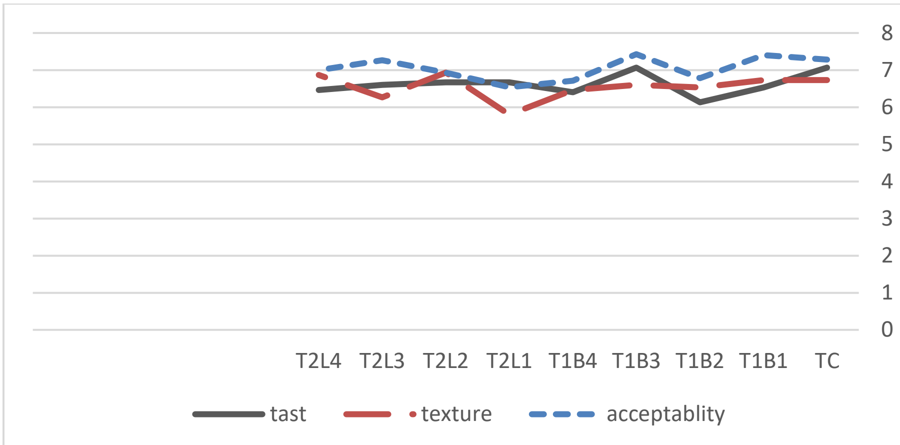

Figure (2): Results of sensory evaluation of treats.

Conclusions

The addition of crushed legume seeds range 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.

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