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Influence of Partial Replacement of Beef Meat by Germinated Lentil on Quality Characteristics of Dry Fermented Sausage

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Abstract: The purpose of the present work was to study the effect of replacement beef meat with 10 and 15% of germinated lentil on the microbiological, physico-chemical and sensory characteristics of dry fermented sausages. Results showed that replacement of beef meat with 15% germinated lentil showed a rapid increase in lactic acid bacteria (LAB) count after 7 days and had the highest LAB count after the 14th day of ripening, while the total viable count was decreased by 5.33 log cfu/g. It could be noted that, gram negative bacteria were totally disappeared on the 7th day of ripening. Whereas, the pH values of sausage samples were rapidly decreased during ripening by increasing replacement level due to the higher lactic acid production. Incorporation of 15% germinated lentil in the sausage formula led to the lowest reduction in diameter, length and weight of the ripened samples. On the other side, protein content was reduced by 24.7%, while crude fiber content was increased by 8.6 folds, when 15% germinated lentil was used in the sausage formula and this trend remained also at the end of ripening. Color parameters of sausage samples such as lightness (L^*) and redness (a^*) were enhanced by increasing replacement level to 10 and 15%, while yellowness (b^*) and chroma were decreased. Infrared analysis of the lentil and sausage samples indicated that germinated lentil showed higher intensity response in all functional groups than those did with ungerminated lentil sample. Replacement of beef meat by 15% of germinated lentil led to formation of many absorption peaks which related to aromatic compounds in rang of 3079 to 710 cm^{-1} rather than other treatments at the end of ripening. Sensory evaluation of the ripened sausage samples showed that the flavor scores of the 15% replacement samples was higher than those of all meat sausage samples due to the formation of the desirable aromatic compounds. Thus, sausage with germinated lentil can be obtained with acceptable sensory properties.

Key words: Sausage ripening · Lentil · Lactic acid bacteria · Physico-chemical analysis · FT-IR spectroscopy

INTRODUCTION

Legume seeds have made a significant contribution to the human diet since ancient times. They are a good and inexpensive source of dietary proteins, carbohydrates, vitamins and minerals [1]. Legumes play an important role in the agriculture and diet of many developing countries and are a major source of dietary nutrients for many people. However, their role appears to be limited because of several factors including low protein and starch digestibility [2], poor mineral bioavailability [3] and high anti-nutritional factors [4]. It has been reported that protein and thiamin [5], mineral bioavailability [6] and protein and starch digestibility [7] increased, whereas phytic acid [8] and tannin decreased during germination of legumes.

Lentils are plants belonging to the legume family and the dried seeds of lentils are considered as pulses. They are bushy annual plants, with a maximum height of around forty centimeters. Like legumes, the lens-shaped seeds of lentils also grow in pods. As compared to legumes, lentils are smaller in size and they are rich in protein, with over 25% of lentils nutritional value being protein [9]. This makes lentils the most popular and inexpensive source for protein, especially for those, who cannot afford meat and for vegetarians. Apart from proteins, lentils have essential amino acids, dietary fiber, folate, other vitamins and minerals [10]. The nutritional value of lentils is gaining considerable interest since its nutritional value /100g dry weight is as follows, energy, 353 kcal, carbohydrates, 60g, sugars, 2 g, dietary fibers, 31 g, fat, 1g, protein, 26 g,

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thiamine (B1), 0.87 mg, folate (B9), 479 μg and iron, 7.5 mg [11]. In common with other legumes, lentils contain a number of components called antinutritional factors which limit the wider use of crop.

Germination appears to be an inexpensive and effective method of achieving desirable changes in nutritious crops and germinated seeds have become a widely accepted food item. Germination causes important changes in the biochemical, nutritional and sensory characteristics of legume seeds [12]. It can be considered as a procedure for improving legume digestibility and reducing flatulence properties [13], which are some of the factors that limit consumption. In other words, germination improved quality of legumes by enhancing the bioavailability and digestibility of nutrients and reducing the antinutrients [14]. It induces the release of hydrolytic enzymes, which produce changes in the physical properties and functionality of seed components. Vidal-Valverde and Frias [13] demonstrated that the nutritive value of lentils may increase with germination processes.

Fermentation of legumes enhances protein digestibility and eliminates partially or completely the antinutritional factors [15]. Fermentation of meat products such as sausages is a traditional method for obtaining keepable products from the meat substrate, which otherwise is extremely sensitive to spoilage [16]. Lactic acid bacteria strains are important microorganisms used as starter cultures in meat fermentations. Their addition to meats may improve safety and stability of the product extending the shelf life and provides diversity resulting in new sensory properties as well as health benefits by probiotic characteristics [17].

Due to their good nutritional quality, germinated lentil was used in this research to study the effect of partial replacement levels of meat by germinated lentil (0, 10 and 15%) on quality characteristics of dry fermented sausages.

MATERIALS AND METHODS

Material: Starter culture *Lactobacillus plantarum* (DSMZ 20174) was obtained from the Egyptian Microbial Culture Collection (EMCC), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Lentils (*Lens culinaris Medikus*) seeds, raw beef meat, fat, natural casing and commercially dried and ground spices were purchased from local market in Cairo, Egypt.

Experimental Methods

Lentil Germination: Lentil seeds were cleaned, washed and soaked in 4-5 volumes of water (~30-35°C) for 8h. After that, the water was drained and the seeds were allowed to germinate in dark under a wet muslin cloth for 36h then, immediately it was used in fresh form to produce dry fermented sausage.

Sausage Manufacture: Three batches of fermented sausage were produced, first batch as a control 100% beef meat, second batch 90% beef meat with 10% germinated lentils and the third batch 85% meat with 15% germinated lentils. The mixture of fermented sausages were made with beef meat 68%, fat tissue 12.83%, sodium chloride 2.31%, glucose 0.5%, spices 0.82%, water as ice 15.49%, sodium ascorbate 0.05%, sodium nitrite 0.005% as well as starter culture of *Lactobacillus plantarum* (7 log cfu/g), which added as active culture. The ingredients except starter culture were processed according to Papavergou *et al.* [18] in mincer equipment until a homogenous distribution was reached, then inoculated with starter culture. The sausage mixtures were stuffed by hand under sanitary conditions into natural casing, the casing was then closed. Afterward, sausage samples soaked for 1 min. dipping in 0.1% sorbic acid solution to prevent any fungal growth on the surface during fermentation. After stuffing, the sausage samples were fermented and ripened in open air at room temperature (~30-35°C) until the end of ripening, (21 days).

Microbiological Analysis: Twenty grams sausage sample were homogenized with 180 ml of sterile peptone water for 1 min. and decimal dilutions prepared then duplicated onto growth media. The following media and incubation conditions were used: MRS agar (sigma) at 30° C, 48-72h, for lactic acid bacteria, nutrient agar (sigma) at 30° C, 48h for total viable count, VRB agar (sigma) at 30° C, 24h for Gram (-) bacteria and Baird-Parker agar (sigma) at 37°C, 48h for *Staphylococci* populations. The results were expressed as log cfu/g [19].

Physico-Chemical Analysis

Chemical Composition: Moisture, protein, dietary fiber and ash were determined according to standard methods [20] (21).

pH Determination: The pH was measured in a filtrate of a homogenized mixture of 1g fermented sausages with 10 ml of water [21].

Weight, Length and Diameter Changes: Two strings of sausages from each treatment were weighed and measured (length and diameter) just after stuffing (0 days). The same strings of sausages were reweighed and re-measured on the 7, 14 and 21st day [22].

Color Measurements: Color parameters of fermented sausage samples were determined according to tristimulus color system described by Françis [23] using a Spectrophotometer (MOM, 100 D, Hungary). Color coordinates X, Y and Z were converted to corresponding Hunter L^*, a^* and b^* color coordinates according to formulas given by the manufacturer. Chroma values were calculated from $(a^2 + b^2)^{1/2}$ and total color intensity $(a^2 + b^2 + L^2)^{1/2}$.

Functional Groups: The functional groups were measured by the infrared spectrum in the samples after being prepared in a powder. The FT-IR (Philips infrared) spectrophotometer available at the micro analytical center, Cairo University, Cairo, Egypt, was used for identifying the functional groups of dry and germinated lentil in addition to all prepared sausage treatments at the first and the end of sausage ripening period according to Bellamy [24].

Sensory Evaluation: Sensory analysis was carried out at the end of ripening by a panel of 10 referees according to Bruna *et al.* [21]. The color, texture, odor, flavor and overall acceptability were also assessed using a non-structured hedonic scale in which samples have given scores of 1 (very poor) to 10 (excellent).

Statistical Analysis: All experimental analysis was carried out in three replicates for all treatments. The data were analyzed by one-way analysis of variance (ANOVA). A multiple comparison procedure of the treatment means was performed by Duncan's multiple range test [25]. Significance of the differences was defined as $P < 0.05$. Analyses were done with Statistical Analysis System SAS computer program [26].

RESULTS AND DISCUSSION

Microbial Flora of Fermented Sausage: The initial count of lactic acid bacteria (LAB) in the sausage mixtures was about 7.83 log cfu/g. The LAB count of dry fermented sausage was significantly affected by replacement levels of germinated lentils (P<0.05) (Fig.1A). Sausages manufactured with germinated lentil showed a rapid increase in LAB count after 7 days of ripening. Sausage batch with 15% of germinated lentils was significantly (P< 0.05) higher than those with 0 and 10% germinated lentils sausage mixtures in LAB count. Such rapid increase in lactic acid bacteria counts is a criterion that the fermentation of sausages precedes correctly [27, 28]. Lactic acid bacteria are considered as desirable microflora (probiotic bacteria) in fermented sausages. The logarithmetic number of LAB starts to decline after the 7th day of ripening due to water loss and hardening of sausage samples. It is worth mentioning that the treatments with germinated lentil had the highest LAB count, especially after the 14th day probably due to their higher germinated lentil contents which contains more available carbohydrates and sugars. However, there were significant (P< 0.05) differences in LAB count between the treatments with germinated lentil and the control sample till the end of ripening period.

The decimal reduction times (D-value) for the declines in LAB count with the progress of the ripening time were calculated from the slopes of the negative part of the LAB count curves in Fig.1A to be 4.53d, 5.97d and

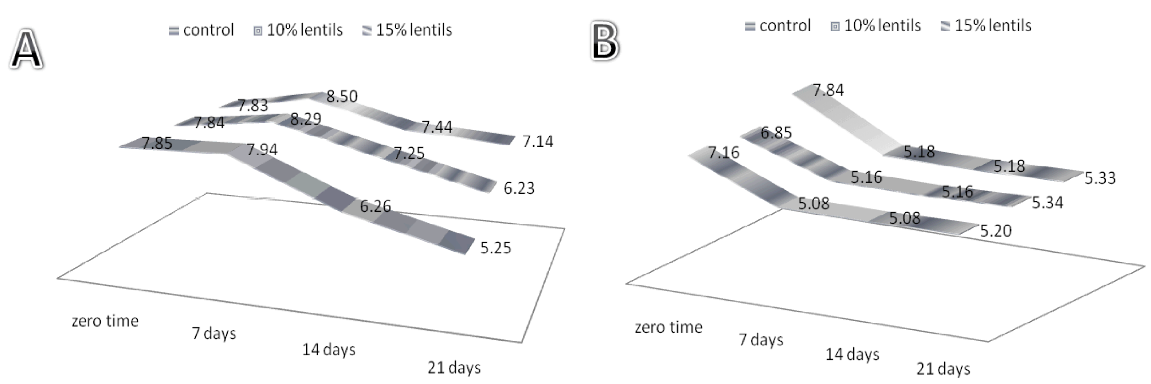


Fig. 1: Changes in log number counts of lactic acid bacteria (A) and total viable count (B) of dry fermented sausage during ripening as affected by lentil levels replacement

Table 1: Changes in pathogenic bacteria of dry fermented sausage during ripening as affected by lentil levels replacement

	Storage time (days)			
Microorganism	0	7	14	21
Gram (-) bacteria				
Control (0% lentil)	2.71[a]	NF	NF	NF
10% lentil	3.31[a]	NF	NF	NF
15% lentil	3.37[a]	NF	NF	NF
Staph. ssp.				
Control (0% lentil)	3.79[a]	NF	NF	NF
10% lentil	3.05[a]	NF	NF	NF
15% lentil	3.79[a]	NF	NF	NF

Values followed by the same small letter (superscript) in the same column for a given character are not significantly different (P>0.05). NF = not found

10.7d for control, 10 and 15% replacement samples, respectively. In this respect, it was clear that increasing the level of germinated lentil by only 5% (from 10 to 15%) led to doubling the D-value from 5.97 to 10.7 d which indicate the important role of germinated lentil in keeping the healthy effect of sausage samples.

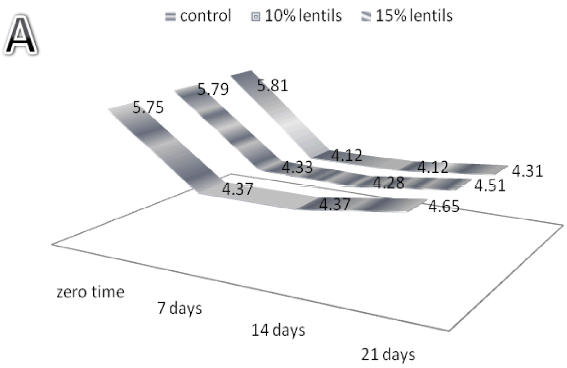
Total viable counts of sausage batches declined during the course of ripening period till the end of the ripening reaching 5.20, 5.34 and 5.33 log cfu/g as 0, 10 and 15% level of germinated lentils was used, respectively (Fig.1B). Statistically, the differences in total viable count between the treatments were not significant (P> 0.05). This means that the replacement of beef meat by germinated lentil did not increase the total viable count of dry fermented sausage and above that there are significant increases in LAB counts with increasing of the replacement level.

Gram (-) bacteria, in which the *Enterobacteriaceae* are included, are generally considered as undesirable microflora for meat products, since they may include pathogenic bacteria, such as *Salmonella* [29]. Therefore, a reduction of gram (-) bacteria is generally desired [27,28]. The initial logarithmic counts of gram (-) bacteria were 2.71, 3.31 and 3.37 in sausage samples with 0, 10 and 15 % germinated lentil replacement levels, respectively (Table 1). Gram (-) bacteria count was not significantly (P> 0.05) affected by germinated lentil replacement levels. It was totally disappeared on the 7th day of ripening period. This data are in agreement with Garcia ***et al.*** [30], where they found that *Enterobacteriaceae* decreased rapidly from initial values of 3 log cfu/g to less than 1 log cfu/g at 5th day of ripening. The same trend was observed with *staphylococcus ssp.* count in all dry fermented sausage treatments. This behavior could be referred probably due to intrinsic factors created in the sausages, where the total count of tested pathogenic bacteria was inversely proportional to the increase in lactic acid bacteria. The development of lactic acid bacteria suppress the growth of gram (-) bacteria by producing organic acids and various antibacterial metabolic products [31, 32]. Also, high populations of LAB inhibit the growth of spoilage and pathogenic bacteria, especially *Staphylococcus aureus* [33]. In addition, it was reported that LAB taken with food have a positive effect on human health [34].

Physico-chemical Properties: The germinated lentil had a significantly effect (P<0.05) on pH of fermented sausages. The initial pH of sausage mixture ranged from 5.75 to 5.81 (Fig.2A). It was rapidly reduced respectively to 4.38, 4.33 and 4.12 as 0, 10 and 15% germinated lentil was used. The lower pH values of treatments with germinated lentil correspond to the higher lactic acid bacteria of the same treatments after 7 days of storage. A pH lowers than 5.0 are sufficient to ensure safety of meat products [35]. Astiasaran ***et al.*** [36] described that at the end of ripening a slow increase in pH values was observed in fermented sausage, according to formation of N-non protein basic compounds and ammonium ions as well as to the buffering action of proteins. The lowering of pH is known to contribute to the inhibition of undesirable microorganisms and reduce the water binding capacity of proteins facilitating drying [25].

Moisture was rapidly decreased from 78.18, 62.58 and 60.74 to 23.85, 24.95 and 26.51 after 7 days of storage as 0, 10 and 15% germinated lentil was used, respectively (Fig. 2B). Afterwards it was decreased slowly with prolonged storage period. The treatment with germinated lentil was significantly (P<0.05) higher in moisture content than the control.

Data presented in Table 2 show that there were no significant differences (P >0.05) between diameter of all sausages strings during the storage period. On the other hand as a result of moisture loss, fermented sausages strings undergone losses in their length and weight during storage (Table 2). Changes in length (as %) show that the control sausage string has lost 10.3% of its original length till the end of the storage period, while sausage strings with 10 and 15 % germinated lentil have lost only 6.35 and 5.19%, respectively of their original length during the same period. The same trend was observed with the weight loss of all fermented sausage samples, where the decrement percentage in weight reached 37.46, 28.81 and 21.18% as 0, 10 and 15 germinated lentil was used, respectively at the end of storage period.



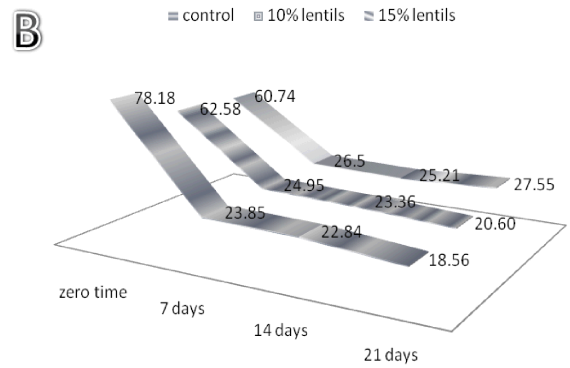


Fig. 2: Changes in pH (A) and moisture content % (B) of dry fermented sausage during ripening as affected by lentil levels replacement

Table 2: Changes in some physical properties of dry fermented sausage strings during ripening as affected by lentil level replacements

Replacement level of germinated lentil	Ripening time (days) zero	7	14	21
Diameter				
Control (0%)	3.80^{a}	2.97^{a}	2.73^{a}	2.93^{a}
10%	3.60^{a}	2.97^{a}	2.90^{a}	2.87^{a}
15%	3.70^{a}	3.20^{a}	2.93^{a}	2.97^{a}
Length				
Control (0%)	11.07^{c}	10.37^{c}	9.87^{c}	9.93^{c}
10%	12.60^{b}	12.20^{b}	12.07^{b}	11.80^{b}
15%	13.87^{a}	13.23^{a}	13.17^{a}	13.15^{a}
Weight				
Control (0%)	115.19^{b}	68.20^{c}	77.73^{c}	72.04^{c}
10%	135.90^{ab}	98.78^{b}	91.34^{b}	96.75^{b}
15%	151.05^{a}	129.41^{a}	122.97^{a}	119.06^{a}

Values followed by the same small letter (superscript) in the same column for a given character are not significantly different (*P* >0.05).

Table 3: Changes in some chemical analysis of dry fermented sausage as affected by lentil levels replacement

Replacement level of germinated lentil	Protein (%)	Ash (%)	Crude fiber (%)
"0" day			
Control (0%)	18.40^{a}	4.51^{c}	0.15^{c}
10%	15.82^{b}	6.75^{b}	0.97^{b}
15%	13.85^{c}	7.58^{a}	1.29^{a}
21st day			
Control (0%)	17.56^{a}	4.69^{c}	0.24^{c}
10%	14.49^{b}	6.62^{b}	1.23^{b}
15%	12.84^{c}	7.64^{a}	1.45^{a}

Values followed by the same small letter (superscript) in the same column for a given day are not significantly different (*P* >0.05).

Protein, ash and crude fiber of the sausages on the day of preparation and the final day of storage were presented in Table 3. Protein contents were 18.40, 15.82 and 13.85 % for the 0 (control), 10 and 15 % replacement with germinated lentil formulations, respectively at the day of preparation and 17.5, 14.49 and 12.84 at the final day of storage with the same order. The protein contents of sausages were significantly decreased (*P* <0.05) with increasing the replacement level of germinated lentil in fermented sausage formulations. The reduction in protein percentage reached 24.73% with the highest level of replacement. At the end of storage period (21 days) further reduction in protein levels was occurred in all sausage samples as a result of the ripening process. The reduction ratio ranged between 4.56 to 8.4% with the replaced sausage samples, which showing the higher loss ratio than the control sample.

On contrary, higher levels of germinated lentil replacement significantly (*P* <0.05) increased the percentage of ash and crude fiber in sausage formulations. The ash and crude fiber contents of replaced samples were higher by, respectively, 1.63 and 8.6 folds than those of the control sausage sample after replacement. Ash contents were almost constant at the end of ripening period , while the amount of crud fibers were apparently increased as a result of the consumption of the simple carbohydrates in the fermentation process during ripening. Water content in the final product was significantly higher with increasing the replacement level may be due to the higher water retention ability of the crude fiber. Similar water retention ability due to the action of fibers has been reported for cereal and fruit fibers in low fat fermented sausages [30].

Replacement level of beef meat by germinated lentil has significantly affected (*P*<0.05) the lightness (L^*), redness (a^*), yellowness (b^*), chroma and total intensity of sausages color (Table 4). On the day of preparation, L^* and a^* value were increased with increasing the replacement level of beef meat by germinated lentil,

Table 4: Changes in color parameters of dry fermented sausage as affected by lentil level replacements

	Color parameters				
Replacement level of germinated lentil	L^*	a^*	b^*	Chroma	Total intensity
"0" day					
Control (%)	35.99^b	4.30^b	16.98^a	17.52^a	40.03^b
10%	38.05^a	4.83^{ab}	15.50^b	16.24^b	41.37^a
15%	38.19^a	5.30^a	13.90^c	14.88^c	40.99^b
21st day					
Control (%)	36.41^c	4.14^c	18.32^a	18.78^a	40.99^b
10%	39.88^b	5.08^b	17.67^b	18.39^a	43.91^{ab}
15%	41.63^a	6.18^a	15.14^c	16.35^b	44.73^a

Values followed by the same small letter (superscript) in the same column for a given day are not significantly different (P >0.05).

whereas b and chroma values were taken the inverse trend. There are no significant differences between the 15% lentil sausage and the control sample in total color intensity. At the end of storage period, L^* and a^* values were significantly increase with increasing the replacement levels in fermented sausages and thus significantly (P <0.05) increase in total color intensity. This may be due to replacement of meat by germinated lentil which containing its seed and husk with the light reddish brown color, especially after germination process, where the sausage samples become more light and red. Because germinated lentil, as expected, contributes more light color to the final product, Ferreira *et al.* [37] pointed that the lightness is the main parameter governing the quality of meat but the importance of red should not be ignored. From another point of view, Ansorena *et al.* [38] stated that redness appears to be the most sensitive parameter for panelists. The yellowness (b^*) were significantly decreased (P<0.05) with increasing the level of germinated lentil replacement. This decrease could be attributed to modifications in myoglobin [39]. Üren and Babayiğit [40] reported a decrease in preference of fermented sausage as the intensity of the yellow color of sausage increases.

From the aforementioned results it can be concluded that the replacement of beef meat by germinated lentil increased significantly (P<0.05) the lightness and redness of fermented sausage samples and also decreased significantly (P<0.05) the yellowness of the same samples.

Functional Groups: The infrared spectrum of the tested lentil and fermented sausage samples is illustrated in Fig. 3. The functional aspects were based on the application of infrared within the range of 400 up to 4000 cm^{-1}. In dry and germinated lentil, the IR spectrum (Fig. 3-1, 2) shows increase in the number of functional group regions with germinated lentil, (20 peaks, compared to 16 peaks with dry lentil). In general, there is an increase in intensity at all absorption regions with germinated lentil compared to dry lentil as in OH stretching from hydroxyl groups, which responsible for water and aromatic compounds at 3390cm^{-1}. Also, The absorption intensity increased at 1540cm^{-1},which referred to amino acids ($-NH_3^+$), from 0.62 to 0.77 as well as at 2925cm^{-1}, which referred to CH stretching from saturated hydrocarbon $- CH_2 - (C = O) CH_2 - C \equiv N$, from 0.66 to 0.96 for dry and germinated lentil, respectively. These results may be due to the effect of germination process on distribution and concentration of functional groups in lentil.

In case of fermented sausage samples at zero time after preparation processes, there is an increase in the number of absorption regions with increasing the replacement level of beef meat by germinated lentil, where the peak numbers of the IR-spectrum were increased from 12 to 13 and 14 for control, 10 and 15% replacement level, respectively as show in (Fig. 3- 3, 5, 7). With 10% replacement level of beef meat, most of absorption peaks increased slightly than the control samples at 3285 cm^{-1}, 2925cm^{-1}, 1650 cm^{-1}, referring to Amide I and 1375 cm^{-1}, referring to nitro-groups, which reached 1.12, 2.84, 1.17 and 1.16 compared to 0.88, 2.59, 0.97 and 1.02 for control sample, respectively. Whereas, at the same pervious wave numbers with 15% replacement level, the absorption was clearly decreased to 0.42, 1.87, 0.52 and 0.58, respectively. This decrease means reduction in aromatic OH, saturated hydrocarbon, Amide I and nitro-groups which lead to reduction the risk of the presence of hydrocarbons as well as nitro-groups in fermented sausage. These findings were in agreement with those by Pedersen *et al.* [41].

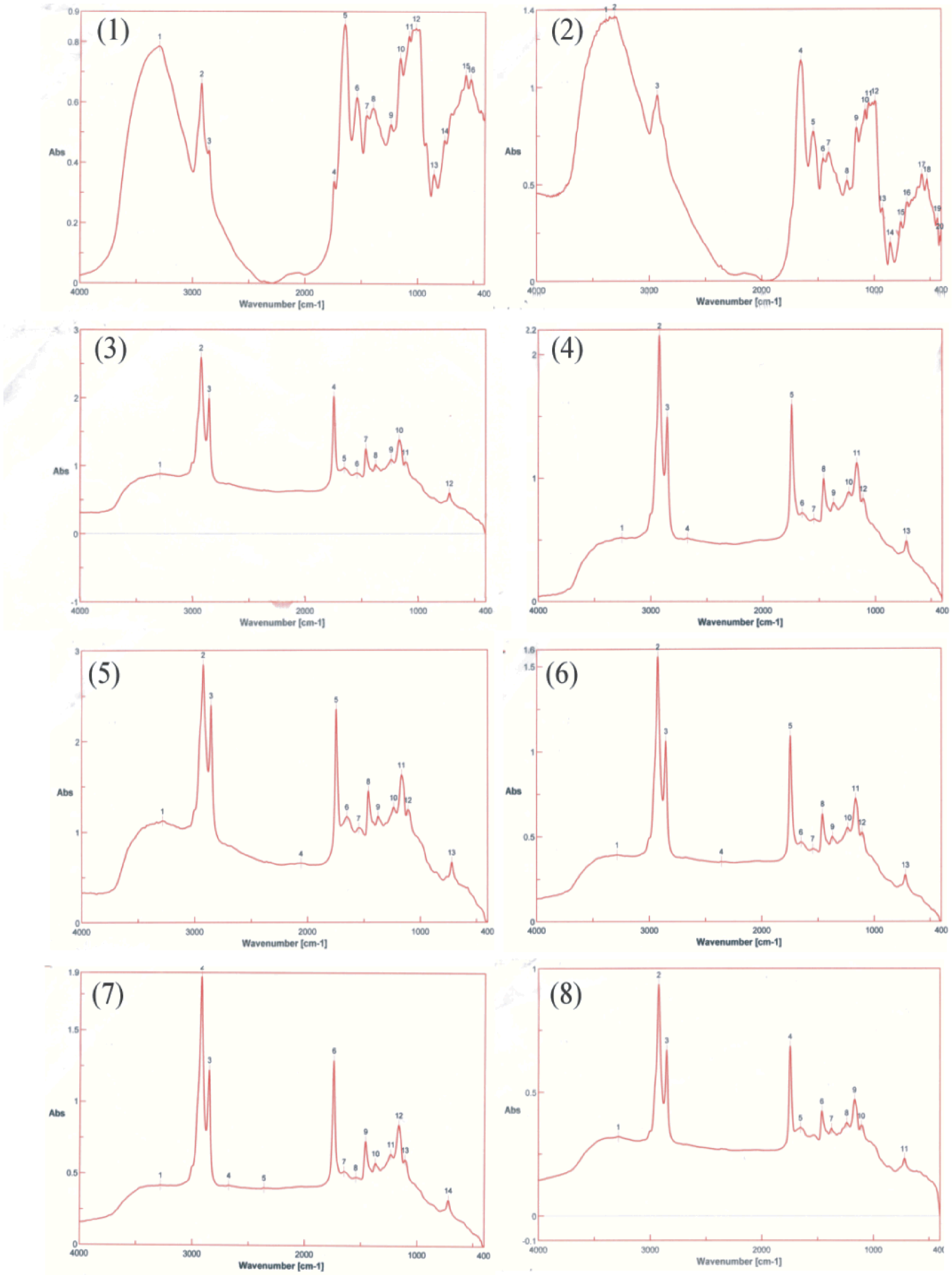


Fig. 3: FT-IR spectrum of: dry lentil (1), germinated lentil (2), zero time of control sausage (3), Final storage of control sausage (4), zero time of 10% germinated lentil sausage (5), final storage of 10% germinated lentil sausages (6), zero time of 15% germinated lentil sausage (7), final storage of 15% germinated lentil sausages (8).

Table 5: Changes in sensory attributes of dry fermented sausage as affected by lentil levels replacement

Replacement level of germinated lentil	Sensory attributes Taste	Odor	Color	Flavor	Texture	Overall acceptability
Control (0%)	7.33^{b}	8.32^{a}	9.14^{b}	7.67^{b}	6.66^{b}	7.66^{b}
10%	8.66^{a}	8.34^{a}	9.00^{b}	8.00^{a}	8.33^{a}	9.00^{a}
15%	8.67^{a}	8.33^{a}	9.43^{a}	8.33^{a}	9.00^{a}	9.33^{a}

Values followed by the same small letter (superscript) in the same column for a given day are not significantly different ($P > 0.05$).

At the end of storage (Fig. 3- 4, 6, 8), there was a decrease in all peaks of absorption with all treatments and the high replacement level one has the lowest values of absorptions. In 15% beef meat replacement level sausages, there are many absorption peaks related to aromatic compounds ranged from 3079 to710cm^{-1} rather than other treatments.

Sensory Evaluation: The influence of germinated lentil replacement levels on sensory attributes of fermented sausages is shown in Table 5. Sausages with 15% germinated lentil had significantly the highest ($P < 0.05$) scores for all sensory attributes. This may be due to increasing the amount of added germinated lentil to the sausage formula, which has positively affected the sensory scores flavor due to increasing the aromatic compounds at the end of storage time. Control sample had the lowest ($P < 0.05$) scores in taste, color, flavor, texture and consequently overall acceptability. These data are in accordance with chemical and physical analysis of sausages, which refer that treatment with germinated lentil was significantly ($P < 0.05$) higher in moisture, fiber content, weight and more lightness and redness than the control. Therefore, samples with germinated lentil become more preferable for panelists in its properties as they become softer than the control.

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