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Physicochemical Properties and Sensory Evaluation of Reduced Fat Fermented Functional Beef Sausage

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Abstract

Background and Objective: Semi-dry fermented sausages were manufactured from beef meat in three types: without starter culture (control), inoculated with *Lactobacillus casei* and inoculated with *Lactobacillus paracasei*. Probiotic fermented sausages are safe and healthy meat products, which receive high commercial interest and growing market shares.

Material and Methods: The physico–chemical characteristics (Protein, Moisture, Fat, Ash, Lactic acid value and pH), microbiological features (total aerobic, total mold and yeast and lactic acid bacteria count) and sensory evaluation (color, flavor, texture and overall acceptability) were analyzed after 0, 10, 20, 30, 40 and 45 days of refrigerated storage at 4°C.

Results and Conclusion: There was a significant difference ($p \leq 0.05$) in moisture content, which decreased in all samples during the period of refrigerated storage. However, all other parameters such as protein, fat and ash increased. The lactic acid produced during the fermentation by lactic acid bacteria resulted in a decrease in the pH value of all samples, and improved sensory evaluation of the fermented sausage inoculated with *Lactobacillus casei* and *Lactobacillus paracasei* during the storage period. The best results were obtained in the fermented sausage inoculated with *Lactobacillus paracasei* in physico-chemical, microbial and sensory characteristics. Also we could preserve the product at 4°C for 45 days.

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1. Introduction

Meat provides high quality protein, consisting of all essential amino acids, minerals and vitamins [1]. Today, demand for meat products is high because they are safe, nutrient, diverse, and attractive in appearance and texture [2]. Fermentation is the chemical conversion of organic substances into primary compounds by the act of enzymes, compound organic catalysts, which are created by microorganisms such as molds, yeasts, or bacteria. It may be aerobic or anaerobic in the environment. It is, generally, considered as an anaerobic method by which sugar is transformed into acid or alcohol [3]. Fermented sausages can be fabricated dry or semi-dry. Fermentation of meat products usually depends on natural microflora. The request for secure products of typical quality and advantageous technological characteristics has led to the use of starter cultures for manufacture of dry-fermented sausages [4]. Starter cultures are used to ensure food safety and superior quality attributes such as sensorial, nutritional, and technological properties [5].

Probiotics, which are live microorganisms mainly from the strains Bifidobacterium and Lactobacillus ssp., are used for both human and animal feeding to enhance their health conditions [6,7]. LAB play an important role in meat preservation and fermentation processes because they affect both the technological properties and the microbial stability of the final product by producing lactic acid as a final product [8,9].

In fact, the main role of LAB is to acidify the sausage [10]. Sausages are prepared from comminution and spiced meat, and are, generally, shaped into regular form; quality of meat products is, generally, assessed by nutritional factor, sensory evaluation and microbiological analysis. The fermented sausage is a good quality product and can be very well stored under cold storage condition [11]. Probiotic fermented sausages are safe and healthy meat products, which receive high commercial interest and growing market shares.

The objectives of the present investigation was production and formulation of a new-low fat functional fermented sausage from beef meat by using two strains of probiotics (*Lactobacillus.(L.) casei and L. paracasei*) to improve the quality and safety of products, and to evaluate the products, physico-chemical, microbiological and sensorial characteristics during the storage time (45 days) at 4°C.The novelty of this research can be related to the lowering of fat content and modifying the safety and quality parameters of the product.

2. Materials and Methods

2.1. Samples preparation

Fresh boneless beef meat was obtained from local market in Karaj, Iran. Other ingredients such as soy oil, sodium chloride, sodium nitrite, red pepper powder, sugar powder, black pepper powder, poly phosphate, garlic, special spices, ascorbic acid, starch and flour were obtained from Kadur Factory (Tehran, Iran)are shown in Table 1.

Table 1. Raw material and common ingredients used in semi-dry fermented sausages' formulation

Ingredients	Quantity (g)
meat	100
soy oil	15
sodium chloride	2.29
sodium nitrite	0.03
sugar powder	0.37
garlic	2
poly phosphate	0.86
red pepper powder	0.26
black pepper powder	0.17
ascorbic acid	0.1
starch	2.6
flour	8.6
special spice	0.54

2.2. Starter culture preparation

Two bacterial strains, *L. casei* (DSM20011) and *L. paracasei* (DSM-20006) were obtained from BioProcess Engineering Laboratory, University of Tehran, Iran. These bacteria were reactivated in de Man Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany), incubated at 37°C for 24 h. Under aseptic conditions, they were transferred to MRS agar by streaked and incubated at 37°C for 48 h. After purification and enumeration of these Lactic acid bacteria, isolated typical colonies were transferred from MRS agar to MRS broth overnight incubated at 37°Cfor 24 h following the same reactivated processing mentioned above, and the number of cells was approximately 1×10^{8} CFU ml^{-1}. Each strain was centrifuged at 4025 ×g, 4°C for 10 min., and the obtained residual was washed two times with 0.85% saline solution water and used for inoculation separately in the product [12].

2.3. Identification of the LAB isolates

The bacteria were identified by microscopic morphological checking tests. LAB isolates were Gram positive, catalase negative, rod shapes, non-spore former, non-motile. LAB Isolates which were used in this study, were kept at 4°C in MRS broth until they were further tested. The purity of cultures was tested periodically and at the starting of each experiment by gram staining, and the strains belonging to the LAB group were identified [8].

2.4. Sausage preparation

Three samples from each types of semi-dry fermented sausage were prepared as following: A control sample was produced without adding starter culture. Two other samples were produced with starter cultures containing one strain from each of the starter cultures *L. casei* or *L. paracasei*. Meat and other above-mentioned ingredients were used in certain percentages per kg batter for production of fermented sausages. The respective starter cultures were added to each sample as a 2 ml wet inoculums per kg of batter. In the control sample, 2 ml of sterile saline water was added per Kg of batter. A Naturin Cutter (Naturin, Germany) was used for preparation of batter; the cutter was sterilized before the preparation of meat mixture for each treatment. The spice mixture and other ingredients including starter culture were added and mixed with minced meat in a cutter for about 20 min. The batter was filled into artificial collagen casings of 20 mm diameter using a filling machine (Naturin, Germany) at 5°C [13]. The produced sausages were fermented at 30°C for 24 h. and then dried at two stages:(60°C for 4 h. and 75°C for 20 min).These heating stages improve the sensory evaluation and inhibit bacterial development. They were, finally, stored in there refrigerator at 4°C as described by Ahmad et al. [11]. Sampling was performed by randomly choosing from each sausage group after 0, 10, 20, 30, 40 and 45 days in order to analyze their physicochemical, microbiological and sensorial properties.

2.5. Microbiological analysis

The samples were analyzed based on the method described by American Public Health Association [14]. After removing the casings from the samples, 1 g from each was taken and transferred under aseptic conditions to a glass tube containing 9 ml of sterile normal saline-peptone water (NaCl 0.85% and 0.1% peptone water). Serial decimal dilutions were prepared (from 10^{-1} to 10^{-8}). Nutrient agar media (Merck, Darmstadt, Germany) were used to count Total Aerobic Counts (TAC), Yeast extract glucose chloramphenicol agar media (Merck, Darmstadt, Germany) were used to mold and yeast counts [(M,.YC)], and (MRS) agar media (de Man, Rogosa and Sharp agar) (Oxoid ltd., Basing-stoke, Hampshire, England)were used for LAB with triplicates. A colony counter was used for counting the colonies grown in the incubated Petri-dishes after being incubated at 37°C for 48 h for TAC and LAB while [(M &YC)] were incubated at 25°C for (3-5) days. Finally means and standard deviations were calculated after counting.

2.6. Physico-chemical analysis

2.6.1. Moisture determination

The moisture content was determined by weighting 5 g of the samples and drying in an oven (SW-90D, Sang Woo Scientific Co., Bucheon, Korea) at 105°C for 24 h until reaching a constant weight as mentioned in the method of AOAC [15].

2.6.2. Fat content

Fat content was measured by the Soxhlet method with a solvent extraction system (SOXTEC Avanti 2050 Auto System, Foss Tecator AB, Hoganas, Sweden) based on the method of AOAC [16].

2.6.3. Protein content

Total protein content was determined according to Kjeldahl method with an automatic Kjeldahl nitrogen analyzer (Kjeltec 2300 Analyzer Unit, Foss Analytical AB, Hoganas, Sweden),which is used to determine the amount of nitrogen (%) and to calculate the ratio of protein by multiplying the amount of nitrogen to the constant factor (6.25) as mentioned in the method of AOAC [17].

2.6.4. Ash content

5 g of each sample was put inside a muffle furnace at 550°C as mentioned in the method of AOAC [18].

2.6.5. pH

The pH value of semi-dry fermented sausage was determined by weighting 10 g of each sample. Then the sausage samples were homogenized in 90 ml of distilled water [19] and their pH value was measured by pH meter (Crison Instruments S.A., Alella, Spain) by submerging the electrode directly into the samples.

2.6. 6. Lactic acid value

Lactic acid value was determined by filtration of the samples and then titration with 0.1N NaOH. (1 ml 0.1N NaOH=0.0090 g lactic acid), which contained phenolphthalein (0.1% in 95% ethanol % w v^{-1}) as the indicator. The results were reported as percent lactic acid [20].

2.7. Sensorial analysis

Sensory evaluation (color, texture, flavor and overall acceptability) was carried out for all the inoculated and control semi-dry fermented sausage samples using 10 trained panelists. According to Al-ahmad [21], for each sample, a score sheet from 1-9; 1 represents very dislike while 9 is very like. Sensory evaluation was done again for the samples stored at 4°C after 0, 10, 20, 30, 40 and 45 days.

2.8. Statistical analysis

SPSS software (ver.17.0) was used to determine the effect of refrigerated storage and the LAB on the quality characteristics of semi-dry fermented sausage. Each trial was repeated 3 times. The obtained data were analyzed by one-way (ANOVA), and significant differences ($p \leq 0.05$) among the means were compared using the Duncan's test.

3. Results and Discussion

3.1. Physico-chemical analysis

Table 2 shows the results of the physical and chemical analyses of semi-dry fermented sausage samples inoculated with *L. casei* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C. The protein content was significantly ($p \leq 0.05$) increased in all samples until it reached 15.42% at the end of storage period. The moisture content in all samples significantly decreased (range 62.78–60.93%) during the storage period. The trend in fat content; it was similar to protein content in a significantly ($p \leq 0.05$) increased in all samples during storage at 4°C. Fat content ranged between 16.38 and 17.51% for semi-dry fermented sausage during the cold storage period. Ash content was almost significantly ($P \leq 0.05$) increased in all samples during the storage period at 4°C. Ash content ranged between 2.29 and 2.54% during the refrigerated storage period.

Table 2. Physicochemical analysis of semi-dry fermented sausages inoculated with *Lactobacillus casei* during refrigerated storage at 4°C

Period of storage (day)	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	pH	Lactic acid (%)
0	13.24±0.01^{f}	62.78±0.06^{a}	16.38±0.01^{f}	2.29±0.01^{d}	5.45±0.01^{a}	0.478±0.001^{f}
10	13.78±0.02^{e}	62.16±0.04^{b}	16.51±0. 01^{e}	2.45±0.02^{c}	5.41±0. 01^{b}	0.546±0.001^{e}
20	14.50±0.02^{d}	61.92±0.24^{b}	16.86±0.02^{d}	2.46±0.02^{bc}	5.40±0.02^{bc}	0.579±0.001^{d}
30	14.61±0.01^{c}	61.86±0.09^{b}	17.00±0.09^{c}	2.48±0. 05^{bc}	5.38±0.02^{c}	0.637±0.001^{c}
40	15.24±0.01^{b}	61.33±0.50^{c}	17.38±0.01^{b}	2.50±0.03^{ab}	5.32±0. 01^{d}	0.671±0.002^{b}
45	15.42±0. 05^{a}	60.93±0.41^{c}	17.51±0.01^{a}	2.54±0.04^{a}	5.18±0.02^{e}	0.760±0.002^{a}

*Values are means of three replicates ± standard deviation.
**Means with different superscript letters in the same column represent significant differences ($p \leq 0.05$)

The results of the physical and chemical analyses of semi-dry fermented sausage inoculated with *L. paracasei* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C are presented in Table 3. The protein content was significantly ($p \leq 0.05$) increased in all samples until it reached 15.475% at the end of storage period, while moisture content decreased in all samples during the storage period,(range 61.40–60.55%). The trend in fat content was similar to protein content which showed a significant ($p \leq 0.05$) increase in all samples during storage at 4°C. Fat content was in the range of 16.40-17.85% for semi-dry fermented sausage during the cold storage period. Ash content was almost significantly ($p \leq 0.05$) increased in all samples during storage at 4°C. Ash content ranged between 2.48 and 2.63% during cold storage period (Table 3).

The results of physical and chemical analyses of semi-dry fermented sausage samples produced without starter culture (control) after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C are shown in Table 4. The storage period effected significantly ($p \leq 0.05$) on protein content in all samples until it reached 17.24% at the end of storage period, while no significant differences were observed in moisture content during the cold storage. The moisture content of the samples was in range of 63.39-61.92% during the storage period. There were significant differences in the samples during 45 days at 4°C. Fat content ranged between 16.37 and 17.35% for the controls during the cold storage period. Ash content was in the almost increased in all samples during storage period at 4°C. Ash content range of 2.56-2.68% during the cold storage period (Table 4). The control sample had higher protein content compared to the samples inoculated with *L. casei and L. paracasei*. The increase in protein content of semi-dry fermented sausages can be due to the proteolytic activities of enzymes produced by microorganisms during the fermentation and ripening, which can cause increase in the bioavailability of amino acids [22]. This increase in protein content in time can be due to the reduction in moisture values. Our results coincide with those of Asmare and Admassu, who reported increase of protein content in all dry fermented sausages [23]; this can be attributed to the decrease of water content and high concentration of nutrients during processing.

Decrease of moisture content led to an increase in protein, fat, ash contents during the storage period (Tables 2, 3 and 4). Our findings are in agreement with those of Ahmad et al. [11], who demonstrated the lowering down of moisture content of semi-dry fermented sausage.

The control sample had lower fat content compared to the fermented sausages inoculated with *L. casei* and *L. paracasei*. Fat contributes to nutritional (source of essential fatty acids, lipo-soluble vitamins, and energy), organoleptic (flavor, texture, and mouth-feel), and technological properties (release of moisture) in meat products [24]. Our results are in consistency with the findings of Asmare and Admassu [23], who reported that fat content was also significantly ($p \leq 0.05$) increased in all dry-fermented sausages.

Table 3. Physicochemical analysis of semi-dry fermented sausages inoculated with *Lactobacillus paracasei* during refrigerated storage at 4˚C

Period of storage (day)	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	pH	Lactic acid (%)
0	13.79 ± 0.02^{f}	61.40 ± 0.03^{a}	16.40 ± 0.02^{f}	2.48 ± 0.02^{e}	5.3 ± 0.01^{a}	0.48 ± 0.002^{f}
10	13.91 ± 0.01^{e}	61.08 ± 0.27^{b}	16.75 ± 0.02^{e}	2.51 ± 0.03^{de}	5.22 ± 0.01^{a}	0.571 ± 0.001^{e}
20	14.69 ± 0.02^{d}	60.91 ± 0.12^{b}	16.91 ± 0.04^{d}	2.54 ± 0.01^{cd}	5.15 ± 0.02^{b}	0.588 ± 0.001^{d}
30	14.92 ± 0.01^{c}	$60.78 \pm 0.20b^{c}$	17.33 ± 0.02^{c}	2.56 ± 0.01^{bc}	5.02 ± 0.08^{c}	0.659 ± 0.001^{c}
40	15.70 ± 0.02^{b}	$60.75 \pm 0.20b^{c}$	17.67 ± 0.03^{b}	2.60 ± 0.03^{ab}	4.98 ± 0.02^{c}	0.728 ± 0.005^{b}
45	15.75 ± 0.01^{a}	60.55 ± 0.08^{c}	17.85 ± 0.02^{a}	2.63 ± 0.03^{a}	4.80 ± 0.01^{d}	0.87 ± 0.002^{a}

*Values are means of three replicates ± standard deviation.
**Means with different superscript letters in the same column represent significant differences ($p \leq 0.05$).

Table 4. Physicochemical analysis of control and semi-dry fermented sausages during refrigerated storage at 4˚C

Period of storage (day)	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	pH	Lactic acid (%)
0	16.74 ± 0.02^{f}	63.39 ± 0.32^{a}	16.37 ± 0.02^{e}	2.56 ± 0.02^{b}	5.51 ± 0.01^{a}	0.388 ± 0.001^{f}
10	16.78 ± 0.02^{e}	62.62 ± 0.59^{b}	16.48 ± 0.03^{d}	2.57 ± 0.02^{b}	5.48 ± 0.03^{ab}	0.510 ± 0.001^{e}
20	16.85 ± 0.02^{d}	62.48 ± 0.09^{b}	16.54 ± 0.12^{d}	2.58 ± 0.005^{b}	5.44 ± 0.04^{bc}	0.572 ± 0.001^{d}
30	16.93 ± 0.02^{c}	62.32 ± 0.42^{b}	16.89 ± 0.02^{c}	2.60 ± 0.03^{b}	5.39 ± 0.01^{cd}	0.631 ± 0.001^{c}
40	17.00 ± 0.02^{b}	62.05 ± 0.07^{b}	17.03 ± 0.04^{b}	2.65 ± 0.05^{a}	5.35 ± 0.02^{d}	0.645 ± 0.001^{b}
45	17.24 ± 0.02^{a}	61.92 ± 0.05^{b}	17.35 ± 0.03^{a}	2.68 ± 0.02^{a}	5.24 ± 0.04^{e}	0.718 ± 0.002^{a}

*Values are means of three replicates ± standard deviation.
**Means with different superscript letters in the same column represent significant differences ($p \leq 0.05$).

The highest ash content (2.56-2.68%) was found in the control sample compared to others (Tables 2, 3 and 4).This increase in ash content could be due to the use of salts (NaCl and $NaNO_2$) in the sausage batters. Salt (NaCl) is one of the major ingredients in dry- fermented sausages: it plays an essential role in assuring the microbiological stability, and influences the final taste, color and texture; these values of ash increase in the end products due to drying process [25]. Our findings are almost in consistency with those of Hemat et al. [26], who reported a significant ($P \leq 0.05$) increase in mineral components among the treatments.

pH and lactic acid values

pH and lactic acid values for the samples inoculated with *L. casei* during storage at 4°C are shown in Table 2. pH value of all samples significantly ($p \leq 0.05$) decreased during the refrigerated storage (range 5.45-5.18)while lactic acid values significantly ($p \leq 0.05$) increased (range 0.478-0.759%) during the cold storage.

Results of pH and lactic acid values for samples inoculated with *L. paracasei* during the storage period at 4°C are indicated in Table 3. pH value in all samples decreased significantly ($p \leq 0.05$) during the cold storage (range5.3-4.80) while lactic acid values significantly ($p \leq 0.05$) increased during this time (range 0.48-0.87%).

Results of pH and lactic acid values for the control samples during the storage period at 4°C are shown in Table 4.pH values in all samples significantly ($p \leq 0.05$) decreased while lactic acid values significantly ($p \leq 0.05$) increased during cold storage at 4°C for 45 days.

The control samples had higher value (5.51-5.24) compared to the samples inoculated with *L. casei* and *L. paracasei*. This decrease in pH values was due to the production of lactic acid during fermentation by lactic acid bacteria. The increase in lactic acid values in all the samples is the result of dropping of pH values during storage at 4°C. The control samples had lower values of lactic acid (0.388-0.718%) compared to those inoculated with *L. casei* and *L. paracasei* during the cold storage period. LAB utilize the carbohydrate portion of the meat as source of energy to produce acids and thus lower the pH, improving the texture of the products, providing stability against the proliferation of food spoilage and pathogen microorganisms and producing some aromatic compounds [27] through production of lactic acid and a number of other antimicrobial and organoleptic compounds (e.g. acetic acid, ethanol, acetoin, carbon dioxide and pyruvic acid) [28]. The results of this study are in agreement with report of Ahmad et al.[11],who demonstrated that refrigerated storage significantly ($p \leq 0.05$) decreased the pH of semi-dry fermented sausage.

3.2. Microbiological analysis

3.2.1. Lactic acid bacteria

LAB resulted in- to increasing the lactic acid, and promoting the color, reducing the enzyme rancidity and improving the sensory properties of the final product [29]. There was a significant increase ($p \leq 0.05$) in the numbers of LAB during the storage at 4°C in all the fermented samples such that they became predominate flora in the final products during the refrigerated period storage (Figure 1). The control samples had lower number of LAB (range 6.47-6.81 log CFU g^{-1}) during the refrigerated storage period comparing to the samples inoculated with *L. casei* and *L. paracasei* (range 6.95 - 7.95 and 7.15-8.28 log CFU g^{-1}, respectively). Increase in LAB numbers (which could be due to the environment of meat) is suitable for the growth of LAB and good adaptation of these bacteria with the fermentation conditions [29]. Our results are in agreement with those of Ferreira et al. in fermented sausages, in which displayed a rapid increase in LAB count was noticed [30]. The number of LAB decreased at the end of refrigerated storage due to the exhaustion of sugar and the low temperature conditions [31], and also may be due to decrease in the moisture and increase in the acidity of sausage during the refrigerated storage [29].

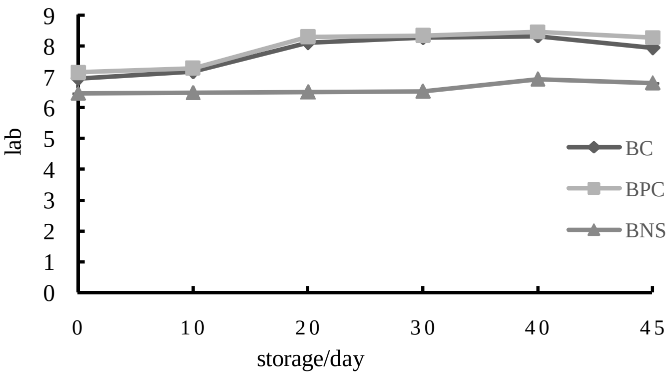

Figure1. Microbial numbers of Lactic acid bacteria counts (LAB) log cfu g^{-1} for semi-dry fermented sausages inoculated with *L. casei*, *L. paracasei* and Control during storage period at 4˚C for 45 days

3.2.2. Total aerobic count

Total aerobic counts (TAC) of semi-dry fermented sausage samples increased in all samples during storage at 4°C (Figure2). The control samples had lower number (range 5.91–6.60 log CFU g^{-1}) during the refrigerated storage period compared to those inoculated with *L. casei* and *L. paracasei* (range 6.55-7.20 and 6.53-6.75 log CFU g^{-1}, respectively). Our results coincide with those of Bacha et al. which showed the same loads of TAC on other sausages [32]. This significant increase ($p \leq 0.05$) in TAC numbers can be due to the initial ingredients and the properties of used meat [33]. Microbial growth during storage is one of the main factors affecting the quality of meat products, leading to the spoilage, and hence, economic losses [34]. Sausage may be contaminated after heat processing and during other processes such as slicing, packaging and peeling [35].

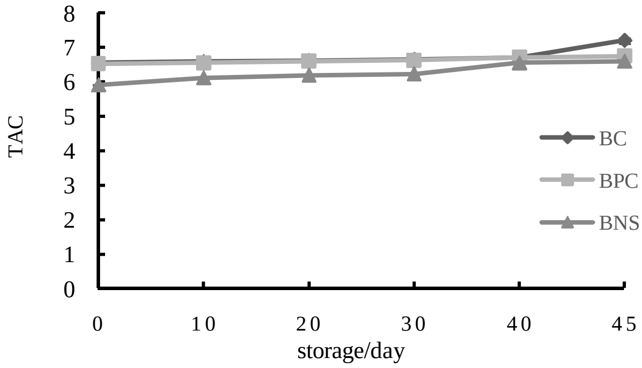

Figure2. Microbial numbers of Total Aerobic counts (TAC) log cfu g^{-1} for semi-dry fermented sausages inoculated with *L. casei*, *L. paracasei* and Control during storage period at 4˚C for 45 days

3.2.3. Yeast and molds count

The numbers of yeasts and molds count were significantly lower (p≤0.05) in the semi-dry fermented sausages and were decreased in all samples during the period of storage at 4°C (Figure3). The control samples had higher number (range 4.17-3.22 log CFU g^{-1}) during the refrigerated storage compared to the samples inoculated with *L. casei* and *L. paracasei* (range 4.14–2.26 and 4.15-2.50 log CFU g^{-1}, respectively). Our results are in agreement with report of Al-ahmad et al. [29], who demonstrated reduced number of yeasts and molds in the starter cultures compared to the control in smoked fermented semi-dry sausages. This decline in the number of yeasts and molds in the samples inoculated with LAB compared with the control can be due to the competition among LAB, yeasts and molds [29].

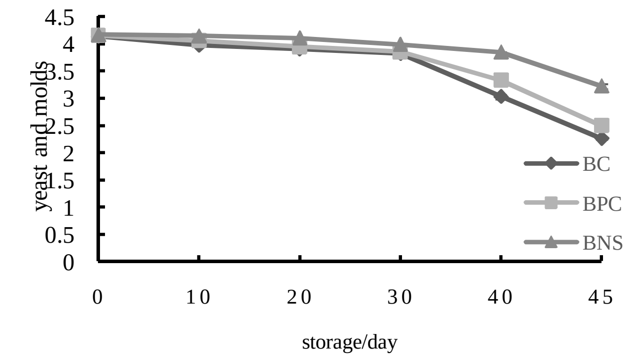

Figure3. Microbial numbers of molds and yeast counts (M&Y) log cfu g^{-1} for semi-dry fermented sausages inoculated with *L. casei*, *L. paracasei* and Control during storage period at 4˚C for 45 days.

3.3. Sensory evaluation

The addition of starter culture improved the sensory properties of samples inoculated with starter culture compared to control. There are many factors affecting the sensory characteristics of meat products such as the meats used as raw materials (genetic type, feed, age, sex, and rearing system), microorganisms selected as microbial starters for the fermentation and type of processing technologies (cooking, drying, ripening, smoking, etc.) [36]. Sensorial analysis included the evaluation of color, flavor, texture and overall acceptability.

3.3.1. Color

Storage time had a significant effect (p≤0.05) on color scores in all the semi-dry fermented sausage samples inoculated with *L. casei*. During the cold storage at 4°C, the color scores significantly (p≤0.05) decreased (range 7.85- 6.75) (Table 5).

As shown in Table 6, storage period had a significant effect (p≤0.05) on the color scores in the semi-dry fermented sausage samples inoculated with *L. paracasei*. The color scores decreased (range 8.07-7.5).Storage period had a significant effect (p≤0.05) on the color scores in all samples of semi-dry fermented sausage (control). The control samples had lower color scores(range 7.75-6.16)during the cold storage period compared to those inoculated with *L. casei* and *L. paracasei* (Table 7).The decrease in color scores during storage may be due to the lipid oxidation and subsequent oxidized compounds reacting with amino acids during the non-enzymatic browning of the product [36]. Our results are in agreement with the findings of Ahmad and Amer [36], who reported that during the cold storage, scores of color significantly (p≤0.05) decreased in the semi-dry fermented sausages.

3.3.2. Flavor

During the cold storage at 4°C, the flavor scores decreased significantly in all samples of semi-dry fermented sausage inoculated with *L. casei* (range 6.83-5.63) during 45 days (Table 5). There were significant differences in flavor scores in all samples of semi-dry fermented sausage inoculated with *L. paracasei*, which had the highest flavor scores during the cold storage, as shown in Table 6. The flavor scores decreased in all samples (range 6.87-5.83) during 45 days at 4°C. There were significant differences in the flavor scores of semi-dry fermented sausage (control) and decrease in all samples during the cold storage (Table 7). The control sample had lower scores (range 6.66-5.60) compared to those inoculated with *L. casei* and *L. paracasei* during the cold storage. The characteristic flavor of fermented sausages mainly originates from the breakdown of carbohydrates, lipids, and proteins through the action of microbial and endogenous meat enzymes [36]. The development of flavor is also influenced by several variables such as product formulation (especially spices), processing conditions, and starter culture [36]. Our results are corresponding with those obtained by Ahmad and Amer [36], who indicated a decrease of flavor during the refrigerated storage.

Table 5. Sensory evaluation of semi-dry fermented sausages inoculated with *L. casei* during storage period at 4˚C for 45 days

Storage/day	Color	Flavor	Texture	Overall acceptability
0	7.85±0.01^{a}	6.83±0.01^{a}	7.82±0.02^{a}	7.33±0.01^{a}
10	7.76±0.01^{b}	6.5±0.03^{b}	7.8±0.04^{a}	7±0.09^{b}
20	7.5±0.03^{c}	6.37±0.02^{b}	7.5±0.02^{b}	6.56±0.01^{c}
30	7.4±0.02^{d}	6.16±0.01^{c}	6.6±0.03^{c}	6.33±0.03^{d}
40	7±0.1^{e}	5.71±0.18^{d}	6.46±0.01^{d}	6.30±0.02^{d}
45	6.75±0.02^{f}	5.63±0.02^{e}	6.13±0.02^{e}	6.22±0.03^{e}

*Values are means of three replicates ± standard deviation.
** Means with different superscript letters in the same column represent significant differences (p≤0.05).

Table 6. Sensory evaluation of semi-dry fermented sausages inoculated with *L. paracasei* during storage period at 4˚C for 45 days

Storage/d	Color	Flavor	Texture	Overall acceptability
0	8.07±0.06^{a}	6.87±0.02^{a}	8±0.1^{a}	8±0.01^{a}
10	8±0.02^{b}	6.53±0.04^{b}	7.86±0.02^{a}	7.8±0.03^{b}
20	7.85±0.01^{c}	6.4±0.04^{c}	7.66±0.03^{b}	7.16±0.01^{c}
30	7.83±0.01^{c}	6.18±0.01^{d}	6.66±0.02^{c}	7±0.09^{d}
40	7.76±0.01^{d}	6.13±0.01^{e}	6.5±0.02^{d}	6.5±0.03^{e}
45	7.5±0.03^{e}	5.83±0.02^{f}	6.16±0.01^{e}	6.44±0.04^{e}

*Values are means of three replicates ± standard deviation.
**Means with different superscript letters in the same column represent significant differences (p≤0.05).

Table 7. Sensory evaluation of control and semi-dry fermented sausages during storage period at 4˚C for 45 days

Storage/d	Color	Flavor	Texture	Overall acceptability
0	7.75±0.01^{a}	6.66±0.01^{a}	7.5±0.03^{a}	7.25±0.02^{a}
10	7.4±0.03^{b}	6.3±0.03^{b}	6.85±0.02^{b}	6.75±0.01^{b}
20	7±0.09^{c}	5.83±0.01^{c}	6.66±0.01^{c}	6.50±0.04^{c}
30	6.83±0.01^{d}	5.66±0.01^{d}	6.5±0.03^{d}	6.30±0.03^{d}
40	6.38±0.03^{e}	5.63±0.03^{d}	6.34±0.02^{e}	6.07±0.12^{e}
45	6.16±0.02^{f}	5.6±0.02^{e}	6.00±0.1^{f}	5.83±0.02^{f}

*Values are means of three replicates ± standard deviation.
** Means with different superscript letters in the same column represent significant differences (p≤0.05).

3.3.3. Texture

Texture is a predominant element of the quality and acceptability of foods. It is perceived from the sensory impressions of the physical properties of a material, its nature, composition and behavior on deformation received from the senses of touch, sight and hearing [36]. As shown in Table 5, texture scores were significantly (p≤0.05) decreased in all samples of semi-dry fermented sausage inoculated with *L. casei* (range 7.82-6.13) during 45 days at 4°C. There were significant differences in the texture scores of semi-dry fermented sausage inoculated with *L. paracasei*. Texture scores decreased in all samples (range 8.0-6.16) during the cold storage (Table 6).

During the refrigerated storage at 4°C, the scores of texture decreased significantly (p≤0.05) in all samples of semi-dry fermented sausage (control) (Table 7). The control sample had lower score (range 7.50-6.00) compared to those inoculated with *L. casei* and *L. paracasei* during the cold storage period**.** The significant decrease in texture during storage may be due to changes in the disulfide bonds and contents of amino acid [36]. Increasing levels of fat constantly improved the scores of texture (as obtained by Ahmad and Amer [36]) during the refrigerated storage (2°C)indicated that the scores of the texture of semi-dry fermented sausages incorporated with 20% and 25% fat significantly (p≤0.05) decreased.

3.3.4. Overall acceptability

According to Table 5, overall acceptability scores decreased significantly (p≤0.05) in all samples of semi-dry fermented sausage inoculated with *L. casei* and ranged between 7.33-6.22 during 45 days at 4°C. The samples of semi-dry fermented sausage inoculated with *L. paracasei* had the highest overall acceptability scores during the storage period. Overall acceptability scores decreased significantly ($p \leq 0.05$) in all samples (range 8.0-6.44) during 45 days (Table 6). The control sample had lower scores (range 7.25-5.83) compared to those inoculated with *L. casei* and *L. paracasei* during the storage period (Table 7). The best sensory characteristics regarding in color, flavor, texture and overall acceptability scores were found in the samples of semi-dry fermented sausage inoculated with *L. paracasei*.

4. Conclusion

It is concluded that the fermentation process with *L. casei* and *L. paracasei* of the low fat semi-dry fermented sausage processed from beef meat led to the dominance of lactic acid bacteria on the microflora in the fermented sausage; it helped to improve the sensorial quality, safety and shelf life by inhibition the spoilage and the growth of pathogenic bacterial and kept them in appropriate condition within 45 days of the refrigerated storage at 4°C. The best sensory evaluation in the color, flavor, texture and overall acceptability scores was obtained in the samples of semi-dry fermented sausage inoculated with *L. paracasei*. The use of *L. casei and L. paracasei* improved the quality and nutritional value of food by presenting functional properties.

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6. Conflict of Interest

The authors declare that there is no conflict of interest.

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خواص فیزیکوشیمیایی و ارزیابی حسی سوسیس فراسودمند تخمیری با چربی کاهش یافته تهیه شده از گوشت گوساله

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چکیده

سابقه و هدف: در این مطالعه سه نوع سوسیس تخمیری نیمه خشک تهیه شده از گوشت گوساله تولید شدند. نمونه‌های فاقد استارتر(نمونه شاهد)، نمونه‌های تلقیح شده با *لاکتوباسیلوس کازئی* و نمونه‌های تلقیح شده با *لاکتوباسیلوس پاراکازئی*. سوسیس‌های تخمیری پروبیوتیک ایمن بوده و از فرآورده‌های گوشتی سالمی به شمار می‌روند که توجه زیاد تجاری و سهم فزاینده ای از بازار را داشته اند.

مواد و روش‌ها: خصوصیات فیزیکوشیمیایی (میزان پروتئین، رطوبت، چربی، خاکستر، لاکتیک اسید و pH)، ویژگی‌های میکروبی (شمارش کلی هوازی ها، کپک و مخمر و باکتری‌های لاکتیک اسید) و ارزیابی حسی (رنگ، عطر، بافت و پذیرش کلی) در روزهای 0، 10، 20، 30، 40 و 45 نگهداری در یخچال در دمای 4^oC بررسی شد.

یافته‌ها و نتیجه‌گیری: در میزان رطوبت نمونه ها، که در تمام نمونه ها در مدت نگهداری در یخچال کاهش یافته بود، تفاوت معنی‌داری ($p \leq 0/05$) مشاهده شد. در حالی که در مورد سایر ویژگی‌ها، مانند میزان پروتئین، چربی و خاکستر، در تمام نمونه‌ها افزایش یافت. همچنین اسید لاکتیک تولید شده توسط باکتری‌های لاکتیکی در طی فرآیند تخمیر، موجب کاهش pH تمام نمونه‌ها و بهبود ویژگی حسی سوسیس‌های تخمیری تلقیح شده با *لاکتوباسیلوس کازئی* و *لاکتوباسیلوس پاراکازئی* در طول مدت نگهداری شد. بهترین نتایج، به لحاظ ویژگی‌های فیزیکوشیمیایی، میکروبی و ارزیابی‌های حسی در سوسیس‌های تخمیری تلقیح شده با *لاکتوباسیلوس پاراکازئی* مشاهده شد. به طوری که می‌توان محصول ذکر شده را در دمای 4^oC به مدت 45 روز نگهداری نمود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافعی وجود ندارد.

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