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Physicochemical Properties and Sensory Evaluation of Fermented Sausage using Probiotic Bifidobacterium

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Abstract

Probiotic food products are very popular on domestic and international markets. The application of probiotic in meat products is still being explored. Six types of sausages were developed from beef sausage (with and without probiotic), mutton sausages (with and without probiotic) and chicken sausages (with and without probiotic) and stored in the refrigerator at 4°C and sampling were performed at1, 10, 20 and 30 days in order to analyze their physicochemical and sensorial properties. During the storage period, ash, dry matter, pH, and titrable acidity, water holding capacity, fat, and protein ($p < 0.05$) were significantly differed among all types of sausages. Beef sausages without probitoic showed high amount of dry matter (52.2±0.60%), protein (24.40± 0.00%) and fat (10.16± 0.35%) at the end of storage. On the other hand, high amount of titrable acidity as(1.22±0.022%) and lower pH value (5.16±0.01) were observed in chicken sausages with probiotic. Water holding capacity was decreasing during storage period. All types of sausages with probiotic had high scores for all attributes, in relation to color, aroma, flavor and overall acceptability while, without probiotic added sausages (beef, mutton and chicken) had higher preferences to accept the texture. Finally, probiotic sausages showed sensory characteristics greatly appreciated by the panelist, with the highest preference except for texture. In addition, beef and mutton sausages (with and without probiotic) were mostly preferred by the panelist for the sensory attributes.

Keywords: **S**ausage, Fermented, Probiotic, Physicochemical, Sensorial properties;

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1. Introduction

Meat provides high quality protein, consisting of all essential amino acids, minerals and vitamins (Hussein et al., 2017). In the mean time consumers are more and more concerned about quality and health-promoting characteristics of meat and meat products like sausages, they are not only looking for certified organic products but also expect confirmation of a greater nutritive value of such products, their prolonged life, and even an additional health promoting effects (Radulovic et al., 2011). Health and wellness is one of the major consumer trends in the food industry. Probiotic lactic acid bacteria are living micro-organisms which have a beneficial effect on the health of the consumer when ingested in certain amounts. Consumption of probiotics has a positive effect on the intestinal microflora, colonization resistance against pathogens and shows beneficial immune responses (Ruiz et al., 2014; Radulovic et al., 2011). One of the main characteristics of these probiotic strains are acid and bile salt resistance (Radulovic et al., 2011; Prasad et al., 1998).

Probiotics are widely used in dairy products, but their application in meat products is still being explored. Meat is generally heated before consumption, which kills probiotic bacteria, but dry sausages are processed by fermenting (Radulovic et al., 2011). The lactic acid bacteria which play a significant role and commonly found in fermented sausages. These microorganisms are used as starter cultures, promoting meat fermentation (Papamanoli et al., 2003). Probiotics are generally beneficiary to human health, most probiotics contain Lactobacillus acidophilus and Bifidobacterium spp, which are frequently used in food products and are normally present in the human gastrointestinal tract (Radulovic et al., 2011; Dali and Davis, 1998). The application of Bifidobacterium spp. and Lactobacillus acidophilus probiotic microorganisms in dry fermented meat products is not yet common. Furthermore, both bacteria synthesize folic acid, niacin, thiamine, riboflavin, pyridoxine and vitamin K, which are slowly absorbed by the body (Tamine et al., 1995). L. acidophilus exhibits antagonistic activity against pathogenic bacteria, which are food borne disease agents (Sanders and Klaenhammer, 2001).

Sausages are prepared from different types of meat and 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 (Ahmad et al., 2012). Probiotic fermented sausages are safe and healthy meat products, which receive high demand among the consumers. There is little information are available to produce fermented sausage from different types meat by using probiotics organism. Therefore, the aim of this work was to develop the probiotic sausages using different types of meat and their effect on the basic chemical, physical composition and sensory quality of fermented sausages.

2. Materials and Methods

2.1 Probiotic culture preparation

Probiotic organism Bifidobacterium (CHR HANSEN, Québec, Canada) was prepared by adding culture (0.33 g) in to one liter of sterilized skim milk (1.5% fat) and stored as 100 ml aliquots in erlene meyer flsk at frozen temperature (-20 ^{o}C) and the cultures were thawed, activated and used for fermentation of sausages batter. Each 1 Kg sausages batter was inoculated with 5 mL of Probiotic organism of Bifidobacterium.

2.2 Sausage preparation

Totally six samples of beef, mutton and chicken fermented sausage were separately prepared as follows: Beef sausages was produces with probiotic organism and without probiotic organism, mutton sausages was produces with probiotic organism and without probiotic organism and chicken sausages was produces with probiotic organism and without probiotic organism.

Fresh boneless beef, mutton and chicken meats were purchased from a local wholesaler and refrigerator at 0^{o} C. Chilled beef, mutton and chicken meat were pre-weighed. Then the chilled meats were chopped and mixed separately using a in a bowl meat cutter and each two kilograms of beef, mutton and chicken sausages batter were separately prepared from those meats. Meat and other above-mentioned ingredients were used in certain percentages per kg batter for production of fermented sausages. The Probiotic organism of Bifidobacterium was added to each sample at 5 ml of inoculums per kg of batter. In the control sample (without probiotic organism of Bifidobacterium) 5 ml of sterile saline water was added per Kg of batter at each sample. Then non-meat ingredients and spices were added and mixed with mixed grinder for about 20 min as described by Hussein et al. (2017). The batter was filled manually into natural casings of goat intestine. The produced sausages were fermented at 30°C for 24 hr and then dried at 60°C for 4 hrs. Finally, they were stored in the refrigerator at 4°C and samplings were performed at1, 10, 20 and 30 days in order to analyze their physicochemical and sensorial properties.

2.3. Physico-chemical analysis

2.3.1 Nutritional Analysis of Sausages

Sausages samples were analyzed to determine the chemical composition of sausages, such as dry matter, ash, fat, protein. The dry matter contents of sausages was determined by oven drying at 105 °C to get constant weight as described by AOAC (1995). Ash content was determined by using muffle furnace at 550 °C for 4 hrs as mentioned in the method of AOAC (1995). Fat content was measured by the Soxhlet method with a solvent extraction system based on the method of AOAC (1995). Total protein content was determined by Kjeldahl method with an automatic Kjeldahl nitrogen analyzer which is used to determine the amount of nitrogen (%) and to calculate the ratio of total protein by multiplying the amount of nitrogen to the constant factor (6.32) as mentioned in the method of AOAC, (1995).

2.3.2 Measurement of pH and lactic acid value

pH of sausage samples were determinate by blending 5g of sausage sample was blended with 5 mL of deionizer water as described by Wang (2000). The pH was measured by a digital pH meter (model: Delta320 pH meter, HANNA Instrument, USA). The pH meter was calibrated with buffer standard pH 4 and pH 7 before measurement. Lactic acid value was determined by filtration of the samples and then titration with 0.1N NaOH as the method described by Hussein et al. (2017).

2.3.3 Determination of Water holding capacity (WHC)

Water holding capacity was determined by the modified centrifugal method using 2.5 g of each sausage sample was wrapped with filter paper (Whatman -3) and centrifuged at 3,000 rpm for 20 min. Water holding capacity was calculated described by Jauregui et al. (1981).

2.3.4. Sensory analysis

Sensory analysis was conducted for organoleptic parameters namely color, taste, flavor, texture and overall acceptability of the products. This was conducted by10 panelist. 9 point hedonic scale, ranging from excellent (score = 9) to very poor (score = 1) as extremes, was used for evaluation. A questionnaire was used for the sensory assessment. Each panelist was asked to evaluate the samples from different treatment which were arranged to assess the organoleptic qualities (Resurreccion, (1998).

2.4. Statistical analysis

Samples were randomly collected and were tabulated. Multivariate Analysis of Variance (MANOVA) test was used to determine the significance level of the treatments, while the Duncan's Multiple Range Test (DMRT) was used for mean separation. Descriptive statistics was done on sensory attributes and the means were compared using the Tukey's test ($P < 0.05$).

3. Results and Discussion

3.1. Physico-chemical analysis

Table 1 shows the results of the dry matter contents of fermented sausage samples inoculated with probiotic culture after 1, 10, 20 and 30 days of cold storage at 4°C.

Table 1: Dry matter contents of sausages during storage period

Treatments	Storage periods			
Dry matter (%)	**Day 1**	**Day 10**	**Day 20**	**Day 30**
T1	39.80 ± 0.30^{efg}	45.20 ± 0.35^{cd}	48.20 ± 0.32^{bc}	52.20 ± 0.60^{a}
T2	31.60 ± 1.45^{ij}	36.30 ± 0.32^{fgh}	38.90 ± 0.36^{efgh}	40.30 ± 0.76^{ef}
T3	41.10 ± 0.27^{efgh}	44.10 ± 0.10^{d}	46.90 ± 0.40^{bcd}	49.30 ± 0.71^{ab}
T4	32.67 ± 0.70^{j}	35.03 ± 0.21^{hi}	36.03 ± 0.15^{gh}	39.67 ± 0.55^{efg}
T5	26.10 ± 0.20^{k}	25.80 ± 0.30^{jk}	27.90 ± 0.30^{k}	30.50 ± 0.88^{j}
T6	37.50 ± 0.87^{efgh}	39.33 ± 0.86^{efg}	40.50 ± 0.51^{e}	44.10 ± 0.93^{d}

T1- Beef sausage, T2- probiotic beef sausage, T3- Mutton sausage, T4- probiotic mutton sausage T5- Chicken sausage, T6- probiotic chicken sausage. Values are means ± standard deviations of replicate determination. Mean with the same letters are not significantly different at ($p < 0.05$).

In this study Significant difference ($p < 0.05$) was observed in dry matter content among all kind of sausages. The amount of moisture content in sausages indicated differences according to different meat and probiotic used. The maximum dry matter content was observed in beef sausages without probiotic (52.2±00.6%) and lowest in chicken sausage probiotic (30.50±0.88 %). On the other hand, among all types of probiotic sausages, higher dry matter content was observed in chicken sausage with probiotic (44.10 ± 3.93%), while lowest dry matter content was observed in mutton sausage with probiotic (39.67±0.55%). Due to the reduction of moisture content in sausages, dry matter content was increased, at the same time production of lactic acid by probiotics has an impact on water losses during the fermentation process. The results were in accordance with (Bacus 1984 ; Kozacinski et al., 2008) who reported similar findings in their study on dry matter contents in fermented sausages. Ash content of different meat types of with or without probiotic sausages increased in an apparently with respect to dry matter content. Due to the moisture reduction and dry matter increment, ash content of sausages was increased. This result was line with Ahmad et al. (2012) results, who observed that slight increment in ash content was observed due to reduction in the moisture content during storage.

The total protein content was significantly ($p<0.05$) increased in all types sausages during storage (Table 2). At the end of storage period, higher protein content was observed in beef sausages without probiotic (24.40± 0.00%) and lower values was observed mutton sausage with probiotic (21.03±0.05%). This increase in protein content in time can be due to the reduction in moisture values (Mauriello et al., 2004). Our results coincide with those of Asmare and Admassu, (2013) who reported increase of protein content in all dry fermented sausages, this can be attributed to the decrease of water content and high concentration of nutrients during processing. Fat content ranged varied between 3.16 and 10.16% for sausage during the cold storage period.

The trend in fat content; it was similar to protein content in a significantly ($p\leq0.05$) increased in all samples during storage (Table 2). Higher fat content was observed in beef sausages without probiotic (10.16± 0.35%) and lowest fat contents was observed in probiotic chicken sausage without probiotic (4.9± 0.05%) at end of the storage. Fat contributes to nutritional, organoleptic and technological properties in meat products (Olivares et al., 2010). Our results are in consistency with the findings of Asmare and Admassu, (2013), who reported that fat content was also significantly ($p\leq0.05$) increased in all fermented sausages.

pH and lactic acid values for the all kind of sausages during storage at 4°C are shown in Table 3. pH value of all samples significantly ($p<0.05$) decreased during the refrigerated storage (range 5.46 -5.16). Higher pH was observed in mutton sausages without probiotic (5.66±0.01) and lower pH was observed in probiotic chicken sausage with Probiotic (5.16± 0.01). Probiotic sausages showed lower pH value than other sausages since production of lactic acid by probiotics. 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. Radulovic et al. (2011) who, reported that fermented sausages showed gradual reduction in pH according to the increment of acidity.

Table 2: Protein and fat contents of sausages during storage period

Treatments	Storage periods			
Protein %	**Day 1**	**Day 10**	**Day 20**	**Day 30**
T1	21.40 ± 0.01^{cde}	22.60 ± 0.10^{bc}	23.20 ± 0.05^{b}	24.40 ± 0.00^{a}
T2	21.33 ± 0.15^{cde}	22.33 ± 0.05^{bc}	23.16 ± 0.05^{b}	24.30 ± 0.05^{ab}
T3	19.40 ± 0.05^{efgh}	19.76 ± 0.05^{efg}	19.96 ± 0.05^{efg}	21.06 ± 0.11^{cd}
T4	19.56 ± 0.05^{defg}	19.90 ± 0.01^{de}	20.83 ± 0.05^{cd}	21.03 ± 0.05^{cde}
T5	18.70 ± 0.01^{efg}	20.76 ± 0.1^{def}	21.36 ± 0.32^{cde}	22.32 ± 0.10^{bc}
T6	18.83 ± 0.05^{efg}	20.70 ± 0.05^{def}	21.20 ± 0.10^{cde}	21.76 ± 0.05^{cd}
Fat %				
T1	8.46 ± 0.05^{def}	8.76 ± 0.05^{bcd}	9.20 ± 0.05^{ab}	10.16 ± 0.35^{a}
T2	8.46 ± 0.05^{def}	8.76 ± 0.05^{bcd}	9.20 ± 0.05^{ab}	10.10 ± 0.10^{a}
T3	8.36 ± 0.11^{ef}	8.63 ± 0.05^{def}	8.90 ± 0.10^{bc}	9.10 ± 0.05^{b}
T4	8.63 ± 0.05^{def}	8.86 ± 0.05^{bcd}	9.06 ± 0.05^{b}	9.30 ± 0.05^{ab}
T5	3.83 ± 0.05^{m}	4.06 ± 0.05^{l}	4.63 ± 0.05^{h}	4.90 ± 0.05^{gh}
T6	3.39 ± 0.05^{m}	4.13 ± 0.05^{k}	4.63 ± 0.05^{h}	5.10 ± 0.10^{g}

T1- Beef sausage, T2- probiotic beef sausage, T3- Mutton sausage, T4- probiotic mutton sausage T5- Chicken sausage, T6- probiotic chicken sausage. Values are means ± standard deviations of replicate determination. Mean with the same letters are not significantly different at ($p< 0.05$).

Lactic acid values significantly ($p<0.05$) increased (range 0.82-1.22%) during the cold storage. During the storage period, titrable acidity was increasing due to catabolism of lactose by probiotic organism. Chicken sausages with probiotic (1.21± 0.02%) showed higher titrable acidity and beef sausage without probiotic (1.08± 0.03%) showed lowest titrable acidity. At the same time, titrable acidity was higher in sausages with probiotic compare to sausages without probiotic. Due to fermentation process by probiotic the titrable acidity was increasing. Similarly, Hussein et al. (2017) were reported in his study that Lactobacilli produce lactic acid which decrease in pH and the increase in acidity in fermented sausages.

3.2 Water holding capacity in sausages during storage period

The water-holding capacity is a crucial property for the quality of meat and may be defined as the capacity of the meat to retain moisture during the application of external process such as cutting, grinding and heating. In this study water holding capacity of sausages was ($p<0.05$) differed among all types of sausages. According to

Table 3: pH and titrable acidity of sausages during storage period

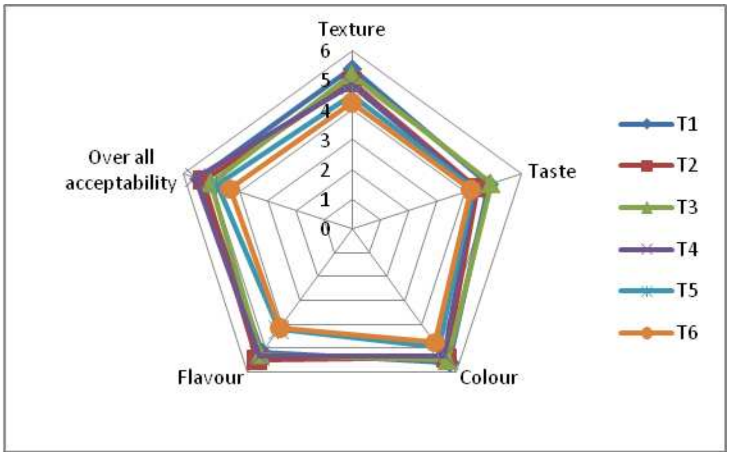
Treatments	Storage periods			
pH	**Day 1**	**Day 10**	**Day 20**	**Day 30**
T1	5.46±0.01^{def}	5.43±0.01^{g}	5.41±0.01^{h}	5.38±0.01^{hi}
T2	5.39±0.00^{hi}	5.37±0.01^{ij}	5.35±0.01^{jk}	5.33±0.01^{k}
T3	5.56±0.01^{a}	5.55±0.02^{b}	5.52±0.04^{c}	5.48±0.01^{de}
T4	5.49±0.01^{d}	5.47±0.01^{de}	5.45±0.01^{fg}	5.44±0.02^{efg}
T5	5.30±0.00^{l}	5.25±0.01^{m}	5.23±0.01^{mn}	5.21±0.01^{n}
T6	5.25±0.01^{m}	5.23±0.01^{mn}	5.21±0.01^{n}	5.16±0.01^{mno}
Acidity %				
T1	0.82±0.01^{m}	0.96±0.01^{kl}	1.03±0.06^{ik}	1.08±0.01^{b}
T2	1.15±0.13^{cdefg}	1.14±0.02^{defgh}	1.21±0.01^{a}	1.05±0.03^{ij}
T3	0.92±0.01^{l}	0.96±1.01^{kl}	1.06±0.02^{hij}	0.97±0.03^{kl}
T4	1.10±0.01^{efghi}	1.13±0.02^{defgh}	1.21±0.01^{a}	1.11±0.02^{efghij}
T5	1.07±0.06^{ghij}	1.12±0.02^{efghi}	1.16±0.03^{cdef}	1.16±0.05^{cde}
T6	1.19±0.03^{c}	1.20±0.03^{mn}	1.21±0.01^{a}	1.22±0.02^{a}

T1- Beef sausage, T2- probiotic beef sausage, T3- Mutton sausage, T4- probiotic mutton sausage T5- Chicken sausage, T6- probiotic chicken sausage. Values are means ± standard deviations of replicate determination. Mean with the same letters are not significantly different at ($p < 0.05$).

this study the decreasing trend of water holding capacity was observed during storage period. It is due to when the pH decline in the in the sausages decrease in water retention capacity occurs, thus facilitating dehydration and, consequently, the reduction in water activity in sausages (Ruiz et al., 2014; Mauriello et al., 2004).

3.3 Change in sensory attributes during storage period

The result showed that significant difference ($p < 0.05$) was observed between treatments throughout the storage period. During the cold storage at 4°C, the texture, taste, colour, flavor and overall acceptability scores decreased significantly in all samples of with and without probiotic sausages. Beef and mutton sausages (with and without probiotic) had the highest attributes scores during the cold storage compare to chicken sausages (with and without probiotic) as shown in Figure 1. The different types of sausages with probiotic had high scores for all attributes, in relation to color, aroma, flavor and overall acceptability. On the other hand, without probiotic added sausages (beef, mutton and chicken) had higher preferences to accept the texture. This may suggest that the fermentation process (sausages with probiotic) changes the texture of sausages, probably due to the reduction in pH and consequent decrease in the water retention capacity after cooking. Similar result was observed by Bomdespacho et al. (2014) and Macedo et al. (2008) in hamburger production. This study revealed that the probiotic sausages showed sensory characteristics greatly appreciated by the panelist, with the highest preference except for texture. In addition, beef and mutton sausages (with and without probiotic) were mostly preferred by the panelist for the sensory attributes.



T1- Beef sausage, T2- probiotic beef sausage, T3- Mutton sausage, T4- probiotic mutton sausage T5- Chicken sausage, T6- probiotic chicken sausage

Figure 1: Variation in sensory attributes during storage period

4. Conclusion

This study, dry matter, ash, acidity, fat and protein contents of all types of sausages were increased simultaneously during storage period. pH and water holding capacity decreased with storage period in all types sausages. The best sensory evaluation in the color, flavor, taste and overall acceptability scores was obtained in the sausage with probitics in all types of meat. Beef and mutton sausages (with and without probiotic) had the highest attrbutes scores during the cold storage compare to chicken sausages (with and without probiotic) The use of probitics improved the quality and nutritional value of sausages by presenting functional properties.

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