Effect of different cultures of lactic acid bacteria fermentation on quality and shelf life of semi dry fermented sausages of buffalo meat

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ARTICLE INFORMATION

ABSTRACT

Four different species of lactic acid bacteria had been used as a starter cultures in six different combinations. Semi dry fermented sausages (SDFS) were prepared using two levels of fat viz. 20% and 25% respectively. Different cultures of LAB fermentation significantly (p<0.05) affected the pH, MPR and TBA number of sausage samples. Refrigerated storage significantly (p<0.05) decreased the pH and MPR while a significant increase (p<0.05) was noticed in TBA number values. Increasing fat level apparently increased pH, TBA and MPR. The total plate count was found to be in between 5.68 and 6.51 log cfu/g, yeast and mold count was found to be in between 3.99-5.21 log cfu/g and coliform count was found to be in the range of 2.26-2.62 log cfu/g on 120th day of storage. While Salmonella shigella was not detected in all samples of semi dried fermented sausages at all during refrigerated storage (2°C) for 120 days. There was no clear noticeable effect of fat level on the microbial properties of the samples.

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1. Introduction

Starter cultures for sausage fermentation are composed of nontoxic, nonpathogenic, and phenotypically and genetically stable microbial strains that possess activities that contribute to the fermentation of the meal, leading to proper acidification, flavor, texture, and color development, and microbial stability of the end-product (Leroy & De Vuyst, 2009). Commercial starter cultures contain LAB are distributed frozen or freeze-dried on suitable carriers (Lucke, 1998). Commercial starter cultures contain LAB are distributed frozen or freeze-dried on suitable carriers (Lucke, 1998). The bacteria which play a significant role and commonly found in fermented sausages are lactic acid bacteria (Coppola et al., 1998). These microorganisms are used as starter cultures, promoting meat fermentation (Papamanoli et al., 2003). Lactic acid bacteria improve safety and stability of the product, enhance colour stability, prevent rancidity and release various aromatic substances (Coppola et al., 1998; Hammes et al., 1995; Nychas & Arkoudelos, 1990; Papamanoli et al., 2003). Lactobacilli are the predominant lactic acid bacteria and among them the most frequently isolated strains are Lactobacillus curvatus, Lactobacillus sakei, and Lactobacillus plantarum (Hammes, 1990). The most promising bacteria for starter cultures are those which are isolated from the indigenous micro-flora of traditional products. These microorganisms are well adapted in the meat environment and are capable of dominating the micro-flora of products. The strains selected as
starter or protective cultures must have the most important technological properties and/or bacteriocin production capabilities (Hammes, 1990).

This study work was carried out to study the effect of different cultures of lactic acid bacteria (LAB) fermentation on quality and shelf life of semi dry fermented sausages of buffalo meat during refrigerated storage (2°C). The quality of sausages developed was evaluated on the basis of physico-chemical characteristics (pH, moisture protein ratio MPR and TBA number) and microbiological characteristics (total plate count, yeast and mold count, coliform count and salmonella shigella count).

2. Materials and Methods

Meat samples collected from the local meat shop in the study were from buffaloes slaughtered according to traditional halal method at slaughter house of Municipal Corporation of Aligarh. The animals were kept in lairage for a period of 18-20 hours. Meat samples from round portion (biceps femoris muscle) of 2.5, 3 and 3.5 years aged female carcasses of good finish were obtained from meat shop within 4 hr. of slaughter. The meat chunks were packed in combination film packaging and brought to the laboratory. Other non-meat ingredients like spices, salt, condiments and combination film were procured from the local market. This fibrous casing (35 mm dia.) was procured from PRS technologies, India. The meat and fat were kept inside ultra low temperature cabinet (Yarco, India) at 2°C.

Fermented sausages were prepared from comminuted mixture of meat; fat salt spices and sugar using bacterial culture and allowed to undergo fermentation under strict conditions of temperature and humidity. Two different lots of semi dry fermented sausages were conducted containing two levels of fat 20% and 25%. The composition of fermented sausages was kept as:- meat (2 kg), fat (400 & 500 g for 20 & 25% fat samples respectively), mix spices (24 g), chilli powder (12 g), condiments (40 g), salt (50 g), sugar (20 g), dextrose (10 g), sodium ascorbate (1000 mg), mono sodium glutamate (2 g), ice (150 g) and 10 ml of starter culture. The buffalo meat and fat were ground on a grinder (PRS Technologies, India). The pH of suspension was recorded with pH meter model PH1500 (Eutech, Singapore).

The quality of sausages developed was determined after homogenizing 10g of the sample with 100 ml distilled water using laboratory grinder (Yarco, India). The pH of suspension was recorded with pH meter model PH1500 (Eutech, Singapore).

Thiobarbituric acid TBA reagent was prepared by dissolving 0.2883 g of Thio barbituric acid in sufficient quantity of 90% acetic acid and by slight warming, the volume being made up 100 ml with 90% acetic acid. 20 g of meat sample were blended in a blender with 50 ml of cold 20% tricarboxylic acid (TCA) for 2 min. The blended contents were rinsed with 50 ml of distilled water, mixed together and filtered through filter paper and the intermediate was collected in a 100 ml capacity-measuring cylinder. The filtrate, termed the TCA extract was used in the estimation of thio barbituric acid (TBA) number.

TBA number was measured by the method described by Strange et al., (1975). Five ml of TCA extract was mixed with 5ml of TBA reagent in test tube. The test tube was kept in a water bath at 100°C for 30 min along with another test tube containing a blank of 10% TCA and 5 ml of TBA reagent. After cooling the tubes in running water about 10 min, the absorbance was measured at 530 nm in a spectrophotometer (Digital spectrophotometer Model 310E, India) and reported as TBA number.

All the samples were evaluated for direct plate count using serial dilution spread plate technique with nutrient agar medium for total plate count, potato dextrose agar for yeast and mold count, MacConkey agar for coliform count and S.S. agar for salmonella shigella count (APHA, 1992). Microbiological characteristics of sausage samples were evaluated in fresh conditions and during refrigerated storage (2°C) after constant intervals. For determination of total plate count, yeast and mold count, coliform count and Salmonella shigella count, the samples were taken with sterile knife,
comminuted to fine particles in a tissue homogenizer (Yarco, India) and then transferred to a test tube containing 9 ml of normal saline solutions. The samples were homogenized in the cyclomixer (mode CM-101, India). Serial dilutions were made by transferring 1 ml of the extract from each dilution and finally the samples were inoculated in the petridishes containing the solid medium. The colonies were counted after 24-48 hr incubation in BOD incubator (York Scientific, India).

\[
\text{TPC} \left( \frac{\text{cfu}}{\text{g}} \right) = \frac{\text{Number of colonies}}{\text{Amount used for inoculation} \times \text{dilution factor}}
\]

Data obtained from experimental observation (n=5), were subjected to analysis of variance (Two ways ANOVA). All statistical analyses were performed using SPSS Version 10.0 for Windows (SPSS Inc., Chicago, IL, USA) as described by Field (2005).

3. Result and Discussion

3.1 pH

a) At 20% fat, pH values of the six samples were found between 4.59 and 4.72 just after product preparation. The reduction in pH was due to formation of lactic acid by the different cultures of lactic acid bacteria (LAB) using carbohydrates (dextrose & sucrose) added in the meat mixture. Different cultures of LAB fermentation significantly (p<0.05) affected the pH of sausage sample. During refrigerated storage at 2°C, pH measurement was carried out every after 15th day, till the end of shelf life and pH values were found to decrease consistently. Refrigerated storage significantly (p<0.05) reduced the pH of fermented sausages. At the end of 120 days of storage, pH values were found to be between 4.32 and 4.41.

b) At 25% fat, pH values of all samples were found between 4.62 and 4.73 (just after product preparation of samples). Different cultures of LAB fermentation significantly (p<0.05) affected the pH of sausage sample. Similar results were obtained by (Ensoy et al., 2010). During refrigerated storage at 2°C, pH values were found to significantly (p<0.05) decrease. At the end of 120 days of storage pH values were found to be between 4.39 and 4.43. Increasing the fat level from 20% to 25% increased the pH of all samples and that agreed with the result of earlier studies (Ahmad, 2005; Liaquati & Srivastava, 2010; Olivares et al., 2010).

The equation of regression lines and correlation coefficient of all samples with two levels of fat have been shown on the regression graph (Fig1). The negative sign in the coefficients of x explains that there was constant decrease of pH during drying. The values of \( R^2 \) for all samples at two different levels of fat were in between 0.9422-0.9973. The values of \( R^2 \) were near to 1, thus the graph may be approximated to a straight line and linear relation well fits between drying period and pH values.

![Figure1](image-url): Regression analysis of pH of semi dried fermented incorporated with 20% fat sausages during refrigerated storage at 2°C

3.2 Thiobarbituric acid number (TBA)

Thiobarbituric acid (TBA) number is important relevant characteristics of meat product that indicate the oxidation state and later on stage rancidity of the product. The semi-dry fermented sausages after preparation were packed in
combination film under atmospheric packaging systems. The samples contained sufficient fat and therefore samples might be oxidized by atmospheric oxygen and may lead to develop warm over flavour (WOF). TBA measurements have been frequently found to give useful correlation with sensory scores, in looking at the development of WOF in cooked meats (Poste et al., 1986). TBA number was determined as mg of malonaldehyde/kg. Malonaldehyde is produced as a result of fat oxidation and it reacts with TBA reagent to produce coloured complex with an absorption max/min 530-532 nm. The red pigment produced is the reaction product obtained from condensation of two moles of TBA reagent with one mole of malonaldehyde (Sinnhuber et al., 1958). 

a) At 20% fat, in fresh condition, TBA number values were between 0.148 and 0.166 mg of malonaldehyde/kg of meat. Samples treated with *L. lactis* ssp. *+ S. griseus* ssp. showed minimum TBA number. Samples inoculated with *L. brevis* + *L. lactis* ssp. had significantly higher accumulation of TBA number as compared to samples inoculated with *L. brevis* + *L. plantarum* and *L. plantarum* + *S. griseus* ssp. or in combination with *L. lactis* ssp. + *S. griseus* ssp. Irrespective of starter cultures employed, there was a significant (p<0.05) increase in TBA number during refrigerated storage. After 120 days of storage, Samples inoculated with *L. lactis* ssp. + *S. griseus* ssp. had significantly higher accumulation of TBA as compared to all remaining samples. Ahmad et al., (2010) and Coşkune et al., (2010) reported that TBA number of SDFS increased during refrigerated storage. Values of R² have been shown in the regression graph itself. The positive sign in the coefficients of x explains that there was constant increase of TBA number during refrigerated storage. Values of R² were very close to 1. Thus the graph may be approximated to a straight line and linear relation well fits between storage period and TBA number values.

b) At 25% fat, the TBA number values were between 0.170 and 0.293 mg of malonaldehyde/kg of meat. Samples treated with *L. brevis* + *L. plantarum* showed minimum TBA number in fresh condition. Refrigerated storage significantly (p<0.05) increased the TBA number of all samples. Samples inoculated with *L. lactis* ssp. + *S. griseus* ssp. had significantly higher accumulation of TBA number (0.748) as compared to all remaining samples. Maurya et. al (2010) reported that pastirma samples inoculated with *Micrococcus varians* (MV) and *Staphylococcus carnosus* (SC) had significantly higher accumulation of TBA as compared to samples inoculated with *Lactobacillus sakei* (LS) alone or in combination with MV and SC. Aksu and Kaya (2002) studied the effect of different commercial starter cultures on fatty acid composition of the pastirma and observed that *Staphylococcus carnosus*, *Staphylococcus xylosus* + *Lactobacillus sakei* had significant (p<0.01) effect on fatty acid composition of pastirma. TBA values of pastirma samples produced with starters were lower than those of the control group. Increasing fat level consistently increased TBA number in all samples of semi dried fermented sausages. That results were similar to the results reported by Ahmad (2005); Ahmad & Srivastava (2007); Liaquati & Srivastava, (2010). However, in both levels of fat, the values of TBA number were under safe limit on the 120th day of storage. Previous reports indicated that the meat samples containing TBA numbers from 0.5 to 1 possess no off odour (Tarladgis et al. 1960). Additionally Watts (1962) reported that values of TBA number of 1-2 mg/kg of malonaldehyde was the minimum detectable level for oxidized flavour in beef and its products for an in-experienced panel (Greene & Cumuze, 1981).

Figures 2 & 3 show the regression analysis of TBA number of semi dried fermented sausages during refrigerated storage at (2°C) incorporated with 20 and 25% fat respectively. The equations of regression and correlation coefficient (R²) have been shown in the regression graph itself. The positive sign in the coefficients of x explains that there was constant increase of TBA number during refrigerated storage. Values of R² were very close to 1. Thus the graph may be approximated to a straight line and linear relation well fits between storage period and TBA number values.  

3.3 Moisture protein ratio (MPR)  

Semi dry sausages have low moisture content (50-35%). The ratio of moisture content and protein is known as moisture protein ratio (MPR) and it has been considered a standard property.
Effect on quality and shelf life of buffalo meat:

**Figure 2:** Regression analysis of TBA number of semi dried fermented sausages incorporated with 20% fat during refrigerated storage at 2°C

**Figure 3:** Regression analysis of TBA number of semi dried fermented sausages incorporated with 25% fat during refrigerated storage at 2°C

a) Table-1 represents the results of moisture protein ratio of buffalo meat semi dry fermented sausage produced by using different cultures of LAB fermentation for 20% fat content. The moisture protein ratio of semi-dry fermented sausage with 20% fat found to be between 1.751 and 1.964 in fresh conditions. Different cultures of LAB fermentation significantly (p<0.05) affected MPR. During refrigerated storage there was a significant (p<0.05) decrease of MPR in all samples. That was
due of loss of moisture content during storage period and increase of protein percentage in SDFS. At the end of storage duration MPR values were in between 1.389 and 1.581.

b) Different cultures of LAB fermentation significantly (p<0.05) affected MPR at 25% fat content. In fresh conditions, the moisture protein ratio of semi-dry fermented sausage with 25% fat found to be between 1.832 and 2.097 (Table 6). During refrigerated storage there was a significant (p<0.05) decrease of MPR in all samples. At the end of 120th days of storage MPR were found in the range of 1.440-1.559. MPR of semi dried fermented sausages was found apparently increased due to increase the percentage of fat. That indicates the decrease in protein content was more than the decrease of moisture content due to increase the level of fat. However, in both levels of fat, the values of MPR obtained in this study were in the range of 1.751-2.097. MPR should be in the range of 3.7-1.0 (AMI, 1982). That was in accordance with FSIS, (1986); Ricke & Keeton, (1997); Doyle, (2001). Guidance from the Food Safety and Inspection Service/United States Department of Agriculture (FSIS/USDA) requires that shelf stable semi-dry and dry sausage be nitrite cured, fermented, and smoked, and have MPR of ≤3:1:1 and ≤1.9:1, with a final of pHs 5.0 (American Meat Institute Foundation, 1997). Figures 4 and 5 show the regression analysis of MPR of semi dried fermented sausages during refrigerated storage at 2°C. incorporated with 20 & 25% fat respectively. The equations of regression and correlation coefficient (R²) have been shown in the regression graph it self. The negative sign in the coefficients of x explain that there was constant decrease of MPR during refrigerated storage. Values of R² were very close to 1. Thus the graph may be approximated to a straight line and linear relation well fits between storage period and MPR values.

Table 1: Evaluation of MPR of semi dried fermented sausages incorporated with 20% fat during refrigerated storage at 2°C

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Values are means of five replicates ±SD; Means with different letters differ significantly (p<0.05)

3.4 Microbiological characteristics of fermented sausages during refrigerated storage 2°C
3.4.1 Total plate count (TPC)

Total plate counts (TCP) of all samples of semi dry fermented sausages were enumerated in fresh condition and periodically after every 15 days during refrigerated storage. There was no count detected till 30 days. However, the countable colonies were noted on 45th day of storage in both levels of fat. Refrigerated storage significantly (p<0.05) increased the TPC of semi dry fermented sausages (SDFS). The total plate count was found to be between 5.68 and 6.41 log cfu/g and 5.83-6.51 log cfu/g on 120th day of storage for samples prepared with 20% and 25% fat respectively. There was no clear noticeable effect of fat level on total plate count. Ahmad (2005), Ahmad & Srivastava (2007) and Liaquati & Srivastava (2010) reported that increasing levels of fat did not significantly (p<0.05) increase the log TPC/g values. Lowest values of log TPC/g have been recorded on samples inoculated by L. brevis + L. lactis ssp. in both levels of fat, while samples inoculated with L. lactis ssp. + S. griseus ssp. scored the highest values of log TPC/g. After 120 days of refrigerated storage (2°C) total plate count of all samples was found to be in the safe limit. Ranken & Kill (1993)
Effect on quality and shelf life of buffalo meat:

Figure 4: Regression analysis of MPR of semi dried fermented sausages incorporated with 20% fat during refrigerated storage at 2°C

Figure 5: Regression analysis of MPR of semi dried fermented sausages incorporated with 25% fat during refrigerated storage at 2°C

described that the spoilage condition which are detected when total plate count in 10^7 per g, the results are also in agreement with Hytainen et al. (1975), Essory et al., (1985). Panda (1971) had also reported that when total viable count in meat tissue exceeds log 10^7/g off odour and slim start. As reported by Brooks et al. (2008) some authors stated that microbial population on raw beef must reach approximately 10^8 cfu/g to show tackiness when touched, whereas others have claimed that proteolytic changes do not occur until bacterial populations are greater than 3.2×10^9 cfu/cm^2 are reached. The ANOVA result indicated that the different cultures of LAB fermentation significantly (p<0.05) affected total plate count of semi dry fermented sausages (SDFS). The results of antimicrobial production by the LAB strains during preliminary experiments showed that some strains
produced higher amounts of organic acids than others (Olaoye, et al., 2011). Figures 6 shows the regression analysis of TPC of semi dried fermented sausages during refrigerated storage at (2°C) produced by 25% fat. The equations of regression and correlation coefficient ($R^2$) have been shown in the regression graph itself. The positive sign in the coefficients of $x$ explains that there was constant increase of TPC during refrigerated storage. Values of $R^2$ were very close to 1. Thus the graph may be approximated to a straight line and linear relation well fits between storage period and TPC. The same trend is observed with samples containing 20% fat.

When log cfu/g of yeast and mold count increase to 4.0, spoilage of food samples starts (COJASA, 2003). The ANOVA tests indicated that the different cultures of LAB fermentation, refrigerated storage and their interaction significantly ($p<0.05$) affected total plate count of semi dry fermented sausages (SDFS). Casaburi et al. (2007) observed that the growth of yeast and molds in Italian style sausages were controlled during storage after inoculation with LAB starter cultures. They concluded that it could be due to the antagonistic activities of the latter. Another study reported similar observations in a Turkish sausage after inoculation with LAB strains as protective cultures (Erkmen, 2008). Similarly, Olaoye & Onilude (2010) noted a reduction in the yeast and moulds counts in fresh beef after inoculation with LAB starters.

3.4.2 Yeast and mold count

Yeast and mold count was not detected in sausage samples till 30 days of refrigerated storage (2°C). A very low count was observed at 45th day of storage. However, the countable colonies were noted on 60th day of storage in both levels of fat. Refrigerated storage significantly ($p<0.05$) increased the yeast and mold count of semi dry fermented sausages (SDFS). The yeast and mold count was found to be in between 4.01 and 5.21 log cfu/g and 3.99-5.19 log cfu/g on 120th day of storage for samples produced by 20% and 25% fat respectively. There was no clear noticeable effect of fat level on yeast and mold count. Ahmad (2005), Ahmad & Srivastava (2007) and Liaquati & Srivastava (2010) reported that increasing levels of fat did not significantly ($p<0.05$) increase the log Y & M C/g values. Lowest values of log Y & M C/g have been recorded on samples inoculated by L. brevis + L. plantarum in both levels of fat, while samples inoculated with L. lactis ssp. + S. griseus ssp. scored the highest values of log Y & M C/g. After 120 days of refrigerated storage (2°C) yeast and mold count of all samples was found to be more than 4 log cfu/g. This particular value of yeast and mold count defines the spoilage condition.

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Figures 6 show the regression analysis of yeast and mold count of semi dry fermented sausages during refrigerated storage at (2°C) prepared with 20% fat. The equations of regression and correlation coefficient ($R^2$) have been shown in the regression graph itself. The positive sign in the coefficients of $x$ explains that there was constant increase of yeast and mold count during refrigerated storage. Values of $R^2$ were very close.
to 1. Thus the graph may be approximated to a straight line and linear relation well fits between storage period and yeast and mold count. The similar trends have been observed for sample containing 25% fat.

**Figure 7**: Regression analysis of yeast and mold count of SDFS incorporated with 20% fat during refrigerated storage at 2°C

**Table 2**: Evaluation of coliform count of semi dried fermented sausages incorporated with 20% fat during refrigerated storage at 2°C

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Values are means of five replicates ±SD; Means with different letters differ significantly (p<0.05)

S1= *Lactobacillus brevis* + *Lactobacillus plantarum*, S2= *L. brevis* + *Lactococcus lactis* ssp. S3= *L. plantarum* + *L. lactis* ssp. S4= *L. brevis* + *Streptomyces griseus* ssp. S5= *L. plantarum* + *S. griseus* ssp. S6= *L. lactis* ssp. + *S. griseus* ssp. F1= 20% fat level. ND = not detected.

3.4.3 Coliform count

Coliform count of semi dried fermented sausages produced with different cultures of LAB fermentation was enumerated and it was found there was no sign of coliform bacteria on plates containing Macconkey agar till 105th day of storage (2°C) in both levels of fat. However coliform count was found to be in the range of 2.05-2.43 log cfu/g after 105 days of storage (Tables-2 & 3). There was no detectable effect of increasing fat level on coliform count. Refrigerated storage significantly (p<0.05) increased the coliform count of all samples at both levels of fat. In the final stage of storage, coliform count was found to be in the range of 2.26-2.62 log cfu/g. There was a significant (p<0.05) difference between samples due to the use of different cultures of LAB. Lowest values of log coliform count/g have been recorded on samples...
inoculated by *L. brevis* + *L. plantarum* in both levels of fat, while samples inoculated with *L. brevis* + *L. lactis ssp.* scored the highest values of log coliform count/g. Adding starter culture accelerated the formation of lactic acid during processing of fermented sausages leading to drop in pH of the products, thus inhabiting the growth of undesirable bacteria. Nazli (1998) investigated the effect of starter culture on the ripening of *sucuk* and reported that coliform bacteria count decreased from initial value of 7.2 log cfu/g to 2.84 log cfu/g after 9 days of fermentation. Reduction in counts of enterobacteriaceae and Staphylococcus in meat has been also reported in other earlier studies (Gomółka- Pawlacka et al. 2004; Kaban & Kaya 2006; Olaoye &Onilude, 2010). Usually, the presence of total coliform in food indicates improper heat treatment or post processing contamination. Coliform are not usually pathogenic. They also indicate inadequate sanitation and disinfection of appliances (CQIASA, 2003).

### Table-3: Evaluation of coliform count of semi dried fermented sausages incorporated with 25% fat during refrigerated storage at 2°C

<table>
<thead>
<tr>
<th>Sample code</th>
<th>No. of Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>S1F2</td>
<td>ND a</td>
</tr>
<tr>
<td>S2F2</td>
<td>ND a</td>
</tr>
<tr>
<td>S3F2</td>
<td>ND a</td>
</tr>
<tr>
<td>S4F2</td>
<td>ND a</td>
</tr>
<tr>
<td>S5F2</td>
<td>ND a</td>
</tr>
<tr>
<td>S6F2</td>
<td>ND a</td>
</tr>
</tbody>
</table>

Values are means of five replicates ±SD; Means with different letters differ significantly (p<0.05)

S1=Lactobacillus brevis + Lactobacillus plantarum, S2=L. brevis + Lactococcus lactis ssp. S3= L. plantarum + L. lactis ssp. S4= L. brevis + Streptomyces griseus ssp. S5= L. plantarum + S. griseus ssp. S6=L. lactis ssp. + S. griseus ssp; F2= 25% fat level. ND = not detected.

3.4.4 *Salmonella shigella* count

*Salmonella shigella* was not detected in all samples of semi dried fermented sausages at all during refrigerated storage (2°C) for 120 days.

### 4. Conclusion

According to the microbiological characteristics of semi dry fermented sausages during refrigerated storage (2°C), samples inoculated with *Lactobacillus brevis* + *Lactobacillus plantarum* had a shelf life of 120 days and samples inoculated with *L. brevis* + *Lactococcus lactis ssp.* and *L. plantarum* + *L. lactis ssp.* had a shelf life of 105 days, while preparation of semi dry fermented sausages samples by using a combined cultures of *L. brevis* + *Streptomyces griseus ssp.*, *L. plantarum* + *S. griseus ssp.* and *L. lactis ssp.* + *S. griseus ssp.* and study during refrigerated storage established a shelf life of 90 days only.

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