Microbial profile of buffalo sausage during processing and storage

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Abstract

A study was made on the microbial levels of buffalo sausage during preparation and storage at 4 ± 1 °C. Microbial counts in raw minced meat were, total plate count (TPC) (log cfu/g) 5.41 ± 0.25; coliforms (MPN/g) 23.2; Staphylococcus aureus (log cfu/g) 1.57 ± 0.11; yeasts and molds (log cfu/g) 2.29 ± 0.07 and lactic acid bacteria (LAB) (log cfu/g) 0.60 ± 0.20. Sausage emulsion showed similar trend in microbial counts with minimal microbial contamination during the preparation of emulsion. Cooked buffalo sausage gave the following microbial counts: TPC (log cfu/g) 3.75 ± 0.31; coliforms (MPN/g) 0.2; LAB (log cfu/g) 0.07 ± 0.01; yeast and molds (log cfu/g) 0.72 ± 0.07. S. aureus, Clostridium perfringens and Bacillus cereus were not detected in cooked sausages. These results indicate that steam cooking for 45 min followed in the study was effective in reducing the microbial counts substantially. The investigation revealed that shelf life of cooked buffalo sausage was 31 days in either vacuum or CO2 at 4 ± 1 °C. The results indicated that spoilage of vacuum packed cooked buffalo sausage was likely due to LAB while microflora other than LAB may be responsible for spoilage of CO2 packed cooked buffalo sausage. The study suggests that measures such as low initial microbial counts, hygienic precautions during preparation of sausage, steam cooking for 45 min, vacuum or CO2 packing and storage at 4 ± 1 °C would control the microbial growth and provide wholesomeness and safety to the buffalo sausage.

Keywords: Sausage; Buffalo meat; Vacuum packing; Microbial profile

1. Introduction

Buffalo meat produced in India is largely exported in frozen condition. Conversion of buffalo meat into a value added product such as sausage would further enhance the foreign exchange earnings. Buffalo meat being comparatively cheaper will have additional advantages over other meats. Sausage is a popular and highly relished meat product world over. Consumers’ awareness has increased in recent times for microbiological quality of sausages. Thus an understanding of microbial profile of sausage is vital. Microorganisms gain access into sausage from meat, spices and other ingredients, from environment, equipment and handlers during processing affecting the microbiological status of the product. Comminuting also adds microbial contamination to sausages. While processing conditions such as heat treatment reduce microbial levels, recontamination takes place during post-processing, handling and storage of sausage. While pathogenic microorganisms would affect the safety, spoilage microorganisms would limit the shelf life of the sausage. Uncooked sausages will have a shorter shelf life since raw ingredients carry high levels of microorganisms. LAB is the major spoilage organism in vacuum-packaged cooked sausages (Korkeala, Alanko, Mäkelä, & Lindroth, 1989; von Holy, Cloete, & Holzapfel, 1991). Green coloration often noticed can be caused by microorganisms such as Lactobacillus viridescens, Leuconostoc, Weissella spp., Carnobacterium divergens, Enterococcus and Pediococcus spp. (Borch, Nerbrink, & Svensson, 1988; Grant, McCurdy, & Osborne, 1988). Vacuum and CO2 packaging enhances the shelf life of sausages (Borch, Kant-Muermans, & Blixt, 1996). Thus, microbial ecology of meat products will mainly depend on the environment, kind of meat and raw materials, equipment, handling practices, processing, packaging and storage temperature. Information on microbiology of buffalo sausage is very limited. The objective of the study was to understand the microbial profiles of buffalo sausage during preparation and also the shelf life of buffalo sausage as...
affected by vacuum, CO₂ and nitrogen packaging and storage temperature.

2. Materials and methods

2.1. Preparation of sausage

A ready-to-eat type of emulsion sausage was prepared from buffalo meat. The composition (%) of sausage was minced meat (57.0), animal fat (11.5), salt (2), sodium tripolyphosphate (0.35), NaNO₂ (0.02), course (80 mesh) wheat flour (6.0), spices (8.13), chilled water (15.0). The minced meat is mixed with all other ingredients in a bowl chopper to obtain sausage batter. The batter is then stuffed into natural casings (from sheep) of diameter 1.4–1.6 cm, using a mechanical stuffer. The stuffed sausages are linked to a length of 10 cm each and steam cooked (saturated air temperature of 80 °C, 25 min).

2.2. Packaging and storage

The cooked sausage was packed in metallised polyester pouches (oxygen transmission rate: 20 ml/m²/24 h at 27 °C; water vapour transmission rate: 1.2 g/m²/24 h at 27 °C, 65% RH) and packed under nitrogen (90%), carbon dioxide (90%) and vacuum (90%). The sausage packed without any modification of atmosphere inside the pouches served as control. The packed sausage is stored at 4 ± 1 °C. The spoilage of the product during storage was assessed by slime formation on surface, off odor and discoloration.

2.3. Microbiological analysis

The samples were drawn for microbiological analysis at each step of processing, namely, minced meat, sausage batter, stuffed sausage and cooked sausage. The samples during storage were drawn once in 4 days and were analysed for microbiological quality. The samples were analysed for microbial profile using standard procedures (APHA, 1992) for total mesophilic count (37 °C, 48 h) and psychrotrophic count (15 °C, 7 days) on plate count agar, yeast and molds on potato dextrose agar (32 °C, 7 days), Staphylococcus aureus on Baird-Parker agar (37 °C, 48 h), coliforms by MPN method in brilliant green bile broth (37 °C, 48 h) and lactic acid bacteria on MRS agar (37 °C, 48 h). Cooked sausage was also analysed for Clostridium perfringens on SPS agar (37 °C, 48 h) and Bacillus cereus on Bacillus cereus agar containing polymixin B (37 °C, 48 h) (Hi-Media, Bombay, India).

2.4. Statistical analysis

The experiments were carried out in six replicates. The significant difference in microbial count during different stages of processing and during storage of sausage was analysed by analysis of variance technique and mean separation was accomplished by Duncan’s multiple range test using statistical software SPSS for windows (SPSS Inc., 1992).

3. Results and discussion

Microbial contamination may be added or reduced at different stages of processing of buffalo sausage. Raw minced meat contained total plate counts (log cfu/g) 5.41 ± 0.25; coliforms (MPN/g) 23.2; S. aureus (log cfu/g) 1.57 ± 0.11; yeast and molds (log cfu/g) 2.29 ± 0.07 and lactic acid bacteria (log cfu/g) 0.60 ± 0.20 (Fig. 1). Microbial counts of minced buffalo meat used for sausage making in the present study were lower than the
counts recorded in a study on the minced meats prepared in the local markets (Narasimha Rao & Ramesh, 1988) and are within the microbiological standards of fresh meat (ICMSF, 1980).

Sausage emulsion did not show any significant (p < 0.05) change in TPC and \( S. \) aureus, but counts of yeast and molds and coliforms increased significantly (p > 0.05) from that of minced meat. Microbial levels of raw sausage stuffed in casing are TPC (log cfu/g) 5.10 ± 0.35; coliforms (MPN/g) 98; \( S. \) aureus (log cfu/g) 1.48 ± 0.03; yeast and molds (log cfu/g) 2.50 ± 0.06 and lactic acid bacteria (log cfu/g) 0.70 ± 0.01. British fresh sausage obtained from shops had been found to contain 1–5000 viable organisms/g, coliforms 1.3 to 2400 ± 10²/g; yeasts 10²–10⁶/g and lactic acid bacteria 10⁷–10⁹/g (Dowdell & Board, 1968).

Pork skin emulsion showed a mean aerobic plate count of 6.3 log cfu/g and a mean lactic acid bacteria of 4.4 log cfu/g (Mäkelä, Korkeala, & Laine, 1990). The raw meat emulsion contained on average 5.0 × 10² cfu Enterobacteriaceae/g; mesophilic count of 1.6 × 10² cfu/g and LAB of 1.0 × 10⁴ cfu/g (Borch et al., 1988). Raw sausage emulsion may contain LAB ranging 10³–10⁴/g (Kempton & Bobier, 1970). It was found that LAB made only a small contribution to the initial population of sausage (Dowdell & Board, 1968; Simard, Lee, Laleye, & Holley, 1983).

Raw sausage emulsion showed aerobic counts 5.0–8.2 log cfu/g and lactic acid bacteria count of <2.0–4.6 (log cfu/g) (Mäkelä et al., 1990). The present study revealed that LAB contribution was markedly very less to the initial microbial contamination of sausage emulsion. The significant increase in yeast and molds and coliforms in the present study may be attributed to the contribution from other ingredients such as starch and spices. However, this increase in counts does not have much relevance from the point of microbial safety, since the cooking process follows.

The cooked sausage had a moisture content of 64.3–66.7%, water activity of 0.86–0.88 and pH of 5.98–6.12. Cooked sausage showed a lower microbial counts of TPC (log cfu/g) 3.75 ± 0.31; coliforms (MPN/g) 0.2; LAB (log cfu/g) 0.07 ± 0.01; yeast and molds (log cfu/g) 0.72 ± 0.07. \( S. \) aureus, \( B. \) cereus and \( C. \) perfringens was not detected in the cooked sausage. These results demonstrate that cooking process followed in the present study was effective in reducing the microbial counts substantially in the sausage. A coliform count of 10¹–10³ per gram was observed in cooked frankfurters and there was no significant change in the number of coliforms during storage at 3 °C (Simard et al., 1983). The presence of low counts of coliforms and yeast and mold may be attributed to recontamination during handling of cooked sausage. Lower mesophilic counts (2.8 log cfu/g) were observed in sausage immediately after heat processing (Borch et al., 1988). Microbial counts recorded in cooked sausage in the present study are within the suggested microbiological standards for cooked meats (ICMSF, 1980). Thus the results indicate that heat treatment employed for processing of sausage was adequate from the point of microbiological quality and safety. It was stated that an effective heat processing is essential during cooking to eliminate heat tolerant lactobacilli such as \( L. \) viridescens for enhancing the shelf life of sausage (Borch et al., 1988) during storage at chill temperature.

The behavior of microbial profile in sausage stored (at 4 °C) separately under vacuum, nitrogen and carbon dioxide is presented in Fig. 2. Total plate counts (TPC) (log cfu/g) increased from an initial level of 4.09 ± 1 to 6.52 ± 0.17 during 0–16 days period in control; to 6.38 ± 0.19 during 0–32 day period in sausage packed in vacuum; to 6.51 ± 0.16 during 0–20 day period in nitrogen packed sausage, and to 6.34 ± 0.21 in carbon dioxide packed sausage. In vacuum-packaged sausage, there was a marginal reduction in TPC during 0–16 day and subsequently TPC increased. In carbon dioxide packed sausage, TPC maintained a slow growth from the initial stages of storage period. In nitrogen packed sausage, increase in TPC was more from 4th day onwards. It was reported that the mean aerobic plate count (APC) of the cooked ring sausages one day after processing was 1.0 × 10³ cfu/g and the APC was 5.60 log/g.
on the sell-by date and 5.84 log/g after 7 days after the sell-by date in vacuum (Korkeala, Lindroth, Suihko, Kuhmonen, & Penttila, 1985). In a study on frankfurter sausage total aerobic count packed in vacuum and stored at 4 °C was found to be 1.7 log cfu/g at 0 day, 2.6 log cfu/g in N₂ and 2.6 log cfu/g in CO₂ after 48 day storage (Blickstad & Molin, 1983).

Psychrotrophic counts increased from a non-detectable level at day 0 to 3.87±0.22 on day 16 in control samples during storage; to 3.72±0.17 on day 32 in vacuum packed samples; to 3.57±0.18 on day 20 in nitrogen packed sample and to 3.62±0.18 in carbon dioxide packed samples. Slow growth rate of psychrotrophs was demonstrated in cooked sausage packed under vacuum or under carbon dioxide during storage. Nitrogen packing showed marginal effect on growth inhibition of psychrotrophs as compared to control samples wherein psychrotrophs grew faster. It was observed that psychrotrophic count (log cfu/g) was 0.5 on day 0, 3.4 on day 48 in vacuum, 2.7 on day 41 in nitrogen packed frankfurter sausage stored at 4 °C (Blickstad & Molin, 1983). The presence of small number of coliforms in the cooked sausage can be attributed to post-cooking contamination. Storage and packaging conditions did not favor the growth of coliforms as the counts increased marginally. With regard to yeast and molds, the counts increased from initial level of 0.85±0.06 on day 0 to 2.37±0.15 on day 16 in control samples; to 2.00±0.13 on day 20 in nitrogen packed sample; to 2.37±0.18 on day 32 in vacuum packed samples and to 2.52±0.15 on day 32 in carbon dioxide packed samples. Both vacuum and carbon dioxide packing have shown substantially inhibitory effects on the growth of yeast and mold during storage but not nitrogen packing. However, Simard et al. (1983) had observed inhibition of yeast and molds in nitrogen packed frankfurters.

Lactic acid bacteria (LAB) counts increased from 0.70±0.01 on day 0 to 1.00±0.06 on day 16 in control samples; to 5.20±0.14 on day 32 in vacuum packed samples; to 2.98±0.15 on day 20 in nitrogen packed samples and to 2.52±0.14 on day 32 in carbon dioxide packed samples. Even though, the number of lactic acid bacteria on fresh meat was generally very low (Kempton & Bobier, 1970), they dominated the flora in vacuum packed meat and meat products and were responsible for the spoilage of the product (Allen & Foster, 1960; Mol, Hietbrink, Mollen, & Van Tinteren, 1971). The results of the present study revealed that carbon dioxide treatment shows highest growth inhibition on LAB in sausage during chill storage. However, this effect has not given additional benefit in extending the shelf life of sausage compared to vacuum packing. It was reported that LAB did not increase during storage of CO₂ packed sausage (Blickstad & Molin, 1983). It was observed that the effect of CO₂ has enhanced when meat was held at low temperature (~1.5 °C) (Greer, Dilts, & Jeremiah, 1993). In a study on frankfurter sausage stored in different gas atmospheres at 4 °C, Blickstad and Molin (1983) observed that LAB counts increased in vacuum and nitrogen packs but not in CO₂ packs. In the present study vacuum packing favored the growth of LAB compared to CO₂ or N₂ packing. The spoilage of vacuum packed cooked sausage as observed by formation of slime, off odor and discoloration occurred when LAB counts reached to 5.20±0.14 log cfu/g. It was observed that spoilage process was started in cooked ring sausage packed in vacuum, when the lactobacilli count was greater than 10⁷ cfu/g (Korkeala et al., 1989). The dominance of LAB in the spoilage flora of emulsion sausages packed in vacuum has been reported (Korkeala et al., 1989).

The low LAB counts recorded in the present study during the storage of cooked buffalo sausage in carbon dioxide indicate that the spoilage of CO₂ packed sausage might be due to microorganisms other than LAB. The off odor development in the stored samples took place on day 16 in control samples; day 32 in vacuum packed sausage; day 32 in carbon dioxide packed sausage and on day 20 in nitrogen packed sausage. The shelf life is 15 days in control samples; 31 days in vacuum or carbon dioxide packed sausage and 19 days in nitrogen packed sausage. Thus, during storage of sausage at 4 °C, vacuum or carbon dioxide packing gave an extra shelf life of 15 days over the control samples while nitrogen packing provided an extra shelf life of 4 days. It was reported that cooked sausages stored aerobically at 5 °C remain in edible condition for an average of 10 days (Pohja, Hermonen, & Nurmi, 1964). In a study on vacuum packed cooked ring sausage during storage, a shelf life of 20–28 days (Korkeala et al., 1985) and 43 days (Korkeala, Lindroth, Ahvenainen, & Alanko, 1987) has been found. A shelf life of 49 days in frankfurter sausage packed in CO₂ was recorded (Blickstad & Molin, 1983).

In conclusion, the study revealed the pattern of microbial profile associated with the preparation of buffalo sausage. Lower initial microbial levels of sausage mix, effective heat treatment during cooking, careful handling of cooked sausage, and maintenance of adequate chill temperature during storage would improve the microbiological quality and enhance the shelf life of buffalo sausage. Application of vacuum or CO₂ would further reduce or inhibit the microbial growth and enhance the shelf life of buffalo sausage.

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References


