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SHORT COMMUNICATION

# Effect of nisin and butylated hydroxy anisole on storage stability of buffalo meat sausage

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**Abstract** The water activity of buffalo meat sausage was adjusted to 0.88 using humectants and by heat treatment. Nisin and butylated hydroxyanisole (BHA) were added to study the shelf life of sausage. The treatments were nisin 100 ppm + BHA 100 ppm ($T_3$); nisin 100 ppm ($T_2$); BHA 100 ppm ($T_1$) and control ($T_0$) without nisin and BHA. The sausages were vacuum packaged in polyethylene terephthalate (PET-poly) pouch and stored for 7 days at ambient conditions (35 ± 2°C, 70–80% RH). The pH of sausage increased during storage whereas the moisture content was higher in treatment $T_1$. Tyrosine value was lowest (18.1 mg%) in $T_3$. There was no significant difference among $T_0$ and $T_1$. The thiobarbituric acid reactive substances (TBARS) number of $T_1$ and $T_3$ were lower than that of $T_0$ and $T_2$. Nisin and BHA together exhibited a significant inhibitory effect on total viable count, staphylococcal, streptococcal and anaerobic counts. There was no significant difference in the yeast and mould counts among $T_1$–$T_3$. $T_3$ had a better appearance, flavour, texture and overall acceptability scores up to 5 days. The product ($T_3$) was acceptable up to 5$^{th}$ day of storage at 35 ± 2°C and 70–80% RH.

**Keywords** Buffalo sausage · Nisin · Butylated hydroxyanisole · Keeping quality

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## Introduction

India has buffalo population of about 97.7 million (FAO 2005) and is the major source of red meat for domestic and export market. Buffalo meat is comparatively cheaper, has no religious taboo in India and mostly consumed as fresh. Meat products typically spoil due to microbial growth or chemical deterioration. The most common form of chemical deterioration is oxidative rancidity (Kanner 1994), which can vary greatly from extensive flavour changes, colour losses and structural damage to proteins (Xiong 1996) to a more subtle "loss of freshness" that discourages repeat purchases by consumers. The most common uses of the synthetic antioxidants in meat products are for processing fresh breakfast sausage and for dried products, such as pepperoni. United States Department of Agriculture (USDA 2000) regulations permit up to 0.01% (based on fat content) each of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in fresh sausage and up to 0.003% (based on total weight) each in dry sausage. Nisin, a small antimicrobial peptide produced by lactic acid bacteria (Hurst 1981), has been tested as a preservative for its antibotulinal effect on bacon (Calderon et al. 1985) and chicken frankfurter emulsion (Taylor et al. 1984). There is a limited information on the effect of nisin on meat products like sausage. The purpose of this study was to evaluate the effect of nisin and BHA on quality of buffalo meat sausages stored under ambient conditions of 35 ± 2°C and 70–80% RH.

## Materials and methods

Hot boned meat and fat from buffalo carcasses were collected from corporation slaughter house, Chennai. They were chilled for 24 h at 4 ± 1°C and then frozen to –18 ± 1°C. Frozen meat was tempered at refrigeration temperature overnight before being minced through a 4.5 mm plate of a meat mincer (OMAS, Model 169789, Electrolux Food Service, Italy).

*Preparation of buffalo meat sausage:* The minced meat and fat were chopped in bowl chopper (Model MTK 662, Maschinenfabrik, Germany). The emulsion was prepared by adding minced meat buffalo meat (80 g), buffalo fat (5 g), refined sunflower oil (15 g), sodium tripoly phosphate (0.3 g), sodium nitrite (120 ppm), NaCl (2 g), spice mix (1.5 g), green condiments (4 g), sugar (1 g), egg powder (1 g), soy protein isolated (3 g), sodium lactate (2 g) and ice (7 g) in control sausage. The BHA (100 ppm) was added for $T_1$, nisin (100 ppm) for $T_2$ and BHA (100 ppm) and nisin (100 ppm) to $T_3$.

Salt, sodium tripolyphosphate and sodium nitrite were premixed and added to the meat mix. Refined vegetable oil (sunflower) was cooled to 4°C before adding to the mixture. During chopping the temperature of the emulsion was maintained at 10–12°C by the addition of slushed ice. The emulsion was then stuffed into 18–20 mm diameter sheep casing using a sausage stuffer (Model MWF 591, Maschinenfabrik, Germany), linked and kept hung in the hot air oven at ~100°C for 60 min to reach the internal core temperature of 72°C as determined by using probe thermometer.

*Packaging and storage:* The cooked sausages (with casings) were vacuum packed using double chamber vacuum packaging machine (Komet Plus Vac, Germany) in multilayer (polyester/ polyethylene pouches of size 6" × 9" with 150

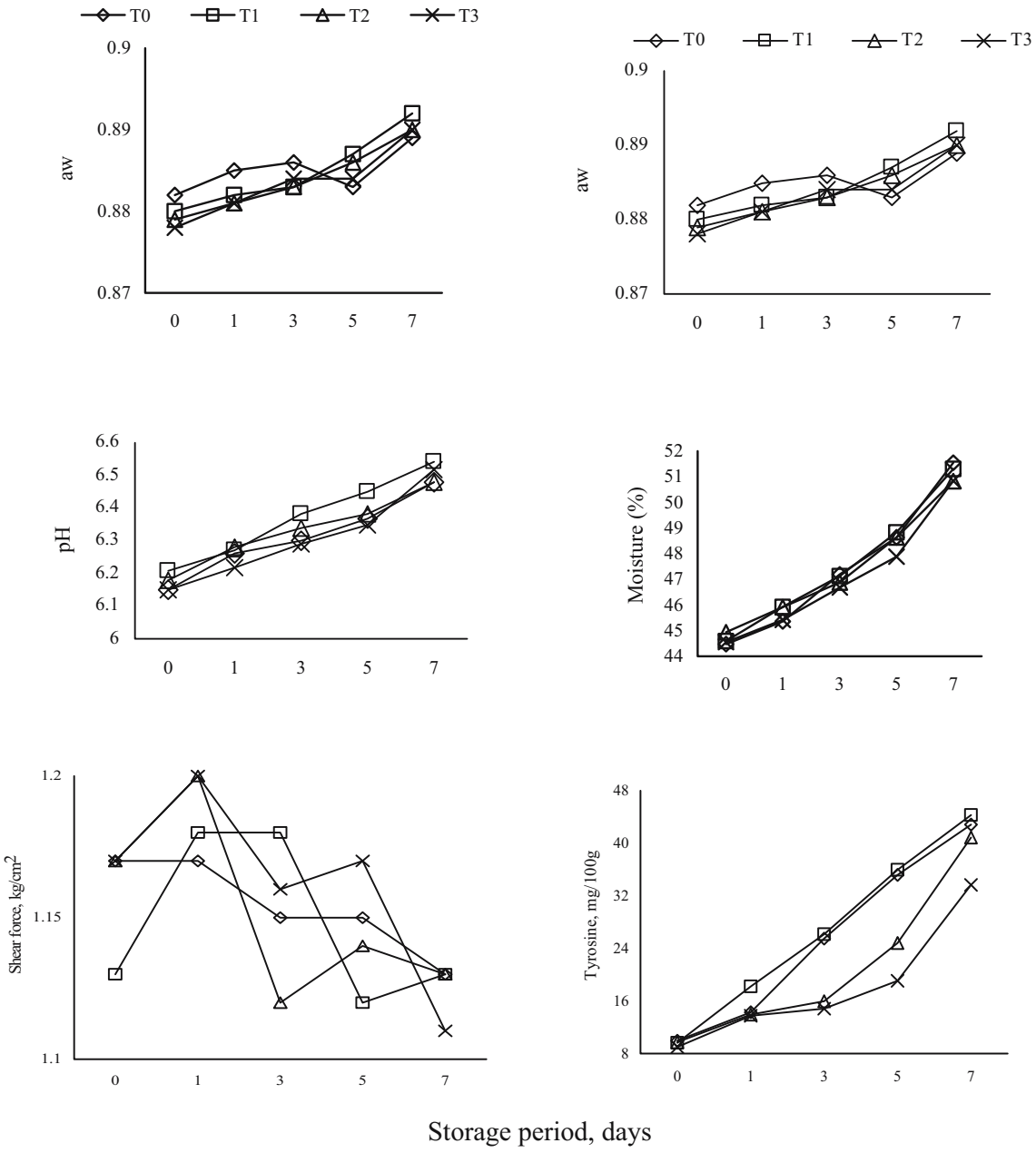


**Fig. 1** Changes in physical and chemical characteristics of buffalo meat sausages during storage at 35 ± 2ºC and 70–80% RH (n = 6)
$T_o$ = Control with out nisin and BHA, $T_1$ = 100 ppm BHA, $T_2$ = 100 ppm nisin, $T_3$ = 100 ppm BHA + 100 ppm nisin

gauge thickness) at 20 psi in 5 pouches for each treatment. The vacuum packaged sausages were served warm to taste panelists on day zero. The packed sausages were stored at 35 ± 2°C and 70–80% RH. The pH, tyrosine values (Strange et al. 1977), thiobarbituric acid reactive substances (TBARS) (Tarladgis et al. 1976), moisture (AOAC 1995) and water activity ($a_{w)}$ (Karthikeyan et al. 2000), total viable count, coliform count, staphylococcal count, yeast and mould count and anaerobic count (APHA 1984) were determined. Sensory attributes were evaluated by semi-trained panelists consisting of 5 members using the 9-point Hedonic scale.

*Statistical analysis:* The experiments were carried out in six replicates. The significant difference in various parameters during different storage periods of sausage were analyzed by multivariate analysis using statistical software SPSS 13.0 for windows.

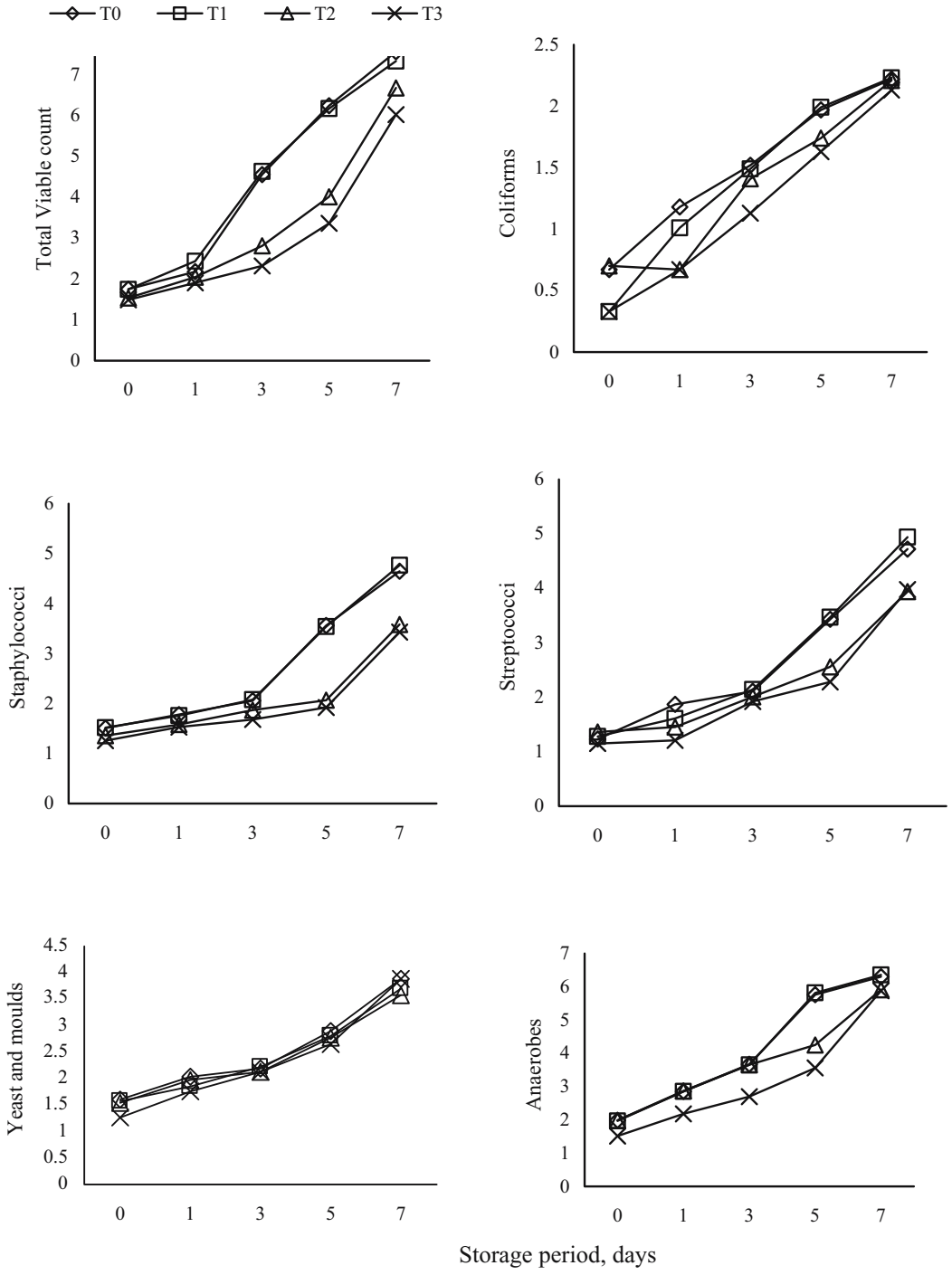


**Fig. 2** Changes in microbiological quality (counts in log cfu/g) of buffalo meat sausages during storage at 35 ± 2ºC and 70–80% RH (n = 6). $T_0$–$T_3$: As in Fig. 1

## Results and discussion

*Physical and chemical characteristics:* Highest pH was in sausages containing BHA alone ($T_1$), while tyrosine and TBARS values were lowest in sausages containing both BHA and nisin ($T_3$) (Fig. 1).The $a_w$, pH, tyrosine value, moisture content and TBARS values increased whereas the shear force values decreased during storage. Aksu and Kaya (2005) also noticed highest pH in beef sausage when treated with BHA alone and nisin. Raju et al. (2003) in fish sausages noticed lowest tyrosine values when treated with both BHA and nisin.

The addition of nisin alone ($T_2$) did not alter the TBARS from that of control. The lowest TBARS values recorded in the sausages containing BHA ($T_1$ and $T_3$) might be due to the ability of BHA to scavenge the free radical which in turn retarded the oxidative rancidity (David et al. 1993). The reduction in TBARS value was in accordance with Aksu and Kaya (2005), Biswas et al. (2004) and Rehman and Salaria

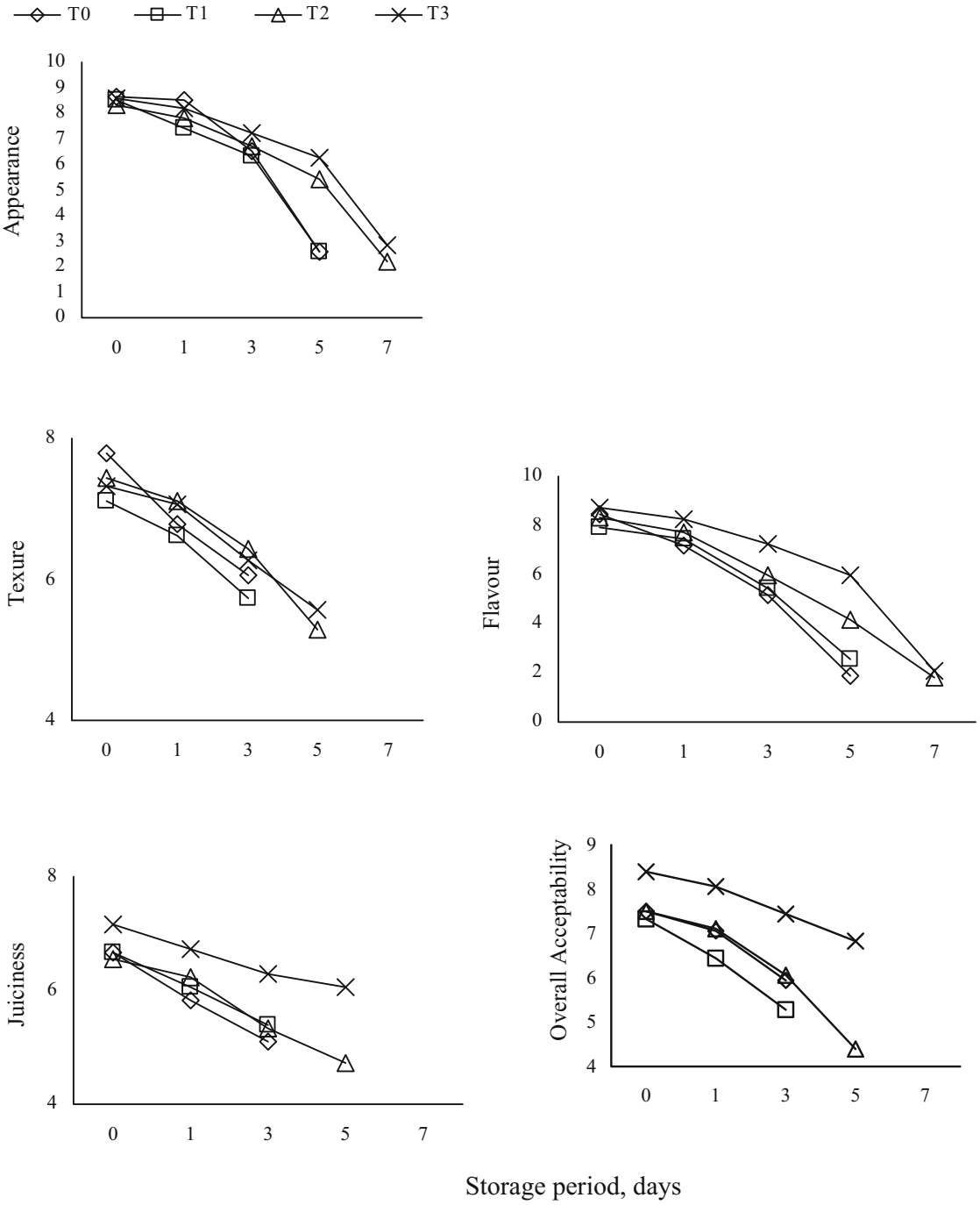


**Fig. 3** Changes in sensory quality (counts in log cfu/g) of buffalo meat sausages during storage at 35 ± 2ºC and 70–80% RH (n = 6). $T_0$–$T_3$: As in Fig. 1

(2006). All the samples except sausages containing BHA either alone or in combination exceeded the threshold level of 2 mg malanoldehyde/kg (Watts 1962) on 5th day of storage, but at the same time the presence of BHA delayed the oxidative rancidity for 2 more days. The increase in TBARS value during storage is in agreement with the findings of Karthikeyan et al. (2000) in caprine keema and Sebranek et al. (2005) in pork sausage.

*Microbiological qualities:* Addition of nisin either alone ($T_2$) or in combination with BHA ($T_3$) significantly reduced the microbial counts when compared to control (Fig. 2). This is in agreement with the findings of Raju et al. (2003) in fish sausage and Sachindra et al. (2005) in buffalo sausage. Similarly nisin alone controlled spoilage in pork (Nattress et al. 2001) and in Bologna-type vacuum-packaged delicatessen sausage (Davies et al. 1999). Nisin also inhibited bacteria on beef surfaces (Cutter and Siragusa 1998). Yeast and mould counts were lower in $T_1$–$T_3$ compared to $T_0$ (control). Even though nisin is least effective against yeast and mould (Marianna et al. 2008), in the present study, the presence of nisin and BHA either alone or in combination retarded growth of yeast and moulds. Chung et al. (1989) found that nisin in combination with nitrite prevented the growth of *Clostridium* spp. In the present study also addition of nisin showed inhibitory effect on anaerobic growth in buffalo meat sausage (Fig. 2).

*Sensory quality:* The highest juiciness and overall acceptability scores were recorded in sausages containing both nisin and BHA ($T_3$) (Fig. 3). This was in accordance with Das and Radhakrishna (2001). But when used alone neither nisin nor BHA showed any significant improvement over the control. The control ($T_0$) and BHA alone ($T_1$) added sausage samples were acceptable only up to 3 days of storage, whereas the presence of nisin ($T_3$) extended the shelf life up to 5 days of storage. The effect of nisin on the sensory characteristics was in accordance with Raju et al. (2003) observed in fish sausage.

## Conclusion

Neither nisin nor BHA alone was effective in extending the shelf life of buffalo meat sausages stored at ambient conditions and their combination extended the shelf life of buffalo meat sausages for 5 days when packed in polyester-polyethylene bags and stored at ambient conditions (35 ± 2°C, 70–80% RH).

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## References

Aksu MI, Kaya M (2005) The effect of α-tocopherol and butylated hydroxyanisole on the colour properties and lipid oxidation of kavurma, a cooked meat product. Meat Sci 71:277–283

AOAC (1995) Official methods of analysis, 16th edn, Association of Official Analytical Chemists, Washington DC

APHA (1984) Compendium of methods for microbiological examination of foods, 2nd edn, Speck ML (ed), American Public Health Association, Washington DC

Biswas AK, Keshri RC, Bisht GS (2004) Effect of enrobing and antioxidants on quality characteristics of precooked pork patties under chilled and frozen storage conditions. Meat Sci 66: 733–741

Calderon C, Collins-Thompson DL, Usborne WR (1985) Shelf-life studies of vacuum-packaged bacon treated with nisin. J Food Prot 48:330–333

Chung K, Dickson JS, Crouse JD (1989) Effects of nisin on growth of bacteria attached to meat. Appl Environ Microbiol 55:1329–1333

Cutter CN, Siragusa GR (1998) Incorporation of nisin into a meat binding system to inhibit bacteria on beef surfaces. Lett Appl Microbiol 27:19–23

Das H, Radhakrishna K (2001) Preservation of mutton as ready-to-eat curry by hurdle technology. J Food Sci Technol 38: 287–289

David M, Horvath G, Schimke I, Mueller MM, Nagy I (1993) Effects of the antioxidant butylated hydroxyanisole on systolic free calcium concentration. Toxicol 77:115–121

Davies EA, Milne CF, Bevis HE, Potter RW, Harris JM, Williams GC, Thomas LV Delves-Broughton J (1999) Effective use of nisin to control lactic acid bacterial spoilage in vacuum-packed bologna-type sausage. J Food Prot 62:1004–1010

FAO (2005) Livestock information, sector analysis and policy research, AGAL Livestock sector brief-India, Food and Agricultural Organisation, Rome, Italy, p 1–21

Hurst A (1981) Nisin. Adv Appl Microbiol 27:85–123

Kanner J (1994) Oxidative processes in meat and meat products: Quality implications. Meat Sci 36:169–174

Karthikeyan J, Kumar S, Anjaneyulu ASR, Rao KH (2000) Application of hurdle technology for the development of caprine keema and its stability at ambient temperature. Meat Sci 54:9–15

Marianna C, Antonietta B, Milena S, Lino F, Rodolfo B, Matteo ADN (2008) Use of nisin, lysozyme and EDTA for inhibiting microbial growth in chilled buffalo meat. Int J Food Sci Technol 43:573–578

Nattress FM, Yost CK, Baker LP (2001) Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. Int J Food Microbiol 70:111–119

Raju CV, Shamasundar BA, Udupa KS (2003) The use of nisin as a preservative in fish sausage stored at ambient (28 ± 2°C) and refrigerated (6 ± 2°C) temperatures. Int J Food Sci Technol 38: 171–185

Rehman Z, Salariya AM (2006) Effect of synthetic antioxidants on storage stability of Khoa -a semi-solid concentrated milk product. Food Chem 96:122–125

Sachindra NM, Sakhare PZ, Yashoda KP, Rao DN (2005) Microbial profile of buffalo sausage during processing and storage. Food Cont 16:31–35

Sebranek JG, Sewalt VJH, Robbins KL, Houser TA (2005) Comparison of a natural rosemary extract and BHA/BHT for

relative antioxidant effectiveness in pork sausage. Meat Sci 69: 289–296

Strange ED, Benedict RC, Smith JC, Swift CE (1977) Evaluation of rapid tests for monitoring alterations in meat during storage. 1. Intact meat. J Food Prot 40:843–847

Tarladgis BG, Younathan BM, Dugan LR (1960) A distillation method for the quantitative determination of malanoldehyde in muscle foods. J Am Oil Chem Soc 37:403–406

Taylor SL, Somers EB, Krueger LA (1984) Antibotulinal effectiveness of nisin-nitrate combinations in culture medium and chicken frankfurter emulsions. J Food Prot 48: 234–239

USDA (2000) Code of federal regulations. Chapter III, part 318.7. Approval of substances for use in the preparation of products, Washington DC

Watts BM (1962) Meat products. In: Sym Food lipids and their oxidation, Shultz HW, Day EA, Sinnbubber RO (Eds), AVI Publ Co. Inc., Westport, USA, p 202–220

Xiong Y (1996) Impacts of oxidation on muscle protein functionality. Proc Reciprocal Meat Conf 49:79–86