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Research Article

DEVELOPMENT AND STORAGE QUALITY OF SMOKED FERMENTED DRY SAUSAGES

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ABSTRACT

The present study was conducted to determine the effect of smoking and/or bacterial starter cultures of *Micrococcus roseus* (MTCC-1532) + *Lactobacillus plantarum* (MTCC-1407) + *Pediococcus acidilactici* (NCIM-2292) in combination on dry sausages. Two variants of sausages were made viz. CA and A (unsmoked sausages), and CB and B (smoked sausages). The smoking effectively inhibited the yeast and mold growth on the surface and provided color and flavour to the dry sausages. Sausages A and B were made with the help of starter cultures while sausages CA and CB were prepared without using starter cultures. The sausages A and B attained the desired final pH 4.9. Application of starter cultures reduced the level of nitrite in dry sausages. The application of starter cultures in sausages A and B reduced the initial load of coliforms and *Staphylococcus aureus* to zero by 10th day of ripening. The dry sausages could be stored successfully for 30 days at ambient temperature.

Key words: Smoking, bacterial starter cultures, fermented dry sausages, buffalo meat, shelf life

Dry sausages are fermented meat products which remain safe and stable without refrigeration. They are well suited to the Indian climate and take care of several health hazards associated with most of the meat and meat products. In a traditional way even today the dry sausage production relies upon natural fermentation which is caused by 'in-house micro flora'. The natural fermentation on occasions leads to development of wild flavors. This necessitates the use of pure or selected starter cultures. Good quality dry sausages have been prepared by ripening sausages at controlled temperature with a combination of pure bacterial cultures such as *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Micrococcus roseus* in equal proportion (Leistner and Gorris, 1995). The micrococci are added for their nitrate reduction and catalase activity which help in the development of color (Berwal and Dinchev, 1993). The lactic acid bacteria ferment the sugars to lactic acid primarily, thus reducing meat pH and providing the prolonged stability against the proliferation of pathogenic microorganisms (Daly *et al*., 1973; Leistner and Gorris, 1995; Karthikeyan *et al*., 2000).

We studied the effect of smoking and bacterial starter cultures on shelf life of dry fermented sausages from pork and buffalo meat blend and investigated their antimicrobial role against the *coliforms*, *Staphylococcus aureus*, *Clostridium botulinum,* yeast and molds in the fermented dry sausages.

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MATERIALS AND METHODS

Bacterial Strains: Bacterial strains *viz*: *L. plantarum* (MTCC-1407) and *M. roseus* (MTCC-1532) were obtained from the Institute of Microbial Technology, Chandigarh, India whereas *P. acidilactici (*NCIM-2292) was obtained from the National Chemical Laboratory, Poona, India. Cultures were revived and activated by sub culturing them three times in nutrient broth. MTCC-1407 and NCIM-2292 were then grown on the MRS agar and MTCC-1532 on M-153 agar. For maintenance, they were transferred in broth once in a fortnight.

The actively growing bacterial cultures were inoculated on MRS/M-153 medium in roux bottles and incubated at 37°C. The bacterial growth was harvested and transferred to a conical flask under aseptic conditions. The optical density of microbial suspension was measured at 660 nm wave length and optical density corresponding to10^3 cells/g was determined.

The cultures were mixed in equal proportion.

Formulation of Dry Sausages: Dry sausages were prepared using ingredients as given in Table 1. Acton and Dick (1976) also suggested the composition of some commercial dry sausages.

Processing of Dry Sausages: Meat was obtained by slaughtering a male buffalo calf aged one year and a boar aged six month according to the procedure outlined by Panda (1995). The carcasses were manual hot deboned and lean and fat were separated. Meat was ground in a meat mincer to a size of 4 mm. The spices and other non-meat additives were added to the comminuted meat. Starter cultures mixed in the ratio of 1:1:1 were used in the form of broth. They were added to the sausage mix and mixed well. The batter was kept for 6 h at 4-6°C. Muslin cloth casing covered by polyethylene casing of 8 cm diameter and 41cm length were used. Average weight of each sausage was 600 g. Polyethylene casings were used for three days to provide anaerobic conditions for fermentation and were removed thereafter leaving behind the muslin cloth casings to facilitate drying. Two groups of sausages were prepared with two variants: unsmoked and without starter cultures (CA), unsmoked and with starter cultures (A), smoked and without starter cultures (CB), smoked and with starter cultures (B).

Fermentation: The fermentation of all variants was carried out at 16±1°C (RH, 85-90%) for 3 days.

Heat Treatment and Smoking: After fermentation, CA and A variants of sausages were processed by heat treatment method (Palumbo *et al*., 1976). The sausages were cooked in a relative humidity temperature control cabinet (60°C). CB and B variants of sausages were processed by smoking. After fermentation, these variants were shifted to the smoke house maintained at 30-40°C (RH, 80-90%) for 16 hours.

Table 1
Ingredients used for the preparation of dry sausages

Ingredients	Per cent
Pork	50.0
Back fat	6.0
Lean buffalo meat	40.0
Salt (sodium chloride)	3.0
Sugar (sucrose)	1.0
Sodium nitrite	0.012
Sodium nitrate	0.08
Black pepper	0.10
Garlic	0.10
Red pepper	0.20
Nutmeg	0.05
Ascorbic acid	0.02

Ripening and Drying: Ripening and drying were carried out in a drying room at 11-15°C. Initially for 5 days, the relative humidity was maintained at 85-90% after that it was reduced gradually.

Product was evaluated by conducting microbiological examination for standard plate count, coliforms, *S. aureus*, *C. botulinum*, yeast and molds at 0, 10, 20 and 30 days intervals following the methods as described by American Public Health Association (APHA, 1984).

RESULTS AND DISCUSSION

The pH plays a major role in determining the physico-chemical qualities of dry sausages. The pH of the batter used for making sausages was 5.77. The sausage variants A and B made with starter cultures attained the desired final pH 4.9 on the 5th day of ripening and remained almost stable during the whole process of ripening (Table 2). The pH reduction of the sausages during ripening was due to lactic acid bacteria (Leistner, 1994). The pH rose around 15th day of ripening which might be due to the split up of soluble

Table 2
Course of pH in sausages during the process of ripening

Sausage variants	pH on days									
	0	1	2	3	5	10	15	20	25	30
CA	5.77^{a}±0.03	5.70^{a}±0.02	5.61^{a}±0.02	5.44^{ab}±0.01	5.41^{ab}± 0.01	5.48^{ab}±0.02	5.49^{ab}±0.03	5.57^{a}±0.02	5.58^{a}± 0.02	5.44^{ab}±0.01
A	5.77^{a}±0.03	5.41^{ab}±0.01	5.22^{ab}±0.02	5.00^{b}±0.03	4.90^{b}±0.02	4.88^{b}±0.01	4.78^{b}±0.02	4.88^{b}±0.01	4.80^{b}±0.02	4.91^{b}±0.02
CB	5.77^{a}±0.03	5.70^{b}±0.02	5.61^{a}±0.02	5.44^{ab}±0.01	5.39^{ab}±0.01	5.50^{ab}±0.02	5.55^{a}±0.02	5.57^{a}±0.02	5.52^{a}±0.02	5.44^{ab}±0.01
B	5.77^{a}±0.03	5.41^{ab}±0.01	5.22^{ab}±0.02	5.00^{b}±0.03	4.60^{bc}±0.02	4.88^{b}±0.01	4.90^{b}±0.02	5.00^{b}±0.03	4.91^{b}±0.02	4.81^{b}±0.02

Days

Means having different superscripts within a row and column (over all mean) differed significantly ($p< 0.05$); n=3; CA=unsmoked, without starter cultures; A=unsmoked, with starter cultures; CB=smoked, without starter cultures; B=smoked, with starter cultures

meat proteins by microbial proteases resulting into rise of free amino acids, volatile fatty acids and production of aldehyde from amino acids contributing to rise in pH (Luke, 1988).

The coliform count of fresh meat used for making sausages was 6.47 log cfu /g which reduced on 10th day to a level of 2.95 log cfu /g in sausages made with starter cultures. Coliforms were not detected in the sausages made with starter cultures on 20th day of ripening as also reported by Caplice and Fitzgerald (1999). Coliforms were present during the ripening period in the sausages made without starter cultures. *S. aureus* was initially present in the fresh meat used for making sausages but was reduced to zero on 10th day of ripening in sausages made with starter cultures (Fig. 1). Similar findings have been reported earlier by Holley *et al*. (1988) and Leistner (1994). *C. botulinum* was found to be absent in all variants of sausages in this study.

Yeast and molds count increased gradually by 30th day of ripening but was still lower than that of controls (Table 3). Yeast has also been found to possess antimicrobial activity (Coretti, 1977; Berwal, 1992). Smoking of meat inhibited the growth of molds. This was probably due to inhibitory effect of smoking on the mold growth (Incze, 1987).

The nitrite level of the sausage mix was estimated to be 130.50 ppm on day 0 (Table 4). On the 15th day of ripening in the sausage variants A and B, there was a significant reduction in the nitrite levels but the reduction in the other two sausage variants CA and

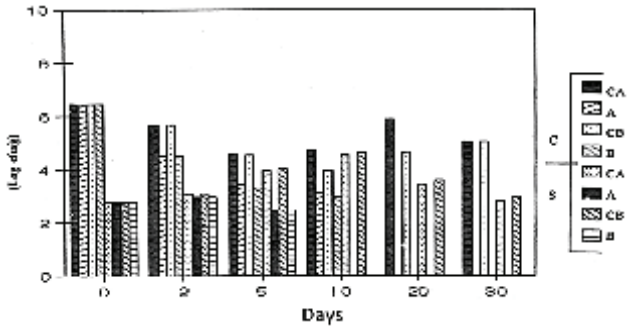

Fig 1. Coliforms and *Staphylococcus aureus* in the dry sausages during process of ripening (P<0.05) CD=1.67. C=coliforms; CA=unsmoked, without starter cultures; A=unsmoked, with starter cultures; CB=smoked, without starter cultures; B=smoked, with starter cultures

CB was little when compared to 0 day values. On the 30th day of ripening, the nitrite content in the sausage variants A and B reduced to almost one fourth compared to 0 day value.

Salahuddin *et al.* (1994) also reported that the residual nitrite content was reduced during ripening in fermented mutton sausages. The reduction took place due to the *Micrococci* which were used as starter culture. Most of the nitrite was utilized in the formation of cured color pigment and was also eliminated as sodium ascorbate by ascorbic acid (Fiddler *et al.*, 1973). While in the sausage variants CA and CB, the unutilized nitrite available to react with the secondary amines might have resulted in the formation of nitrosamine which is carcinogenic and mutagenic (Bacus, 1986). The present study revealed that good quality dry sausages could be prepared by smoking and

Table 3
Number of molds and yeast in dry sausages during ripening (N = 3, Mean±S.E.)

Sausage variants	Molds (log cfu /g) C.D. =0.516						C.D.=0.211
	0 Day	2	5	10	20	30 days	Overall mean
CA	4.03^{cd}±0.05	3.77^{a}±0.00	4.56^{b}±0.02	4.63^{b}±0.01	5.49^{a}±0.01	5.95^{a}±0.02	4.74^{a}±0.17
A	4.03^{b}±0.05	2.53^{c}±0.08	3.72^{b}±0.05	4.63^{a}±0.01	4.07^{b}±0.03	4.96^{a}±0.06	4.00^{c}±0.17
CB	4.03^{d}±0.05	2.77^{b}±0.00	4.07^{b}±0.00	4.32^{b}±0.02	5.29^{a}±0.06	5.53^{a}±0.01	4.50^{b}±0.15
B	4.03^{ab}±0.05	2.52^{c}±0.08	3.60^{b}±0.00	4.47^{a}±0.00	3.86^{b}±0.03	4.52^{a}±0.03	3.84^{c}±0.15
	Yeast (log cfu/ g) C.D.=0.229						Overall mean C.D. =0.093
CA	2.32^{f}±0.02	3.61^{a}±0.01	4.03^{d}±0.05	4.83^{c}±0.02	5.32^{b}±0.03	6.25^{a}±0.00	4.39^{a}±0.29
A	2.32^{f}±0.02	3.23^{e}±0.08	3.61^{d}±0.01	4.54^{c}±0.01	5.44^{b}±0.08	5.06^{a}±0.03	4.20^{b}±0.29
CB	2.32^{e}±0.02	3.61^{d}±0.01	3.83^{d}±0.02	4.44^{c}±0.07	5.24^{b}±0.03	5.92^{a}±0.00	4.22^{b}±0.27
B	2.32^{f}±0.02	3.23^{e}±0.08	3.70^{d}±0.05	4.03^{c}±0.05	5.48^{b}±0.01	6.06^{a}±0.02	4.16^{b}±0.40

Means having different superscripts within a row and column (over all mean) differed significantly ($p< 0.05$); CA=unsmoked, without starter cultures; A=unsmoked, with starter cultures; CB=smoked, without starter cultures; B=smoked, with starter cultures

Table 4
Residual nitrite in the dry sausages during ripening

Sausage variants	Nitrite (ppm)			
	0 day	15 days	30 days	Overall mean
CA	130.50^{a}±1.50	120.66^{a}±0 .81	103.65^{a}±0.40	118.0^{a}±3.61
A	130.50^{a}±1.50	73.00^{b}±0.02	31.00^{c}±0.70	77.88^{b}±13.48
CB	130.50^{a}±1.50	116.00^{ab}±1.41	102.33^{b}±0.40	116.00^{a}±3.74
B	130.50^{a}±1.50	66.33^{b}±1.47	27.00^{c}±0.70	74.33^{b}±0.02

Means having different superscripts within a row and column (over all mean) differed significantly ($p< 0.05$); CA=unsmoked, without starter cultures; A=unsmoked, with starter cultures; CB=smoked, without starter cultures; B=smoked, with starter cultures

ripening using artificial casing with selected pure bacterial cultures *L. plantarum+P. acidilactici + M. roseus* at controlled temperature and relative humidity.

The metabolic activity of starter cultures caused acidification of products and reduced the nitrite concentration. The application of starter cultures brought the initial load of coliforms and *S. aureus* to zero by 10th day of ripening. *C. botulinum* was found to be absent in all variants of sausages at all stages of processing and storage. It was also not detected from the fresh meat samples. Smoking inhibited the mould growth and played an important role in color development of sausages. The dry sausages were found to be storable without refrigeration for 30 days

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