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Development of Bacterial Colonies in Dry Fermented Smoked Buffalo Sausages

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ABSTRACT

Texture and juiciness of fermented sausages directed by the distribution of bacterial colonies in the meat system. A study was conducted to assess the localization of bacterial cultures in fermented smoked and unsmoked dry sausages by employing light microscopy. Scanning electron microscopy was also carried out to determine the morphological processes that take place during manufacture of raw ripened dry sausages from pork and buffalo meat blend. *Lactobacillus plantarum (*MTCC-1407*), Pediocoocus acidilactici (*NCIM-2292*)* and *Micrococcus roseus (*MTCC-1532*)* were used as starter cultures in combination of 1:1:1 for fermentation of sausages. On light microscopy, bacterial cultures were seen localized in nests in the fermented sausages. Scanning electron microscopy revealed that closely bound muscle fibres were surrounded by protein net work.

Key words: *Fermented sausages, light microscopy, scanning electron microscopy, starter cultures.*

INTRODUCTION

Dry sausages are shelf stable fermented meat products which are made with the help of microbial starter cultures. The primary genera of micro organisms which have been successfully utilized as meat starter cultures are *Lactobacillus sp., Micrococcus sp., Pediocoocus sp*., yeasts and moulds. The micro cocci are added for their nitrate reduction and catalase activity which help in the development of color in the meat products. The lactic acid bacteria during processing ferment the sugars primarily to lactic acid, thus reducing meat pH and providing prolonged stability by inhibiting the proliferation of food spoilage micro-organisms. Thomas *et al* (2008) also studied the effect of different levels of emulsion pH adjusted with lactic acid and glucono-delta-lactone on the quality of Pork Sausages. Sureshkumar *et al* (2006), Huda *et al* (2010) and Dharamveer *et al* (2007) evaluated the quality of buffalo meat sausages, Malaysian Commercial Chicken Sausages and chevon smoked sausages, respectively.

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Katsaras and Leistner (1991) studied the distribution and development of bacterial colonies in fermented sausages and reported that the bacterial colonies were located in cavities called nests and are unevenly distributed in the sausages matrix not only during natural fermentation but also if freeze dried starter cultures were added to the sausage mixture. They stated that a small and even distance between nests of desirable bacteria in a sausage matrix is advantageous as it fosters the necessary ripening process. Liepe (1987) investigated the distribution of different micro-organisms in the fermented sausage at the beginning of ripening process and concluded that no significant difference in the bacterial numbers existed between samples taken from the edge or the core of the product. Sokolov and Tschechowskaga (1976) reported that added salt in fermented sausages brought about a swelling of the myofibrils and at the same time some proteins were solubilized. Soluble proteins consisting of actomyosin cover the meat and fat particles and act as binder. Such sausage mix was called “Sol state” because it is still soft and the soluble proteins

form reversible coagulable structure. While studying the morphology of fermented sausages, Rabeur and Kiessling (1982) found that due to the removal of water caused by the drying of sausage as well as the production of lactic acid, the coagulation structures become stable and hence "Sol state" changes into "Gel state". Katsaras and Budras (1989) observed that both gel formation and water release result in the formation of sausage matrix and consequently the product has a firm texture and becomes sliceable.

MATERIALS AND METHODS

Bacterial strains

*Lactobacillus plantarum (MTCC- 1407), Pediococcus acidilactici (*NCIM-2292*)* and *Micrococcus roseus (MTCC-1532)* were used in combination of 1:1:1 for production of dry sausages.

Formulation of dry sausages

Ingredients	Per cent
Lean buffalo Meat	50.0
Back fat	10.0
Pork meat	40.0
Salt (sodium chloride)	3.0
Sugar (sucrose)	1.0
Sodium nitrite	0.012
Sodium nitrate	0.08
Black pepper	0.23
Garlic	0.10
Red pepper	0.20
Nutmeg	0.05
Ascorbic acid	0.02

***Preparation of stock cultures*:** Cultures were revived and activated by sub culturing them 3 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 once in a fortnight.

***Preparation of innoculum*:** The actively growing bacterial cultures were inoculated on MRS/M-153 medium in roux bottle and incubated at 37^0C. The bacterial growth was harvested from the roux bottles with the help of sterile saline solution containing glass beads and transferred under aseptic conditions to conical flask. 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 the ratio of 1:1:1.

***Processing of dry sausages*:** Meat was obtained by slaughtering a male buffalo aged one year and a boar in the slaughter house of the Department of Animal Products Technology, CCS, Haryana Agricultural University, Hisar. The carcasses were hand deboned and lean and fat were separated.

Grinding: Meat was ground in meat mincer to a size of 4mm.

***Mixing*:** 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 ^{0}C.

***Filling*:** Muslin cloth casing covered by polyethylene casing of 8 cm diameter and 41cm length were used. Average weight of each sausage was 600g. 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.

***Sausage variants*:** Two groups of sausages were made each having two variants.

Sausage variants:

Fermentation: The fermentation of the sausages of all variants was carried out at 16 +1^0C (RH, 85-90%) for 3 days.

Heat treatment: After fermentation CA and A variants of sausages were processed by following heat treatment method used by Palumbo *et al.* (1976). The sausages were cooked in a relative humidity temperature control cabinet 60^0C which was recorded by digital temperature probe.

Smoking: CB and B variants of sausages were processed by smoking. After fermentation, these variants were shifted to the smoke house maintained at 30-40^0C (RH, 80-90%) for 16 h.

Variants	Recipe	Starter cultures	Smoked	Heat treatment
CA	As given above	—	—	+
A	- do-	+	—	+
CB	- do-	—	+	—
B	- do -	+	+	—

CA: control for **A: CB** control for **B**. For fermentation and ripening of CA and CB the natural micro flora was relied upon.

Ripening and drying: Ripening and drying was carried out in the drying room at 11-15^{0}C. Initially for 5 days, the relative humidity was maintained at 85-90 % after that it was reduced gradually.

Morphology of Dry Sausages Light microscopy: Samples of different sausage variants were taken after 20 days of ripening and they were fixed overnight in 10 % formalin. Tissues were dehydrated in the ascending series of alcohol i.e. 50%, 60%, 70%, 80%, 90% and absolute for half an hour in each set. The sections were cleared by dipping in 3 sets of alcohol: benzene in equal volume by keeping tissues for half an hour in each set. After this the infiltrated sections were embedded in the mould having melted paraffin wax and allowed the wax to solidify. The solidified blocks were ready for sectioning for which the block holder was stuck with the blocks and fixed in manual microtome (Spencer-20) and adjusted to obtain section of 6 micron. Continuous ribbons floated on lukewarm water in a water bath. A glass slide coated with a solution of glycerin and egg albumin in equal ratio was dipped in the water bath by bringing it under the floating section and picked it on the slide and kept the slide in oven for 30 min. at 45^{0}C. The slide was stained by using Taylors (1966) method for g +ve and g -ve bacteria and slides were examined under light microscope. Blue to black circles with regular cell wall were g +ve organisms and bright red were g -ve organisms. Yellow color was for tissue mass and other debris and black dots, non uniform in clusters were carbon particles.

Electron microscopy: Scanning electron microscopy (SEM) of sausage samples was conducted at Regional Scientific Instrumentation Centre, All India Institute of Medical Sciences, New Delhi. Sausage tissue samples of the size 10 mm^2 were fixed immediately after treatment in 3% buffered gluteraldehyde (pH 7.2) for 6 h at 4+ 1^0 C . Samples were washed three times for half an hour each with 0.1 M phosohate buffer (pH 7.2) and the temperature during washing was kept at 4+1^{o}C. After washing, the samples were fixed in 1% osmium tetraoxide for 2 h at 4+ 1^{0}C and again washed thrice with 0.1M phosphate buffer for 30 min. each. Samples after washing were dehydrated with specific grade acetone, 30%, 50%, 70%, 80%, 90%, 95% and with acetone for 30 min. each at 4+ 1^0 C. After dehydration, critical point drying was done by 'Jumbo' critical point dryer and Thermo- regulator (Polaron Equipment Ltd.). The critical point dried specimens were mounted on aluminium stubs using 'conducting –paint'. These stubs were shifted to the Sputter coating unit (Balzer Union SCDO 20) for metal coating (Gold) for scanning electron microscopy. Stubs were then examined on Scanning Electron Microscope (Philips SEM-501 B) at different magnifications and photomicrographs were taken.

RESULTS AND DISCUSSION

Morphology of dry sausages Light Microscopy: The two variants of sausage A and B which were manufactured with starter cultures using two different lines of processing i.e. smoked and unsmoked, were subjected to morphological studies under light microscopy. The g +ve bacteria were found to be blue stained cocci, singly or in clusters in form of pockets / nests in the sausage matrix (Fig 1). This confirmed the finding of Katsaras and Leistner (1988) who found that the bacterial cultures used were located in nests in the fermented sausages. In the smoked sausage (variant B) the presence of carbon particles was

observed in the muscle cell which indicated the impact of smoking. Some irregular shaped black colored structures with uneven margin indicated the spices ingredients used in the sausage formulation (Fig 2). Our findings confirm the study of Katsaras and Leistner (1991) who reported that the bacterial colonies or nests were unevenly distributed in the sausage matrix, not only during fermentation process but also during ripening.

Fig 1. Localization of bacterial cultures in nest (N) Surrounded by firm sausage matrix. (X400)

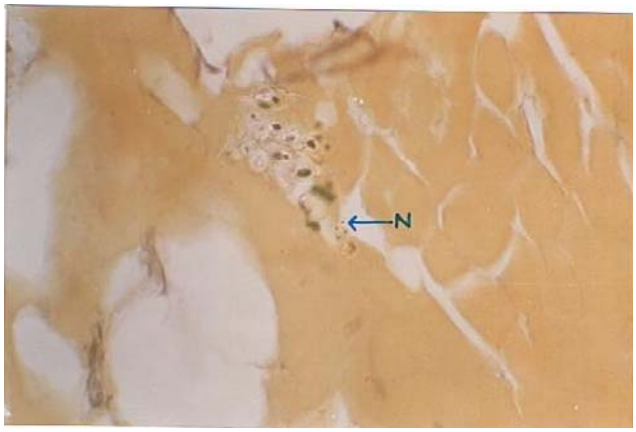
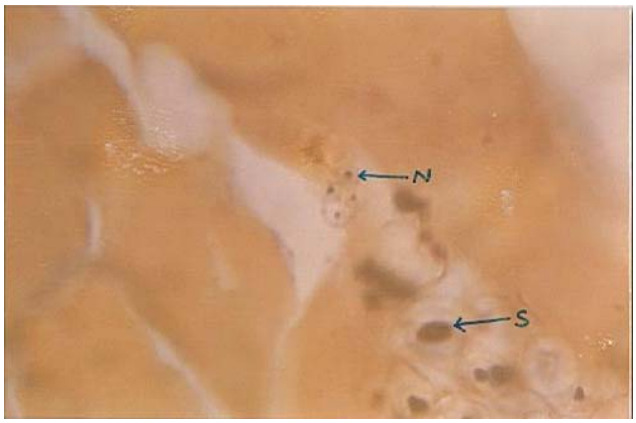


Fig 2. Nest of bacteria (N) and spices (S) in a fermented dry sausage. (X400)



Scanning Electron Microscopy: The full ripened dry sausages were subjected to morphological studies to understand the binding system by mean of SEM. Comminution process destroyed the architecture of muscle up to a large extent Closely bound muscle fibres surrounded or interwoven with a protein network were seen. This

Fig 3. Meat particles surrounded by soluble proteins resulting in bind in fermented dry sausage. (SEM, X1600)

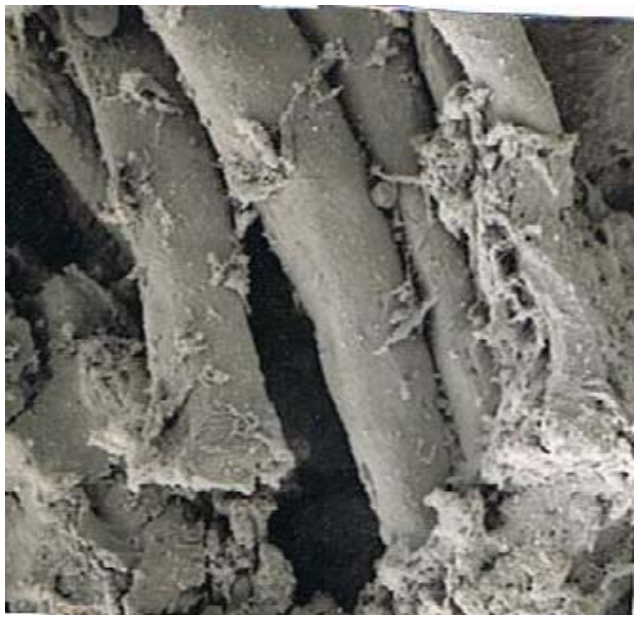


Fig 4. Muscle fibers interwoven with protein network in dry fermented sausage. (SEM, X3125)

network enclosed meat and non meat additives in sausages and provided the structural bind. It can be seen more clearly at a higher magnification in (Fig 3, 4). The added salt brought about a swelling of the myofibrils and at the same time the structure of the protein changed and some protein were

Fig 5. Scanning Electron micrograph of fermented dry sausage after ripening showing "Gel State" of muscle proteins (SEM, X 6250)

solublised (Katsaras and Leistner, 1991). These soluble proteins, consisting of actomyosin, covered the meat and fat particles and acted as binders. The sausage mix which was in "Sol State" when prepared (Sokolov and Tschechowskaya, 1976) got coagulated. On production of lactic acid and with the progress in drying "Sol State" changed to a stable "Gel State" (Rauber and Keissling, 1982). Fig 5 also justified the findings of Katsaras and Budras, (1989) that gel formation and water release resulted in the formation of the sausage matrix, and consequently the product had a firm texture and become sliceable.

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