Fate of Bacillus spp. from spices in fermented sausages

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In the older literature, the spoilage of fermented sausages has frequently been ascribed to the growth of Bacillus spp.. Spices often contain $>10^7$ Bacillus spores per gram and thus are an important source of contamination. In order to evaluate the necessity to use decontaminated spices for the processing of fermented sausages, we studied the behaviour of Bacillus spp. from spices during ripening of salami.

In order to make conditions most favourable for growth of bacilli, sausages were prepared with addition of nitrate rather than nitrite, and without starter cultures. Even if the ripening conditions were faulty (high temperature, humidity too low), the Bacillus spores naturally occurring on black pepper were unable to develop. Only few of them germinated and subsequently died. Bacillus spp. capable of anaerobic growth in a model substrate (pH 6.0) containing 5 % NaCl and 300 mg KNO_3/l were usually detected on spices only after selective enrichment. If high numbers (10^6/g) of these bacilli (B. licheniformis strains) were inoculated into salami mixture, a large proportion of their spores germinated, but vegetative cells rapidly lost viability without multiplicating to any significant extent. Experiments with model substrates confirmed that Bacillus spp. are inhibited in fermented sausage by a combination of low water activity, low pH and low oxygen supply. In the early stage of ripening, nitrite may also contribute by delaying the outgrowth of the spores as long as the pH is still high.

In conclusion, the use of decontaminated spices for the manufacture of fermented sausages is not necessary.

Introduction

Spores of Bacillus spp. form the majority of the microflora of spices: their number frequently exceeds 10^7/g (see PIVNICK, 1980). Thus, spices are an important source of contamination of foods with Bacillus spores. If they are capable of growth in a food, a microbicidal treatment of spices may increase the microbiological stability and safety of the product. All the methods available for inactivation of bacterial spores on spices have certain disadvantages, e.g. the formation of chlorhydrines by ethylene oxide treatment or problems with respect to legislation and consumer's acceptance of the use of ionizing irradiation. Therefore, it seems worthwhile to evaluate the necessity of a microbicidal treatment of spices for various foods. In the older literature (see, for example, SCHÖNBERG and WALZ, 1954) the spoilage of fermented sausages has frequently been ascribed to Bacillus spp., although opposite views have also been expressed (e.g. by CORETTI, 1958, POHJA and NIINIVAARA, 1960). This situation led us to reinvestigate the role of Bacillus spp. from spices in the spoilage of fermented sausage.

Materials and Methods

Salami mixtures were formulated with 3.3 kg each of pork shoulder, lean beef and pork back fat, 260 g sodium chloride, 3 g potassium nitrate, 40 g "ERKOPUR" (a starch hydrolysate containing about 25 % each of mono- and disaccharides) and 40 g ground black pepper which was used untreated or decontaminated with ethanol vapour (NEUMAYR, 1983). The mixtures were filled into vapour permeable casings (NATURIN R2) of 60 or 75 mm diameter. "Normal" ripening conditions were 3 days at 22°C and 90 - 92 % RH, 3 - 4 days at 20°C and 88 - 90 % RH, 3 days at 18°C and 85 % RH and 3 days at 14 - 16°C and 80 % RH. Subsequent storage was at 8 - 12°C and 75 % RH. The sausages - except those inoculated with vegetative Bacillus cells - were lightly smoked during the first two days of ripening.

For the microbiological examination, samples were homogenized in a "stomacher" with nine volumes of 0.85 % NaCl solution. Dilutions were plated on suitable nutrient media as specified by NEUMAYR (1983). Before analysis, spices were chilled with liquid nitrogen and ground in a WARING laboratory blender. When only spores were to be enumerated, the homogenates were pasteurized (80°C, 10 min). To determine the number of Bacillus spp. presumably capable of

growth in raw sausage mixtures and to obtain vegetative Bacillus cells for inoculation of sausages mixtures, a medium ("RM") was used which contained per liter 20 g peptone from meat, 10 g meat extract, 5 g glucose, 50 mmoles lactic acid, 50 g NaCl and 300 mg KNO_3, and had a pH of 6.0. For solid media, 1.2 % agar was also added. RM adjusted to various pH and a_w values (NaCl as solute) was also used for model growth experiments with Bacillus spp.

Batches of raw sausage mixtures were inoculated with vegetative cells or spores of Bacillus spp. at levels of approximately 10^6/g. Spores were produced on Nutrient Agar (MERCK) containing 10 mg $MnSO_4 x H_2O$/l. Two B. cereus strains (B 50, B 51), one B. subtilis strain (B 74) and two B. licheniformis strains (B 49, B 101) were from the culture collection of our Institute, the other strains (all identified as B. licheniformis by the method of CLAUS, 1978) were isolated from various spices after enrichment in RM.

Results

First, we compared the effect of the microflora of black pepper (ca. 5×10^7 Bacillus spores per g) and the ripening conditions ("normal" and "faulty", i.e. 26°C, 60 % RH throughout the ripening period) on the properties of salami. Figs. 1 and 2 show that the Bacillus spores introduced with the pepper did not develop: they did not even lose heat resistance. Likewise, the overall properties of the sausages were not influenced by the microflora of black pepper. "Faulty" ripening at high temperature and low humidity led to the formation of a dry, shrinked surface layer (case hardening), a rancid aroma and to a faster inactivation of the vegetative microflora (gram-negative rods and lactobacilli) during the drying period (Fig. 2).

We then enumerated the Bacillus spp. in 21 batches of 12 spices used in the manufacture of fermented sausages (black and white pepper, ginger, marjoram, chillies, nutmeg, coriander, caraway, paprika, allspice) by using optimal culture conditions (pH 7.0; 0.5 % NaCl; aerobic) and a model substrate (RM Medium) containing 5 % NaCl, pH 6.0. If determined under optimal conditions, counts of Bacillus spores ranged from 3×10^3 to 10^8/g (with a geometric mean of 7×10^5/g). A considerable proportion (10 % or more) of these bacilli grew in RM under aerobic conditions and in optimal medium under anaerobic conditions, but only one spice sample (coriander) contained more than 100 Bacillus spores per g which were capable of anaerobic growth in RM. From the other samples, such bacilli could only be isolated after several transfers in liquid RM medium. All of the isolates which grew were identified as B. licheniformis. In RM

medium containing 250 mg $NaNO_2$/l rather than nitrate, no growth of bacilli under anaerobic conditions was observed.

To maximize the risk of development of bacilli in fermented sausages, 11 of these selected B. licheniformis strains were inoculated into salami mixture at a level of 10^6/g. In the first experiment, vegetative cells adapted to growth in RM were used and the sausages were ripened at 24°C and low relative humidities (80 % for 2 days, 70 % for another 2 days and 60 % for another 10 days). Fig. 3 shows that the cells did not grow and - after an initial lag of ca. 2 days - rapidly lost viability. When spores rather than vegetative cells were used and the sausages ripened under "normal" conditions, a large proportion of the spores germinated and subsequently died (Fig. 4). Although the two B. cereus strains from our culture collection were capable of anaerobic growth in RM, their spores did not even lose heat resistance during ripening of inoculated salamis.

In order to clarify the mechanism of inhibition of B. licheniformis in fermented sausages, growth experiments were carried out in RM medium with various pH and a_w values and additives. All strains grew anaerobically in unmodified RM which represents the conditions prevailing in fresh raw sausage mixture prepared with nitrate. If 10^4 vegetative cells per ml were inoculated, the count reached 10^7 within 1 - 2 days at 25°C. Growth of B. licheniformis could not be inhibited by simultaneous inoculation of lactobacilli (Lactobacillus sake, L. plantarum, L. curvatus, all isolated from fermented sausages), but the addition of 125 mg $NaNO_2$/kg rather than nitrate caused a lag of 6 and 8 days until initiation of aerobic and anaerobic growth, respectively. At the end of this lag, no more nitrite was detectable in the medium. Within one week of ripening, the conditions in the salami mixture would have become too unfavourable to allow growth of bacilli at any nitrite level.

Discussion

We found Bacillus spp. from spices unable to multiply in fermented sausage during ripening. This was also true if large numbers of bacilli capable of growth at low a_w values and low oxygen tensions were inoculated into the sausage mixture, even if slightly faulty ripening conditions were chosen. The inhibition of Bacillus spp. in fermented sausage is mainly due to the combination of low a_w, low pH and low oxygen tension. However, additional "hurdles" contribute to the

inhibition of the development of bacilli in the early ripening stages when neither a_w nor pH are low enough. Our experiments with model substrates (RM medium) indicate that nitrite - added to the mixture or formed from nitrate by the competitive microflora - is one of these factors. Germination of Bacillus spores may also be delayed by unfavourable temperature and by direct antagonistic effects of vegetative bacteria in fresh sausage mixture.

Other authors (REUTER et al., 1968; HOFMANN and SCHARNER, 1980; GERIGK and GOSSLING, 1981) did not find any multiplication of bacilli in fermented sausages, either. In contrast, SCHÖNBERG and WALZ (1954) stated that Bacillus spp. (of the so-called "Mesentericus-Subtilis-Group") are a prominent cause of the spoilage of raw sausages. What could have led them to this conclusion? Especially under faulty ripening conditions (high temperature, low humidity), the number of vegetative cells in a fermented sausage decreases fairly rapidly during drying (compare Fig. 2). Furthermore, the nutrient media used by SCHÖNBERG's group did not support good growth of lactobacilli. Since most of the Bacillus spores do not even germinate in fermented sausage (see Figs. 1 and 2), it is not surprising that bacilli were found to be dominant in the final product. SCHÖNBERG and coworkers may have also been misled by the fact that, when growing in optimal nutrient media, their Bacillus isolates often produced similar spoilage symptoms (proteolysis, lipolysis, degradation of heme pigments) as observed in spoiled fermented sausages, especially when ripened at elevated temperatures. The bacilli from spices, however, failed to do so when they were tested on media similar to raw sausage mixture (POHJA and NIINIVAARA, 1960).

Untreated spices may also significantly increase the number of clostridia and of moulds in raw sausage. Clostridia, however, are unable to develop in European-style fermented sausage (LÜCKE et al., 1983). Moulds only develop on the surface of raw sausages which is almost inevitably contaminated also with mould spores from the environment. Therefore, care has to be taken to avoid growth of undesirable moulds, irrespective of the use of sterilized or untreated spices.

We conclude that a microbicidal treatment of spices used for the manufacture of fermented sausages (not heated at any stage of manufacture) is not necessary.

References

CLAUS, D. (1978): 152. KIN-Informationsseminar, Neumünster

CORETTI, K. (1958): Arch. Lebensmittelhyg. 9, 32-35

GERIGK, K., and GOSSLING, U. (1981): Fleischwirtschaft 61, 1124-1128

HOFMANN, H.-P., and SCHARNER, L. (1980): Nahrung 24, 285-293

LÜCKE, F.-K., HECHELMANN, H., and LEISTNER, L. (1983):Proc. 29th Europ. Congress of Meat Research Workers, Salsomaggiore/Parma

NEUMAYR, L. (1983): Thesis, Technische Universität München; 173 p.

PIVNICK, H. (1980), in: ICMSF (ed.): Microbial ecology of foods, Vol. II, pp. 731-751 Academic Press, New York

POHJA, M.S., and NIINIVAARA, F.P. (1960): Fleischwirtschaft 12, 932-934

REUTER, G., LANGNER, H. J., and SINELL, H.-J. (1968): Fleischwirtschaft 48, 170-176

SCHÖNBERG, F., and WALZ, E. (1954): Fleischwirtschaft 6, 33-35

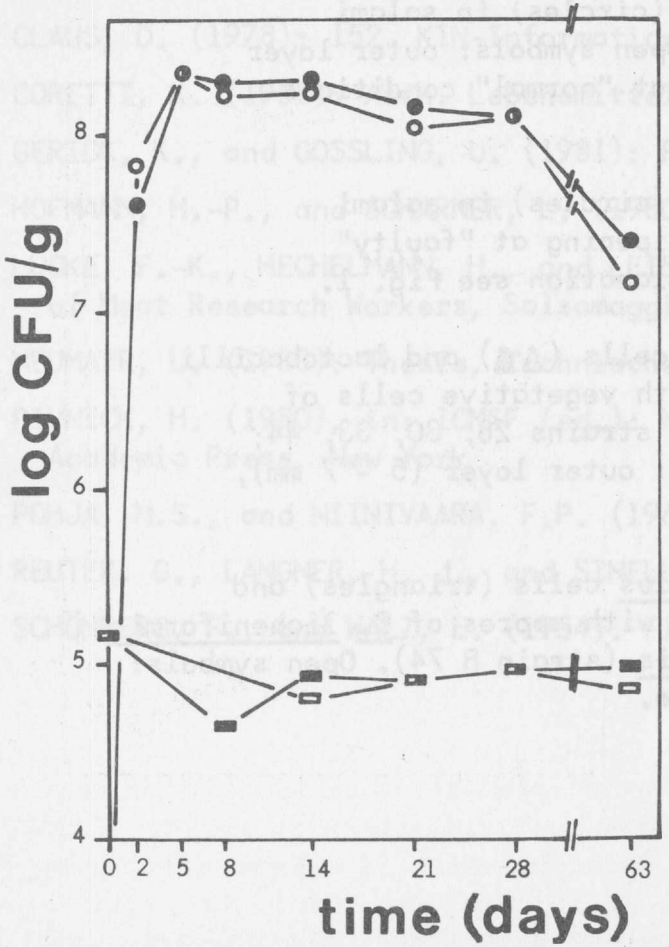
Legends to Figures:

Fig. 1: Bacillus spores (squares) and lactobacilli (circles) in salami manufactured with untreated black pepper. Open symbols: outer layer (5 - 7 mm), closed symbols: core. Ripening at "normal" conditions (see Methods).

Fig. 2: Bacillus spores (squares) and lactobacilli (circles) in salami manufactured with untreated black pepper. Ripening at "faulty" conditions (26°C, 60 % RH). For further explanation see Fig. 1.

Fig. 3: Bacillus spores (▽▼), vegetative Bacillus cells (△▲) and lactobacilli (circles) in salami inoculated primabely with vegetative cells of B. licheniformis (A: strains 29, 32, 47; B: strains 28, 30, 33, 44; C: strains 31, 34, 43, B 101). Open symbols: outer layer (5 - 7 mm), closed symbols: core.

Fig. 4: Bacillus spores (squares), vegetative Bacillus cells (triangles) and lactobacilli (circles) in salami inoculated with spores of B. licheniformis (strains 5, 15, 21, 24, B 49) and B. subtilis (strain B 74). Open symbols: outer layer (5 - 7 mm), closed symbols: core.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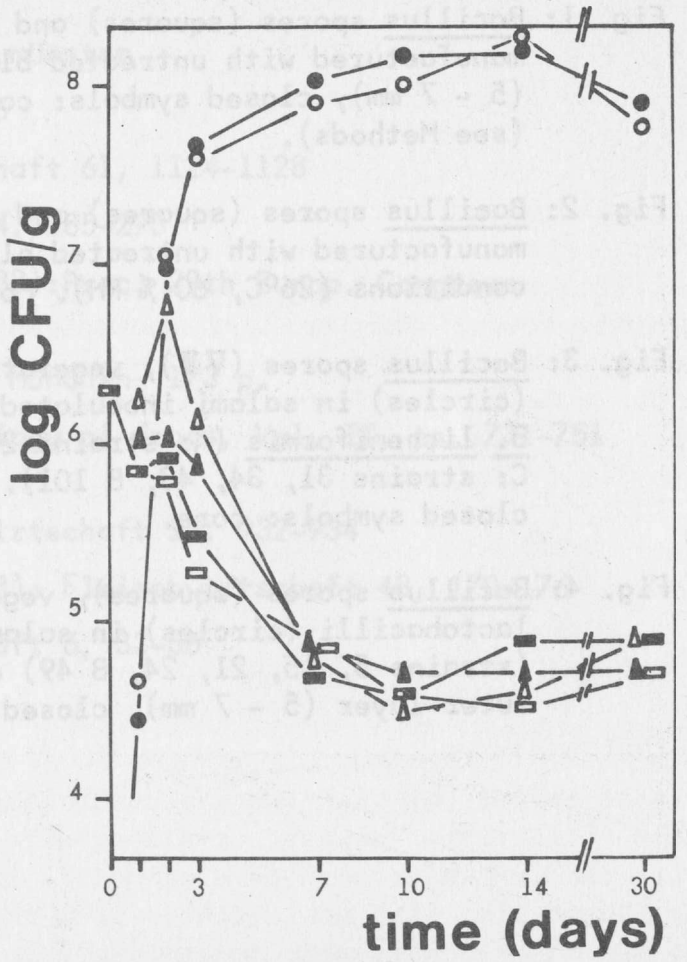
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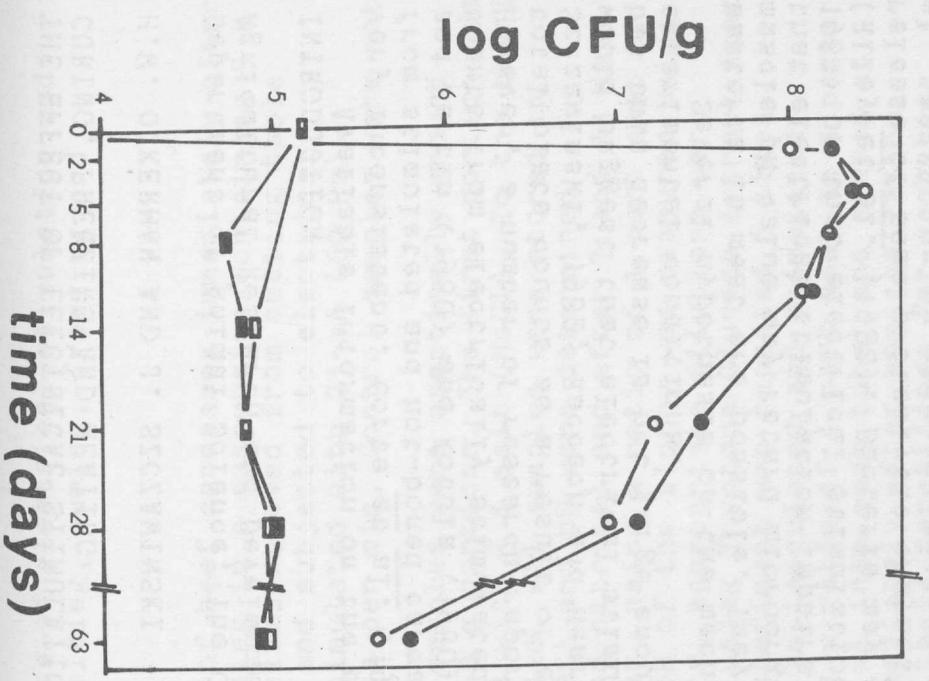
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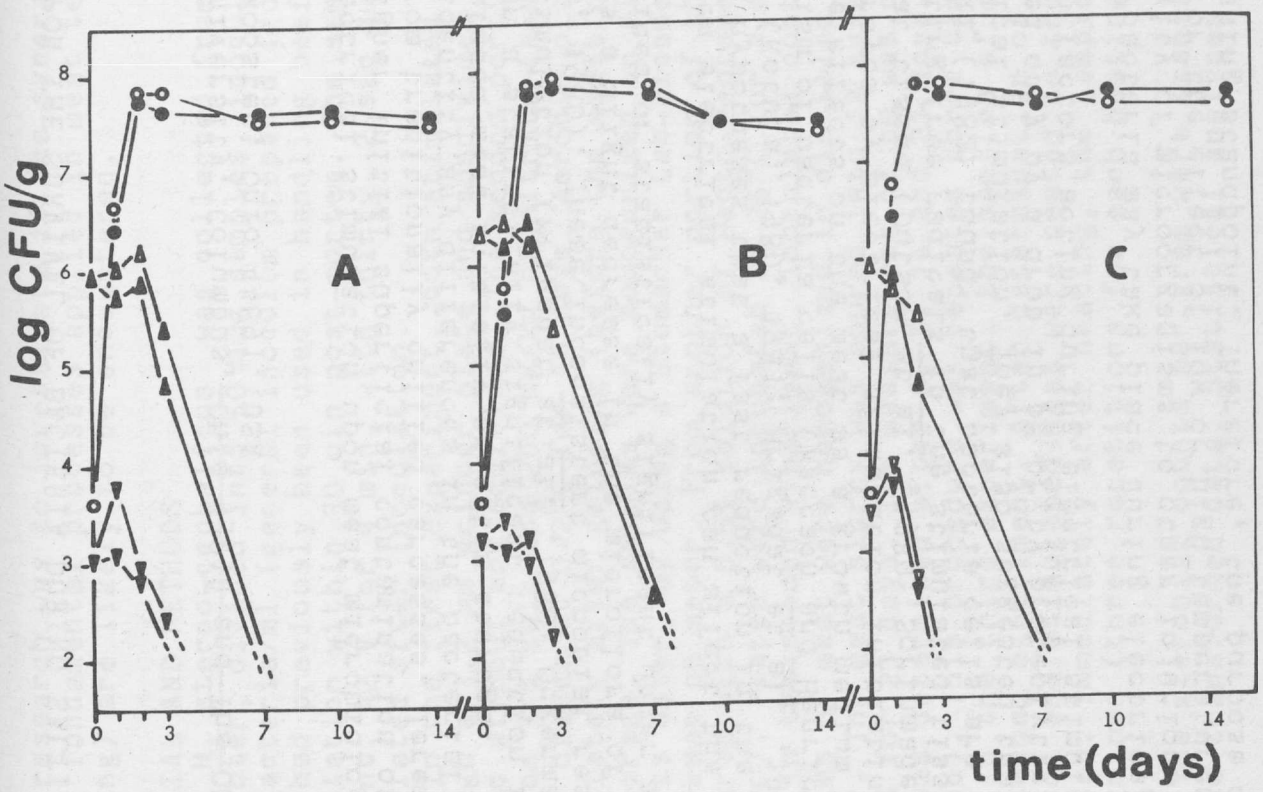
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