7 Growth of Salmonellae in fermented sausages manufactured using starter cultures

R. BRANKOVA, B. DINEVA, A. KRASTEV, AND I. KALOYANOV*

Meat Technology Research Institute, Sofia, Bulgaria
*Central Research Institute of Veterinary Medicine, Sofia, Bulgaria

The application of starter cultures in the manufacture of fermented meat products is important not only for the acceleration and the control of ripening processes, but also for ensuring the safety of the products from the microbiological point of view.

There are apprehensions about the safety of fermented sausages with respect to Salmonellae, because of the shortened terms of production (Scharner, 1980). Contradictory data are available in literature on the possibilities of growth for salmonellae under the existing technologies. Some authors feel that production conditions contribute to the discontinuance of the growth of those microorganisms (Goepfert and Chung, 1970), and others, that the lengthening of the production process to as many as 105 days cannot ensure a salmonellae-free product, when the salmonellae were introduced during production (Scharner, 1980).

The chances of salmonellae finding their way into the meat of regularly slaughtered healthy animals are rather limited (Ivanov et al., 1983; Dimitrova et al., 1982). They can be reduced to single cells per gramme of sausage meat upon blunders in sanitation, additional contamination, or presence in lymph nodes of host animals. Stecchini et al. (1982) pointed out that no presence of salmonellae in raw materials should be admitted. They found that the lengthening of the ageing period did not contribute to salmonellae destruction, although that their levels were considerably reduced.

Goepfert and Chung (1970) introduced salmonellae as 24-hour broth cultures and manufactured sausages according to the regular technological requirements, using starter microorganisms. After a 14-day ageing of the sausages, no salmonellae were isolated from samples with starter cultures introduced, while in control samples, without starter microorganisms, their counts remained high.

Sinell and Hentschel (1977) contaminated sausages with Salmonella typhimurium and a fortnight later could demonstrate no presence of salmonellae.

Yonova and Mladenov (1979) studied the growth of salmonellae in fermented sausages with GDL. They felt that reduced pH-values were very important for the discontinuance of salmonella growth. Intensive ripening brings about the destruction of salmonellae in a shorter time.

In the experiments carried out by us which were related to the application of pure microbial cultures in meat industry, no salmonellae were isolated from any of the experimental lots or from the sausages manufactured under industrial conditions (Brankova et al., 1983).

The objective of the present work was to follow the growth of different Salmonella species in fermented sausages produced using a preparation of starter cultures.

Materials and Methods

Strains of S. senftenberg, S. enteritidis, S. typhimurium isolated from meat products were used in the experiments. Fast-ripening sausages were manufactured using a preparation containing freeze-dried L. plantarum and M. varians and produced at the Meat Technology Research Institute.

The sausages were contaminated with a suspension of a 24-hour agar culture of salmonellae in two ways: (a) by an even mixing in the sausage meat before its stuffing into casings (a method similar to the one proposed by Deibel et al., 1961), and (b) by injecting the sausages filled already.

Fast-ripening meat products were prepared by the generally adopted technology for their manufacture, requiring a temperature rise in the ripening chambers up to 24-26°C during the first 36-48 hours, and a relative air humidity of 90-95%. In most of the experiments, two sausage products were employed, differing in formulation.

All the raw materials were analysed in advance for the presence of salmonellae in 30 g of product.

At intervals during ripening and in the finished product, determinations were made of salmonellae per gramme and of the total counts of lactobacilli (MRS-agar), micrococci (MSA), and the coli-titre (Brilla broth and Endo). Salmonellae were determined by direct counting on BPL-agar and Gasner agar and upon enrichment in selenite-F broth after Leifson and repeated inoculations on BPL agar and Gasner agar (Bulgarian State Standard 6835-74).

Different experimental variants (shown in Tables 1 and 2) were developed in the different experimental schemes.

1. Contamination of sausages with Salmonella senftenberg

Table 1. Experimental variants

Variant No.	Salmonella counts per g of sausage meat: 2,8 x 10^4	2,4 x 10^2	0 (Control)	Starter preparation
1	X*	-	-	-
2	-	X	-	-
3	-	-	X	-
4	X	-	-	X
5	-	X	-	X
6	-	-	X	X

* Participation in the variant.

Salmonellae were introduced by mixing the suspension into the sausage meat prior to stuffing.

2. Contamination of sausages with S. typhimurium and S.enteritidis

Two series of experiments were conducted: (I) with contamination at the level of 2,5 x 10^2 cells; and (II) with 1,6 x 10^4 cells/g of sausage meat. The variants are entered in Table 2.

Table 2. Experimental variants

Variant No.	Sausage type*: I	II	Salmonella species: S. typhimurium	S. enteritidis	Starter preparation
1	X**	-	X	-	-
2	X	-	X	-	X
3	-	X	X	-	-
4	-	X	X	-	X
5	X	-	-	X	-
6	X	-	-	X	X
7	-	X	-	X	-
8	-	X	-	X	X

* Sausage type according to formulation.
** Participation in the variant.

In these experiments, salmonella suspensions were injected into sausages after their filling.

3. Contamination of sausages with S. typhimurium and S. enteritidis at the level of 1,4 x 10^2 cells/g by an even mixing with sausage meat. The same variants as in the previous experiments were developed (Table 2), but only sausage I was employed.

Results and Discussion

Changes in the counts of salmonellae in the individual experimental variants upon ageing for 7 or 14 days are shown in Table 3 (experimental scheme 1, Table 1). It can be seen from the table that, upon the introduction of a starter preparation, salmonellae are found on day 7 with only the higher level of contamination ($2,8 \times 10^4$). After two weeks, no salmonellae are isolated from any of the experimental variants. In the sausages ripening without starter cultures, a certain growth of salmonellae and an increase in their counts were found on day 7. After two weeks, however, no salmonellae were isolated from those variants either. This suggests that the very technology, in which sausage pH-values are reduced, water activity decreases and sodium chloride concentration rises, that technology contributes to the self-cleaning of the product of this group of microorganisms.

The coli titre and enterococci counts decreased also in the variants containing a starter preparation. Lactobacilli counts, from $3,4 \times 10^3$ in control variants initially, rose to 10^7 after 7 days, and in experimental samples, from 10^5 initially, to 10^8 after 14 days.

Table 3. Changes in salmonella counts in experiments with the contamination of fermented sausages with S.senftenberg (cells/g)

Variant No.	Initially: BPLA	Gasner	After 7 days: BPLA	Gasner	After 14 days: BPLA	Gasner
1	2,7 x 10^4	3,2 x 10^4	7,1 x 10^4	3,4 x 10^5	0*	0
2	1,1 x 10^3	4,1 x 10^3	1,3 x 10^5	8,9 x 10^4	0	0
3	0	0	0	0	0	0
4	8,5 x 10^3	1,2 x 10^4	9,0 x 10^2	8,3 x 10^2	0	0
5	2,4 x 10^3	4,0 x 10^3	0	0	0	0
6	0	0	0	0	0	0

*'0' designates samples in which no S.senftenberg was found in 30g of product.

Results indicate that pH reduction resulting from the life activities of lactobacilli (control samples have pH values of 5,6-5,8, and variants with a starter preparation, 4,8-4,9) can have an effect similar to that of GDL found by Yonova and Mladenov (1979) on enterobacteria in ripening meat products.

For the determination of the growth of S. typhimurium and S. enteritidis in fermented sausages, experiments were conducted by injecting suspensions into sausages or by their mixing with sausage meat (Table 2).

Results from the variants with injecting salmonellae are shown in Figure 1. As obvious, S. enteritidis counts multiplied in only sausage I, control variants: without the introduction of a starter preparation. In those variants, lactobacilli and micrococci were at lower levels than in the other variants and this can explain, to a degree, the multiplication of this Salmonella species. In all of the remaining variants for both sausage products, a decrease in counts was found, and those were reduced to 40-50 cells per gramme of product. In these experiments, the presence of salmonellae can be explained by the method of contamination. Upon injection, a cluster distribution of salmonella cells in the sausage is obtained. The great accumulations of Salmonellae at definite spots prevent direct contact with the remaining sausage meat and the other microorganisms and contribute to the former's preservation. In practice, no such cluster distribution can be obtained.

Coliform counts in the variants with a preparation introduced decreased also; after 15 days, coli bacteria could only be isolated from 10 g of product, and in controls, from 1 g. None were isolated from 0,1 g of sausage.

Micrococci, 10^4 in initial variants, reached 10^4-10^5 cells/g in controls, and in experimental variants kept within the range of 10^5 cells/g.

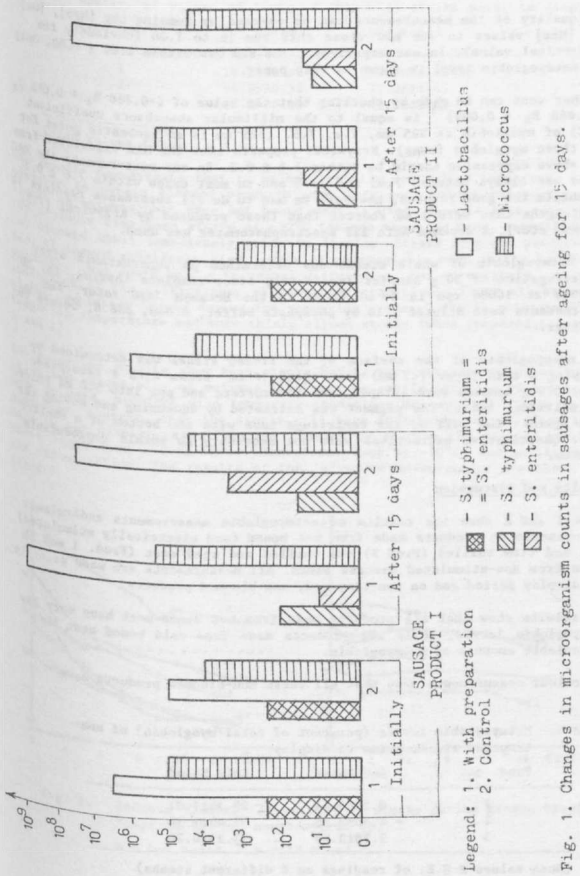

Fig. 1. Changes in microorganism counts in sausages after ageing for 15 days.

In other experiments with the injection of higher levels of salmonellae (10^4 cells/g), up to 10^3 cells/g were found after 15 days (i.e., growth was discontinued and counts decreased by one log cycle). A reduction of salmonella counts down to 10^2 cells/g was found in the variants with a starter preparation.

These experiments indicated that, in order to obtain a model closest to what may eventually happen in practice, it is necessary to effect an even mixing of a definite number of salmonella cells with the sausage meat. Experiments were conducted, where the initial contamination with S. typhimurium was at the level of 10^2 cells/g, and with S. enteritidis, $1{,}4 \times 10^2$ cells/g of sausage meat. After 12 or 20 days, Salmonellae of both species were only isolated from control variants out of 30 g of product after an enrichment, and none were isolated from the sausages with a starter preparation. This indicates that, with mixing salmonella contaminants with the sausage meat, what actually may happen in practice, the chances of contacts with starter cultures are higher, and the antagonistic effect is manifested. The results obtained are in line with those of Goepfert and Chung (1970) and Sinell and Hentschel (1977).

These data suggest that the very technological conditions in the manufacture of fermented meat products promote the discontinuation of reproduction and counts reduction in the enterobacteria that may eventually have found their way there. The introduction of starter preparations of a definite antagonistic activity contributes to the self-cleaning of these products.

Regardless of the results obtained, our standpoint coincided with that of Stecchini et al. (1982), that it is necessary to strictly observe all sanitary requirements in the manufacture of fermented meat products, so as to prevent their contamination with pathogenic enterobacteria.

References

Dimitrova, N. et al., 1982, Mesopromishlenost Bulletin, 15, 4, 6-8.
Ivanov, I. et al., 1983, Mesopromishlenost Bulletin, 16, No. 4, 75-77.
Yonova, I., M. Mladenov, 1979, Mesopromishlenost Bulletin, 12, No. 2, 12-15.
Bulgarian State Standard (BDS) 6835-74, "Meat Products. Methods of bacteriological analysis".
Brankova, R. et al., 1983, 12th Int. Symp. Microbial associations and interactions in food, Budapest.
Deibel, R. et al., 1961, Appl. Microbial., 9:239-243.
Goepfert, J., K. Chung, 1970, Milk & Food Technology, 33:185.
Scharner, E., 1980, Zpravodaj Masného Průmyslu ČSR, 3/4, 54-58.
Sinell, J., S. Hentschel, 1977, Fleischwirtschaft, 57, 7, 1317-1320.
Stecchini, M. et al., 1982, Ind. alimentari, 21, 7, 521-523.