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THE MICROBIOLOGY OF SOUTH AFRICAN DRIED SAUSAGE

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OPSOMMING: DIE MIKROBIOLOGIE VAN SUID-AFRIKAANSE DROËWORS

Dertig droëworsmonsters is mikrobiologies en fisies-chemies ontleed. Tien individuele of groepe van organismes is kwantitatief bepaal. Die aanwesigheid van Salmonella is kwalitatief ondersoek. Groot verskille in die kwaliteit van die worse is gevind, en word o.m. aangetoon deur die totale tellings wat tussen $3{,}5 \times 10^4$ en $1{,}11 \times 10^9$ selle per gram gewissel het. *Microbacterium thermosphactum* kon in 37% van die monsters aangetoon word. Geen konsekwente verwantskap tussen die getalle van die verskillende organismes kon gevind word nie. Salmonella is uit 23% van die monsters geïsoleer. Die wateraktiwiteit het gewissel tussen 0,40 en 0,85 en die pH tussen 4,61 en 7,09. Daar word aan die hand gedoen dat 'n maksimum toelaatbare waarde vir wateraktiwiteit neergelê word, en dat mikrobiologiese kontrole ingestel word, veral ten opsigte van patogene bakterieë.

SUMMARY:

Thirty dried sausage samples were examined microbiologically and physico-chemically. Ten specific organisms or groups of organisms were quantitatively determined and the presence of Salmonella only qualitatively. Large variations in the quality of the samples were found and are sufficiently demonstrated by the total counts which varied between $3{,}5 \times 10^4$ and $1{,}11 \times 10^9$ viable bacterial cells per gram. In 37% of the samples *Microbacterium thermosphactum* could be detected. No apparent relationship between the numbers of the different organisms could be found. Salmonellas were isolated from 23% of the samples. The water activity varied between 0,40 and 0,85 and the pH between 4,61 and 7,09. It is suggested that a maximum value for the water activity of dried sausage be specified. Furthermore, microbiological control should be introduced, especially for pathogenic bacterial.

Dried sausage is a traditional and popular South African meat product. According to the definition of Sedimeier and Kotter (1953) the term "raw sausage" truly applies to this product, since it is distributed and consumed in a raw and non-fermented state. In contrast to the U.S.A. and Europe, dried sausage is produced and consumed in South Africa during the winter. The production overseas of dried or semi-dried sausage usually involves a fermentation process (Everson, Danner and Hammes, 1970) whereby a flavoursome product is obtained which can be consumed with safety, even after prolonged storage.

When preparing dried sausage in South Africa, initial preservation to a large extent is obtained by means of the curing ingredients (about 0,8% sodium chloride in addition to spices such as coriander and cloves). The quick drying process which is made possible by the relatively dry winter months in the greater part of the country, enhances the preservation process. Furthermore, the low winter temperatures to some extent prevent spoilage of the product during the early stages of its preparation. Additional preservatives like smoke or nitrite and/or nitrate (salpetre) are rarely used.

The basic ingredients used in the preparation of the product may vary considerably, depending on the personal taste and experience of the manufacturer as well as the availability and value of different cuttings from the carcass. As a result, dried sausage often contains a fairly high amount of connective tissue. Although beef normally constitutes the greater part of the basic ingredients, different amounts of mutton and, to a lesser extent, venison and pork may be included. Fat contributes about 20% (m/m) of the product. Since practice has shown that lard may cause rancidity in the product, suet or mutton-fat is normally used.

It is thus obvious that South African dried sausage is not a standardised product. The lack of control over any group of microorganisms (especially the enteropathogenic group of bacteria) in this raw meat product constitutes a possible health hazard.

This report primarily deals with the general microbiological quality of dried sausage collected in the Pretoria area with a view to establishing a basis for future work towards a standardised, safe and flavoursome product.

Procedure

Samples

A total of 30 dried sausages, "ready for consumption", were bought from 25 different butcheries in the Pretoria area over a period of 12 months. One sample was a fermented sausage of the "Landjäger" type.

Microbiological analysis

Sample preparation

A two-hundred and fifty gram sample of each sausage was aseptically ground and mixed in an M.S.E.

Atomix Blender. A suitable amount was diluted ten-fold in a sterile salt-peptone-solution (0,85% NaCl + 0,1% peptone) and homogenised according to the ISO-method of Barraud, Kitchell, Labots, Reuter and Simonsen (1967) in a Waring type MSE Atomix Blender at 6 000 rpm for 30 sec. and 12 000 rpm for 60 sec. Serial dilutions were made in $\frac{1}{4}$ strength Ringer solution. About 200 g of the ground sample was required for the detection of salmonellas.

Microbial counts

Total viable counts were made by plating on Plate Count Agar (PCA) (Merck) and a modified Tomato-Dextrose agar (TDA) (Robertson, 1964). The modification constituted the replacement of tomato juice by an equal volume of cheese-cloth filtered "V8" vegetable juice. The plates were incubated at 30°C for 3 days.

Total aerobic counts were made on PCA according to Barraud *et al* (1967). Incubation was at 30°C for 3 days.

Counts of lactobacilli, leuconostocs and pediococci (LLP-group) were made on Rogosa-agar (Rogosa, Mitchell and Wiseman, 1951). Since an anaerobic atmosphere (e.g. 10% H_2 : 90% CO_2 or 10% CO_2 : 90% N_2) yielded lower counts than aerobic conditions, plates were incubated aerobically for 5 days at 30°C. From each sample at least 30 colonies were randomly selected and microscopically examined to determine the relative proportions of the three groups.

Coagulase-positive staphylococci were enumerated on two different media. Mannitol-Salt-agar (MSA), recommended by Gilbert (1972) for counting coagulase-positive staphylococci in foods, was used for detecting coagulase-positive staphylococci specifically and micrococci in general. Incubation was at 37°C for 24h. Satisfactory results were also obtained with Baird-Parker medium (BP) (Baird-Parker, 1962) with 5% egg yolk emulsion and 3,5% potassium tellurite. Plates were incubated at 37°C and were evaluated after 24 and 48 h. Colonies were isolated from MSA and BP on Nutrient Agar (Merck) and tested with rabbit plasma (Miles-Ceravac) for coagulase by means of the slide-test (Harrigan and McCance, 1966). Attempts to determine coagulase-positive staphylococci with a modified MPN-method (Giolitti and Cantoni, 1966) were less satisfactorily.

Enterococci (group D streptococci) were determined by surface-plating of suitable dilutions on Citrate Azide Tween Carbonate agar (CATC agar) (Merck) and counted after 24h at 37°C.

Proteolytes were enumerated on a medium (PMA) consisting of beef extract (3 g), meat peptone (3 g), sodium chloride (5 g), skimmilk power (10 g), $Ca(OH)_2$ (0,15 g), $CaCl_2$ (0,05 g) and 13,5 g agar, made up to a litre with distilled water. After sterilisation the pH was adjusted with HCl or NaOH to 7,0 ± 0,2. Plates were incubated at 30°C and were investigated daily for clear zones around colonies for 2 to 3 days.

The *Klebsiella-Enterobacter*-group was enumerated on Violet-Red-Bile agar (VRB) (Merck) according to the double-layer technique (Harrigan & McCance, 1966). Incubation was at 37°C for 24h.

Pseudomonads were counted on a modified CETCH-agar according to the method of Solberg, O'Leary & Riha (1972). The CH3565 was replaced by a suitable amount of Irgasan DP300 (Ciba-Geigy).

Microbacterium thermosphactum was enumerated on Streptomycin sulphate-Thallous acetate-Actidione medium (STAA) according to Gardner (1966).

Total anaerobes were determined on Brewer anaerobic agar (Difco) by means of the surface-plating method using 0,1 ml of a serial dilution. Incubation was at 37°C for 3 to 5 days in an anaerobic incubator (Forma) using a gas mixture of 10% H_2 and 90% CO_2.

Yeasts and molds were enumerated on Potato-Dextrose agar (PDA) (Merck). The pH of the medium was adjusted to 3,7 after sterilisation by means of a sterile 10% solution of tartaric acid. The plates were incubated at 25°C for 5 days.

The presence of *Salmonella* spp. was determined qualitatively. The methods of Georgala & Boothroyd (1970) were modified in order to detect any possible salmonella. Enrichment was achieved by suspending 50 g of the ground sample in 450 ml of each of selenite and tetrathionate enrichment broth. Enrichment broths were incubated at 37°C and 43°C respectively. After both 24 and 48 h enrichment cultures were streaked out on Kauffmann Brilliant-green Phenol-red Lactose agar (BPL) (Merck) and Bismuth Sulfite agar (BSA) acc. to Wilson and Blair (Merck) respectively. Suspected positive colonies were purified on Brain-Heart Infusion plates (BHI) (Difco) and single colonies were transferred to BHI-slants. Identification was carried out by means of the "Enterotube" method (Roché), followed by serological classification.

Physical and chemical analyses

The pH of each sample was determined by means of a standardised pH-meter, five minutes after mixing approximately 10 g of the ground sample with 5 ml of distilled water.

The water-activity of the samples (a_w) was determined by use of an a_w-meter (Lufft, Stuttgart) according to the technique of Rödel, Ponert and Leistner (1975).

Moisture content was determined gravimetrically after drying 10 g of a ground sample at 100°C for 24 h.

The ash content was determined gravimetrically by incineration of a preweighed sample at 550°C for 16 h.

Results and Discussion

Basic statistical information on the distribution of some of the most important bacterial groups is given in Table 1. It will be noted that the coefficients of variation (CV) are very high; this fact most surely

Table 1

Population distribution of some bacterial groups in dry sausages (n = 30)

Count	Medium	Minimum	Maximum	Mean ($\bar{x}$)	Standard Deviation (S)	CV (%)
Total population	PCA	*6,0 x 10^4	6,9 x 10^8	7,6 x 10^7	1,48 x 10^8	195
Total population	TDA	3,5 x 10^4	1,11 x 10^9	1,13 x 10^8	2,72 x 10^8	240
Aerobic bacteria	PCA	1,9 x 10^4	6,3 x 10^8	7,82 x 10^7	1,54 x 10^8	196
Anaer. bacteria	Brewer's	1 x 10^1	4,7 x 10^7	3,33 x 10^6	9,93 x 10^6	298
Klebsiella-Enterobacter	VRB	**0	9,0 x 10^3	3,03 x 10^2	1,65 x 10^3	545
LLP-Group	Rog.	1,6 x 10^3	6,4 x 10^8	3,24 x 10^7	1,17 x 10^8	361
Pseudomonads	CETCH	0	5,9 x 10^4	3,28 x 10^4	1,08 x 10^5	329
Proteolytes	PMA	0	5,0 x 10^5	6,5 x 10^4	1,28 x 10^5	197

* Counts per gram **Not detectable in 1 gram

Table 2

Relative proportion of coagulase-positive staphylococci to total bacterial counts of dried sausage

Sample No.	Medium			
	Mannitol-salt agar		Baird-Parker medium	
	Count[1]/g	%[2]	Count[1]/g	%[2]
3	6,0 x 10^5	2,63	6,3 x 10^5	2,72
7	4,3 x 10^6	16,73	<5 x 10^5	–
8	3,0 x 10^7	8,8	1,4 x 10^7	4,23
15	4,1 x 10^7	6,5	1,2 x 10^6	1,9
16	7,1 x 10^5	11,26	<5 x 10^5	–
21	3,7 x 10^6	14,74	<5 x 10^5	–
23	<5 x 10^5	–	8,5 x 10^5	0,44
24	2,2 x 10^6	0,6	<5 x 10^5	–

1) The higher value of total counts on either TDA or PCA was used in each case.

2) Only those samples with counts exceeding 500 000 coagulase-positive staphylococci per gram were taken into account.

Table 3

Salmonellas isolated from dried sausage samples of various bacteriological qualities

Sample	a_w	Isolate	Enrichment medium	Total count (on TDA)	"Coliforms" per gram	Selective medium
4	0,685	*Salmonella linton*	*S	4 x 10^6	**ND	BPL[1]
4		*S. glasgow*	***T			BPL
5	0,66	*S. glasgow*	T	1,7 x 10^6	ND	BSA[2]
5		*S. glasgow*	T			BPL
7	0,40	*S. glasgow*	T	1,2 x 10^7	ND	BSA
17	0,715	*S. typhimurium*	T	1,1 x 10^6	ND	BSA
17		non-typable (rough)	S			BSA
23	0,64	*S. linton*	T	1,6 x 10^8	2,7 x 10^1	BSA
		S. linton	T			BPL
24	0,649	*S. cerro*	T	3,5 x 10^8	ND	BSA
24		*S. cerro*	S			BPL
24		non-typable (rough)	T			BSA
25	0,645	*S. cerro*	S	2,7 x 10^7	ND	BSA
25		*S. cerro*	T			BSA

* Selenite enrichment broth acc. to Leifson; ** Not detected; *** Tetrathionate broth
1) Kauffmann Brilliant-green Phenol-red Lactose agar; 2) Bismuth sulfite agar acc. to Wilson and Blair

Table 4

Distribution of some physico-chemical values in dry sausages (n = 30)

Determination	Minimum	Maximum	Mean ($\bar{x}$)	Standard deviation (S)	CV (%)
pH	4,61	7,09	5,67	0,445	7,85
Moisture (%)	8,68	34,47	19,08	6,563	34,40
s_w	0,40	0,85	0,692	0,114	16,50

refers to large differences in the bacteriological quality of the analysed dry sausage samples. The total bacterial counts varied between 3,5 x 10^4 and 1,11 x 10^9 per gram on TDA, and 6,0 x 10^4 and 6,9 x 10^8 per gram on PCA. In both cases the lowest total counts were found in sample no. 14 (a fermented sausage). The aerobic counts corresponded to a large extent with the total counts and varied between 1,9 x 10^4 and 6,3 x 10^8 per gram. The geometric means, calculated for results on these media (TDA, PCA and aerobes on PCA) agreed closely, and were respectively 1,13 x 10^8, 7,6 x 10^7 and 7,82 x 10^7 per gram. Although the majority of the samples (83% and 77% on TDA and PCA respectively) contained less than 10^8 viable bacteria per gram (Fig. 1), this distribution might have been different during the initial drying period of the sausages. Also, as will be shown later, the total counts do not necessarily reflect the hygienic conditions under which the product was prepared. Total counts of up to 10^9 per gram during the first two weeks after preparation of raw sausage seem to be a common phenomenon (Coretti, 1956) whereas Takacs and Zukal (1961) even consider the rise in total count to be independent of the ripening process in fermented sausages.

Since dried sausage samples were obtained at a stage when the product was sold for consumption, it was expected that the a_w-values would be low enough to prevent bacterial growth, even during prolonged storage. Although the a_w-value in no case exceeded 0,85, samples (37%) had a_w-values of between 0,70 and 0,80 (Fig. 2). An average a_w-value of 0,692 was calculated (Table 4). Since aerobic staphylococci may still be active at a_w-values of 0,85 and most common molds and even halophilic bacteria grow at an a_w-value of 0,75 (Nickerson and Sinskey, 1972), approximately 50% of the samples can not be considered truly preserved. Consequently, storage, especially under moist conditions, might render at least 50% of the samples susceptible to yeast and mold growth. Although no visible mold growth was detected, at least 26% of the samples contained more than 1 million yeasts and molds per gram (Fig. 3).

It may be expected that a lowering of the water-activity of dried sausage causes a decrease in bacterial numbers, especially of the gramnegatives. The possible correlation between total counts and water-activity was therefore analysed statistically according to the method of Steel and Torrie (1960). The null hypothesis (Ho) that the population correlation coefficient is zero, was tested against the one sided alternative that the correlation coefficient is significantly greater than zero, using the 5% level of significance. For this analyses 29 samples were used, leaving out no. 14 (a fermented type of sausage). With n = 29, $t_{0,05;\ 29-2} = 1,703$. Therefore Ho would be rejected if r is greater than 0,311 at the 5% confidence level. Although the results show that the water-activity of the samples influenced the total bacterial counts, the correlation coefficient even at the 5% level was very low (0,372 for TDA, 0,380 for PCA and 0,233 for anaerobic counts on Brewer's agar).

The correlation coefficient between total counts on TDA and PCA was simultaneously tested. In this case the correlation (coefficient = 0,734) was highly significant. It can therefore be concluded that TDA and PCA are equally suitable for determining total bacterial counts in dried sausage. The distribution of the total bacterial population as determined on TDA and PCA are compared diagrammatically in Fig. 1. In addition to the statistical information in Table 1, the population distribution of some bacterial groups, especially where a high CV is expected, is presented diagrammatically in Figs. 3–8.

Organisms responsible for organoleptic spoilage of meat and possibly of dried sausage are mainly the pseudomonads (which are to a certain extent psychotrophic), proteolytes, *M. thermosphactum*, yeasts and molds and, to a lesser extent, anaerobes. Total numbers of any specific group usually must exceed 100 000 per gram before a detectable influence on the quality of the product might be exptected. Only two samples contained more than 100 000 pseudomonads per gram whereas 30% of the samples had counts lying between 100 and 1 000 per gram; the average was 3,28 x 10^4 per gram (Table 1). Proteolytes exceeded the 100 000/g level in only 17% of the samples, whereas in another 17% no proteolytes were found. In 63% of the samples *M. thermosphactum* could not be detected (Fig. 4), whilst only two samples (with a_w-values of 0,75 or higher) contained more than 100 000 viable cells per gram. Since it seems that *M. thermosphactum* mainly causes spoilage of fresh or uncured meat products (Gardner & Patton, 1969), it probably constitutes a minor factor in the preparation of dried sausages.

The total anaerobic count averaged 3,33 x 10^6 (Table 1), and in 26% of the samples exceeded 1 million per gram. However, the anaerobic populations largely consisted of lactobacilli and anaerobic staphylococci.

The micrococci, pediococci, streptococci (especially Lancefield's group N), lactobacilli and even leuconostocs may be beneficial to the quality of fermented sausages (Deibel, Niven and Wilson, 1961; Everson *et al.*, 1970; Eriksson, 1960; Reuter, 1970, 1972). It may be expected that these groups at least play some part towards the organoleptic quality of dried sausage, especially when their numbers are taken into account. According to Niinivaara & Pohja (1957) the population is dominated by micrococci during the first stages of ripening of raw meat products. Although we found two samples without any detectable micrococci, at least 36% of the samples contained more than 100 000 micrococci per gram and in 23% the numbers exceeded 1 million per gram (Fig. 5). In comparison with other bacteria, micrococci seem to be relatively tolerant to a lowering of the a_w towards 0,90 and even 0,85 (Sulzbacher, 1969). Our results may therefore be a reflection of the population trend during the drying process, at least as far as micrococci are concerned.

The LLP-group (lactobacilli, leuconostocs and pediococci) seems to be of definite interest in dried sausage. No sample contained less than 10^3 LLP organ-

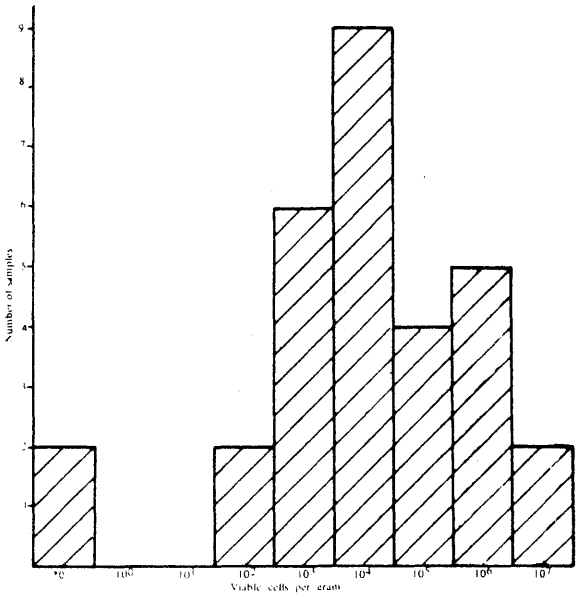

Fig. 5. – *Distribution of numbers of micrococci in dry sausages*
(not detectable in 1 gram)*

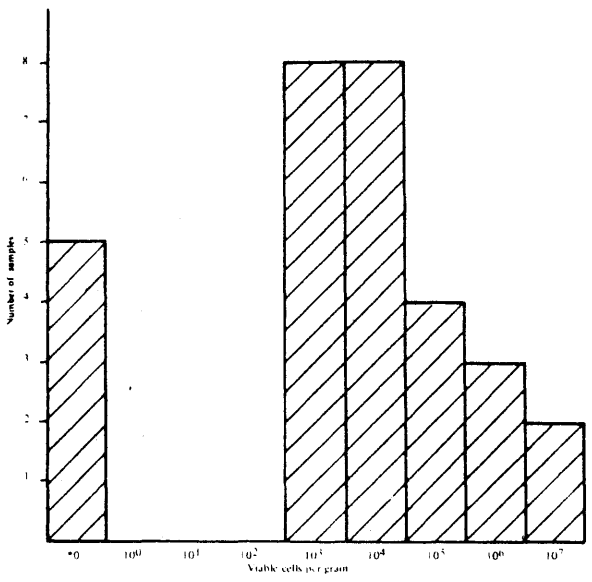

Fig. 7. – *Distribution of coagulase-positive staphylococci in dry sausages*
*(*not detectable in 1 gram)*

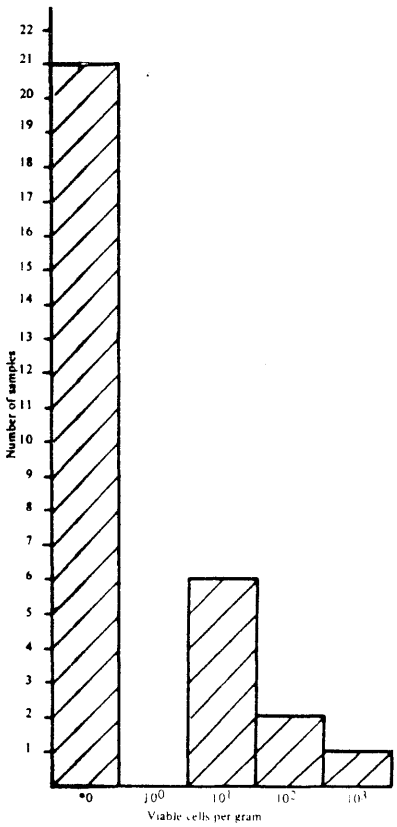

Fig. 6. – *Distribution of the* Klebsiella-Enterobacter-*Group in dry sausages*
(not detectable in 1 gram)*

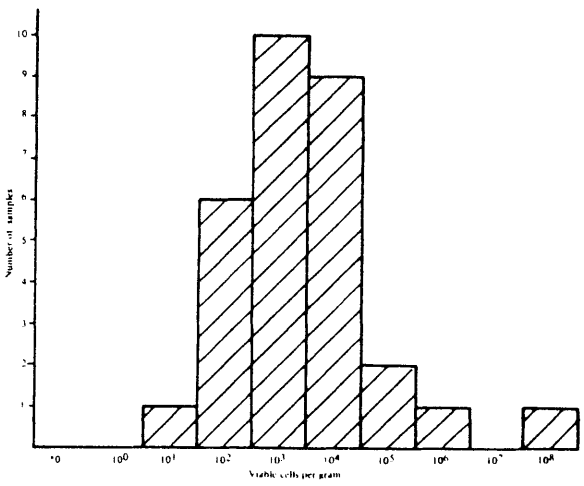

Fig. 8. – *Distribution of enterococci (group D streptococci) in dry sausages*
(not detectable in 1 gram)*

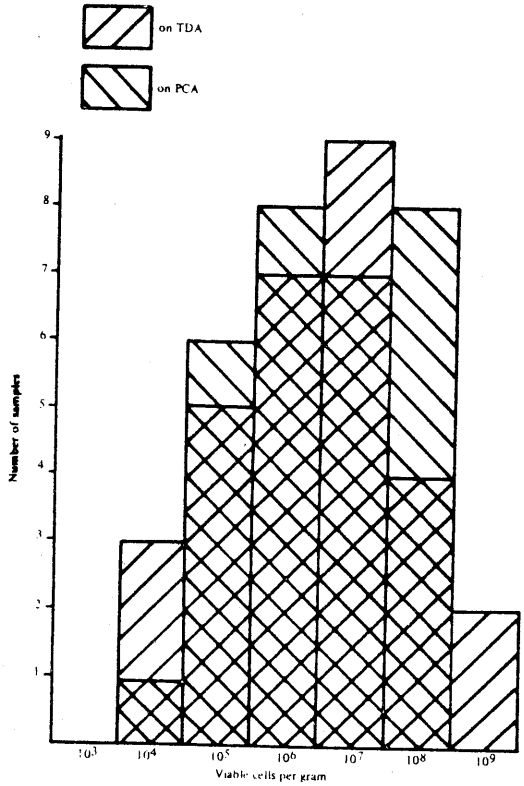

Fig. 1. – *Distribution of total microbial population (as determined on PCA and TDA) in dry sausage samples*

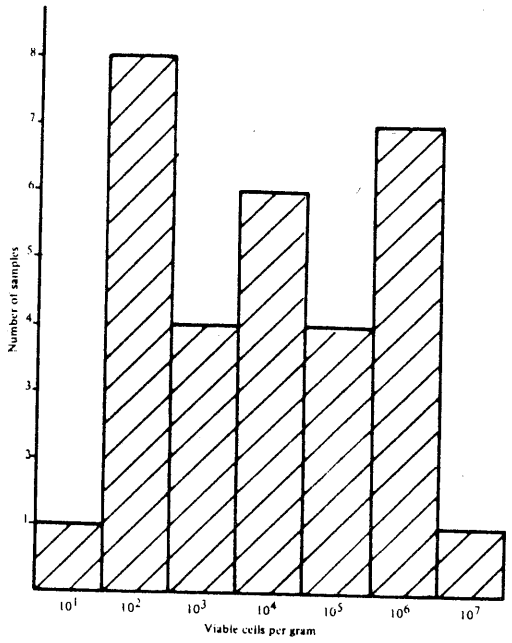

Fig. 3. – *Distribution of yeasts and molds in dry sausages*

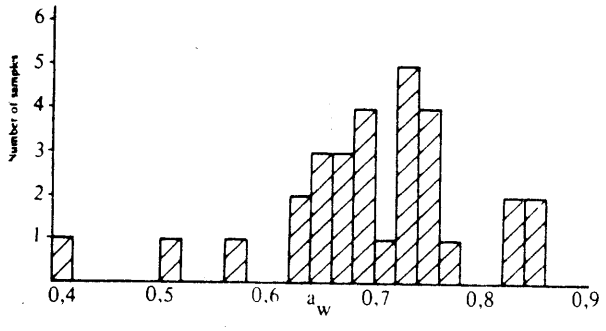

Fig. 2. – *Distribution of water-activity values in dry sausages*

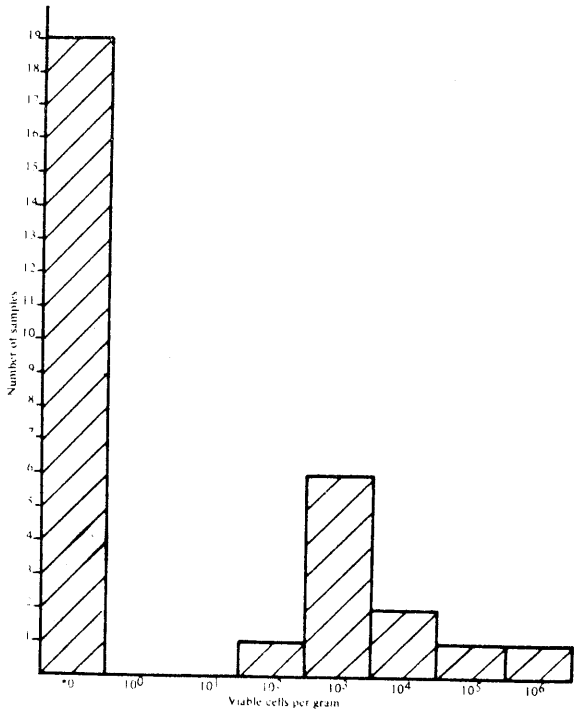

Fig. 4. – *Distribution of numbers of* Microbacterium thermosphactum *in dry sausages*
*(*not detectable in 1 gram)*

isms per gram. In 60% of the samples the counts exceeded 1 million per gram; the average was 3,24 x 10^7 per gram (Table 1). This group constituted 50% or more of the total population in at least 26% of the samples. In 43% of the samples numbers exceeded 40% of the population. The LLP-group constituted between less than 10% (17% of the samples) and 87% of the total viable counts. However, no relationship seems to exist between the total count and the percentage of LLP-organisms in the population. No pediococci or leuconostocs could be detected at the dilution being used, and it is concluded that the lactobacilli constitute at least 97% of the LLP-group.

From a hygienic point of view the *Klebsiella-Enterobacter*-group (especially *E. coli* and salmonellas), coagulase-positive staphylococci and Lancefield's group D streptococci (enterococci) are considered to be most important. In South African dried sausage the presence of these groups seems to be of special significance since this product does not undergo any fermentation process and is consumed raw. The population distribution of the *Klebsiella-Enterobacter*-group is shown in Fig. 6 (see also Table 1). The fact that 70% of the samples contained less than 1 detectable cell per gram, certainly is encouraging. However, it must be borne in mind that the *Klebsiella-Enterobacter*-group normally has a low survival rate in dehydrated products, and is furthermore inhibited by low pH-values (Niinivaara & Pohja, 1957). On the other hand 30% of the samples contained more than 10 viable cells per gram. This lies above the limit suggested by Tackacs, Jirkovsky and Hegyi (1963) for raw sausages.

Apparently no obvious relationship existed between the presence of the *Klebsiella-Enterobacter*-group and the pH of a sample. Detectable numbers (less than 10/g) were found in sausages with pH-values varying between 4,97 and 6,25. However, it may be expected that the decrease in these numbers will be more pronounced at pH-values below 5,5. The fact that pH-values of 43% of the samples were lower than 5,5 (average 5,67; see Table 4) suggests that at least some kind of fermentation may take place during the processing of dried sausage. This seems to correlate with the relatively high numbers of lactobacilli. The possible advantages of a "safety factor" of this kind must be kept in mind if any attempts are to be made towards improvement of the quality of raw, dried sausage.

The importance of coagulase-positive staphylococci is often overlooked. Levels of 500 000/g or higher are considered dangerous (Allison, 1949), but much depends on the amount of food ingested. Although coagulase-positive staphylococci are rarely detected in raw sausages (Deibel *et al.*, 1961) the contrary seems to be true for dried sausage (Fig. 7). The majority (83%) of the samples contained less than 1 000/g, but in 26% the limit of 500 000/g was exceeded. In at least three samples, depending on the medium, coagulase-positive staphylococci constituted more than 10% of the total population (Table 2).

The majority (83%) of the samples contained between 100 and 100 000 anterococci per gram and two samples contained more than 1 million per gram (Fig. 8).

Ten *Salmonella* spp. were isolated from 7 different samples, representing positive recovery from 23% of the samples (Table 3). The presence of salmonellas was neither related to high bacterial counts nor to the presence of countable numbers of the *Klebsiella-Enterobacter*-group. The presence of coagulase-positive staphylococci and salmonellas suggests that dried sausage may constitute a public health hazard. Dried sausages seem to be of an unsatisfactory quality in most cases, bot from a health and a technological point of view.

The variation in hygienic quality is sufficiently demonstrated by differences in numbers and composition of the microbial populations. It is furthermore emphasized by differences in pH-values (varying between 4,61 and 7,09), a_w-values (Fig. 2) (varying between 0,40 and 0,85) and the moisture content (between 8,68 and 34,47%) as is shown in Table 4. In addition the ash content varies between 3,59 and 7,95%. Since the latter two factors largely determine the a_w-value, scatter diagrammes were made to compare a_w-values with ash (mineral) and moisture content respectively. As expected the moisture content in particular seems to have a definite relation to water-activity.

The efficient preservation of dried sausage depends on a low water-activity. However, it must be kept in mind that this only prevents possible spoilage of the product as long as it is kept under dry conditions. Also it is important to note that a low water-activity simultaneously guarantees some form of preservation of the micro-organisms in the product. These organisms are in a viable state, and in the case of salmonellas may constitute a threat to public health, even over long periods. In the presence of safety factors like nitrite, smoke or starter cultures, *Salmonella* spp. disappear within a few weeks from fermented types of sausage (Erikson, 1960). Since none of these safety factors are involved in the production of dried sausage, it is recommended that a maximum a_w-value be laid down before the product is regarded as acceptable for consumption. In addition, dried sausage should be free from any detectable enteropathogenic bacteria, whereas numbers of coagulase-positive staphylococci should never exceed 100 000/g. This can only be achieved if meat of the highest quality is used, and as long as the strictest hygienic principles are practiced during production. The introduction of a ripening or fermentation process, especially one involving selected strains of lactic acid bacteria (i.e. starter cultures), would certainly contribute to a solution of the problems.

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