The microbiological quality and safety of locally produced commercial sausages in Ethiopia

Hirut Assaye and Mogessie Ashenafi*

Addis Ababa University, Ethiopia
E-mail: hirutas2000@yahoo.com
E-mail: mogessie@gmail.com
*Corresponding author

Abstract: Small-scale sausage production is recent in Ethiopia. This study aimed to determine the microbiology of the products made available to the consumer. The microbiological quality of 120 samples of sausages (pork, beef, veal and chicken) collected from various supermarkets in Addis Ababa were investigated. The mean microbial counts ranged from 4.41 to 6.07 log cfu/g for aerobic mesophiles, 2.02 to 4.31 log cfu/g for Enterobacteriaceae, 1.06 to 3.41 log cfu/g for coliforms, 2.30 to 3.91 log cfu/g for enterococci, 2.42 to 4.93 log cfu/g for staphylococci, 5.16 to 7.50 log cfu/g for lactic acid bacteria and 2.70 to 4.69 log cfu/g for yeasts. *Bacillus* spp. dominated the aerobic mesophilic bacterial flora. *Salmonella* was isolated from two samples. Commercial sausages spoiled within 3 to 4 days under ambient storage and after 12 days under refrigeration storage. The pH value was around 6 and the moisture content values were higher than 30%. Time/Temperature abuse during processing and post-cooking contamination could result in the high count. Producers should follow basic hygienic procedures during processing.

1 Introduction

The process of preserving meats by stuffing salted, chopped meats flavoured with spices into animal casings dates back to the ancient Greeks and Romans (Hudnall, 1999). Sausages and sausage products have since evolved into a wide variety of flavours, textures and shapes resulting from variations in ingredients and manufacturing processes. Sausages are popular and relished worldwide (Sachindra *et al.*, 2005). The main ingredients used in these comminuted products are meat trimmings and cuts not marketed or consumed as fresh meat, lower-grade carcasses and certain specialised meat by-products such as tripe, liver, blood or blood plasma and nonmeat ingredients called 'fillers' or 'binders' (Jelen, 1985). Food additives are used for their colouring and antimicrobial, antioxidative and preservative properties, as well as for improved nutrition, increased emulsification and altered flavour.

Growing consumer interest in foodstuffs of high nutritional value that are safe and hygienically prepared has prompted interest in sausages. On the other hand, these products, due to specific recipes, very short shelf life, storage conditions and inappropriate management in the meat warehouse or shop, might be rendered undesirable for consumption (Domanska and Rozanska, 2003).

Raw meat contains a variety of microorganisms and is an ideal growth medium for many organisms. Sausages, in addition to the meat components, have additional sources of organisms in the seasoning and formulation ingredients. Comminuting also adds microbial contamination to sausages (Sachindra *et al.*, 2005). Microorganisms may also gain access to sausages from the environment, equipment and handlers, which affect the microbiological status of the product. Even though processing conditions such as heat treatment reduce microbial levels, recontamination takes place during the post-processing handling and storage of sausages. Thus, the microbial ecology of meat products will mainly depend on the environment, the kind of meat and raw materials, equipment, handling practices, processing, packaging and storage temperature (Sachindra *et al.*, 2005).

Although traditional sausage making is common among some communities in Ethiopia, commercial-scale sausage production started very recently. Initially, sausages were produced mainly from pork, but consumer demand was low due to the religious taboos related to the consumption of pork. Currently, various emulsion-type sausages, separately made from veal, chicken, beef or pork, are made available to consumers in various retail shops. Consequently, there is a growing consumer interest in these processed meat products. There is, however, no available information regarding the microbiological status of these products. The aims of this study were, therefore, to assess the microbial load and safety of these commercially produced sausages and evaluate their keeping quality when maintained at ambient and cold temperatures.

2 Materials and methods

2.1 Samples

A total of 120 vacuum-packed emulsion-type sausage samples consisting 30 samples each of pork, beef, veal and chicken were collected from various supermarkets in Addis Ababa, Ethiopia, and immediately brought to the laboratory for microbiological analysis. Microbiological analysis was conducted within 1 to 3 h of sample collection.

2.2 Microbiological analysis

A 25 g sample of each sausage type was placed aseptically in a sterile stomacher bag and homogenised in 225 ml of sterile 0.1% (w/v) peptone water using a Stomacher lab blender (model 400, Seward JAC, London). Serial tenfold dilutions were prepared to count the following microbial groups:

- *Aerobic mesophilic bacteria* – from appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on pre-dried surfaces of Plate Count Agar (Oxoid) plates. Colonies were counted after incubation at 30°C–32°C for 48 h.
- *Coliforms* – a volume of 0.1 ml of appropriate dilutions was spread plated in duplicates on pre-dried surfaces of Violet Red Bile Agar (Oxoid) plates. The plates were incubated at 30°C–32°C for 24 h, after which purplish red colonies surrounded by a reddish zone of precipitated bile were counted as coliforms.

- *Enterobacteriaceae* – a volume of 0.1 ml of appropriate dilutions was spread plated in duplicates on pre-dried surfaces of Violet Red Bile Glucose Agar (Oxoid) plates. The plates were incubated at 30°C–32°C for 20–24 h, after which pink to red purple colonies with or without halos of bile precipitation were enumerated as members of Enterobacteriaceae.
- *Staphylococci* – a volume of 0.1 ml of appropriate dilutions was spread plated in duplicates on pre-dried surfaces of Mannitol Salt Agar (Oxoid) plates. The plates were incubated at 30°C–32°C for 36 h. Yellow colonies were counted as staphylococci.
- *Enterococci* – a volume of 0.1 ml of appropriate dilutions was spread plated in duplicates on pre-dried surfaces of Bile Aesculin Agar plates consisting of (g/l distilled water) peptone 8, bile salts 20g, ferric citrate 0.5g, aesculin 1g, agar 15g, pH 7.1$\pm$0.2). The plates were incubated at 30°C–32°C for 24 h. The colonies surrounded by a blackened zone were counted as enterococci.
- *Lactic acid bacteria* – a volume of 0.1 ml of appropriate dilutions was spread plated in duplicates on pre-dried surfaces of MRS (DeMan, Rogasa, Sharpe) (Oxoid) agar plates. The inoculated plates were incubated anaerobically at 30°C–32°C for 48 h. All colonies were counted as lactic acid bacteria.
- *Yeasts* – a volume of 0.1 ml of appropriate dilutions was spread plated on Chloramphenicol-Bromophenol blue agar consisting of (g/l distilled water) yeasts extract (Oxoid) 5.0, glucose 20, choramphenicol 0.1, Bromophenol-blue 0.01, agar 15, pH 6.0–6.4. The plates were incubated at 25°C–28°C for 4–5 days. Smooth, nonhairy colonies lacking extensions at margins under a stereoscopic microscope were counted as yeasts.

2.3 Flora analysis

About seven colonies were picked randomly from countable Plate Count Agar plates and transferred to Nutrient Broth (Oxoid). These were incubated at 30°C–32°C overnight. The cultures were purified by repeated plating and characterised to the genus level and various bacterial groups using the following tests:

- cell morphology
- the potassium hydroxide (KOH) test (Gregerson, 1978)
- the Oxidation Fermentation (O/F) test (Hugh and Leifson, 1953)
- the Catalase test
- the Cytochrome Oxidase test (Kovacs, 1956).

2.4 Isolation of Salmonella spp.

The sausage samples (25 g) were homogenised in 225 ml of buffered peptone water using a Stomacher lab blender and incubated at 37°C for 24 h. From this, 1 ml of culture was transferred into separate tubes, each containing 10 ml of Selenite broth (Oxoid), 10 ml of Tetrathionate broth (Oxoid), 10 ml of Mannitol Selenite broth (Oxoid) or 10 ml of

Muller-Kauffman Tetrathionate broth (Oxoid). A volume of 0.1 ml of culture was also transferred into a separate tube containing 10 ml of Rappaport Vassiliadis (RV) broth (Merck). The Selenite and Mannitol Selenite broths were incubated at 37°C for 24 h and the Tetrathionate, RV and Muller-Kauffman Tetrathionate broths were incubated at 43°C for 48 h in a water bath. The culture from each enrichment broth was separately streaked on plates of MacConkey Agar, Salmonella-Shigella (SS) Agar and a Xylose Lysine Desoxycholate (XLD) medium (all from Oxoid). The characteristic colonies from each selective medium were picked, purified and tested biochemically on Triple Sugar Iron Agar (Oxoid), Lysine Iron Agar (LIA) (Oxoid), Urea Agar (Oxoid), Simmons Citrate Agar (Oxoid) and SIM Medium (Oxoid). The ability of *Salmonella* to ferment mannitol, glucose or sucrose was assessed using a fermentation broth containing the corresponding sugars. The fermentation tubes contained inverted Durham tubes to detect gas production.

Presumptive *Salmonella* isolates were further confirmed by using an API 20E (BIOMERIEUX) identification system as described by the manufacturer.

2.5 Determination of the keeping quality of sausages during aerobic storage at ambient and cold temperatures

The sausages were collected from supermarkets immediately after delivery from factories and were separately stored at ambient and refrigeration temperatures. The storage conditions were intended to reflect what normally would happen in routine food handling in home kitchen environments and food service establishments. The samples were aseptically removed from packages at periodic intervals and counts of aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts were monitored during the storage time. Counting was done at 8 h intervals for sausages stored at the ambient temperature and at 48 h intervals for those stored at the refrigeration temperature.

2.6 Measurement of pH and moisture content (%) values

The pH of each sausage sample was determined by blending a 10 g sausage sample in a stomacher with 100 ml of distilled water. The pH of the homogenate was then measured using a digital pH meter. The moisture content of the sausages was determined by allowing the samples to dry to constant weight at 35°C.

2.7 Statistical analysis

The Coefficient of Variation (CV) was calculated to see if there was significant variation in counts within the samples of sausages.

3 Results and discussion

The mean aerobic mesophilic counts of the various commercial emulsion-type sausages in this study ranged between log 4 and log 6 cfu/g (Table 1). This was much higher than the value (log 2 cfu/g) reported for commercial Frankfurters in Argentina (Sabbag *et al*., 2005). The mean aerobic mesophilic count for the chicken Frankfurters collected from retail markets in Egypt (El-Khateib *et al*., 1988) was markedly higher than that observed in our chicken Frankfurter samples.

Table 1 The microbial counts (log cfu/g) of commercial Frankfurters (n = 120)

	Pork sausage			*Beef sausage*			*Veal sausage*			*Chicken sausage*		
Bacterial groups	*Mean*	*SD*	*%CV*	*Mean*	*SD*	*%CV*	*Mean*	*SD*	*%CV*	*Mean*	*SD*	*%CV*
Aerobic mesophiles	5.19	1.41	27.16	5.61	0.77	13.72	6.07	2.32	38.22	4.41	1.09	24.71
Enterobacteriaceae	2.94	1.29	43.88	3.46	1.40	40.46	4.31	2.97	68.91	2.02	1.12	55.51
Coliforms	2.73	1.20	43.95	2.98	1.43	47.98	3.41	2.74	80.35	1.06	0.29	27.36
Enterococci	2.51	1.59	63.34	3.91	1.01	25.83	3.46	2.19	63.21	2.30	0.98	44.78
Staphylococci	3.73	1.84	49.32	4.93	0.93	8.86	3.82	2.79	73.03	2.42	1.54	64.63
Lactic acid bacteria	7.50	1.00	13.33	7.30	0.96	13.15	5.89	2.29	38.87	5.16	1.44	27.90
Yeasts	4.69	0.85	18.12	4.62	0.60	12.99	3.00	1.77	59.00	2.70	1.82	67.40

Notes: SD = standard deviation; CV = coefficient of variation.

There are no standards or guidelines regarding the microbial load of sausages in Ethiopia. Shapton and Shapton (1991) stated an aerobic mesophilic count of <log 5 cfu/g for cooked sausages. In our samples, 59% of all sausages (53% of pork, 87% of beef, 57% of veal and 40% of chicken Frankfurters) exceeded the typical aerobic mesophilic count value set for cooked sausages (Table 2). A high aerobic mesophilic count is not in itself a health risk, but in a cooked product, it indicates an overall lack of hygiene (Little and de Louvois, 1998). The long-term storage of sausages either in factories or shops may also contribute to an increase in the aerobic mesophilic counts of sausages, particularly if there is temperature abuse during storage. Thus, the high aerobic mesophilic count recorded in commercial Frankfurters might not only reflect the sanitary quality, but also the possible continued growth of microorganisms during storage in supermarkets.

Table 2 Frequency distribution (%) of counts (log cfu/g) of microbial groups in all sausage samples

	Sausage samples (%)			
Microbial groups	*<2.00*	*2.00–4.99*	*5.00–7.99*	*>8.00*
Aerobic mesophilic bacteria	0	41	52	7
Enterobacteriaceae	25	58	15	2
Coliforms	44	46	10	0
Enterococci	24	61	15	0
Staphylococci	28	38	34	0
Lactic acid bacteria	1	23	53	13
Yeasts	20	60	20	0

The counts of Enterobacteriaceae were higher than log 2 cfu/g in 75% of our sausage samples and over half of the beef and veal sausages had counts of ≥log 4 cfu/g (Table 2). The veal sausage samples with Enterobacteriaceae counts as high as log 7 and log 8 cfu/g were also encountered (data not shown). Coliforms were also frequently encountered in 56% of the sausage types at counts as high as >log 2 cfu/g. As these groups of bacteria are supposed to be eliminated in the cooking process usually applied to Frankfurters, their presence at this level is indicative of post-processing contamination.

In 76% of our sausage samples, enterococci were encountered at levels higher than log 2cfu/g. The count was particularly higher in the beef and veal sausage samples (data not shown). In processed meat products, enterococci are not generally desirable because they cause spoilage (Giraffa, 2002). Enterococci can be found as spoilage contaminants in processed meats, either by surviving heat processing due to their thermoduric nature (Franz *et al.*, 1999) or by cross-contamination at the final stages of processing, such as slicing and packaging (Hugas *et al.*, 2003).

The mean staphylococcal counts recorded for the pork, beef and veal Frankfurters were around log 4 cfu/g (Table 1). Moreover, the samples which had counts of staphylococci at ≥log 5 cfu/g were also frequently encountered in this study (Table 2). Hill (1972) stated that cooked meat products such as bologna and Frankfurters should be free of staphylococci. Staphylococci are common in unprocessed animal products and products that are handled by hand. Heat processing, however, should reduce their numbers or eliminate them.

The counts of lactic acid bacteria in our sausage samples were notably high, ranging from log 5 to log 8 cfu/g in 66% of the various sausage types (Table 2). Lactic acid bacteria are known to dominate the spoilage flora of emulsion sausages and the spoilage process usually starts when the counts reach values of >log 7 cfu/g (Korkeala *et al.*, 1989). In our sausage samples, however, no observable spoilage was detected, although over 70% of pork, 60% of beef and about 40% of veal Frankfurter samples had lactic acid bacteria counts of $\geq 10^7$cfu/g (data not shown). In fact, Samelis and Georgriadou (2000) reported that the shelf life of vacuum or modified atmosphere-packed cooked meats should be preferably defined by an acceptable off-odour/off-flavour or appearance rather than a certain bacterial level.

The majority of sausage samples (80%) had yeast counts ranging from log 2 to >log 8 cfu/g (Table 2). Yeasts were described as the spoilage organisms of sausages (Jay, 1996). Palumbo *et al.* (1974) also reported that yeasts were the dominant spoilage flora of the Frankfurters produced in their plant. Thus, the yeasts in our samples, if and when they proliferate and reach higher numbers, could contribute to the products' spoilage.

The CV for counts of all bacterial groups within the samples of a sausage type was high (CV > 20%). This showed that the level of contamination within the different samples of a sausage type varied significantly (Table 1). This was indicative of the lack of quality control in the processing, storage and handling of sausages. In fact, most of the processing plants had no system of microbiological control of the raw materials (meat and other ingredients) or the final product at any stage of production. In addition, none of the processing plants stated the production and sell-by date on product packages. Thus, the variation in the counts of the different microbial groups among our sausages might arise from the level of initial contamination, lack of control steps during processing, extent of post-heating contamination or the length of time the products were maintained in supermarkets.

Of the 487 bacterial strains isolated from commercial Frankfurters, 95% belonged to Gram-positive organisms (Table 3). Among our isolates, *Bacillus* was the most dominant species. The dominance of Gram-positive spore-forming rods in commercially processed Frankfurters was also observed by other workers (Palumbo *et al.*, 1974). Spices are considered possible sources of large numbers of aerobic spore-forming bacteria in sausages (IOM, 1985). The isolation of members of this genus from several raw and processed foods all over the world is attributed to their virtue of having resistant endospores that confer tolerance to adverse conditions and various stresses.

Table 3 Frequency distribution (%) of dominant bacteria in commercial Frankfurters

Sausage type	*No. of isolates*	*Bacillus*	*Micro-coccus*	*Staphylo-coccus*	*Strepto-coccus*	*Lacto-bacillus*	*Entero-bacteriaceae*	*Other Gram-positive rods*
Pork sausage	107	40	13	18	10	8	4	7
Beef sausage	133	28	15	23	14	8	8	2
Veal sausage	129	25	21	18	13	7	9	7
Chicken sausage	118	57	20	12	7	5	–	–
Total	487	37	17	18	11	7	5	5

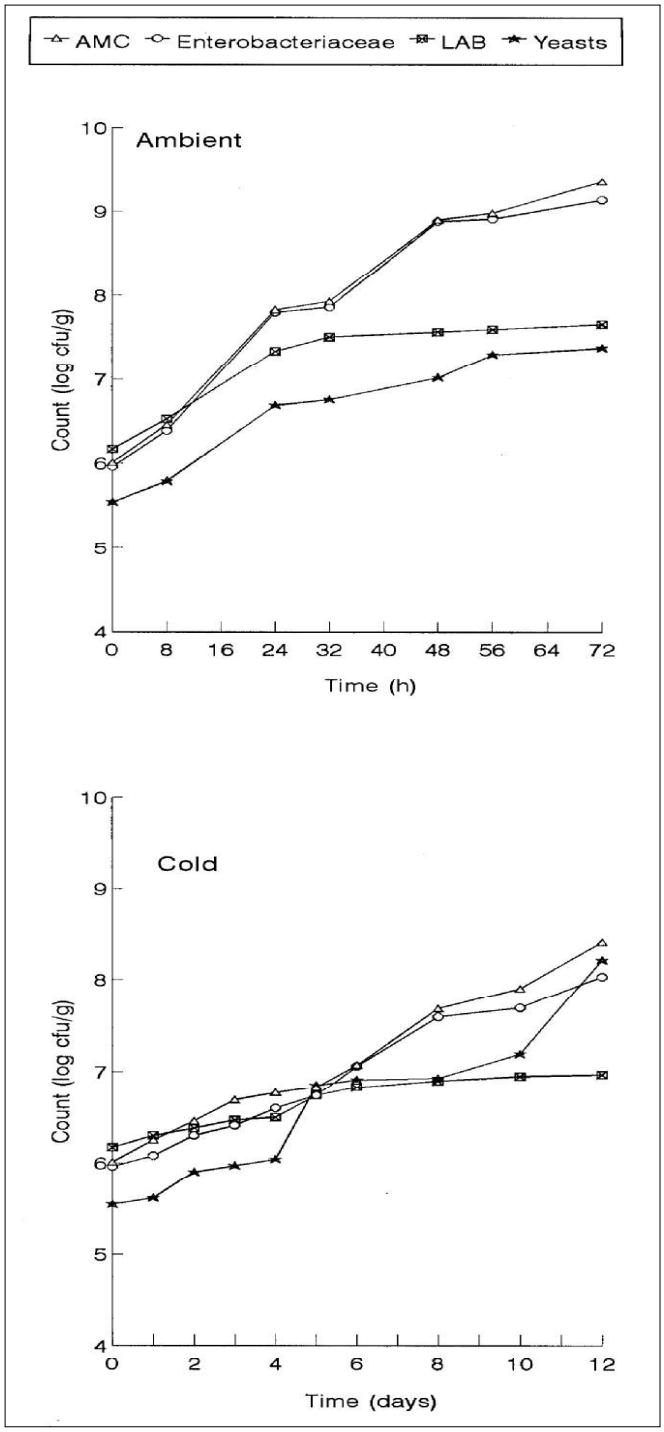
Micrococci constituted the second dominant group in veal and chicken Frankfurters and third in the other sausage types. The dominance of micrococci in these sausages might be due to their heat-resistant nature. Palumbo *et al.* (1974) indicated that the predominant organisms in the Frankfurters produced in their plant that survived the heating step were micrococci and increased to substantial numbers in the product during storage at 5°C. Micrococci have been shown to cause spoilage in packaged Frankfurters.

The presence of staphylococci in raw meat and their known heat resistance suggest that they could be a problem in heat-processed meat products. It is reported that other microflora present in meat have an adverse effect on the growth of staphylococci and that staphylococci grow better in cooked meat and in fresh meat treated with salt (Jay, 1996).

The frequency of isolation of *Salmonella* spp. in this study was low (1.7%). *Salmonella* was not detected in any of cooked chicken Frankfurt sausage samples taken from a sausage factory in the state of São Paulo, Brazil (Luiz *et al.*, 2004). It was stated that *Salmonella* are not usually found in Frankfurters because they cannot survive the heat process (Palumbo *et al.*, 1974). *Salmonella* are usually killed by temperatures greater than 50°C, with the death rate increasing as the temperature increases (Doyle and Mazzotta, 2000). The detection of *Salmonella* in the investigated sausage samples in our study, thus, indicates the use of raw materials contaminated with *Salmonella*, accompanied by an insufficient cooking process (underprocessing) or recontamination after cooking and prior to packaging. In fact, salmonellae are known to be present in the raw meat used in sausage manufacturing (Palumbo *et al.*, 1974). Luiz *et al*. (2004) also isolated *Salmonella* strains from 4 of the 30 samples of mechanically deboned chicken meat used as a raw material in making a Frankfurt sausage and even in 2 of the 15 samples of sausage emulsion after the addition of preservatives. If sausages are cooked adequately prior to consumption, any *Salmonella* present would be killed and there would be no risk of food infection. Therefore, the delivery of adequate heat during the cooking of comminuted meat products is important to ensure food safety, although some cooking processes (frying, grilling or barbecuing) may not completely kill *Salmonella* (Mattick *et al.*, 2002). The presence of *Salmonella* spp. in the sausage samples investigated in our study thus indicates the possible public health risk from eating sausages, especially undercooked ones. It also shows the need for the close supervision of processing and sanitation practices.

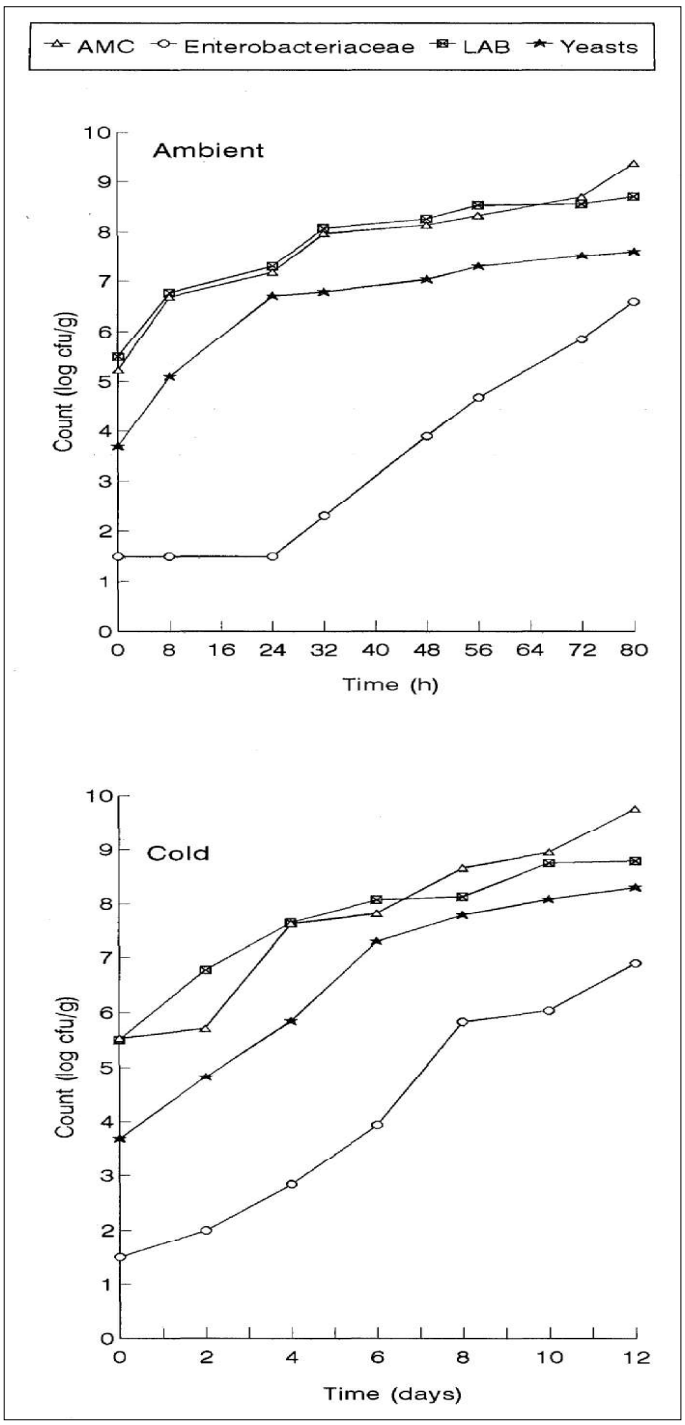
Commercial Frankfurters spoiled within three to four days during aerobic storage at an ambient temperature. At the refrigeration temperature, the sausage samples spoiled after 12–16 days of storage (Figure 1). Spoilage was manifested as slime formation on the surface of casings and the production of off-odour. All microbial groups reached high counts on the day spoilage was detected (Figure 1). Bayne and Michener (1975) indicated that Frankfurters could be spoiled by nonhazardous spoilage microorganisms if they are subjected to temperature abuse. These organisms can survive heat treatment in moderate numbers and can also be present as a result of post-processing contamination. In this study, even the microbial groups (Enterobacteriaceae and yeasts) which were in low counts (<log 2 cfu/g) on the collection day of some sausages grew faster and reached high counts on the day spoilage was detected. In fact, the significant contribution of yeasts to sausage spoilage under aerobic and temperature-abused conditions was also observed by Samelis and Georgriadou (2000). Cayre *et al.* (2005) reported that, independent of temperature and oxygen permeability, lactic acid bacteria counts increased from log 2 to log 8 cfu/g in cooked meat emulsions.

Figure 1 The microbial dynamics of commercial pork (A), beef (B), chicken (C) and veal (D) sausages during aerobic storage at ambient and cold temperatures



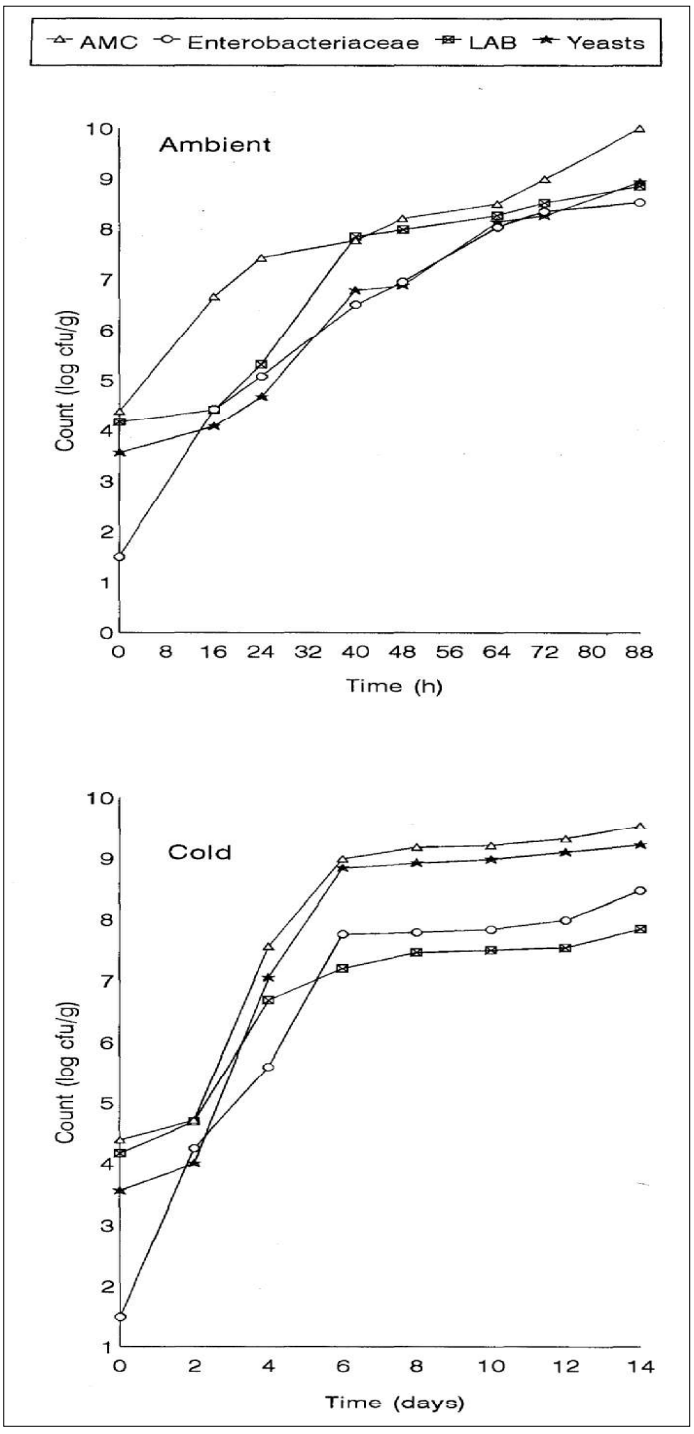
(A)

Figure 1 The microbial dynamics of commercial pork (A), beef (B), chicken (C) and veal (D) sausages during aerobic storage at ambient and cold temperatures (continued)



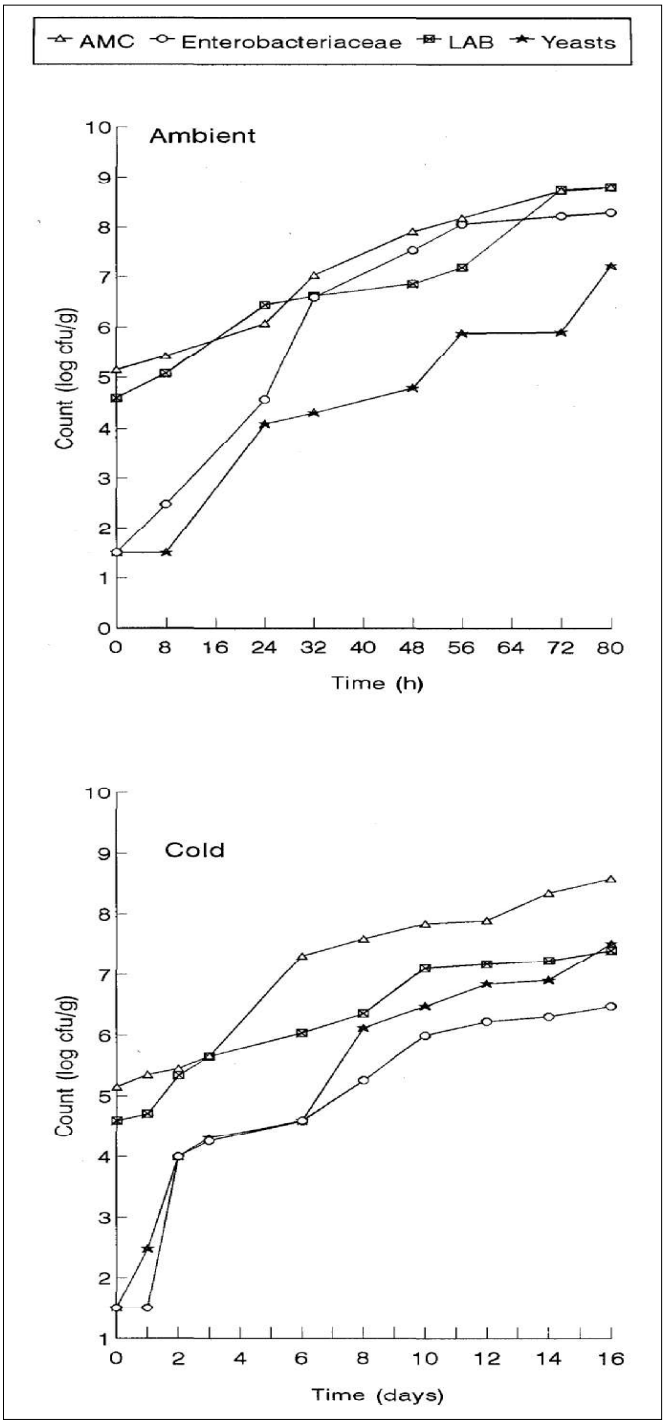
(B)

Figure 1 The microbial dynamics of commercial pork (A), beef (B), chicken (C) and veal (D) sausages during aerobic storage at ambient and cold temperatures (continued)



(C)

Figure 1 The microbial dynamics of commercial pork (A), beef (B), chicken (C) and veal (D) sausages during aerobic storage at ambient and cold temperatures (continued)



(D)

The majority of commercial Frankfurters analysed in our study had pH values above 6. The variability in the pH values among the samples in each type of Frankfurter sausage was low (Table 4). The pH would support the proliferation of spoilage flora (Jay, 1996) and allow the multiplication of several bacterial pathogens. The moisture content values of the Frankfurters investigated in our study ranged from 32% to 46% (Table 4). These values were significantly lower than that reported for the Frankfurters in other studies (>60%) (Metaxopoulos *et al.*, 2002; Sabbag *et al.*, 2005). Even though our Frankfurters had a lower moisture content, fillers or binders such as wheat flour, salt, milk solids, oil and others added in their formulations might still allow the multiplication of many types of bacteria and yeasts.

Table 4 The pH and moisture content (%) values of commercial sausages

Sample type	*pH*					*Moisture content (%)*				
	Min	*Max*	*Mean*	*SD*	*% CV*	*Min*	*Max*	*Mean*	*SD*	*% CV*
Pork sausage	5.88	6.52	6.27	0.15	2.39	40	46	43	1.9	4.40
Beef sausage	5.81	6.51	6.27	0.16	2.56	38	45	41	2.12	5.16
Veal sausage	5.77	6.39	6.11	0.13	2.13	32	37	35	1.40	4.06
Chicken sausage	5.36	6.62	6.27	0.25	3.98	32	40	36	2.68	7.55

In general, the majority of commercial Frankfurters considered in this study had a high microbial load and, in some cases, even pathogens were isolated. Time/Temperature abuse during processing and/or post-cooking contamination due to the improper handling of the products may contribute to high microbial counts. Furthermore, the absence of a microbiological control system on the ingredients, process and end product and the poor sanitary condition of some of the processing plants revealed inadequacies concerning the quality and safety of these products.

Acknowledgements

Hirut Assaye acknowledges the financial support of SIDA/SAREC, obtained through the Graduate Programme of Addis Ababa University. Mogessie Ashenafi thanks the Howard Hughes Medical Programme (Dr. W. Anderson) for its financial support and the Biology Department of Howard University, USA, for making the required facilities and support available during the preparation of the manuscript.

References

Bayne, H.G. and Michener, H.D. (1975) 'Growth of *Staphylococcus* and *Salmonella* on Frankfurters with and with out sodium nitrite', *Appl. Microbiol.*, Vol. 30, pp.844–849.

Cayre, M.E., Garro, O. and Vignolo, G. (2005) 'Effect of storage temperature and gas permeability of packaging film on the growth of lactic acid bacteria and *Brochothrix thermosphacta* in cooked meat emulsions', *Food Microbiol.*, Vol. 22, pp.505–512.

Domanska, K. and Rozanska, H. (2003) 'Microbiological quality of Polish edible offals processed meat products during storage. Influence on N – nitrosamines content', *Bull. Vet. Ins. Pulawy.*, Vol. 47, pp.217–223.

Doyle, M.E. and Mazzotta, A.S. (2000) 'Review of studies on the thermal resistance of salmonellae', *J. Food Prot.*, Vol. 63, pp.779–795.

El-Khateib, T., Abd El-Rahman, H., Hamdy, M. and Lotfi, A. (1988) 'Poultry meat products in Egypt "proximal chemical composition and microbiological quality"', *Fleischwirtsch.*, Vol. 68, pp.756–757.

Franz, G.M., Holzapfel, W.H. and Stiles, M.E. (1999) 'Enterococci at the cross road of food safety?', *Int. J. Food Microbiol.*, Vol. 47, pp.1–24.

Giraffa, G. (2002) 'Enterococci from foods', *FEMS. Microbiol. Rev.*, Vol. 26, pp.163–171.

Gregerson, G. (1978) 'Rapid method for distinction of Gram-positive from Gram-negative bacteria', *European J. Appl. Microbiol.*, Vol. 5, pp.123–127.

Hill, W.M. (1972) 'The significance of staphylococci in meats', *Proceedings of the 25th Annual Recip., Meat Conference of the American Meat Science Association, Ames, Iowa*, National Live Stock Meat Board, Chicago, USA, pp.300–305.

Hudnall, J.W. (1999) 'Safe practices for sausage production', *Distance Learning Course Manual*, Washington, DC, USA.

Hugas, M., Garriga, M. and Aymerich, M.T. (2003) 'Functionality of enerococci in meat products', *Int. J. Food Microbiol.*, Vol. 88, pp.223–233.

Hugh, R. and Leifson, E. (1953) 'The taxonomic significance of fermentative versus oxidative Gram – negative bacteria', *J. Bacteriol.*, Vol. 66, pp.24–26.

IOM (1985) *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*, Washington, DC: National Academies Press.

Jay, J.M. (1996) *Modern Food Microbiology*, 5th ed., New York: Chapman and Hall.

Jelen, P. (1985) *Introduction to Food Processing*, Reston, VA: Reston Publishing Company Inc.

Korkeala, H., Alanko, T., Makela, P. and Lindroth, S. (1989) 'Shelf life of vacuum-packed cooked ring sausages at different chill temperatures', *Int. J. Food Microbiol.*, Vol. 9, pp.237–247.

Kovacs, N. (1956) 'Identification of *Pseudomonas pyocyanae* by the oxidase reaction', *Nature*, Vol. 178, p.703.

Little, C.L. and de Louvois, J. (1998) 'The microbiological examination of butchery products and butchers' premises in the United Kingdom', *J. App. Microbiol.*, Vol. 85, pp.177–186.

Luiz, A.F., Moreira, F.C., Correa, E.F. and Falcao, D.P. (2004) 'Monitoring of the dissemination of *Salmonella* in the Chicken Frankfurt-sausage production line of a sausage factory in the State of São Paulo, Brazil', *Mem. Inst. Oswaldo Cruz*, Vol. 99, pp.477–480.

Mattick, K.L., Bailey, R.A., Jorgenson, F. and Humphrey, T.J. (2002) 'The prevalence and number of *Salmonella* in sausages and their destruction by frying, grilling or barbecuing', *J. Appl. Microbiol.*, Vol. 93, pp.541–547.

Metaxopoulos, J., Mataragas, M. and Drosinos, H.E. (2002) 'Microbial interaction in cooked cured meat products under vacuum or modified atmosphere at 4°C', *J. Appl. Microbiol.*, Vol. 93, pp.363–373.

Palumbo, S.A., Huhtanen, C.N. and Smith, J.L. (1974) 'Microbiology of the Frankfurter process: *Salmonella* and natural aerobic flora', *Appl. Microbiol.*, Vol. 27, pp.724–732.

Sabbag, N.G., Aringoli, E.E., Santiago, L.G., Costa, S.C. and Gonzalez, R.J. (2005) 'Quality evaluation of commercial Frankfurter by deterioration index method', *J. Food Quality*, Vol. 28, pp.467–478.

Sachindra, M.N., Sakhare, Z.P., Yashoda, P.K. and Narasmiha, R.D. (2005) 'Microbial profile of buffalo sausage during processing and storage', *Food Cont.*, Vol. 16, pp.31–35.

Samelis, J. and Georgiadou, K.G. (2000) 'The microbial association of Greek taverna sausage stored at 4 and 10 degrees C in air, vacuum or 100% carbon dioxide, and its spoilage potential', *J. Appl. Microbiol.*, Vol. 88, pp.58–68.

Shapton, D.A. and Shapton, N.F. (1991) *Principles and Practices for the Safe Processing of Foods*, Oxford, UK: Butterworth-Heinemann.