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MICROBIOLOGICAL QUALITY OF BAKED MEAT PIES

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INTRODUCTION

Meat pies have become quite a popular food item in Ghana especially in the urban areas. They are normally sold with Fan Milk products by push-bike retailers, at rest stops, canteens and Super Markets.

Although meat pie has hitherto not been associated with reported mass food poisoning, it has on occasion been a source of infection in other countries. Outbreaks have been recorded in Britain in the following areas: West Lancashire and Preston in 1953 (U.K. Ministry of Health, 1955), Manchester in 1950 and Northampton and Prestatyn in 1949 (U.K. Ministry of Health, 1953). These reports have prompted an investigation into the microbiological quality of meat pies in Ghana because there had been occasions when mouldy pies as well as undercooked pies had been offered for sale to the public.

Undercooking may leave undestroyed endospores of pathogenic bacteria such as Clostridia in the initial microbial load of the meat used in the stuffing. Furthermore, pies undergo spoilage with storage or mishandling very quickly. This spoilage is always accompanied by extensive microbial growth.

During this familiarization programme in food bacteriology, an investigation into these problems will afford an opportunity of putting various microbiological techniques into practice besides providing valuable information.

MATERIALS AND METHODS

Materials

Meat pies were purchased from three Stores in Accra : Kingsway, G.N.T.C. (Ghana House) and U.T.C. on the day of delivery by the bakers to the Stores. They were conveyed to the laboratory in sealed sterile polythene bags and kept in a room at $20 \pm 1^{\circ}\text{C}$.

Two batches of samples were obtained from U.T.C. and Kingsway Stores and four from G.N.T.C. Two of the batches from G.N.T.C. and those from U.T.C. consisted of pies packed in small polythene bags. The pies from Kingsway Stores and two batches from G.N.T.C. were not covered but were kept in small cabinets from which they are picked with forceps and sold to the public.

Methods

Twelve pies were withdrawn from each batch of samples at 2-day intervals over a period of 10 days for microbiological examination.

Six pies were examined for the estimation of total viable bacteria, coliform organisms and fungi. Two samples were taken separately from 3 pies pooled together. Each sample weighing 10 grams was blended in a homogenizer in 90 ml. of sterile quarter strength Ringer's solution. Serial dilutions were made and used in inoculating agar media for viable counts.

Total viable bacteria counts and coliform organisms were obtained using Miles and Misra Surface Count Method (Miles and Misra. 1938). This method consists of placing very small drops of the serial dilutions on the surface of well dried agar plates, ~~after a specific period.~~

Enumeration of viable fungi was by Pour Plate technique where measured amounts of known serial dilutions were mixed with cool molten agar medium in petri dishes, and the media allowed to set.

Media used in plating included Blood Agar, made up of Oxoid Plate Count Agar enriched with sterile defibrinated human Blood, for the estimation of total viable bacteria; Oxoid MacConkey Agar, pH 7.4 as described by Report, 1956, for enumeration of coliform organisms; Rose

Bengal Agar as given by Martin (1950) for enumeration of fungi.

Plates for estimation of total viable bacteria were incubated aerobically at 30°C and anaerobically at 37°C for 72 hours. Those for enumeration of coliforms and fungi were incubated aerobically only at 37°C and 30°C for 24 hours and 5 days, respectively.

The meat content of the other 6 pies was sampled separately from the pastry for the presence of Clostridium botulinum. The detection of this organism requires an enrichment technique as given in "Recommended Methods for the Microbiological Examination of Foods" (Sharf, 1966). This technique involved an enrichment of a portion of the blended meat content of the pies in Cooked Meat Medium. Six large boiling tubes of freshly heated Cooked ^{Meat} Medium were each heated in a water bath at 80°C for 20 minutes to kill all vegetative cells. All six tubes were then incubated anaerobically for 4 days at 35°C in an anaerobic jar. After incubation the material from the tubes were stained by Gram's stain and examined microscopically. Tubes showing presence of Gram-positive bacilli were then subcultured into Blood Agar plates, and isolated in pure cultures. Cultures were Gram-stained and tested for catalase reaction.

An attempt was made to identify the Bacillus species isolated using the media and methods for such identification given in "Manual for the identification of Medical Bacteria" (Cowan and Steel, 1970).

Some of the tests used include -

- i) Starch hydrolysis (p. 163).
- ii) Citrate utilization (p. 152-153).
- iii) Indole production (p. 158).
- iv) Nitrate reduction (p. 161).
- v) Voges-Proskauer (V-P) reaction (p. 160).
- vi) Fermentation of sugars (p. 22-23).

Fermentation tests were carried out using carbon sources at a concentration of 1.0%. All inoculations were made using young cultures grown on nutrient agar slopes. The catalase test was carried out on a slide and confirmed by flooding with hydrogen peroxide on a culture growing on nutrient agar slant.

RESULTS

Total Viable Counts

Tables 1 - 8 show that the meat pies examined from all three Stores have low initial total counts of both bacteria and fungi. No coliform organisms were found in any of the pies examined. There was generally an increase in microbial numbers as the pies aged.

Pies from U.T.C.

The first batch of pies from U.T.C. purchased on September 4, 1972 (Table 1) was sampled for only a period of 6 days. Bacterial numbers increased sharply from 0.0006×10^6 per gram on the first day of sampling, to 296.0×10^6 per gram on the fourth day. Fungal populations were however few and there was a dominant species of Penicillium on all the plates.

The second batch of pies showed a similar pattern of increase in bacterial numbers but on the 10th day there was a decline in numbers of both bacteria and fungi, probably due to autolysis. Penicillium was again the most dominant fungus isolated.

Pies from G.N.T.C. (Ghana House)

The results in Tables 3 and 4 are for meat pies packed in polythene bags. Results for unpackaged pies are shown in Tables 5 and 6.

Little or no microbial growth was obtained from pies sampled on the day of delivery to the stores. By the second day numbers have increased considerably. This increase in numbers is shown throughout the period of storage for pies purchased on 28th December (Table 6). In Tables 3, 4, 5 and 7, an increase in numbers with time is not obvious but very high microbial population levels were obtained. Penicillium sp. and Aspergillus niger were the prevalent fungi isolated from all four batches of pies. In Table 4 where the pies were packaged in polythene bags, no fungal growth was obtained until after the sixth day of storage.

Pies from Kingsway Stores

The general pattern of ~~increase~~ increase in microbial numbers with aging is shown in Table 8. Microbial determination of pies purchased in November (Table 7) followed the same trend as some of the pies from G.N.T.C. The most dominant fungal species were again, Penicillium and Aspergillus Niger. A third fungus, a member of the Mucorales and identified as Rhizopus sp. was isolated from all the samples and occurred in large quantities.

Generally most of bacterial colonies were obtained from plates incubated aerobically. This indicates that they are mostly aerobic organisms.

TABLE I

Effect of aging on microbial population levels of meat pies from U.T.C. Stores
(Pies purchased on 4th Sept. 1972)

Age in days	BACTERIA Total Viable Counts per Gram (x 10 ⁶)		FUNGI Total Counts per Gram (x 10 ³)
	Aerobic	Anaerobic	
0	0.0006	0.00005	0.0
2	0.0278	0.00844	0.0
4	296.0	0.0	10.0
6	327.0	0.0944	17.8
8	* -	-	-
10	-	-	-

* - No records were taken

TABLE 2

Effect of aging on microbial population levels of meat pies from U.T.C. Stores
(Pies purchased on 28th Dec. 1972)

Age in Days	BACTERIA Total Viable Counts per Gram (x 10 ⁶)		FUNGI Total Counts per Gram (x 10 ³)
	Aerobic	Anaerobic	
0	0.001	0.0	0.02
2	0.012	0.035	0.02
4	400.0	3.5	19.5
6	600.0	375.0	20.3
8	650.0	70.0	30.2
10	52.0	80.0	18.0

TABLE 3

Effect of aging on microbial population levels of meat pies from GNTC (Ghana House)
(Pies purchased on 28th Sept. 1972)

Age in days	Sample No.	BACTERIA Total Viable Counts per Gram (x 10 ⁶)		FUNGI Total Counts per Gram (x 10 ⁴)
		Aerobic	Anaerobic	
0	01	0.0	0.0	0.0
	2	0.0	0.0	0.0
2	1	0.05	0.0	0.0
	2	2.0	0.0	0.0
4	1	0.7	0.0	0.0
	2	1.4	0.0	0.0
6	1	20.0	50.0	0.0
	2	450.0	60.0	0.0
8	1	50.0	-	2.0
	2	* -	-	0.066
10	1	-	-	-
	2	-	-	-

* - No records were taken

TABLE 4

Effect of aging on microbial population
levels of meat pies from GNPC (Ghana House)
(Pies purchased in October, 1972)

Age in days	Sample No.	BACTERIA Total Viable Counts per Gram (x 10 ⁷)		FUNGI Total Counts per Gram (x 10 ³)
		Aerobic	Anaerobic	
0	1	0.0	0.0	0.01
	2	0.0045	0.008	0.03
2	1	12.5	-	0.2
	2	95.0	-	0.2
4	1	80.0	185.0	0.3
	2	280.0	240.0	0.4
6	1	40.0	10.0	1400.0
	2	5.0	26.0	500.0
8	1	150.0	180.0	30000.0
	2	220.0	260.0	12000.0
10	1	1.8	0.24	18000.0
	2	1.3	0.22	8000.0

TABLE 5

Effect of aging on microbial population levels
of Meat pies from GNPC (Ghana House)
(Pies purchased in November, 1973)

Age in days	BACTERIA Total Viable Counts Per Gram ($\times 10^6$)		FUNGI Total Counts Per Gram
	Aerobic	Anaerobic	
0	0.08	0.07	30
2	350.0	0.0008	10
4	145.0	110.0	10
6	125.0	15.0	32.0×10^3
8	19.75	37.0	140.0×10^3
10	330.0	180.0	*-

*- No record was taken.

TABLE 6

Effect of aging on microbial population levels
of meat pies from GNPC (Ghana House)
(Pies purchased on 28th Dec. 1972)

Age in days	BACTERIA Total Viable Counts Per Gram (10^7)		FUNGI Total Counts Per Gram
	Aerobic	Anaerobic	
0	0.0005	0.0	10
2	0.95	0.004	10
4	120.0	0.003	0.02×10^4
6	95.0	8.75	7.2×10^4
8	10.0	14.0	416.0×10^4
10.	145.0	90.0	$7,200.0 \times 10^4$

TABLE 7

Effect of aging on microbial population levels
of meat pies from Kingsway Stores
(Pies purchased in November, 1972).

Age in days	Sample No.	BACTERIA Total Viable Counts Per Gram ($\times 10^6$)		FUNGI Total Counts Per Gram
		Aerobic	Anaerobic	
0	1	0.0	0.0	0.0
	2	0.0	0.0	0.0
2	1	3.6	0.0	0.0
	2	1,300.0	0.0	0.0
4	1	200.0	0.0	0.0
	2	0.35	0.2	0.0
6	1	90.0	-	1.82×10^3
	2	1,400.0	90.0	10.0
8	1	5.0	5.0	7.6×10^3
	2	12.0	8.0	0.35×10^3
10	1	12.0	0.03	$3,600.0 \times 10^3$
	2	4.5	1.0	4.0×10^3

TABLE 8

Effect of aging on microbial population levels
of meat pies from Kingsway Stores
(Pies purchased on 28th Dec. 1972)

Age in Days	BACTERIA		FUNGI
	Total Viable Count Per Gram		Total Counts Per Gram (x 10 ⁴)
	Aerobic (x 10 ⁶)	Anaerobic	
0	0.0005	0.0	0.02
2	0.0035	0.00008	0.006
4	25.0	-*	0.16
6	22.5	1.5	13.2
8	30.5	2.2	312.0
10	2,000.0	500.0	9,300.0

* - No record was taken.

Test for the presence of Clostridium botulinum

Three hundred and seventy-two (372) pies from the three Stores were examined for the presence of Clostridium botulinum. The characteristics given by Cowan and Steel (1970) were used as a basis to distinguish the bacterial colonies isolated.

Of the colonies examined from blood agar plates none were Clostridia, all being mostly Bacillus spp. Some catalase positive gram positive, Staphylococci and a few non-sporing gram positive, catalase positive rods were also isolated.

Fourteen species of Bacillus were isolated in pure cultures on the basis of morphological characteristics. Most of these were commonly isolated from samples from all three stores.

Tables 9 to 16 show the representative organisms grown from the samples and the effect of heating at 80°C for 20 minutes on the types of colonies. Heating at this temperature kills all vegetative cells and only spores of heat resistant organisms survive. Hence a mixture of both rods and cocci were isolated from tubes that were not heated and heated tubes gave only spore-forming Bacillus species. A few colonies of gram positive catalase positive Staphylococci were isolated from heat treated samples as shown in Table 12.

It was not possible to identify all the Bacillus spp. found because facilities were not available to perform all the tests given in Cowan and Steel (1970). Table 17 shows the few tests performed. All the isolates except one produced acid from glucose. Five isolates produced acid from mannitol and 3 produced acid from arabinose. None of the isolates produced indole from tryptophan, but most hydrolysed starch and only 3 utilized citrate as a sole source of carbon. Ten out of fourteen isolates reduced nitrate to nitrite and most were V-P positive i.e., they produce acetylmethyl carbinol from glucose.

TABLE 9

BACTERIAL TYPES ISOLATED FROM MEAT
CONTENT OF PIES FROM UTC STORES

(Pies purchased on 4th Sept., 1972)

Age in days	Tubes heated at 80°C for 20 minutes			Unheated tubes		
	No. of isolates of		Total No. of Isolates	No. of isolates of		Total No. of Isolates
	Rods Gram + ve Catalase +ve	Cocci Gram + ve Catalase +ve		Rods Gram + ve Catalase +ve	Cocci Gram + ve Catalase +ve	
0	0	0	0	3	0	3
2	6	0	6	4	2	6
4	5	0	5	5	3	8
6	3	0	3	5	1	6

TABLE 10

BACTERIAL TYPES ISOLATED FROM MEAT
CONTENT OF PIES FROM UTC

(Pies purchased on 28th Dec. 1972)

Age in days	Tubes heated at 80°C for 20 mins.			Unheated tubes		
	No. of Isolates of		Total No. of Isolates	No. of Isolates of		Total No. of Isolates
	Gram +ve Catalase +ve Rods	Gram +ve Catalase+ve Cocci		Gram +ve Catalase +ve Rods	Gram +ve Catalase +ve Cocci	
0	3	0	3	3	3	6
2	3	1	4	2	3	5
4	2	0	2	1	2	3
6	2	2	4	3	3	6
8	1	0	1	2	2	4
10	2	0	2	3	1	4

TABLE 11

Bacterial Types isolated from meat
content of pies from G.N.T.C. (Ghana House)
(Pies purchased on 28th Sept., 1972)

Age in days	Sample No.	Tubes heated at 80°D for 20 minutes			Unheated Tubes		Total No. of Isola- tes
		No. of Isolates of		Total No. of Isola- tes	No. of Isolates of		
		Gram + ve Catalase+ve Rods	Gram + ve Catalase+ve Cocci		Gram + ve Catalase+ve Rods	Gram + ve Catalase+ve Cocci	
0	1	6	1	7	6	0	6
	2	2	1	3	3	2	5
2	1	5	0	5	4	0	4
	2	2	2	4	1	1	2
4	1	3	0	3	5	1	6
	2	2	0	2	2	0	2
6	1	9	0	9	8	1	9
	2	2	0	2	1	2	3
8	1	6	0	6	5	0	5
	2	4	3	7	3	1	4
10	1	3	0	3	5	1	6
	2	4	1	5	2	2	4

TABLE 12

Bacterial Types isolated from meat
 content of pies from G.N.T.C. (Ghana House)
 (Pies purchased in October, 1972)

Age in Days	Sample No.	Tubes heated at 80°C for 20 minutes			Unheated Tubes		
		No. of Isolates of		Total No. of Isolates	No. of Isolates of		Total No. of Isolates
		Gram + ve Catalase + ve Rods	Gram + ve Catalase + ve Cocci		Gram + ve Catalase + ve Rods	Gram + ve Cat. + ve Cocci	
0	1	6	0	6	7	0	7
	2	5	1	6	5	0	5
2	1	4	2	6	6	0	6
	2	4	2	6	4	3	7
4	1	1	4	5	1	6	7
	2	3	2	5	2	3	5
6	1	7	0	7	7	4	11
	2	7	1	8	5	5	10
8	1	4	●	4	5	3	8
	2	5	1	6	7	4	11
10	1	-*	-	-	-	-	-
	2	-	-	-	-	-	-

*-No records were taken.

TABLE 13

BACTERIAL TYPES ISOLATED FROM MEAT
CONTENT OF PIES FROM
GNTC (GHANA HOUSE)

(Pies purchased in Nov. 1972)

Age in days	Tubes heated at 80°C for 20 mins.			Unheated tubes		
	No. of Isolates of		Total No. of Isolates	No. of Isolates of		Total No. of Isolates
	Gram +ve Catalase +ve Rods	Gram +ve Catalase +ve Cocci		Gram +ve Catalase +ve Rods	Gram +ve Catalase +ve Cocci	
0	2	0	2	2	1	3
2	1	0	1	2	2	4
4	2	0	2	3	2	5
6	1	0	1	1	2	3
8	1	0	1	1	1	2
10	1	0	1	2	2	4

TABLE 14

BACTERIAL TYPES ISOLATED FROM MEAT
CONTENT OF PIES FROM G.N.T.C.

(Pies purchased on 28th Dec.1972)

Age in days	Tubes heated at 80°C for 20 mins.			Unheated tubes		
	No. of Isolates of		Total No. of Isola- tes	No. of Isolates of		Total No. of Isolates
	Gram +ve Catalase +ve Rods	Gram +ve Catalase +ve Cocci		Gram +ve Catalase +ve Rods	Gram +ve Catalase +ve Cocci	
0	1	1	2	4	1	5
2	1	0	1	2	4	6
4	4	0	4	1	2	3
6	2	0	2	2	3	5
8	3	0	3	4	1	5

TABLE 15

Bacterial Types isolated from meat
content of pies from Kingsway Stores
(Pies purchased on 25th October, 1972)

Age in Days	Sample No.	Tubes heated at 80°C for 20 minutes			Unheated Tubes		
		No. of Isolates of		Total No. of Isolates	No. of Isolates of		Total No. of Isolates
		Gram + ve Catalase + ve Rods	Gram + ve Catalase+ve Cocci		Gram + ve Catalase+ve Rods	Gram + ve Catalase+ve Cocci	
0	1	1	0	1	4	0	4
	2	1	0	1	4	1	5
2	1	2	0	2	2	2	4
	2	4	1	5	3	1	4
4	1	2	0	2	2	3	5
	2	4	0	6	1	2	3
6	1	4	0	4	2	3	5
	2	5	0	5	4	1	5
8	1	3	0	3	3	2	5
	2	3	0	3	3	3	6
10	1	2	0	2	1	4	5
	2	3	0	3	3	2	5

TABLE 16

BACTERIAL TYPES ISOLATED FROM MEAT
CONTENT OF PIES FROM KINGSWAY STORES

(Pies purchased on 28th Dec., 1972)

Age in days	Tubes heated at 80°C for 20 minutes			Unheated tubes		
	No. of isolates of		Total No. of isolates	No. of isolates of		Total No. of isolates
	Gram + ve Catalase + ve Rods	Gram + ve Catalase +ve Cocci		Gram + ve Catalase + ve Rods	Gram + ve Catalase +ve Cocci	
0	1	0	1	2	0	2
2	4	1	5	2	3	5
4	3	0	3	2	1	3
6	3	0	3	1	1	2
8	2	0	2	2	1	3

TABLE 17

CHARACTERISTICS OF BACILLUS SPECIES ISOLATED FROM
MEAT CONTENT OF PIES FROM GNTC., KINGSWAY STORES
AND UTC.

Isolate No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gram Reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Morphological group	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Citrate Utilization	+	-	-	-	-	-	-	+	+	+	-	-	-	-
Starch Hydrolysis	+	-	-	-	+	+	+	+	+	-	+	+	+	+
Glucose (acid)	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Arabinose (acid)	-	-	-	-	-	-	+	+	-	-	-	-	+	-
Mannitol (acid)	+	-	+	+	+	-	-	-	-	+	-	-	+	-
Indole	-	-	-	-	-	-	-	-	-	+	-	-	-	-
V-P	-	+	+	+	+	+	+	+	+	-	-	-	+	+
Nitrate Reduction	-	+	-	+	+	+	+	+	+	-	-	+	+	+

DISCUSSION

The data obtained within the time available are too few to permit any firm general conclusions but some comments may be made.

By the fourth day of storage pies from all three stores have developed large fungal and bacterial populations. Even though no pathogenic organisms were found, pies with such high counts are not good for human consumption because a figure of 10^7 per gram means that a gram of pie contains bacterial debris. The danger of the pies accumulating mycotoxins becomes great as the pies age.

No Clostridium botulinum was found in any of the pies examined but the numerous species of Bacillus isolated from the meat content indicate that the baking temperatures do not kill bacterial spores. The inside of the pie is often moist enough to permit bacterial multiplication and might be sufficiently anaerobic to permit growth of Clostridium botulinum which is not an unduly strict anaerobe (Meyer, 1959) and its growth together with other Bacillus species might be favoured.

Meat pies should advisably not be kept for more than 3 days in stores under conditions they are stored at present as spoilage sets in after this period.

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