Microbial Spoilage of Meat Pies Sold In Jos Metropolis

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Abstract: A total of 450 meat pies were collected from five locations in Jos metropolis. The three components of meat pies; paste, meat filling and potato topping were screened for total plate count, staphylococcal count, streptococcal count and fungal count under two storage temperatures of 4° C for 7days and 37° C for 3 days. A total of 12 organisms belonging to 7 genera were isolated from different components of the meat pies as follows: Bacillus cereus, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus pyogens, Streptococcus viridians, Escherichia coli, Lactobacillus fermentum, Aspergillus oryzae, and Penicillum roqueforti with a relative frequency of 16.9%, 10.2%, 12.6%, 13.5%, 7.6%, 7.0%, 8.2%, 6.3%, 5.0%, 6.1%, 4.2% and 3.4% respectively. Significant difference (P>0.05) was observed in the relative frequency of the microorganism under study. The sample bought from site 'D' had the highest total aerobic count of 5.5 x 10⁶ cfu/g while the samples from the meat pie stand in the University of Jos, had the least aerobic plate count of 3.2 x 10⁵ cfu/g. The fungal count of samples collected from site 'D' was highest while that of sample from the location designated 'A' was the least. The study revealed that the sanitary conditions of the meats pies sold at these locations were below acceptable standard and need an improved production and handling procedures.

I. Introduction

Food is defined as any solid or liquid which when ingested or eaten or consumed and absorbed by the body produces energy, promotes tissue growth and repair or regulate these processes (Pampalon-Roger, 2006). A spoiled food is therefore one which has under gone some damages or some organoleptic changes which makes it unfit for human consumption. It is simply "a food that is unacceptable to a consumer for reasons of smell, taste, appearance, texture or the presence of foreign bodies" (Garbutt, 1997). To some extent; the spoilage of food is subjective being influence by cultural and economic factors as well as by the sensory acuity of the consumer and the intensity of the defect. However, despite variations in expectations, most consumers would agree that gross discoloration, strong off odours and the development of slime constitute spoilage (Gill, 1993).

Meatpie is a popular bakery product consisting of a pastry casing enclosing a meat or vegetable filling which may or may not be capped with mashed potato (Thomas and Masters, 1988).

Meat pies are made from wheat flour of the species Triticum vulgare and contain other ingredients such as fat and oils; potato of the species Solanum tuberosum and meat mostly beef. With the high nutritive content of meat used in making meat pies it is no wonder that meat pie is a highly favourable environment for microbial growth and as a result is subject to rapid spoilage (Gill, 1996)).

Spices and other food condiment are commonly used to garnish food in Nigeria. The spices commonly used in meat pies include curry, thyme, black pepper (piper guineese) and red pepper (Capsicum annum). Apart from adding to the aesthetic quality of meat pies spices have been shown to harbor bacteria and toxigenic fungi, there by acting as a source of contamination of food (Conner, 1993).

There is a great increase in the consumption of meat pies due partially to increasing numbers of working mothers, more school age children obtaining their own meals and refreshment and a highly mobile population (Matz, 1984), since most spoilt meat pie, does, not have the off-odour normally associated with many spoiled foods, it is of public health interest to carry out this study to determine the spoilage characteristics of meat pie at different storage conditions.

Collection of Samples

II. Materials And Methods

The meat pies used for this study were collected randomly from five different locations within Jos metropolis. The locations were designated A,B,C, D and E respectively with codes names as:

- MB
- JG
- BD
- HW

MS

The samples were collected with sterile forceps and put in sealed clean polythene bags and brought to the laboratory for analysis. Ninety (90) samples each were collected from the different sampling sites at different occasions. On each occasion, three meat pies were bought from each of the five locations. One was analysed immediately as fresh sample, One was stored at 4° C for 7 days and one was stored at 37° C for 3 days.

Preparation of Samples

The different components of the meat pie, meat filling, pastry and potato were separated as eptically, using a pair of sterile scissors and forceps. One gram of each component was weighed out using the weighing balance and homogenized in 9ml of 0.1% w/v peptone water in sterile glass pestle and mortar.

Enumeration and isolation of organisms

Three ten-fold serial dilutions $(10^{-1}, 10^{-2}, 10^{-3})$ of each of the meat pie component were made using 0.1% w/v peptone water as diluents. 0.1ml of homogenized component + peptone water was placed in duplicate by pour plate method on

- Nutrient agar for total aerobic plate count
- Blood agar for streptococcal count
- Manitol salt agar for staphyl ococcal count
- Malt extract agar for fungal count

All plates for bacterial count were incubated at 37° C for 48 hrs and those for fungal count were incubated at 25° C for 7 days.

Characterization and identification of isolates

Plates were examined macroscopically and microscopically. Morphological examination such as gram staining reactions and motility tests were carried out according to the method of Bradshaw (1999). Identification of isolates to species level was according to scheme outlined by Bergey's Manual of determinative Bacteriology (Buchanan and Gibbons, 1994).

Colonies on malt extract agar were examined using few drops of lactophanol in cotton blue dye on slides and then covered with cover slip and examined under the microscope for morphological feature of fungi. This method was adopted from Samson <u>et al</u> (1992). All data generated were subjected to statistical analysis using 2-way ANOVA.

III. Results

The results revealed that there were no obvious outward sign of biodeterioration on the meat pies. When the interior of the meat pies were examined it was discovered that they had offensive odours. The significant spoilage odour normally associated with spoilt foods was absent in meat pies stored at 4° C when physically examined. It was also noticed that the meat filling and the potato topping had become sticky.

Microscopic examination of gram stained smears prepared from each meat pie component showed extensive growth of gram positive cocci, gram positive bacilli, a few gram negative rods and some fungi species. The isolated organisms were distributed among five genera of bacteria and two genera of fungi. They included Bacillus, Lactobacillus, Streptococcus, Staphylococcus, Escherichia, Aspergillus and pencillium (Table 1).

Sampling site	Fresh pies	At 37°C	At 4 ^o C
А	Bacillus	Lactobacillus, Bacillus	Bacillus
В	Lactobacillus, Bacillus	Bacillus, Staphylococcus	Lactobacillus
С	Staphylococcus, Bacillus	Streptococcus	Bacillus
		Aspergillus Spp, Penicillum Spp	Staphylococcus
D	Streptococcus, Bacillus	Escherichia coli	Bacillus
		Aspergillus, Penicillum	Aspergillus
E	-	Staphylococcus	Bacillus
		Aspergillus Spp, Bacillus	

Table 1: Microorganisms isolated from meat pies stored at different Temperatures

The results of the total plate counts of fresh meat pies obtained from the five sampling sites revealed that the samples from the site designated `D' had the highest total aerobic count of 5.5×10^6 while sample from the site designated `E' had the least aerobic plate count of 3.2×10^5 (Table 2). The highest viable bacterial counts of stored meat pies were obtained in meat pies stored at 4° C for 7 days (table 3) while the least viable bacterial counts were obtained from fresh meat pies (Table 4).

Bacillus had the highest percentage frequency of occurrence (89.3%), followed by Streptococcus (82%) while E. coli (18%) had the least percentage frequency of occurrence (Table 5).

Sampling site	Total aerobic	Streptococcal	Staphylococcal
	count (Cfu/g)	count (cfu/g	count (cfu/g
А	5.2×10^{6}	$4.7 \text{ x } 10^4$	4.6×10^5
В	3.4×10^6	$1.1 \ge 10^4$	3.0×10^5
С	4.8 x 10 ⁶	2.6 x 10 ⁵	3.6 x 10 ⁵
D	$5.5 \ge 10^6$	4.9 x 10 ⁶	3.4×10^6
Е	3.2×10^5	$4.0 \ge 10^4$	6.2 x 10 ⁴

 Table 2: Total bacterial counts of fresh meat pies obtained from the five sampling sites

Table 3: Viable bacterial counts of meat pies stored at 4^oC for 7 days

Sampling	Component of pies	Total plate	Streptococcal	Staphylococcal
Site		count (cfu/g)	count (cfu/g)	count (cfu/g)
А	Paste	1.6 x 10 ⁸	$7.0 \ge 10^7$	3.0×10^7
	Meat filling	2.7×10^8	$5.0 \ge 10^7$	$1.8 \ge 10^7$
	Potato topping	$1.8 \ge 10^8$	$4.0 \ge 10^7$	3.0×10^7
В	Paste	2.8×10^8	7.3×10^7	$6.0 \ge 10^7$
	Meat filling	5.2 x 10 ⁸	9.0 x 10 ⁷	2.0×10^7
	Potato topping	2.4×10^8	6.2×10^7	$4.0 \ge 10^7$
С	Paste	2.1×10^7	2.2 x 10 ⁶	2.4 x 10 ⁶
	Meat filling	2.7 x 10 ⁹	$1.0 \ge 10^7$	$4.0 \ge 10^6$
	Potato topping	1.1 x 10 ⁹	$1.6 \ge 10^7$	$4.4 \ge 10^7$
D	Paste	1.6 x 10 ⁹	1.3×10^7	2.0×10^7
	Meat filling	2.4 x 10 ⁹	$6.4 \ge 10^7$	2.2×10^7
	Potato topping	2.8 x 10 ⁹	$5.0 \ge 10^7$	2.0×10^7
Е	Paste	3.8×10^7	$4.7 \ge 10^6$	2.0×10^6
	Meat filling	2.4×10^8	8.5×10^7	$6.0 \ge 10^7$
	Potato topping	6.4 x 10 ⁸	9.9 x 10 ⁷	4.2×10^7

Table 4: Viable Bacterial counts of fresh meat pies obtained from the five sampling sites

Sampling Site	Component of pies	Total plate	Streptococcal	Staphylococcal
	Bt-	$\frac{\text{count (cfu/g)}}{2.9 \times 10^6}$	$\frac{\text{count (cfu/g)}}{3.2 \text{ x } 10^5}$	$\frac{\text{count (cfu/g)}}{8.4 \text{ x } 10^6}$
Α	Paste			
	Meat filling	4.8×10^6	4.3×10^5	3.0×10^6
	Potato topping	3.2×10^6	2.0×10^6	5.1×10^5
В	Paste	$5.0 \ge 10^5$	1.7 x 10 ⁶	2.4 x 10 ⁵
	Meat filling	4.1 x 10 ⁵	3.4 x 10 ⁶	$6.2 \ge 10^5$
	Potato topping	$1.2 \ge 10^6$	$1.0 \ge 10^6$	5.7 x 10 ⁵
С	Paste	$4.6 \ge 10^5$	2.1×10^6	$4.0 \ge 10^5$
	Meat filling	4.2×10^{6}	3.0×10^6	6.3×10^5
	Potato topping	3.2×10^6	3.4×10^6	3.2×10^5
D	Paste	$4.1 \ge 10^6$	3.0×10^5	2.0×10^5
	Meat filling	$6.4 \ge 10^6$	$4.6 \ge 10^5$	3.2×10^5
	Potato topping	5.4 x 10 ⁶	3.4×10^5	3.1 x 10 ⁵
Е	Paste	$1.8 \ge 10^5$	2.7×10^4	$1.7 \text{ x } 10^4$
	Meat filling	3.6×10^5	3.4×10^4	2.8×10^4
	Potato topping	2.3 x 10 ⁵	3.1 x 10 ⁴	1.9 x 10 ⁴

 Table 5: Frequency of isolations of organisms from 150 samples of fresh meat pies

Organism	No. of time isolated	Relative frequency
Bacillus	134	89.3%
Escherichia coli	27	18%
Staphylococcus	120	80%
Streptococcus	123	82%
Lactobacillus	108	72%
Aspergillus	45	30%
Penicillium	40	26.7%

IV. Discussion And Conclusion

The results obtained from fresh meat pies indicate that there were considerably high mean aerobic counts which range from 3.2×10^5 to 5.5×10^6 . A similar result of high mean aerobic count was reportedly obtained by Thomas and Masters (1988) in a work carried out on luncheon meat to determine the succession of spoilage micro flora at two different storage temperatures. Other works by Nigerian researchers, such as that done on `fura by Umoh <u>et al</u> (1989) reported aerobic plate count of $5.6 \times 10^5 - 2.7 \times 10^6$ cfu/ml.

Since aerobic counts give a fair picture of the bacterial load of foods and the sanitary conditions of the production and handling (Jay, 1996), the results obtained in this work indicate that sanitation in the production of meat pies from the area sampled need to be improved in order to obtain low count that might not be significantly detrimental to public health.

Apart from Bacillus species that are isolated in large numbers, there are other bacterial species such as Staphylococcus, Streptococcus, Lactobacillus, Escherichia coli. According to Kraft, (1992) Escherichia coli is a normal inhabitant of the gut but the enteropathogenic strains has been found to cause disease which manifest itself in form of diarrhea sometimes with or without fever.

The presence of E. coli on meat pies is probably due to recontamination from water. This is because E. coli is an indicator of sewage pollution of water and unhygienic methods of preparation. (Hobbs & Roberts, 1998).

It has been shown that the presence of E. coli to a significant amount in food is of public health importance, the fact that the present result did not show any significant number of E. coli is of public health interest. Apergillus and Penicillium are fungi species known to cause food spoilage. Their presence on the meat pies under study maybe due to contamination from air because spores of these organisms are easily air borne. Statistically, it was found that the microbial load of sample at 37⁰C storage has significant (P>0.01) difference from samples stored at 4°C. This maybe due to the unfriendly environment created for this organism by the refrigeration temperature of 4^oC as opposed to 37^o C which supports most mesophiles.

Conclusion V.

The microbiological quality of the meat pies currently under study is below acceptable standard. This is because the total aerobic count was as high as 1.2×10^7 in contrast to the accepted standard maximum of 1.8×10^7 10^3 cfu/g (WHO, 2006).

VI. Recommendation

The fact that the bacteriological quality of meat pies is mostly dependent on sanitary practices during preparation, the following recommendations are necessary:

- 1. It is important to sanitize the water to be used for mixing the flour as well as washing the utensils
- 2. Ready to eat meat pies should be kept or packed aseptically into sanitized containers before retailing
- 3. Persons suffering from communicable diseases and carriers should not be allowed to handle foods which are meant for human consumption.

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