Vet.Med.J.,Giza. Vol.45,No.3.(1997):361-368.

MICROBIOLOGICAL EVALUATION OF PIZZA WITH SPECIAL CONCERN TO FOOD-BORNE PATHOGENS

A. M. ABOUZEID and M. M. T. EMARA

Dept. of Food Hygiene and Control, Fac. of Vet. Med., Cairo Univ.

SUMMARY

Thirty samples of pizza randomly collected from different restaurants and pastries in Cairo and Giza governorates were investigated to evaluate their microbiological quality. The obtained results indicated that the mean Aerobic plate, Enterobacteriaceae, Aerobic sporeformers, Bacillus cereus, Staphylococci, Staph. aureus, Enterococci, Pseudomonas, Aeromonas, Coliforms, Enteropathogenic Escherichia coli (EPEC) and Yeast & Mold counts per gram were $1.5x10^5$, $2x10^4$, $6.1x10^2$, $2.1x10^2$, $2.9x10^3$, $6.1x10^2$ $3.2x10^4$, $2.4x10^3$, $1.2x10^2$, $9.8x10^2$, $1x10^2$ and $6.5x10^3$ respectively.

Pizza was found to be contaminated with newly emerging food-borne pathogens such as Aeromonas hydrophila, as well as Salmonella species. Escherichia coli, Enterobacter agglomerans, E. coloacae, Citrobacter diversus, C. freundii, Klebsiella ozaenae and K. rhinoschleromata were isolated in percentage ranged from 3.3 to 20%. However, neither Listeria monocytogenes nor Yersinia enterocolitica could be isolated from the examined samples.

Different genera of molds including; Aspergillus, Penicillum, Cladosporium, Alternaria, Mucor, Fusarium and Scopulariopsis were isoalted from the examined samples in percentages ranged from 0.94 to 31.13%.

The public health hazards of the isolated organisms, as well as suggested control measures were fully discussed in order to improve the quality of pizza.

INTRODUCTION

Food of animal origin is considered as the main reservoir for a variety of zoonotic diseases rather than many diseases transmitted from man to man through the contamination of food during preparation, distribution and storage (Hassan, 1986 and Yassien & El-Essawy, 1990).

Pizza is considered one of the most popular products which has been recently introduced to the Egyptian markets and does not follow any standard operating procedures. Its price and production depends on the individual standard controlled by private producers (Shaltout et al., 1992).

Pizza may be exposed to contamination with

different types of microorganisms from the time of preparation till consumption, probably due to the absence of good hygienic practice and personal cleanliness specially in small restaurants. Furthermore, the ingredients used in manufacturing as; eggs, milk, meat, meat products, poultry and poultry products may be among the major sources of contamination if they are producted under bad hygienic conditions.

Food of animal origin may be responsible for certain cases of illness among consumers specially when organisms of public health hazards existed. Therefore, strict hygienic supervision in all steps of production, as well as the use of sound ingredients, clean equipment, hygienic stores and healthy handlers are essential to produce wholesome food (Gork, 1985).

Few attempts have been made to evaluate the extent of contamination of pizza, so this study has been caried out to throw light on the microbial quality of pizza in Cairo and Giza governorates.

MATERIALS AND METHODS

1- Collection of the samples:

Thirty samples of pizza were randomly collected from different restaurants and pastries in Cairo and Giza governorates. The collected samples were transferred in sterile containers to the laboratory with a minimum of delay to be microbiologically examined.

2- Preparation of the samples:

Samples were prepared according to the technique recommended by ICMSF (1978). Ten grams from each sample were weighed into sterile polyethylene bag, then 90 ml of 0.1% strength Ringer solution were added and the bag was blended in the stomacher for about one minute. The bag was then thoroughly shaken just before examination.

3- Enumeration of different types of organisms:

3.1- Aerobic plate count (APC): using the drop technique recommended by ICMSF (1978).

3.2- Enterobacteriaceae count: The technique adopted by Gork (1976) was applied using the drop plate method on Violet red bile glucose agar.

3.3- Aerobic sporeformers count: The technique recommended by Harrigan and McCance (1976) was adopted..

3.4- Bacillus cereus count: The method described by Holbrook & Anderson (1980) was followed using Bacillus cereus selective agar medium.

3.5- Staphylococci count: Baird-Parker medium was used, and the suspected colonies were identified according to Balley & Scott (1982).

3.6- Enterococci count: Enterococci selective differential media (ESD) developed by Efthymiou & Joseph (1974) was performed.

3.7 Pseudomonas and Aeromonas counts: Using GSP medium and the technique described by Kielwein (1969). Suspected Aeromonas

colonies were isolated, purified and identified according to Palumbo et al. (1992).

3.8- Coliforms: The Most Probable Number (MPN) technique described by APHA (1985) was used, and the suspected colonies were isolated, purified and identified according to Krieg & Holt (1984).

3.9- Enteropathogenic Escherichia coli count: The recommended method of ICMSF (1978) was applied.

3.10- Yeast and Mold counts: The method recommended by ICMSF (1978) was performed using Sabouraud's dextrose agar with Chloramphenicol. The isolated Molds were identified according to Domasch et al. (1980) and Samson et al. (1981).

4- Isolation and identification of specific food-borne pathogens:

4.1- salmonella species: The technique adopted by Flowers et al. (1992) was used by pre-enrichment in trypticase Soya broth with yeast extract (TSBYE) followed by selective enrichment in Rappaport medium, and selective plating on both S. S. and XLD agar. Suspected colonies were purified and identified according to Krieg & Holt (1984).

4.2- Yersinia enterocolitica: The technique described by Donald & George (1992) was used by selective enrichment in modified Rappaport broth before selective plating onto CIN agar. The suspected colonies were purified and identified according to AOAC (1984).

4.3- Listeria species: The prepared samples were plated onto modified Oxford agar (MOX) following selective enrichment in Fraser broth as the technique recommended by APHA (1992). Suspected purified colonies of Listeriea species showed positive Gram stain and catalase test were identified as described by Lovett (1987)

RESULTS AND DISCUSSION.

Resutls recorded in table (1) revealed that the aerobic plate count ranged from $2x10^2$ to $7.2x10^5$ with a mean value $1.5x10^4 \pm 0.6x10^4$, these results were nearly similar to those reported by Yassien & El-Essawy (1990).

Table (1) indicated that the mean Enterobacteriaceae, Enterococci, Coliforms and EPEC counts were $2x10^4 \pm 0.3x10^3$, $3.2x10^4 \pm 0.4x10^3$, $9.8x10^2 \pm 0.6x10^2$ and $1x10^2 \pm 0.2x10^1$ respectively. Yassien & El-Essawy (1990); Sallam et al. (1991) and Idris & Ibrahim (1995) obtained similar results.

Aerobic sporeformers and Bacillus cereus were present with mean values of $6.1x10^2 \pm 0.4x10^2$ and $2.1x10^2 \pm 0.1x\ 10^2$ respectively, which were in agreement with those reported by Janewyatt & Guy (1981) and Sallam et al. (1991) . Food contaminated with aerobic sporeformers and stored for long period at improper temperature may be able to cause illness to the consumers. The ubiquitous nature of B. cereus and its role in food poisoning are well documented by Trundle et al

(1979).

From the results recorded in table (1), it is clear that the Staphylococci count ranged from $1x10^2$ to $1.2x10^4$ with a mean value of $2.9x10^3 \pm 0.8x10^2$ while the mean Staph. aureus count was $6.1x10^2 \pm 0.4x10^2$ with a minimum of $1x10^2$ and a maximum of $6.4x10^3$, nearly the same results were recorded by Idris & Ibrahim (1995).

Pseudomonas and Aeromonas species could isolated as obvious from results reported in (1) with values ranged from $1x10^2$ to $3x10^4$, $1x10^2$ to $3x10^3$, and mean values of $2.4x10^3$ $0.8x10^2$ and $1.2x10^2$ and $1.2x10^2 \pm 0.2$ respectively.

Table (1): Statistical analytical results of microbiological counts of the examined pizza samples

Counts	Minimum	Maximum	Mean	ESM ±
APC	$2.0x10^2$	$7.2x10^5$	$1.5x10^5$	$0.6x10^4$
Enterobacteriaceae	$1.0x10^2$	$8.0x10^2$	$2.0x10^4$	$0.3x10^3$
Aerobic sporeformers	$1.0x10^2$	$7.2x10^4$	$6.1x10^2$	$0.4x10^2$
Bacillus cereus	$1.0x10^2$	$1.7x10^3$	$2.1x10^2$	$0.1x10^2$
Staphylococci	$1.0x10^2$	$1.2x10^4$	$2.9x10^3$	$0.8x10^2$
Staph. aureus	$1.0x10^2$	$6.4x10^3$	$6.1x10^2$	$0.4x10^2$
Enterococci	$1.0x10^2$	$9.2x10^5$	$3.2x10^4$	$0.4x10^3$
Pseudomonas	$1.0x10^2$	$3.0x10^4$	$2.4x10^3$	$0.8x10^2$
Aeromonas	$1.0x10^2$	$3.0x10^3$	$1.2x10^2$	$0.2x10^2$
Coliforms	$2.3x10^2$	$4.3x10^2$	$9.8x10^2$	$0.6x10^2$
EPEC	$2.3x10^2$	$2.4x10^3$	$1.0x10^2$	$0.2x10^1$
Yeast and Mold	$1.0x10^2$	$1.3x10^4$	$6.5x10^3$	$0.1x10^3$

ESM ± = Standard Error of the Mean

Table(2) : Incidence of isolated coliforms in the examined pizza samples

Coliforms	No.	% *
Escherichia coli	6	20.0
Enterobacter agglomerans	3	10.0
E. coloacae	4	13.3
Citrobacter diversus	2	6.60
C. freundii	1	3.30
Klebsiella ozaenae	1	3.30
K. rhinoschleromata	3	10.0

* Percentage was calculated to the total samples

Table(3) : Incidence of Listeria, Salmonella and Aeromonas species isolated from the examined pizza samples

Isolates	No.	% *
Listeria species	3	10.00
L. welshimeri	2	06.67
L. ivanovi	1	03.33
Salmonella species	4	13.33
S. enteritidis	3	10.00
S. rubidae	1	03.33
Aeromonas species	6	20.00
A. caviae	3	10.00
A. hinshawii	2	06.67
A. hydrophila	1	03.33

* Percentage was calculated to the total samples

Table(4) : Incidence of isolated Molds in the examined pizza samples

Isolates	No.	% *
1- Aspergillus	60	56.60
A. flavus	33	31.13
A. niger	25	23.58
A. fumigatus	2	01.89
2- Penicillum	18	16.98
3- Cladosporium species	19	17.92
C. herbarium	10	09.43
C. resinae	9	08.49
4- Alternaria	3	02.38
5- Mucor	1	00.94
6- Fusarium	1	00.94
7- Scopulariopsis	1	00.94
8- Unidentified	3	02.83

* Percentage was calculated to the total isolates (106)

Although Aeromonas group was detected in 6 samples (20%), only one isolate was identified as A. hydrophila (3.33%), the other isolates were identified as A. caviae (10%) and a. hinshawii (6.67%) (table 3). The obtained results are closely similar to those reported by Palumbo et al (1985).

Table (2) revealed that Escherichia coli, Enterobacter agglomerans, E. coloacae, Citrobacter diversus, C. freundii, Klebsiella ozaenae, K. rhinoschleromata were isolated in percentage of 20, 10, 13.3, 6.6, 3.3, 3.3 and 10% respectively.

The coliforms seem to be implicated in food illness, E. coli induces severe diarrhea in infants, cystitis, pyelonephritis and peritonitis as well as food poisoning among consumers (Park et al., 1974 and Sinell et al., 1989). Klebsiella species were incriminated in upper respiratory tract infection (Banwart, 1979). In addition, Enterobacter species have been implicated in acute and chronic diarrheal diseases (Twedt & Boutain, 1979).

Results tabulated in table (3) revelaed that Listeria monocytogenes could not be isolated from any of the examined samples. Other Listeria species including L. welshimeri and L. ivanovi were isolated in percentages of 6.67 and 3.33% respectively. However, none of the Yersinia species could be isolated.

Salmonella enteritidis and S. rubidae could be isolated from pizza in a percentage of 10% and 3.33% respectively (table 3). Salmonella is one of the most important microorganisms which is incriminated in food poisoning (Fraizer Westhoff, 1988).

The presence of indicator organisms in pizza frequently reliable indication of faul preparation, handing and sanitation. Moreov presence of Staph. aureus reflects the degree contamination of ingredients added after cookin (Varnam & Evans,1991). Staph. aureus is usuall associated with food poisoning and produce thermostable enterotoxin resulting in sever mainfestations (Sankaran & Leela 1983) and Olfa 1983).

Inspection of the results in table (1) indicated that the mean value of Yeast and Mold count was $6.5x10^3 \pm 0.1x10^3$. Most of the isolated molds are very important from the public health point of view. Aspergillus. Penicillum and Fusarium species which can produce mycotoxin (Kiemeir, 1980) could be isolated from the examined samples in percentages of 56.6, 16.98 and 0.94% respectively. Cladosporium which is responsible for the development of the black coloration on meat preserved at low temperatures (Lowey, 1980 and Mansour, 1986) was isolated in a percentage of 17.92%. Alternaria, Mucor and scopulariopsis could be isolated from the examined samples in percentages of 2.83, 0.94 and 0.94% respectively (table 4). The incidence of isolated molds is similar to that recorded by Saudi & Mansour (1990).

In conclusion, in order to obtain a finished product of good keeping quality and safe for human consumption; strict hygienic measures should be adopted in all steps of preparation till

consumption. These measures require the use of fresh raw ingredients of good sources, clean equipment and adequate storage conditions, as well as personal hygiene and educational training programs for employers engaged in food processing and handling .

REFERENCES

American public Health Association "APHA" (1985): Standard Methods for the Examination of Dairy Products. 15 th Ed. Washington, DC, USA.

American public Health Association "APHA" (1992): Compendium of Methods for the Microbiological Examination of Foods, 3 rd Ed. Washington, DC., USA.

Association of Official Analytical Chemistry "AOAC" (1984): Bacteriological Analytical Manual. 6th Ed., FDA. USA.

Balley, W. R. and Scott, E. G. (1982): "Diagnostic Microbiology". A textbook for the isolation and identification of pathogenic microorganisms. 6th Ed., Mosbay company Saint Louis.

Banwart, G. J. (1979): Basci Food Microbiology, AVI publishing company. INC. West port. Connecticut.

Domasch, K. H.; Gams, W. and Anderson, T. H. (1980): Compendium of Soil Fungi. Academic press, London.

Donald, A. S. and George, W. (1992): Yersinia. Chapter 27. Compendium of Methods for Microbiological Examination of Food. Vanderzant, C and Splittoesser, D (Eds). 3 rd Ed. American Public Health Association. Washington, DC, USA.

Efthymiou, C. J. and Joseph, S. W. (1974): Development of selective Enterococci medium based on manganese deficiency, Sod. azide and alkaline pH. Appl. Microbiol, 28: 411-416.

Flowers, R. S.; Jean-Yves D'Aoust; Anderwa, W. H. and Bailey, J. S. (1992):Salmonella. Chapter 25, Compendium of Methods for Microbiological Examination of Food. Vanderzant, C and Splittoesser, D (Eds), 3rd Ed. American Public Health Association, Washington , DC, USA.

Fraizer, W. C. and Westhoff, D. C. (1988): Food Microbiology . 4th Ed. McCeraw-Hill, book Company, USA.

Gork, F. P. (1976): Uber die Ursachen von qualitatsmangeln bei tiefgefroren fertiggerichten auf Fleischbasis in der fluggast Verpflegung. D. Ing. Diss, TU. Berlin.

Gork, F. P. (1985): Personal hygiene, a basic requirement for hygiene production. LSG safe food in airline catering. Feb 13/14 th, 1985, Frankfurt, Germany.

Holbrook, R. and Anderson, J. M. (1980): An improved selective and diagnostic medium for the isolation and enumeration of Bacillus cereus in food. Canadian J. of microbiology, 26: 753-759.

Harrigan, W. F. and McCance, M. E. (1976): Laboratory Methods in Food and Dairy Microbiology. Academic press. London, New York and San Francisco.

Hassan, A. (1986): Microbiological quality of ready to eat meat meat in air catering. M. V. Sc. Fac. of Vet. Med. Cairo Univ.

Idris, A. M. and Ibrahim, M. Hematt (1995): Microbial profile of ready to eat pizza. Alex. J. Vet. Sci., 11 (3) 1st Scientific conference.

International Commitee of Microbiological Specifications for Food "ICMSF" (1978): Microorganisms in Foods. I Univ. of Toronto, Canada.

Janewyatt, C. and Guy, V. H. (1981): Incidence of growth of B. cereus in retail pumpkin pies. J. of Food Protec. 44 (6): 422-424.

Kielwein, G. (1969): Ein Nährboden zur selective Züchtung von Pseudomonaden und Aeromonaden - Arch. f. Lebensmittelhyg, 20: 131-133.

Kiemeir, F. (1980): Mykotoxine in Milch und Milchprodukten. J., Mykotoxine in Lebensmitteln. Verlag Fischer . Stuttgart.

Krieg, N. R. and Holt, J. G. (1984): Bergey's Manual of Systemic Bacteriology, vol. 1. Williams and Willkins. Baltimore, London.

Lovett, J. (1987): Listeria isolation pp. 2901-2909 in FDA Bacteriological Analytical Manual, supplemented to 6th Ed. Association of Officical Analytical Chemistry, Arlington, VA, USA.

Lowey, P. D. (1980): Black spot. 21 st. Meat Res. Conf. Hamilton, No. 748, 47 Meat Ind. Res. New Zealand.

Mansour, N. K. (1986): Zum Vorkommen von Schimmelpilzen der Gattung Cladosporium linkex fries auf schaffleisch. Vet. Med. Diss. München.

Olfat, M. S. (1988): Microbiological studies on some bakery products with special reference to spoilaage and food poisoning microorganisms. M. V. sc. thesis, Microbiology, Fac. of Agric. Cairo, Univ.

Palumbo, S. A.; Carlos, A. and Gerard, S. Jr. (1992): Aeromonas hydrophila group. Chapter 30. Compendium of Methods for Microbiological examination of Food. Vanderzant, C and Splittoesser, D. (Eds). 3rd Ed. American Public Health Association, Washington, DC., USA.

Palumbo, S. A.; Flicisisma, M.; Williams, A. C.; Buchanam, R. L. and Thayer, D. W. (1985): Quantitative delection of Aeromonas hydrophila. Appll. Environ. Microbiol, 50: 1027-1030.

Park, C. E.; Tood, E. C. D.; Purvis, V. and Laidey, R. (1974): Diseases transmitted by cheese. Lancet 1: 27.

Sallam, S. S.; El-sherif, Amal M. and Yassien, N. A. (1 Bacteriological quality of meals from avitation k Assiut Vet. Med. J. 24 (8).

Samson, R. A.; Hoeksta, E. S. and Van orrschot, C. A (1981): Introduction to Food Fungi. Centraalbureau Schimmelultures, Baarn, Holland.

Sankaran, R. and Leela,, R. K. (1983): Prevalenc enterotoxegnic Staphylococci in bakery product Food Protec, 46, 2: 95-97.

Saudi, A. M. and Mansour, N. K. (1990): Fun contamination of ready to eat meals in airline cateri Fleischwirtsch. 70 (5): 563-564.

Shaltout, O.; Zeina, H. M. and Yossef, M. M. (199 Chemical and nutritional evaluation of pizza on th Egyptian markets. Proceeding of Alex. conf. of Foo Science and Technology, pp. 77-90.

Sinell, H. J.; Wigner, J.; Klingbeil, H.; Mall, A.; Heitmann M. and Hildebrandt, G. (1989): Hygiene monitoring o deep frozen pizza. Archiv für Lebensmitteln- Hygiene 39(6): 151-155.

Trundle, P. c. P.; Karmer, J. M.; Jorgensen, K.; Gilbert, R. J. & Melling, J. (1979): Properties and production characteristics of vometing, diarrheal with virology and immunology, 2 ND Ed. MIR., Moscow.

Twedt, R. M. and Boutain, B. K. (1979): Potential public health singificance of non Escherichia, Coliforms in food. J. Food Protec. 42 (2): 101-103.

Varnam, A. H. and Evans, M. G. (1991): Food Pathogens. An illustrated text. Wolf publishing Book house, 1-26, London, England.

Yassien, N. A. and El-Essawy, H. A. (1990): Bacterial quality of ready to eat meals in air catering . Vet. Med. J. Giza, 38 (1): 39-45.