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BEHAVIOR OF PSEUDOMONAS AERUGINOSA DURING MANUFACTURE AND STORAGE OF DAMIETTA CHEESE AND MINCED MEAT

(With 1 table & 5 Fig.)

By

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دراسة سلوك ميكروب السيدوموناس ايروجينوزا أثناء تصنيع وتخزين الجبن الدمياطى واللحم المفروم

نجـــاح سعـــد ، شوكـــت فتحـــى

يعتبر ميكروب Pseudomonas aeruginosa أحد الميكروبات المحبة للبرودة والتي تلعب دوراً خطيراً في فساد الاغذية مؤدية الي خسارة اقتصادية كبيرة بالاضافة الي خطورة هذا الميكروب علي صحة الانسان لما يسببه من تسمم غذائي نتيجة تناول أغذية ملوثة بهذا الميكروب. لذلك تم تصنيع جبن دمياطى مع اضافة الملح بنسب ٥٪ ، ١٠٪. وقد تم حقن اللبن بعد بسترته بميكروب السيدوماناس ايروجينوزا قبل اضافة الملح والمنفحة وتم تخزين الجبن في الشرش عند درجة حرارة الغرفة العادية، ثم اخذت عينات من الجبن مباشرة بعد التصنيع ثم اسبوعياً لمدة ٦ اسابيع. وتبين من الفحص ان الميكروب ينمو في المراحل الاولي من التصنيع، ثم بدأ عدد ميكروب السيدوموناس ايروجينوزا يتناقص اثناء فترة التخزين حيث لم يمكن اكتشافه مع نهاية الاسبوع الثالث في الجبن المعد من لبن مضاف اليه ملح بنسبة ٥٪ وفي نهاية الاسبوع السادس في الجبن المعد من لبن مضاف اليه ملح بنسبة ١٠٪. كذلك تناقص الرقم الهيدروجيني للجبن أثناء فترة التخزين ، بينما تناقصت نسبة الرطوبة وتزايدت نسبة الملح. وكذلك تم تصنيع لحم مفروم حقن بميكروب السيدوموناس ايروجينوزا وقسم الي ثلاثة أجزاء. وتم تخزين جزئين ببصل وبدون بصل في درجة حرارة الثلاجة، أما الجزء الثالث فتم تخزينه في الديب فريزر. وقد وجد أن الميكروب قد تناقص في العد أثناء التخزين الا انه ظل متواجد في اللحم المفروم المضاف اليه بصل والغير مضاف اليه بصل والمخزون في الثلاجة لمدة ١٢ ، ٩٠ أيام علي التوالي ، ولمدة ٤٧ يوم في اللحم المفروم المخزن في الديب فريزر. وقد ناقش الباحثان الخطوات الصحية التي يجب اتباعها عند تصنيع وتداول الاغذية للحفاظ علي صحة المستهلك.

SUMMARY

Two portions of laboratory pasteurized milk artifically contaminated with approx. 10^6 pseudomonas aerugionsa/ml were used to manufacure Damietta cheese after addition of 5% and 10% sodium chloride and rennet. Cheese samples were stored in their whey at room temperature (30 $\pm$ 1 C) and examined periodically for Ps. aerugionsa count, pH value, moisture and salt contents. Pseudomonas aerugionsa increased in numbers during the initial stage of manufacture of both types of cheeses. However, the organism was not detected after 3 and 6 weeks in these cheeses, respectively. Progressive reduction in Ps. aerugionsa count in cheeses was associated with a significant drop in pH and moisture content, while salt content increased during storage period.

Also three samples of prepared minced meat were inoculated with approx. 10^6 Ps. aerugionsa/g. Two samples, one with added 5% onion and the other one without onion were stored at chilling temperature (4$\pm$ 1 C), while the third one without onion was stored at deep freezing temperature (24 $\pm$ 1 C), and tested periodically for Ps. aerugionsa count and pH value. The organism decreased gradually in numbers but can not be eleminated from minced meat. However the organism was detected after 9 and 12 days in minced meat without and with 5% onion at chilling temperature, respectively untill decomposition occur, and after 47 days in minced meat stored at deep freeezing temperture. There was a gradual decrease in pH value followed by gradual increase in three types of minced meat. The public health significance of these findings are discussed.

INTRODUCTION

Pseudomonas aeruginosa is wide spread in nature and is a soil and water pollutants. Its presence in food items could also be taken as indication of faecal contamination, as it is present in the intestinal tract of animals and human beings (Hoadley and McCoy, 1968 and Bergan, 1975).

Pseudomonas aeruginosa is now recognized by public health authorities and veterinarian ecologists as it plays an important role in

causing many infections in man and anmimals such as otitis, meningoencephalitis, pneumonia, pyelitis, eye infections, septicaemia and skin infections (Clement and Millard, 1953; Winso, 1957; Burlina, 1962 and Chernosky and Dukes, 1963).

Pseudomonas aeruginosa was reported as a causative organism of severe forms of acute gastroenteritis (SUTTER et al., 1966 and PERERRA et al., 1977). Several cases of food poisoning have been reported in Tanta and Assiut city due to consumption of dairy products (Abdel-Aziz, 1979 and Ahmed, 1980). Moreover, the organism is responsible for several cases of mastitis and remain in the udder for a number of years (Howell, 1972). The study conducted by Ahmed et al. (1989) reported several cases of food poisoning outbreaks due to consumption of cheese contaminated with Ps. aeruginosa. Furthermore, DAVIS and BABEL (1954) proved that the organism was responsible for slime formation on cottage cheese. LUKASOVA and MRAZ (1986) found that 3% NaCl reduced the growth of Ps. aeruginosa. Also lactic acid fermentation decrease the organism counts to degree dependent on the environmental acidity and freezing at -18 C reduced Ps. aeruginosa counts, but did not result in complete destruction of the organism.

Raw minced meat is a good medium for the rapid growth of Ps. aeruginosa. It has been mentioned that this organism has the ability of causing spoilage of meat held under chilling condition and led to several outbreaks of food poisoning (PERERRA et al., 1977). YOUSSEF et al. (1984) found Ps. aeruginosa in 1.7% of examined minced meat samples.

There are little available literatures which discuss the behavior of Ps. aeruginosa in some food items, thus, this study was planned to determine the behavior of the organism during manufacture and storage of Damietta cheese and minced meat.

MATERIAL AND METHODS

Strain of Ps. aeruginosa used in this study was isolated and well identified from kareish cheese in Food Control Dept. Fac. of Vet. Med. Assiut Univ.

A culture of Ps. aeruginosa was prepared using cetrimide broth as described by LOWBURY (1951).

Manufacture of Cheese :

Raw milk from the dairy farm of Faculty of Agriculture, Assiut

Univ. was pasteurized at 63 °C for 30 min. Broth culture of Pseudomonas aeruginosa was added to the warmed (40 °c) pasteurized milk to yeild approx. 10^6 cells/ml. A sample was taken after inoculation to determine pH value and the initial Ps. aeruginosa count. The inoculated pasteurized milk was divided into two portions which were salted by addition of sodium chloride to give concentration of 5% and 10%. The procedure described by FAHMY and SHARARA (1950) was used to manufacture Damietta cheese. Two control blocks of cheese were prepared from pasteurized milk. Samples from the curd and finished cheese were examined for Ps. aeurginosa counts and pH values. Finished cheeses were examined for moisture and salt contents. Cheeses with their controls were stored at room temperature (30 ± 1 °C) and tested wekly for Ps. aeruginosa count, pH value, moisture and salt contents.

Preparation of minced meat:

Minced meat was prepared from red meat. Enough of the broth culture of Ps. aeruginosa was added to minced meat to give 10^6 organism/g. The inoculated minced meat was divided into three equal samples. Onion was added to a sample at a level of 5% and the three samples were put in presterilized polyethylene containers. Two samples with 5% onion and without onion were kept at chilling temp. (4 + 1 °C) and tested daily for Ps. aeruginosa count and pH values. The third sample was stored at deep freezing temp. (-24 ± 1 °C). The frozen minced meat was examined after 24h, 96h, then weekly up to 6 weeks for Ps. aeruginosa count and ph values. Three control samples were prepared from minced meat.

Preparation of samples for examination:

Cheese samples were prepared for testing according to Standard Method (MARTH, 1978). In case of minced meat, 10 g of each sample were weighed aseptically into a sterile blender jar and 90 ml of peptone water were added. The sample was homogenized at low speed (2000 r.p.m.) for 2 minutes. Subsequent '0 fold serial dilutions of the homogenate were prepared (ICMSF, 1978).

Enumeration of Pseudomonas aerugionsa:

Surface plating technique for each sample and its dilutions was done using Cetrimide agar plates as described by LOWBURY (1951). The plates were incubated at 42C for 48 h, and typical colonies of those formed by Ps. aeruginosa were counted. Confirmatory tests were done on each isolate thought to be Ps. aeruginosa as described by Chruickshank et al. (1975).

Measurement of pH, moisture and salt:

The pH of cheese and minced meat was determined using a pH meter (an Orion model 701) equipped with standard electrode. Moisture content of cheese was subjected according to Standard methods (MARTH, 1978). The technique of ATHERTON and NEWLANDER (1977) was used to determine NaCl in the cheese.

RESULTS

The obtained results are presented in Table 1 and Fig. 1,2,3,4 and 5.

DISCUSSION

The data presented in Table (1) emphasize that there was a gradual increase in salt contents of cheese during storage and a decrease in moisture contents occurred during this period. These findings were noted by SALEEM et al. (1978) and MOUSTAFA et al. (988)

Numbers of Ps. aeruginosa increased during manufacture of cheese prepared from milk with added 5% salt (Fig. 1) with a maximum population (10^8 cells/g) by the end of cheese making, and reduced in numbers during storage until they become undetectable by direct plating by the end of third week. The pH value after manufacture was 6 and decreased gradually during storage. A low pH value of 4.2 was obtained by the end of the third week.

The corresponding results obtained from cheese prepared from milk with added 10% salt (Fig. 2), Ps. aeruginosa achieved its maximum population (10^8 cells/g) by the end of cheese making. The organism reduced in numbers during storage and could not be detected from cheese by the end of sixth week. The pH of cheese after manufacture was 5.8 and decreased during storage to 4 at the end of sixth week.

Results in this study indicated that, if present in milk, Ps. aeruginosa can grow during the initial stage of manufacture of Damietta cheese until the pH of cheese is reduced to 4.2 or below. The obtained results are in agreement with the conclusion of LUKASOVA and MRAZ (1986) that the failure of Ps. aeruginosa to grow during storage of cheese apparently resulted from lactic acid fermentation and the lowering of pH. Pseudomonas aeruginosa could be detected in Damietta cheese by AHMED (1980), SAAD (1983) and KORASHY (1992). The results proved that pH is the

principle factor which govered the surviral of Ps. aeruginosa in Damietta cheese, while sodium chloride has little or no effect.

Concerning the results obtained from minced meat, Ps. aeruginosa decreased gradually in count in minced meat without onion during chilling until meat decomposed by the end of 9th day (Fig. 3). A low pH value of 5.6 during storage at the end of 6th day, then the pH increased till 7.2 by the end of 9th day. Addition of 5% onion to minced meat previously infected with Ps. aeruginosa increased the keeping quality till the 12th day during chilling (Fig. 4). Results in Fig. (5) showed that Ps. aeruginosa decreased in number from 10^6/g to 1 x 10^3 /g at deep freezing by the end of the 47th day. The pH value of minced meat after preparation was 6.9 and decreased to 5.5 by the end of 12th day, then increased to 6.8 by the end of storage period. Pseudomonas aerugionosa can flourish during extended periods of storage in cold temperature (RICHTER, 1981).

These findings indicate that numbers of Ps. aeruginosa were reduced during chilling and deep freezing but was not completely eleminated from minced meat. These results are in agreement with the results obtained by LUKASOVA and MRAZ (1986).

These findings emphasize the tenacity of Ps. aeruginosa during storage even in unfavourable conditions (e.g. presence of high salt content and low temperature). Consequently, one can not depend on the environment of food products to promptly inactivate the organism during storage. Thus, to ensure Ps. aeruginosa free food products, the organism must be kept out of any product during preparation and subsequent handling of the product. Use of adequate hygienic practices are needed to accomplish this goal.

REFERENCES

Abdel-Aziz, A.F. (1979): Fourth Annual Report on Causative Agents of food Poisoning, Project No. 14-75, C-1002.

Ahmed, S.H. (1980): Studies on acute gastrointestinal infections in Assiut Governorate. Ph. D. Thesis, Assiut Univ.

Ahmed, S.H.; Ahmed, A.A-H; Moustafa, M.K. and El-Tahatawy, M.Y. (1989): Microbiological studies on Pseudomonas aeruginosa food poisoning associated with consumption of milk and milk products with reference to pyocine typing. Assiut Med. J., Vol. 13(1): 71-80.

Atherton, H.V. and NEw Lander, J.A. (1977): Chemistry and testing of dairy product. 4th Ed. AVI Publishing Companhy Inc. Westport Connecticut.

Bergan, T. (1975): Resistance of Pseudomonas aeruginosa. (2nd) John Wiley and Sons, London.

Burlina, A. (1962): Patobgia polmonare del Lattante da Pseudomonas aeruginos. Riv. Anat. Pat Oncol., 22: 1095–1123.

Chernosky, M.E. and Dukes, C.D. (1963): Green nails, Importance of Pseudomonas aeruginosa in onychia. Archs. Derm. 88: 548–553.

Chruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H.A. (1975): Medical microbiology, 12th Ed. Churchill Livingstons Ltd., London.

Clement, R. and Millard, L. (1953): L'infection a bacille pyocyaninque chez L'enfant–Press mad, 61: 661–662.

Davis, P.A. and Babel, F.J. (1954): Slime formation on cottage cheese. J. Dairy Sci., 17:176.

Fahmy, A. H. and Sharara, H. A. (1950): Studies on Egyptian Domiati cheese. J. Dairy Res. 17: 312–327.

Hoadley, A.W. and McCoy, E. (1968): Some observations on the ecology of Pseudomonas aeruginosa and its occurrence in the intestinal tracts of animals. Cornell Veterinarian, 58: 354–363.

Howell, D. (1972): Survey on mastitis caused by environmental bacteria. Vet. Rec. 90:654.

ICMSF, (1978): Microorganisms in Foods, their significance and methods of enumeration. 2nd Ed. Univ. of Toronto Press, Toronto, Candada.

Korashy, E. (1992): Incidence of Pseudomonas aeruginosa in milk and some milk products. M.V.Sc. Thesis, Fac. Vet. Med. Assiut Univ.

Lowbury, E.J.L.(1951): Improved culture methods for the detection of Ps. pyocyanea. J. Clin. Pathol., 4: 66–72.

Lukasova, J. and Mraz, O. (1986): Effects of some technological processes on the survival of Pseudomonas aeruginosa in foods. Acta Veternaria Brono, 55 (4): 353–358.

Marth, E.H. (1978): Standard methods for the examination of dairy products 14th Ed. American Publich Health Association, Washington, D.C.

Moustafa, K.M.; Ahmed A–H–Ahmed and Nagah; M.S. (1988): Fate of Enteropathogenic Escherichia coli (EEC) during manufacture and storage of Domiati cheese. Assiut Vet. Med. J. 20: 39, 104–109.

Pererra, P.P.; Mathan, S.M.; Bhat, M.; Albert, P. and Baker, J. (1977): Aetiology of acute gastroenteritis in infancy and early childhood in Southern India. Arch. Dis. Child., 52 (6) 482.

Richter, R. (1981): Microbiological problems in dairy food. J. Food Prod. 44 (6): 471–475.

Saad, N. (1983): Psychrotrophic bacteria in milk and milk products. M.V.Sc. Thesis, Fac. of Vet. Med., Assiut Univ.

Saleem, R.A.; Abdel–Salam; Nagmoush, M. R. and El–Abd, M. M. (1978): White pickled soft cheese from concentrated milk. ii. Effect of concentration of brine and calcium chloride added. Egyptian J. Dairy Sci. 6: 207–220.

Sutter, V.I.; Hurst, V.; Gressman, M. and Caonje, R. (1966): Source and significance of Pseudomonas aeroginosa in sputum. J. Am. Med. Assoc. 197: 854–958.

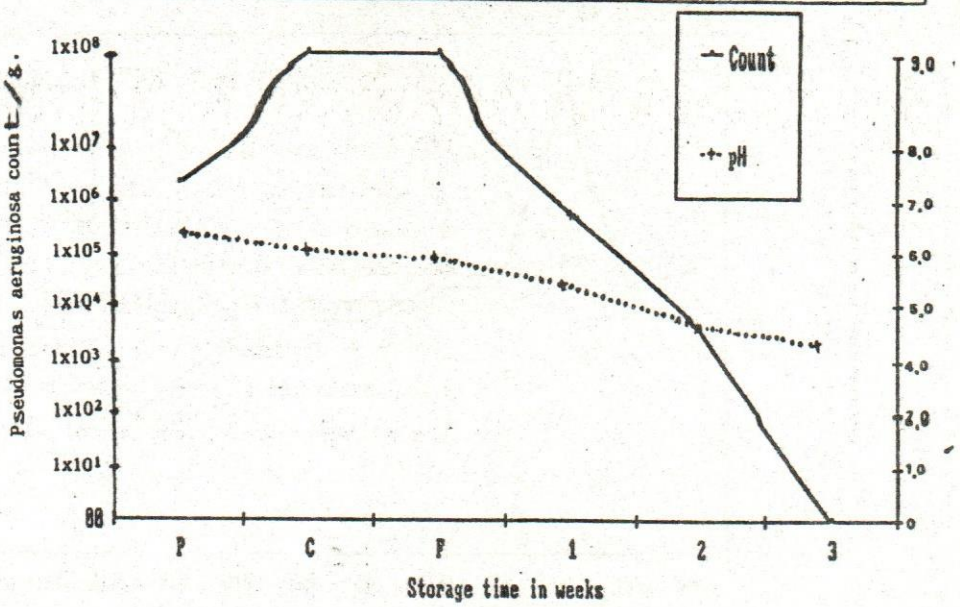
Winso, S. (1957): Fall au Pseudomonas enterit hos svin Medlemabl. Sver. Vet. Forb. 9, 104.

Youssef, H. Hefnawy, Y.; Ahmed, S. H. and Abdel–Rhaman, H. (1984): Bacteriological evaluation of raw minced meat in Assiut city. Fleischwirtsch. 64(5): 590–592.

Table (1): Changes in chemical composition of Damietta cheese during storage.

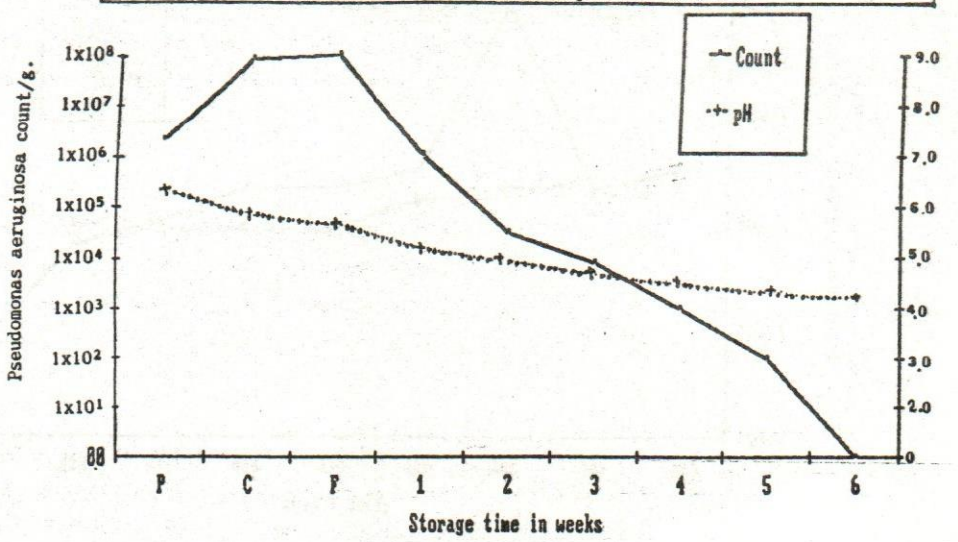
Time of storage / week	Cheese prepared from milk with 5% salt			Cheese prepared from milk with 10% salt		
	T.S%	Moisture	Salt %	T.S%	Moisture	Salt %
0	35.30	64.70	4.80	34.10	65.90	8.80
1	37.90	62.10	5.60	35.20	64.80	9.30
2	40.80	59.20	6.20	37.80	62.20	9.80
3	41.70	58.30	6.40	41.00	59.00	10.00
4				43.20	56.80	10.50
5				45.00	55.00	11.00
6				46.00	54.00	11.30

Fig. (1): Count of Pseudomonas aeruginosa in Damietta cheese prepared from milk containing 5% salt and stored at room temperature (38±1°C).



P = Inoculated pasteurized milk.
C = Curd.
F = Finished cheese.

Fig. (2): Count of Pseudomonas aeruginosa in Damietta cheese prepared from milk containing 10% salt and stored at room temperature (38±1°C).



P = Inoculated pasteurized milk.
C = Curd.
F = Finished cheese.

Fig.(4): Influence of chilling on Pseudomonas aeruginosa count in minced meat with 5% onion.

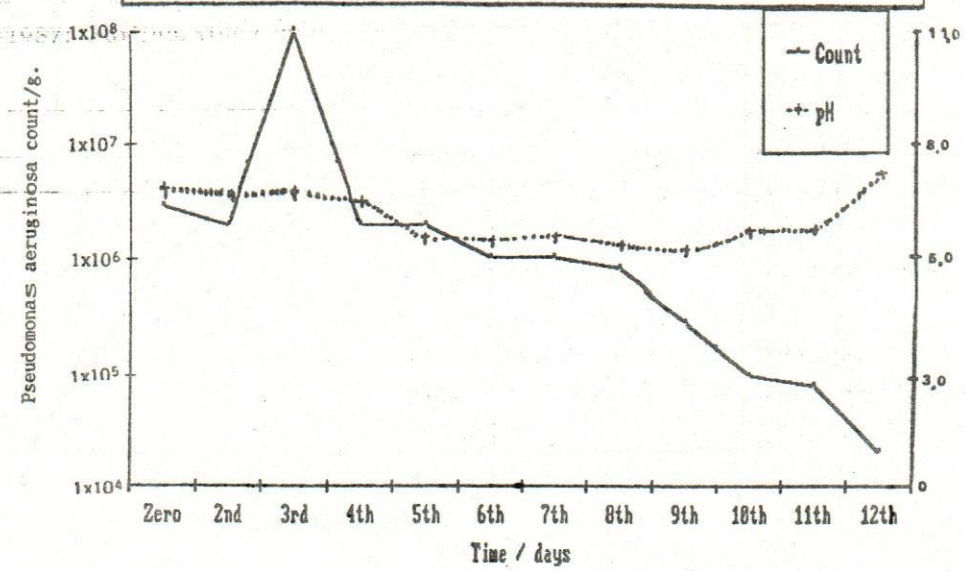


Fig. (3): Influence of chilling on Pseudomonas aeruginosa inoculated in minced meat without addition of onion.

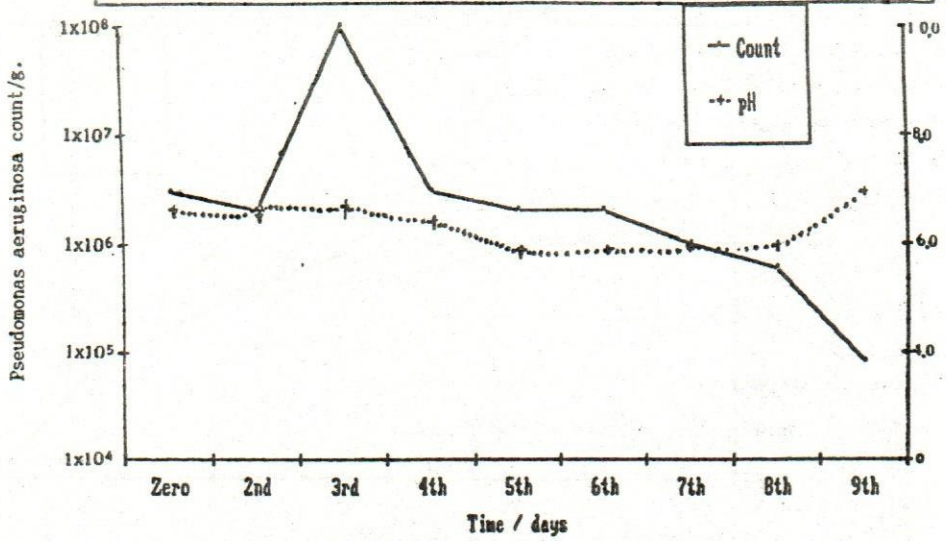


Fig. (5): Influence of deep freezing on Pseudomonas aeruginosa count in minced meat.