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A STUDY ON THE EFFECT OF FREEZING STORAGE ON THE VIABILITY AND ENTEROTOXIN "A" PRODUCTION OF TOXIGENIC STAPHYLOCOCCUS AUREUS INOCULATED IN FLUID MEDIUM AND MINCED MEAT.

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SUMMARY: The effect of freezing storage at -18°C on the viability of toxigenic S.aureus known to produce enterotoxin A (2 ug/ml culture supernatant fluid) when experimentally inoculated (with $6x10^7$ organism/ml or g) into Brain Heart Infusion (BHI) fluid medium and minced meat was studied. The recovered S. aureus cells were examined for their ability to produce enterotoxin A by Cellophane-membrane - over - agar method and the toxin was assayed serologically by reversed passive latex agglutination technique. Exposure of toxigenic S.aureus to freezing storage at- 18°C resulted in a gradual loss of viability in fluid medium and in minced meat. The difference in the decline of viable S. aureus count in both inoculated media was negligible at start, but it became progressive by time. The rate of cell death of S.aureus was faster in minced meat than in fluid medium and minimum count (100 viable cells/g or ml) of S. aureus was recorded at 23th week and 27th week post-storage in minced meat and fluid medium respectively. One week later no viable organisms could be detected in both tested fluid medium and in minced meat. The capability of recovered S. aureus to produce enterotoxin A after transferring to toxin production medium remained constant (2 ug/ml culture supernatant fluid) both in BHI broth and in minced meat.

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INTRODUCTION

The physiological damage of bacteria by low temperatures as in cases of freezing, chilling and freeze drying has been extensively investigated (Straka and Stokes, 1959; Postgate and Hunter, 1963; Ray et al., 1971 and Jackson, 1974).

The exposure of S. aureus in contaminated foods to low temperatures has been reported to decrease their viable count (Raj and Liston, 1961; Jackson, 1974; Farrell and Upton, 1978; Hamdy et al., 1986 and Niazi et al., 1987). However, it seems that some of the cells can escape the injurious of effect of freezing and multiply at favourable temperatures. This is substantiated by the success of Gilbert and Wieneke (1973), Wieneke (1974) and Sedik (1982) in isolating enterotoxigenic S.aureus, implicated in several food poisoning outbreaks, from frozen foods such as frozen meats (chicken, turkey, beef, pork and ham). Moreover, Wieneke, (1974) found that staphylococcal enterotoxins could still be demonstrated in staphyloccal culture filtrates stored for 4 years at 4°C.

Therefore, the present investigation was initiated to determine how far the storage at freezing temperature (-18°C)affect the viability and enterotoxigenicity of enterotoxigenic S.aureus artificially inoculated in Brain Heart Infusion (BHI) broth and in minced meat.

MATERIAL AND METHODS

1. Organism and preparation of inoculum:

Staph. aureus strain S-53 g isolated from a sample of minced meat in the Unit of Bacterial Toxins, Animal Health Research Institute and known to produce enterotoxin type A was used in this experiment.

The strain was inoculated on the surface of nutrient agar plates and incubated aerobically at 37°C for 24 hours. The growth was washed 3 times with sterile physiological saline and then the sediment was suspended in sterile saline and the number of cells was adjusted by the optical density at a wavelength of 600 nm using a Spectronic-20 spectrophotometer to contain $6x10^9$ viable cells/ml.

2. Artificial inoculation of Brain Heart Infusion (BHI) broth:

One litre of sterile BHI broth (Bergdoll, 1962) was inoculated with 10 ml of the organism suspension, thoroughly mixed and contained $6x10^7$ Staph. aureus/ml. Inoculated BHI broth was distributed in sterile test tubes each contained 10 ml amount.

3. Artificial inoculation of minced meat:

One kg of minced meat proved to be free from coagulase positive staphylococci was inoculated by 10 ml of the suspended organism and regularly distributed to have $6x10^7$ organism/g., then divided into parts each weighing 10 grams. Inoculated broth and minced meat were stored at-18°C and examined every 7 days for determining the viable count of S. aureus until the organism disappeared.

4. Enumeration of the organism:

Two samples of 10-g minced meat were used at each time, each sample was blended in 90 ml of 0.1 % peptone water using high speed blender. The number of Staph aureus present in the suspended sample was determined by spreading 0.1 ml of tenfold dilutions of the meat homogenate over the surface of BHI agar plates. The typical colonies were counted after 48 hours incubation at 37°C. The same procedure was applied for counting staph aureus in BHI broth tubes (2 tubes at each time).

5. Recovery and detection of enterotoxin A:

The representative colonies of staph aureus at each time of enumeration were picked up and transfered to BHI agar slants and incubated for 18-24 hours at 35°C. An aqueous suspension of S.aureus prepared from the agar slant cultures (contained approximately 300 million organisms per ml), were used for production and estimation of enterotoxin A by the Cellophane-membrane-over-agar method (Hallander, 1965 and Jarvis and Lawrence, 1970) and the serological procedure using Oxoid SET-RPLA(a kit for the detection of staphylococcal enterotoxin A) by reversed passive latex agglutination technique.

RESULTS

The effect of freezing storage at -18°C on the viability and enterotoxin A production by toxigenic staph. aureus inoculated in fluid culture medium (BHI broth) and in minced meat is presented in (Fig. 1). Counts showed that the gradual decrease in viability was greater in inoculated minced meat than in fluid medium. The difference can be neglected at the start (8.41 and 11.19 % in the BHI broth and in minced meat respectively) but it became in close contact for considerable time. After a period of 12 weeks, the rate of cell death of Staph. aureus became faster particularly in samples of minced meat than in fluid medium as the viability decreased by 16.85 and 27.92 % respectively. Complete disappearance of Staph.aureus, manifested by a progressive loss of its ability to form colonies on BHI agar, was demonstrated in minced meat samples after 24 weeks of storage at -18°C. In contrast, at the same period of storage the number of Staph.aureus counted in fluid medium decreased only by 51.48 % and then progressively reduced by 75 % after 27 weeks. Complete diminish in fluid medium was recorded after 28 weeks of storage and the organism had no power of being viable.

Concerning the productivity of enterotoxin A, it was observed that the capability of recovered S.aureus (300 million cells/ml) to produce enterotoxin A after transferring to toxin production medium remained constant (2 ug/ml of culture supernatant fluid) in both BHI broth and in minced meat until the organism diminished to the lesser minimum level.

DISCUSSION

The present work demonstrates that exposure of S.aureus to freezing storage at -18°C resulted in loss of its viability as manifested by the drop of viable count both in BHI broth and in minced meat. This observation is consistent with the known effects of temperatures on the biological system as metabolic injury (Raj and Liston, 1961; Jackson, 1974; Farrell and Upton, 1978). In addition, Pereira et al. (1982) observed that a lowering of temperature would result in slower growth of toxigenic S.aureus in casein hydrolysate fluid medium. Furthermore,

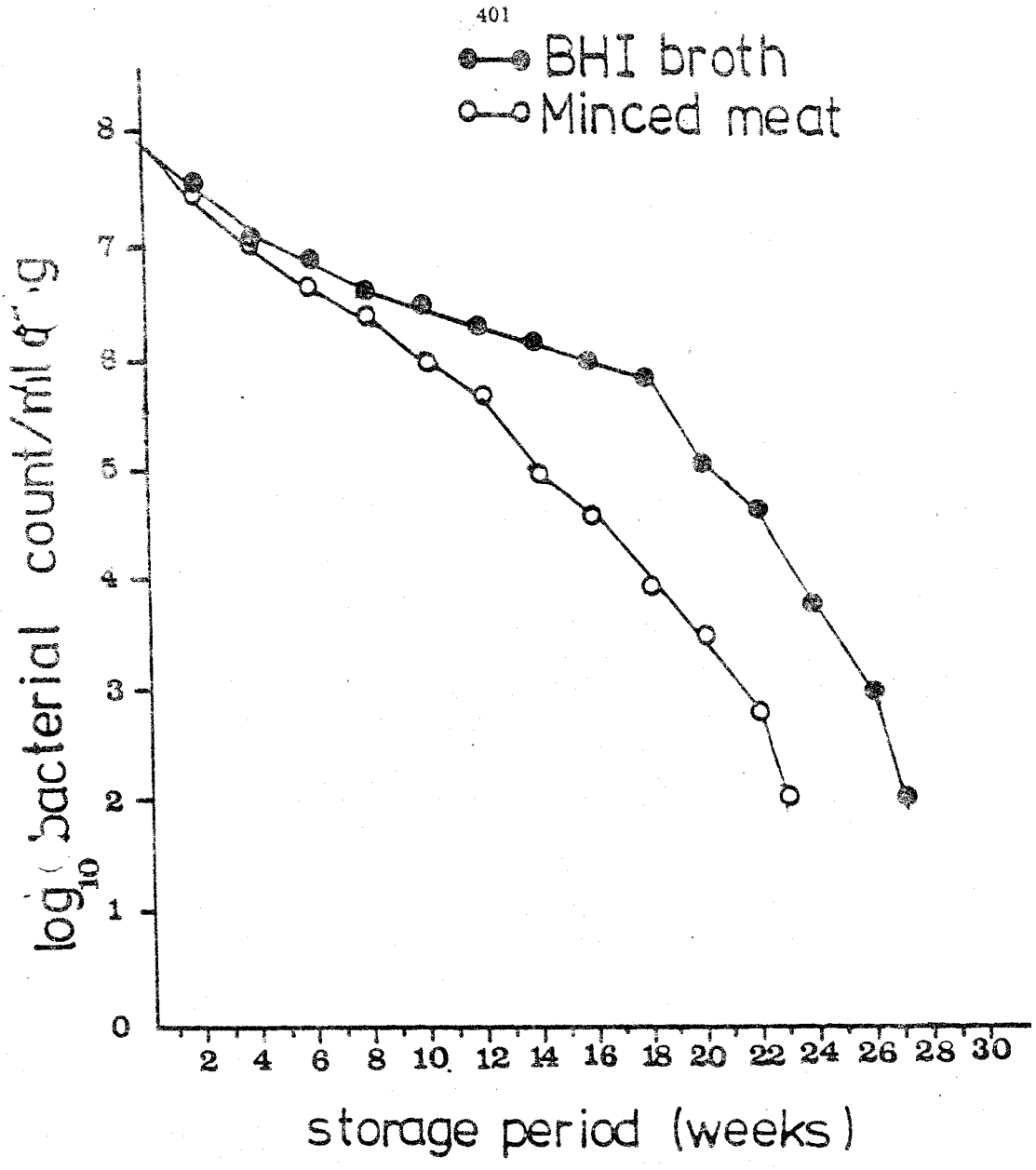

Fig. 1: Effect of freezing storage at-18°C on the viability of enterotoxigenic Staphylococcus aureus in fluid culture medium and minced meat.

Jackson (1974) reported that certain functions such as multiplication and cell devision can obviously not occure below the minimum growth temperature of S.aureus, however, even below the minimum growth temperature it was well established that metabolism of S.aureus does not cease completely.

Hamdy et al. (1986) found that freezing at-18°C caused continous decrease in the S.aureus contaminating dressed broilers and the minimum count in skin and muscle (2.9, 1.9 $\log_{10}$ respectively) was recorded in samples examined after 120 days of freeze storage. In the present investigation, the minimum count (100 viable cells/g or ml) of toxigenic S.aureus was recorded at 161 days (23 weeks) in minced meat stored at-18°C and at 189 days (27 weeks) in fluid culture medium and one week later no viable organisms could be detected either in minced meat or in broth. In contrast, Raj and Liston (1961) found that a temperature of -18°C for 393 days decreased the number of S.aureus contaminating sea foods by ten folds. Previous reports had discussed the influence of freeze storage on bacteria. In this respect, Frey and Greaves (1951) reported that during the freeze-drying process the immediate death-rate of organisms is often high. Ingram and Mackey (1976) recorded that many organisms may perish during the process of storage, particularly between temperature of 0°C till-30°C. They are mechanically crushed or injured by extracellular ice crystals.

They added that at low temperature the damage of the organisms was caused by rise in concentration of electrolytes within the cells when the water separates out as ice. The faster rate in the decline in viability of toxigenic, coagulase positive S. aureus inoculated in minced meat stored at-18°C and demonstrated in the present investigation than that observed in case of fluid culture medium, might be explained by the variation in water electrolytes and nutrients in both cases. The explanation of Straka and Stokes (1959) that the injured Staph aureus cells can no longer grow in a simple, glucose-salts agar medium but can develop on a rich, complex medium as trypticase soya agar and injured S.aureus cells may constitute as much as 40 % of bacterial population, may be still valid in explaining our results. Furthermore, Casman et al. (1963) reported that the inability to obtain a good growth in ground raw meat may have been due to competition with other acid forming bacteria which could be more effective under anaerobic

condition than under the aerobic conditions preferred by staphylococcal organisms.

On the other hand, the storage of tested strain of S.aureus in fluid medium and in minced meat appeared to have no effect on the production of enterotoxin A.These findings substantiate the claim of Ostovar and Bremier (1975) who found that prolonged thawing of frozen convenience food items resulted in an increase in number of S.aureus and could possibly lead to the production of enterotoxins which would be impossible to eliminate by ordinary cooking practices. Moreover, Troller (1976) reported that low staphylococcal counts recovered from frozen foods constitutes no potential danger as long as food was kept under refrigeration and not allowed to thaw for a long time at room temperature. Regarding the public health significance of S.aureus in frozen food, Casman et al.(1967) that 3 % out of 260 strains isolated from frozen foods produced enterotoxin A and 1 % formed enterotoxin B. Ostovar and Bremier (1975) reported that most isolates of coagulase positive staphylococci produced type A or B enterotoxins. Sedik (1982) recorded that 132 samples (58.4 %) of 225 frozen beef products contained less than 100 viable S.aureus/g. and 36 % of isolants were enterotoxigenic.

In conclusion, hygienic measure should be taken in consideration to prevent food contamination with toxigenic staph aureus particularly during processing of foods and before freeze storage, since the organism is able to survive freezing for a longer time and also does not loose its capability to produce enterotoxins. In addition, the injured S.aureus cells may recover again and grow during thawing of such foods and become able to produce enterotoxin.

REFERENCES

1. Bergdoll, M.S. (1962): The chemistry and detection of staphylococcal enterotoxin. Am. Meat Inst. Found. Circ. 70, p. 47.

2. Casman, E.P.; McCoy, D.W. and Brandly, P.J. (1963): Staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. **11**, 498-500.

3. Casman, E.P.; Bennet, R.W.; Dorsey, A.E. and Issa, J.A. (1967): Identification of a fourth staphylococcal enterotoxin D.J. Bacteriol. **94**, 1875-1882.

4. Farrell, G.M. and Upton, M.E. (1978): The effect of low temperature on the growth and survival of Staphyloccus aureus and Salmonella typhimurium when inoculated on to bacon. J. Food Technol. **13**, 15-23.

5. Fry, R.M. and Greaves, R. (1951): Bacterial resistance, disinfection and sterilization. P. 56-96, In: Topley and Wilson's Principles of Bacteriology, Virology and Immunity. 7th Ed. Vol. 1. Butler, Tanner Ltd. Forme and London.

6. Gilbert, R.J. and Wieneke, A.A. (1973): Staphylococcal food poisoning with special reference to the detection of enterotoxin in food. pp. 273-285. In B.C., Hobbs and J.H.B., Christian (eds). The microbiological safety of food. Academic Press, London and New York.

7. Hallander, H.O. (1965): Production of large quantities of enterotoxin B and other staphylococcal toxins on solid media. Acta Pathol. Microbiol. Scand **63**, 299-305.

8. Hamdy, M.M.; Nouman, T.M. and Hatem, M. (1986): Influence of cold storage on Staphylococcus aureus contaminate of dressed broilers. J. Egypt. Vet. Med. Ass. **46** (1), 13-21.

9. Ingram, M. and Mackey, B.M. (1976): In: Inhibition and Inactivation of Vegetative Microbes, p. 111. Ed. by F.A. Skinner and W.B. Hugo, Academic Press, London.

10. Jackson, H. (1974): Loss of viability and metabolic injury of Staphylococcus aureus resulting from storage at 5°C.J. Appl. Bacteriol. **37**, 59-64.

11. Jarvis, A.W. and Lawrence, R.C. (1970): Production of high titers of enterotoxins for the routine testing of staphylococci. Appl.Microbiol **19**, 698-699.

12. Niazi, Z.M.; Refai, M. and El-Sawah, H. (1987): Detection of staphylococcal enterotoxin A in experimentally infected cheese. J. Egypt. Vet. Med. Ass. 47 (3), 607-618.

13. Ostovar, K. and Bremier, M.J. (1975): Effect of thawing on growth of staph.aureus in frozen convenience food items. J. Milk Food Technol. 38, 337-339.

14. Pereira, J.L.; Salzberg, S. and Bergdoll, M.S. (1982): Effect of temperature, pH and sodium chloride concentrations on production of staphylococcal ent erotoxins A and B. J. Food Protection, 45 (14), 1306-1309.

15. Postgate, J.R. and Hunter, J.R. (1963): Metabolic injury in frozen bacteria. J. Appl. Bacteriol. 26. 405-409.

16. Raj, H.D. and Liston, J. (1961): Survival of bacteria of public health significance in frozen sea foods. Food Technol.15, 429-434.

17. Rav, B.; Jezeski, J.J. and Busta, F.F. (1971): Effect of rehydration on recovery, repair and growth of injured freeze-dried salmonella anatum. Appl. Microbiol. 22, 184-190.

18. Sedik, M.F. (1982): Incidence of enterotoxigenic staphylococci in frozen meat and chicken products. J, Egypt. Vet. Med. Ass. 42 (2), 31-36.

19. Straka, R.P. and Stokes , J.L. (1959): Metabolic injury to bacteria at low temperatures. J. Bacteriol. 78, 181-185.

20. Troller, J.A. (1976): Staphylococcal growth and enterotoxin production factors for control. J. Milk Food Technol. 39 (7), 499-503.

21. Wieneke, A.A. (1974): Enterotoxin production by strains of Staphylococcus aureus isolated from foods and human beings. J. Hyg. (Camb). 73, 255-262.