Journal of Food Protection, Vol. 50, No. 12, Pages 1023-1024 (December 1987)

A Research Note

Effects of Yeasts on Survival of *Staphylococcus aureus* in Pickled Cheese Brine

AMOS NUSSINOVITCH[1*], BARUCH ROSEN[2] and RUTH FIRSTENBERG-EDEN[1]

Department of Food Engineering and Biotechnology, Technion, Haifa, Israel and Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

(Received for publication March 31, 1987)

ABSTRACT

The interactions between staphylococci and yeasts in pickled cheese brine were investigated. Above pH 5, *Staphylococcus aureus* grew in pickled cheese brine. Acid-consuming yeasts increased the pH of the brine to a level which enabled development of staphylococci. This indicates the need to monitor yeast contamination in cheeses preserved by the combination of acid and high salt.

Pickled cheeses are a traditional product of the Balkans and the Eastern Mediterranean *(8)*. Lately there has been an increasing interest in these cheeses not only in their countries of origin but elsewhere *(3,4,6,11)*. In Greece *(2)*, in Israel *(5)* and apparently in the Sudan *(4)* pickled cheese has been implicated as a suspected vector of *Staphylococcus aureus* food poisoning.

Minor and Marth *(7)* indicated that the metabolites produced by lactic starter cultures in dairy products tend to suppress growth of, and toxin production by *S. aureus*. Studies by Yanai et al. *(11)* demonstrated that Israeli pickled cheese was "a well controlled ecological system." This control was effected through the salt and lactic acid produced in these cheeses and in their brine by the lactic acid bacteria. The present study was intended to define the growth parameters of *S. aureus* in Israeli pickled cheese brine and to study the effects of yeasts on these parameters.

MATERIALS AND METHODS

Synthetic sterile pickled cheese brine (SPCB) was produced in the following fashion: 50 g of fat-free milk powder and 90 g of NaCl were dissolved in 500 ml of distilled water and sterilized at 121°C for 12 min. Another 500 ml of sterile solution (121°C, 12 min) containing 3% lactic acid and enough Na_2HPO_4 to achieve the desired pH, were added.

The toxin-producing staphylococci used in this research were isolated from fresh raw milk of mastitic cows and identified as typical *S. aureus* by conventional methods *(9)* (thermonuclease-positive, toxin production-positive). The *S. aureus* culture was maintained on tryptic soy agar slants.

All counts were made on Baird Parker agar *(9)*. Dilutions before counts of all microorganisms were in 0.1% sterile peptone water. The level of *S. aureus* inoculum was adjusted to about 1×10^4-1×10^5 of initial CFU/ml in the SPCB.

Yeasts were isolated from commercial pickled cheese brine on oxytetracycline-glucose yeast extract agar (OGY) having the following composition: yeast extract, 0.5%; glucose, 1.0%; agar, 2.0%; and oxytetracycline, 0.01%. Yeasts were also enumerated on OGY.

Formol-number was expressed as ml of 0.1 *N* NaOH needed to readjust a 20-ml sample of brine back to pH 7 after 4 ml of neutralized solution of 40% formaldehyde had been added.

RESULTS

Figures 1 and 2 demonstrate growth of toxin-producing staphylococci in SPCB at 10 and 20°C, respectively. Between pH 5 and 6.5 there was some apparent growth after introduction of the *S. aureus* inoculum, which was later followed by stabilization and decline. At 20°C growth of staphylococci in SPCB at pH 5.5 and pH 6.0 was more pronounced than at 10°C.

Yeasts isolated from PCB were introduced in SPCB and produced pH changes shown in Fig. 3 and 4. Both at 10 and 20°C pH increased over a long period in the presence of several yeast isolates.

Figure 5 demonstrates the effect of yeast inoculation into PCB on the formol number of the brine. From data in the figure it can be deduced that the pH of the brine was increased more by consumption of acids than by production of amine bases from the soluble proteins.

Figure 6 demonstrates the interaction of yeasts and staphylococci growing together in the brine. Yeasts were

[1]*Technion.*
[2]*Bar-Ilan University.*

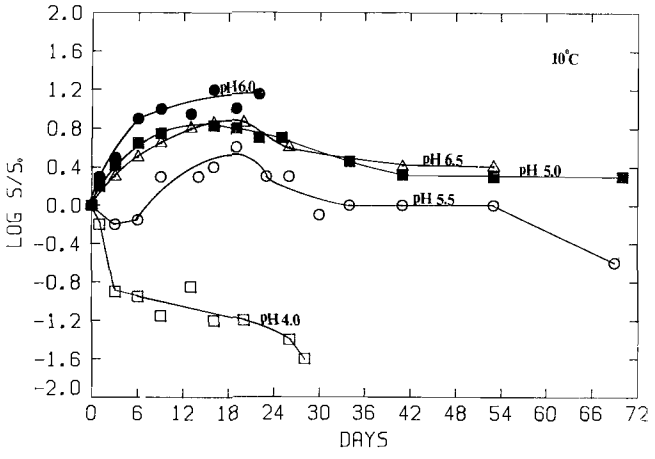

Figure 1. *Growth/decline of toxigenic staphylococci in pickled cheese brine, in the pH range of 4-6.5, at 10°C. (S/So is the ratio of the CFU at any given time to the CFU at the beginning of the experiment, S-staphylococci).*

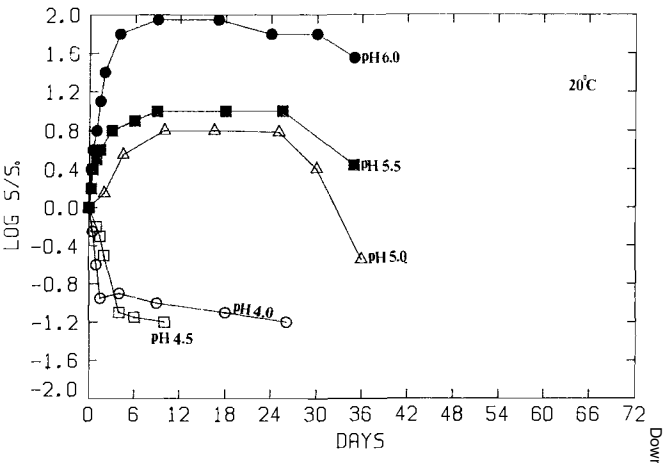

Figure 2. *Growth/decline of toxigenic staphylococci in pickled cheese brine, in the pH range of 4-6.0, at 20°C.*

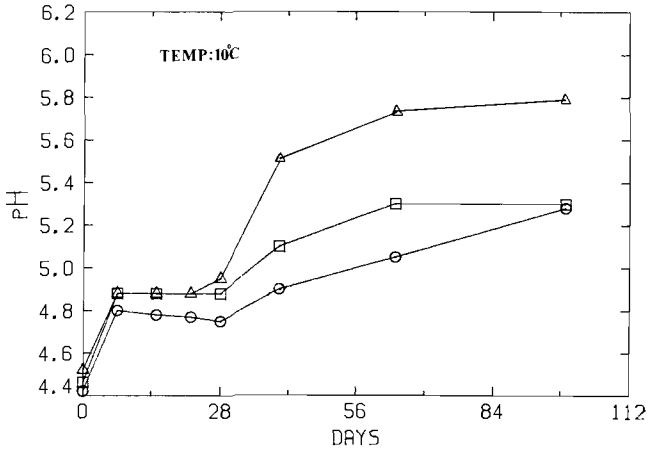

Figure 3. *Acid-consuming yeasts raising the pH of pickled brine at 10°C.*

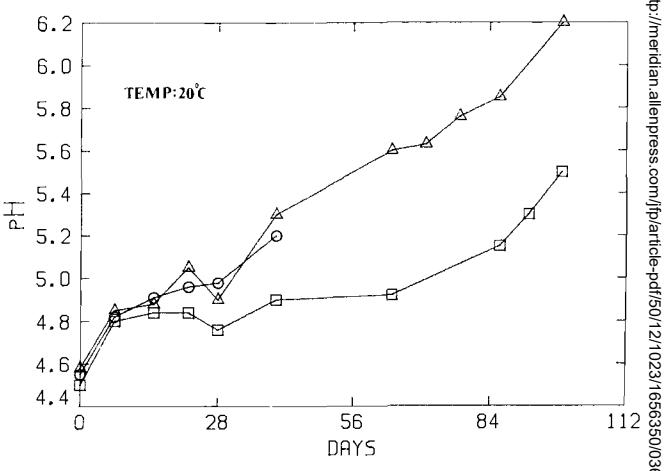

Figure 4. *Acid-consuming yeasts raising the pH of pickled cheese brine at 20°C.*

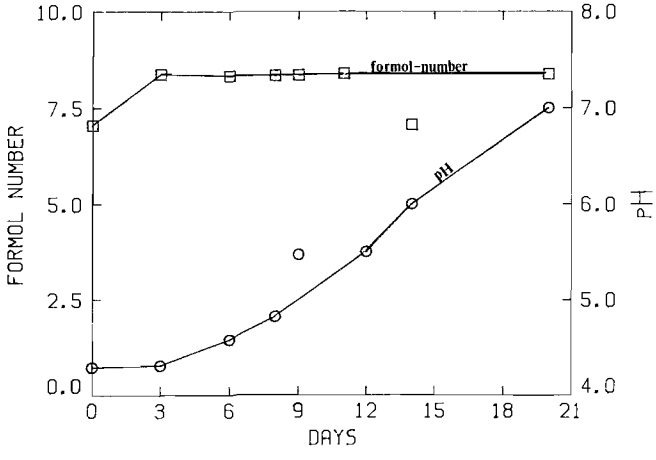

Figure 5. *Change in acidity and formol number of pickled cheese brine during the growth of acid-reducing yeast.*

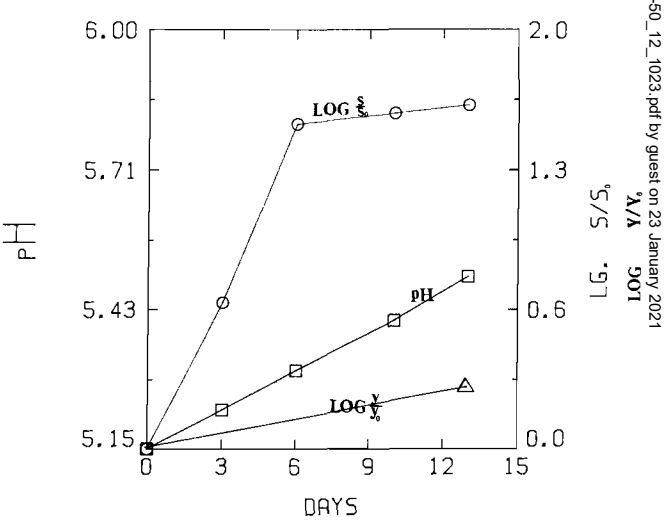

Figure 6. *Growth of* S. aureus *in pickled cheese brine containing active acidconsuming yeasts. (S/So, Y/Yo are the ratios of the CFU at any given time to the CFU at the beginning of the experiment, S-staphylocci, Y-yeasts).*

inoculated into the brine at pH 4.7, and when the pH rose to 5.15 staphylococci were also introduced. No inhibition of either species was apparent. Both staphylococci and yeasts grew well in the presence of each other.

con't. p. 1043

18. Juhl, K. J. 1979. Lipid and pigment oxidation in raw ground beef systems. M. S. Thesis, Iowa State Univ., Ames.
19. Kastner, C. L. 1983. Optimal hot-processing systems for beef. Food Technol. 37(5):96-104.
20. Marth, E. H. (ed.). 1978. Standard methods for the examination of dairy products, 14th ed. Am. Public Health Assoc. Inc., Washington, D.C.
21. Mol, J. H. H., J. E. A. Hietbrink, H. W. M. Mollen, and J. Van Tinteren. 1971. Observations on the microflora of vacuum packed sliced cooked meat products. J. Appl. Bacteriol. 34:377-397.
22. Molins, R. A., A. A. Kraft, H. W. Walker, and D. G. Olson. 1985. Effect of poly- and pyrophosphates on the natural bacterial flora and inoculated *Clostridium sporogenes* PA 3679 in cooked vacuum packed bratwurst. J. Food Sci. 50:876-880.
23. Nielsen, H. J. S., and P. Zeuthen. 1983. Influence of phosphate and glucose addition on some important spoilage bacteria in vacuum packed bologna-type sausage. J. Food Prot. 46:1078-1083.
24. Pulliam, J. D., and D. C. Kelly. 1965. Bacteriological comparisons of hot processed and normally processed hams. J. Milk Food Technol. 28:285-286.
25. Reagan, J. O., S. L. Pirkle, D. R. Campion, and J. A. Carpenter. 1981. Processing, microbial and sensory characteristics of cooler and freezer stored hot-boned beef. J. Food Sci. 46:838-841.
26. Rhee, K. S., R. N. Terrell, M. Quintanilla, and C. Vanderzant. 1983. Effect of addition of chloride salts on rancidity of ground pork inoculated with a *Moraxella* or a *Lactobacillus* species. J. Food Sci. 48:302-303.
27. Schwartz, W. C., and R. W. Mandigo. 1976. Effect of salt, sodium and tripolyphosphate and storage on restructured pork. J. Food Sci. 41:1266-1269.
28. Snedecor, G. W., and W. G. Cochran. 1980. Statistical methods, 7th ed. The Iowa State University Press, Ames, IA.
29. Wagner, M. K., and F. F. Busta. 1984. Inhibition of *Clostridium botulinum* growth from spore inocula in media containing sodium acid pyrophosphate and potassium sorbate with or without added sodium chloride. J. Food Sci. 49:1588-1594.
30. Waldman, R. C., D. O. Westerberg, and S. Simon. 1974. Influence of curing ingredients and storage time on the quaity of preblended sausage meats and frankfurters. J. Food Sci. 39:718-722.
31. Whiting, R. C., R. C. Benedict, C. A. Kunsch, and D. Blalock. 1985. Growth of *Clostridium sporogenes* and *Staphylococcus aureus* at different temperatures in cooked corned beef made with reduced levels of sodium chloride. J. Food Sci. 50:304-307.
32. Witte, V. C., G. F. Krouse, and M. E. Bailey. 1970. A new extraction method for determining 2-thiobarbituric acid values for pork and beef during storage. J. Food Sci. 35:582-585.
33. Yasosky, J. J., E. D. Aberle, I. C. Peng, E. W. Mills, and M. D. Judge. 1984. Effects of pH and time of grinding on lipid oxidation of fresh ground pork. J. Food Sci. 49:1510-1512.

Nussinovitch et al., *con't. from p. 1024*

DISCUSSION

The SPCB simulated the situation in commercial pickled cheese brine which is difficult to standardize. Our studies show that toxigenic *S. aureus* from bovine sources can grow in pickled cheese brine under the proper conditions (temperature 10 or 20°C, salt content 9% and pH above 5.0). Presence of growing yeast cells increased the pH of the medium, thus spurring the growth of *S. aureus*. Both *S. aureus* and yeasts showed continued growth in the presence of each other. The yeasts were not identified at this stage. Our studies show that acid-consuming yeasts are quite prevalent in Israeli pickled cheese brine.

It is interesting to note that Minor and Marth *(7)* stressed the possible role of *S. aureus* from bovine source in recontaminating dairy products and practically predicted part of the results of the present study. We see no reason why *S. aureus* from human sources will behave differently. A reintroduction of human *S. aureus* into pickled cheese brine is quite possible as much of this cheese is "fished" by the vendor from the brine in which it is marketed.

Hofi et al. *(3)* graded Egyptian market Domiati cheese and showed that there was a good relationship between pH and organoleptic quality good cheese, average pH 4.2 (4.0-4.4); medium quality, pH 4.6 (4.5-4.8); fair, pH 4.9 (4.6-5.6). Their cheese was much saltier than our system. Nevertheless, it seems that their grading system is valid also from the point of view of staphylococcus food poisoning.

It has been shown here that growth of yeasts in pickled cheese brine will increase the pH, thus stimulating the growth of *S. aureus*. The levels of *S. aureus* counts reached in our study may be below the numbers reported to produce damaging levels of toxins *(10)*. However, the model suggested here seems to be a valid avenue by which previously considered safe products may become a vehicle of *S. aureus*-produced enterotoxin.

REFERENCES

1. Cox, H. E., and D. Pearson. 1962. The chemical analysis of foods. New York.
2. Elikis, C. G. 1966. Food poisoning due to cheese produced in Greece. Acta Microbiol. Hellen. 11:181-183.
3. Hofi, A. A., S. El-Shibiny, G. A. Maharan, S. M. Farahat, and A. A. Abdelbaky. 1975. The quality and chemical composition of market Domiati cheese. Egyptian J. Dairy Sci. 2:135-138.
4. Khalid, A. S., and W. F. Harrigan. 1984. Investigation into the effect of salt level on growth of, and toxin production by *S. aureus* in Sudanese cheese. Lebensm.-Wiss. Technol. 17:99-103.
5. Komarov, M., and R. Seligmann. 1975. Enterotoxigenic staphylococci in meat, other market foods and food-poisoning. Refuah Veterinarith (Quarterly Israel Vet. Med. Assoc.) 32:88-98.
6. Lloyd, G. T., and E. H. Ramshwaw. 1979. A manufacture of Bulgarian-style feta cheese - A review. Austral. Jour. Dairy Technol. 34:180-183.
7. Minor, T. E., and E. H. Marth. 1976. Staphylococci and their significance in foods. Elsevier, New York.
8. Sanders, G. P. 1953. Cheese varieties and descriptions. U.S.D.A., Washington.
9. Speck, M. L. (ed.). 1976. Compendium of methods for the microbiological examination of foods. American Public Health Assoc., Washington, D.C.
10. Tatini, S. R., W. D. Wesala, J. J. Jezeski, and H. A. Morris. 1973. Production of staphylococcal enterotoxin in blue, brick, mozzarella and Swiss cheese. J. Dairy Sci. 56:429-435.
11. Yanai, Y., B. Rosen, A. Pinskey, and D. Sklan. 1976. Microbiology of Israeli pickled cheese. J. Milk Food Technol. 39:4-6.