Food Poisoning Potential of Pathogens Inoculated onto Bologna in Sandwiches

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ABSTRACT

Bologna sandwiches inoculated separately with low levels (100 to 1,000 per g) of specific pathogens at time of sandwich preparation to simulate conditions that might occur in home or food service preparation, were stored at 4, 21 and 30 C for 0, 4, 8 and 25 h and monitored for growth of pathogens. All pathogens, except Clostridium perfringens, were capable of significant growth after more than 8 h of incubation at 30 C, but not at 4 or 21. Significant growth at 21 C only occurred with Staphylococcus aureus after 25 h of incubation. C. perfringens failed to grow on bologna in all sandwiches. All other pathogens, except S. aureus, failed to grow on bologna with low pH (pH <6.1). Growth of S. aureus was retarded on bologna at pH 5.5, and inhibited at pH 5.1. Only gram negative pathogens (enteropathogenic Escherichia coli and Salmonella typhimurium) were adversely affected by increased bacterial competition. Results indicated that bologna in sandwiches under these experimental conditions would only become a potential vehicle for food poisoning under almost unrealistic conditions of handling and storage.

Storage abuse and improper handling are major factors involved in food poisoning outbreaks in North America (2, 3). Sandwiches are products which could be exposed to considerable abuse before consumption. Despite this potential for food poisoning from sandwiches, there are few reported cases of sandwich-borne food poisonings. This study was undertaken to investigate the food poisoning potential of sandwiches. However, to limit the scope of the study, bologna was chosen as the only sandwich filling.

Bologna supports growth of microorganisms, but may not be an ideal medium for bacterial growth. A variety of inhibitory and selective agents present in bologna can affect survival of specific microorganisms. The most important inhibitory agents include: nitrite, pH, salt concentration, competition, oxygen and carbon dioxide partial pressures and storage temperature. The combination of factors in cooked, cured meats are reported to select gram positive, salt tolerant microorganisms, such as Bacillus, Micrococcus, Sarcina, Lactobacillus and Microbacterium spp. (1, 10, 11, 12, 16, 18, 19, 25, 26, 29). On this basis, it may be expected that potential food poisoning organisms such as Bacillus cereus and Staphylococcus aureus can develop in cooked, cured meats.

An earlier study (20) indicated that bologna could be obtained in the retail marketplace with either a high pH (near pH 6.5) or a low pH (below pH 5.5), and that pH change was not a reliable function of product age, since certain manufacturers’ product had a high pH with high microbial counts after 30 days shelf-life. This study was designed to evaluate the effects of differences in competition with other bacteria (age of product), pH and storage temperature on growth of several selected pathogens.

MATERIALS AND METHODS

Freshly sliced, but not packaged, bologna was obtained directly from two manufacturers, selected for differences in product pH at the end of the product’s shelf-life. The product was returned to the laboratory and vacuum packaged in aluminum-nylon-polypropylene pouches (Cryovac Division, Grace Chemicals, Mississauga, Canada) under 26 lb/sq. in. vacuum. Packaged bologna was stored at 4 C for 2 and 30 days, representing “new” (low competition) and “old” (high competition) bologna, respectively.

Sandwiches were prepared by placing one slice of bologna between two slices of enriched, white bread, which had been spread with one teaspoonful of soft margarine (Parkay). The bread with margarine was equilibrated to 21 C before bologna was added. Triplicate samples of the three bologna types (high pH, low competition; high pH, high competition; low pH, high competition) were evaluated for their ability to support growth of selected pathogens: S. aureus S-6, Salmonella typhimurium (ATCC 13311), enteropathogenic Escherichia coli 0124 DM, B. cereus B4AC and Clostridium perfringens 8239-H. (All cultures, except S. typhimurium 13311, were obtained from Dr. A. Hauschild, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada).

At the time of sandwich preparation the bologna slice was inoculated with low levels (100 to 1,000 per g) of test organisms, by spreading 0.05 ml of appropriately diluted overnight culture on one side of each slice, using a sterile, glass hockey stick. Inoculated sandwiches were placed in plastic bags (Zip Lock Seal, Dow Chemicals) and incubated at 4, 21 and 30 C for 0, 4, 8 and 25 h.
Following incubation, counts of test organisms were determined on the following media: *S. aureus* on Difco Baird-Parker medium, incubated 36 C for 48 h; *S. typhimurium* on Difco Brilliant-Green agar, incubated 36 C for 24 h; *E. coli* on Difco Violet Red Bile agar, overlaid and incubated 36 C for 24 h; *B. cereus* on Mossel et al. (17) Mannitol, Egg-Yolk, Phenol Red, Polymyxin (MYP) agar, incubated 30 C for 24-20 h, but in the case of heavily contaminated plates for 40 h; *C. perfringens* on egg-yolk free Tryptose-Sulfite-Cycloserin (TSC) agar, incubated anaerobically (using BBL "Gas-Paks" to obtain a H₂/CO₂ atmosphere) 36 C for 24 h.

Counts were confirmed as follows: *S. aureus* using Difco coagulase plasma; *S. typhimurium* using MacConkey agar, TSA slants, and serological screening with Salmonella O Antiserum Poly A-I and Group B factors 1, 4, 5 and 12; *E. coli* using MacConkey agar and gas production in EC medium at 45.5 ± 0.05 C; *B. cereus* using gram stain and catalase production; and *C. perfringens* using gram stain, nitrate reduction and motility. Confirmation was also inferred from the absence of growth of typical organisms from uninoculated control sandwiches.

**RESULTS AND DISCUSSION**

Microflora, pH and moisture content of the bologna samples were measured. New (low competition), high pH product had total counts ranging from 1.2 × 10² to 4.3 × 10⁴ organisms per g, pH range 6.4 to 6.7 and moisture content 58.0 to 59.4%. Old (high competition), high pH product had total counts above 1 × 10⁶ organisms per g, pH range 6.3 to 6.7 and moisture content 55.8 to 57.3%. Old, low pH product had total count above 1 × 10⁴ organisms per g, pH range 5.1 to 5.5 and moisture content 53.5 to 55.2%. An additional old sample with pH 6.1 was analyzed. Salt content was approximately 2.4% (data supplied by manufacturers) and levels of other inhibitors, with the possible exception of nitrite, were controlled. Nitrite levels in old bologna were probably 15 - 20% less than in new product, as a result of storage depletion (24); however, breakdown products of nitrite might also be involved in microbial inhibition (13, 21, 22).

Growth of test organisms on bologna in sandwiches is shown in Fig. 1 - 5. Growth is expressed in terms of relative population change by plotting log₁₀ of the ratio of the number of pathogens recovered after incubation to the number inoculated. The most striking observation was that significant growth only occurred after more than 8 h of incubation at 30 C. All test pathogens, except *C. perfringens*, grew on high pH bologna (new and old) after 25 h of incubation at 30 C. *C. perfringens* generally failed to grow, and declined in numbers after exposure to more abusive storage conditions, especially in low pH product (Fig. 5).

![Figure 1. Influence of time and temperature of incubation, age and pH of bologna on growth of *S. aureus*.](image1)

![Figure 2. Influence of time and temperature of incubation, age and pH of bologna on growth of *S. typhimurium*.](image2)

![Figure 3. Influence of time and temperature of incubation, age and pH of bologna on growth of *E. coli*.](image3)

![Figure 4. Influence of time and temperature of incubation, age and pH of bologna on growth of *B. cereus*.](image4)
Relative increases of \( E. \ coli \) were 1,200- to 6,300-fold and for \( S. \ typhimurium \) were 50- to 640-fold on new bologna, compared to 1- to 74-fold and 0.5- to 80-fold relative increases on old bologna, respectively. These results support the observations of Heiszler et al. (11) that gram negative bacteria could not compete with the natural flora of cooked, cured meats.

The lack of increased inhibition of \( S. \ aureus \) and \( B. \ cereus \) in older product was surprising because both organisms are reportedly inhibited by the normal saprophytic flora of most foods (4, 5, 7, 8, 9, 15, 23, 27). Lactic acid bacteria and group D streptococci are even more antagonistic to \( S. \ aureus \) and \( B. \ cereus \) (4, 5, 8, 9), but some of the test product had lower lactic and group D Streptococcus counts than expected. Competitive inhibition may occur even when low bacterial populations are present (4), however, such an eventuality was not indicated in, nor could it be determined from this study.

Subjective evaluation of the bologna in the sandwiches (i.e. cursory evaluation of appearance and odor) was made after incubation. Gross changes were not observed in any new product samples, even after 25 h incubation at 30 C. This was in accordance with normal expectations that, most food poisoning bacteria grow in foods without visible signs of deterioration. In contrast, however, deterioration of bologna could be detected before significant pathogen growth occurred in old product samples. Typical forms of deterioration were off-odors and greening.

**CONCLUSIONS**

These studies indicated that bologna in sandwiches contaminated with low levels of bacterial pathogens at time of preparation was only likely to be a vehicle of food poisoning under almost unrealistic conditions. Only product contaminated with \( S. \ aureus \), \( B. \ cereus \), enteropathogenic \( E. \ coli \) or salmonellae exposed to highly abusive storage (i.e. more than 8 h at 30 C), with low initial bacterial counts and high initial pH had food poisoning potential. Reasonable handling of sandwiches, especially with refrigerated storage, should give a large margin of safety for such products contaminated with low levels of typical food poisoning pathogens at time of sandwich making.

Although pH and age (microbial competition) are reported and interpreted as the factors affecting the ability of pathogens to grow, other factors might also be related. Older product was less likely to support pathogen growth than new product, even under abusive conditions, and detectable spoilage occurred on old product before, or at the same time as pathogen growth. This study indicated that restrictions on the total microbial load of a vacuum packaged, sliced luncheon meat such as bologna might be totally unrealistic, since the older, high competition product appeared even safer for consumer handling than newer, low competition product.
REFERENCES


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**Report of the Journal Management Committee 1978**

The Journal Management Committee met on August 14, 1978 and discussed the actions taken on previous recommendations as well as new ideas for the improvement of the Journal.

The Committee decided to reaffirm a few of its previous recommendations to the Executive Board and to make new recommendations for the Board's consideration as follows:

1. Although there has been improvement in the amount of material published that is of direct interest to the practicing sanitarian, further progress would be desirable. To this end, the Committee recommends that the Assistant Managing Editor of the Journal review material in other publications to identify and obtain appropriate articles for reprinting in the Journal.

2. The Committee recommends that the Instructors to Authors material be expanded by adding a section specifically for the writing of articles for the practicing sanitarian.

3. The Committee recommends that the Assistant Managing Editor proceed with the plan to appoint a committee of knowledgeable sanitarians and a committee of dairy fieldmen whose responsibilities would be to develop lists of subjects of general concern that should be developed and published in the Journal as well as to identify people who could prepare these articles.

4. The Committee recommends that the Assistant Managing Editor be charged with the review of the Federal Register and similar publications to obtain information of interest to the membership and to prepare appropriate articles on this for the Journal. Similarly, the Morbidity and Mortality Reports of the Center for Disease Control should be reviewed for information of interest to the membership and this information published in the Journal.

5. The Committee recommends that the possibility be investigated of advertisement for the Journal being placed in other journals on a reciprocal basis.

6. The Committee recommends that the listing of articles titles and authors on the title page of the Journal be set up on a 2 column format.

7. The Committee recommends that the source of the material printed as filler in the main section of the Journal and in the News and Events section be identified at the end of each article.

R. B. Read, Jr., Ph.D. Chair

Chairman