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Growth of *Staphylococcus aureus* and Enterotoxin Production in Homemade Mayonnaise Prepared with Different pH Values

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ABSTRACT

The ability of *Staphylococcus aureus* to grow and produce enterotoxins in homemade mayonnaise prepared at different pH values was studied. Ten enterotoxigenic strains, producing one or two enterotoxin types (A, B, C, or D) were inoculated into mayonnaise samples with pH adjusted to values ranging between 4.0 and 5.8, and incubated at 37°C for 7 d. Counts were made on days 1, 3, 5, and 7 and extracts were prepared on day 7 to detect enterotoxin by ELISA. An important difference was seen between those samples prepared with pH below or equal to 4.9 and those over or equal to 5.0; in the range of pH between 4.0 and 4.9 the average of staphylococcal population was 100 CFU/g; at pH 5.0 it was 1.6×10^5, and at pH 5.15 and above it was at least 8×10^6 CFU/g. Enterotoxin was detected only at initial pH over 5.15 and when final pH was not less than 4.7. The highest amount of enterotoxin corresponded to 157.8 ng of SEB/100 g of mayonnaise.

Several staphylococcal food poisoning outbreaks involving different types of salads are recorded every year by the Centers for Disease Control (USA) *(12)*. Although acidity is important in limiting microbial growth in foods, the low pH of salad dressings is partially neutralized when the dressing is used as an ingredient in salads *(12)*. In the occidental world, salad dressings are used frequently with foods. Mayonnaise (a salad dressing emulsion prepared with oil, eggs and either vinegar or lemon juice) is the prototype of these dressings. Eggs, an important ingredient of the dressing, have been reported to promote the synthesis of enterotoxin B (SEB) *(7,10)*. On the other hand, lysozyme present in eggs may inhibit growth of staphylococci *(2,15)*; also, oils because of their acidity may affect microbial growth *(20)*. Commercial mayonnaise has been reported to prevent growth of *Staphylococcus aureus (4)*; however, in many countries mayonnaise for daily consumption is mainly homemade, with the pH values somewhat higher than those of the commerical product. In addition, foods containing mayonnaise may be maintained at ambient temperatures; for example, school children and workers do not always have the opportunity to keep their foods refrigerated before consumption. Also, foods for picnics and banquets often have to be prepared a number of hours before consumption. In some countries, it is customary to exhibit food in a protected but unrefrigerated glass cabinet. The purpose of the present investigation was to study the ability of mayonnaise, prepared in a range of pH values between 4.0 and 5.8, to support staphylococcal growth and enterotoxin production.

MATERIALS AND METHODS

Staphylococcal strains

A variety of staphylococcal strains, kindly provided by M. S. Bergdoll (Food Research Institute, Madison, WI), which produce enterotoxin A (SEA), enterotoxin B (SEB), enterotoxin C (SEC), and enterotoxin D (SED), either alone or in combination were used to inoculate mayonnaise with different initial pH values. These strains were S6 (SEB plus SEA), FRI-100 (SEA), FRI-137 (SEC1), FRI-350 (SEB), FRI-379 (SEB), FRI-472 (SED), FRI-913 (SEA plus SEC), 1143 (SEC), 1173 (SEB) and 1183 (SEC). *S. aureus* strains were grown on BHI (Micro = Adsa, Spain) at 37°C for 24 h.

Mayonnaise samples

Mayonnaise was prepared in the laboratory following the homemade formulation. Two eggs were emulsified with a mixer (Braun, Germany) in 500 ml of sunflower seed oil (maximum acidity, 0.2%), adding salt in enough quantity to render the mayonnaise desirable for sensory attributes. The pH was adjusted by addition of vinegar to the values shown in Table 1. Moreover, 20 other samples were prepared at pH values varying between 4.0 and 4.9 (2, 6, 6, and 6 samples adjusted to pH 4.0, 4.5, 4.8, and 4.9, respectively). To facilitate sample handling and distribution of microorganisms within the emulsion and minimally affecting water activity, mayonnaise was diluted 2:1 in a 62.5% (w/v) sugar syrup and pH re-checked for stability. The mixture was distributed in 200-ml plastic bottles (100 g/bottle) and tyndalized. After sterility controls, samples were stored at 4°C and used before two weeks.

Inoculation

Mayonnaise inoculation was done at a rate of 10^5 CFU/g (+/- 15%). Two inocula were tested with strain 472 (1×10^4

TABLE 1. *Growth and enterotoxin production by the staphylococcal strains inoculated into mayonnaise prepared at different pH values.*

Strain	SET	Initial pH	Final pH	Log	Enterotoxin (ng/100 g)
S6	SEB	5.2	4.7	7.2	4.0
S6	SEB	5.6	5.2	6.9	NS
FRI-100	SEA	5.15	5.1	7.2	45.5
FRI-137	SEC	5.2	5.0	6.5	12.5
FRI-350	SEB	5.8	5.0	6.8	62.9
FRI-379	SEB	5.5	5.2	6.8	157.8
FRI-379	SEB	5.7	4.3	7.9	0
FRI-472	SED	5.0	4.5	4.7	0
FRI-472	SED	5.8	4.8	6.1	42.9
FRI-913	SEA and SEC	5.2	4.3	7.6	0 and 0
FRI-1143	SEC	5.8	5.0	8.1	129.5
FRI-1173	SEB	5.0	4.2	4.3	0
FRI-1183	SEC	5.3	4.6	8.3	0

and 1×10^5 CFU/g). All inoculations were done in duplicate samples. Every strain was inoculated in two mayonnaise samples prepared with pH under 5.0. Samples were incubated at 37°C for 7 d.

Bacterial enumeration and pH measurement

One-gram samples were taken on days 1, 3, 5, and 7. Enumeration was achieved by decimal dilutions using 1% peptone water (Difco) plus 0.1% Tween 20. Bacterial counts were done using duplicate plates and the spread plate technique. Both Baird-Parker agar (BP) (Micro = Adsa, Spain) and Plate Count Agar (PCA) (Micro = Adsa, Spain) were used for staphylococcal counts. The pH, both initial and final, was determined with a pH meter type 414 (Crison, Barcelona, Spain).

Enterotoxin detection

Mayonnaise samples were examined on day 7 for the presence of SEA, SEB, SEC, and SED by the ELISA method as described by Freed et al. *(5)*. Enterotoxin was extracted according to the method proposed by Miller et al. *(11)*, but concentration was achieved by dialysis against polyethylen glycol 20 *M* (Serva). Recovery of entrotoxin added to non-inoculated mayonnaise ranged between 65 and 72%; the minimum enterotoxin detected was 2.0 ng/100 g of mayonnaise.

RESULTS AND DISCUSSION

Cell counts were done in two different media, a selective one, Baird-Parker Agar (BP), and a non-selective medium (PCA), to compare the ability of the two media to support growth of pH-stressed cells. Several investigators have found BP to be adequate for recovery of stressed cells *(3,8,13,16)*, whereas other investigators reported that media containing tellurite were inadequate for recovery of staphylococci from foods *(19)*. However, if egg yolk is present, the effect of tellurite may be counteracted *(18)*. In our experiments with salad dressing, the results obtained with the two media were similar.

An important difference was seen between those samples prepared with the pH below or equal to 4.9 and those over or equal to 5.0. At an initial pH of 4.0 no live cells were recoverable (Fig. 1). Less than 100 CFU/g

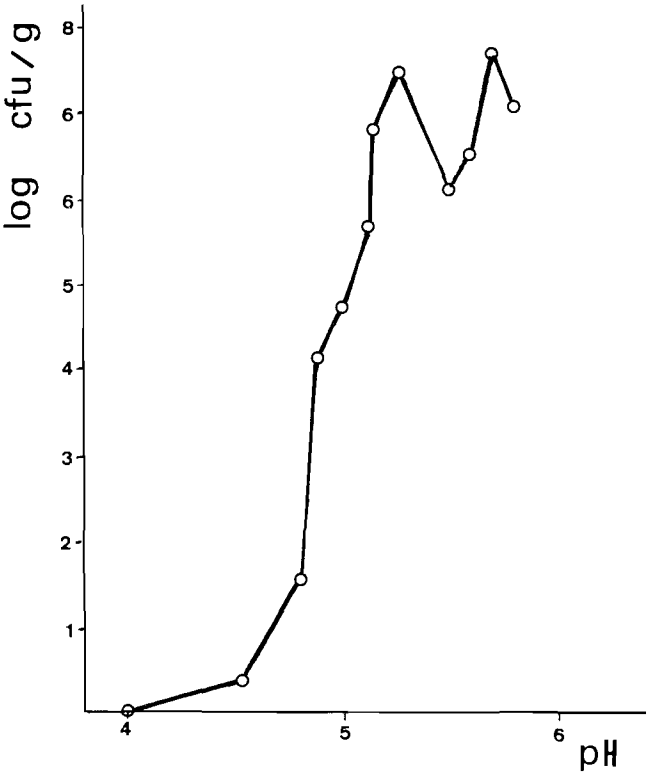

Figure 1. *Average values (log_{10} CFU/g) for staphylococcal populations at different pH values independent of strain.*

were recoverable after 7 d of incubation at pH values between 4 and 4.9. At pH 5.0, the average recovery was 1.6×10^5 CFU/g (log_{10}, 5.2), whereas at pH 5.15, the recovery was 8×10^6 CFU/g (log_{10}, 6.9). Staphylococcal growth did not occur at initial pH values of 4.9 or below; in some instances the staphylococci were inactiviated within a few hours. On the other hand, multiplication of *S. aureus* did occur in 48 to 72 h, even as much as 1000-fold for strains FRI-1183 and FRI-1143, at initial pH values above 5.0, with counts staying above 10^6 CFU/g throughout the incubation period. It is indicated from these results that in mayonnaise the pH may

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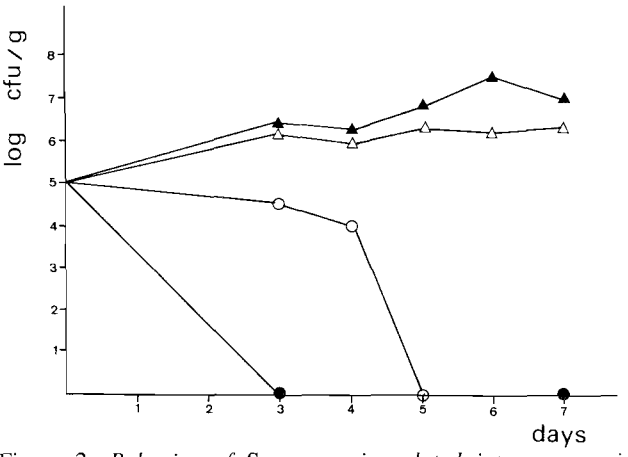

Figure 2. *Behavior of* S. aureus *inoculated into mayonnaise samples prepared with pH 5.6 (▲——▲), 5.2 (Δ——Δ), 4.8 (○——○), and 4.5 (●——●).*

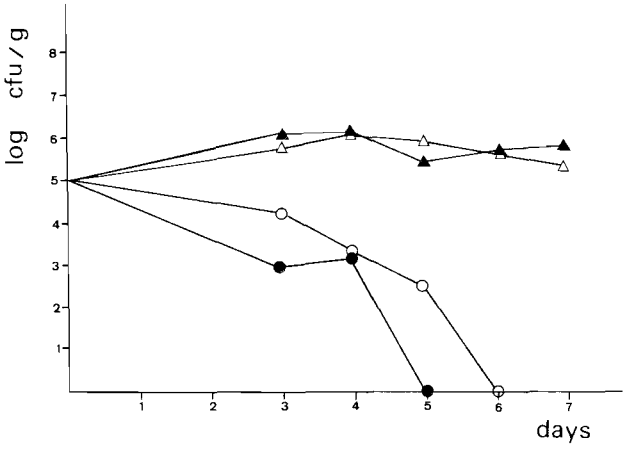

Figure 3. *Behavior of* S. aureus *FRI-137 inoculated into mayonnaise samples prepared with pH 5.5 (▲——▲), 5.2 (Δ——Δ), 4.8 (○——○), and 4.5 (●——●).*

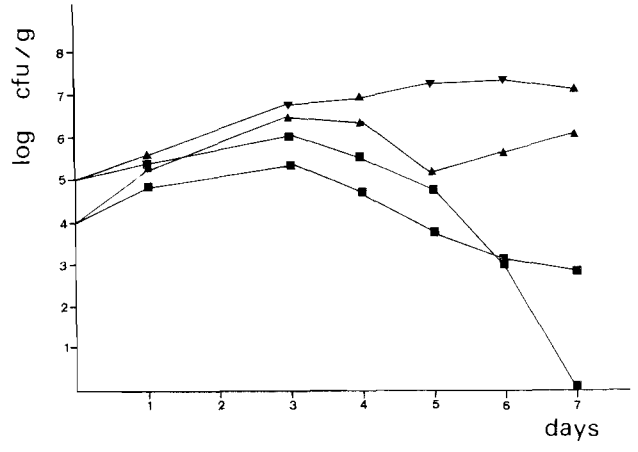

Figure 4. *Behavior of* S. aureus *FRI-472 inoculated into mayonnaise samples prepared with pH 5.8 (▲——▲) and 5.0 (■——■), as influenced by inoculum.*

be a critical factor in the range between pH 4.9 and 5.1. This is in agreement with the results of Barber and Deibel *(1)* who reported that in aerobiosis staphylococcal growth was possible at pH values equal to or above 5.1. The differences in cell counts above pH 5.1 are statistically insignificant; the data are in disagreement with those of Magrini et al. *(9)* who found that the growth rate of strain 137 was less at pH 5.4 than at pH 6.0.

The daily cell counts in inoculated samples prepared at two pH values are shown in Fig. 2, 3, and 4. The counts remained stable when the pH was above 5, but decreased gradually at lower pH values. The effect on inoculum size is presented in Fig. 3. No enterotoxin was detected in the mayonnaise when the initial pH was under 5.0; however, all enterotoxins were produced at initial pH values above 5.1. The enterotoxin production by the different strains at pH values over 5.0 is shown in Table 1. The increased production of enterotoxin a higher pH values can be attributed to more active staphylococcal growth.

The fact that no enterotoxin was detected in the presence of good growth in some instances (Table 1) has been observed by other investigators. Genigeorgis et al. *(6)* reported that growth of strain 137 was possible in the pH range of 5.0 to 6.5, although SEC could be detected only at pH values above 5.5. It has been reported also that pH affects differently the synthesis of the enterotoxins; SEA was produced at most pH values allowing growth *(14,17)*, whereas production of SEB and SEC was decreased at low pH values. However, we were unable to detect production of either SEA or SEC by strain 913 although good growth was observed. In this instance and in those in which good growth was observed but no enterotoxin was detectable, the final pH of the mayonnaise was 4.6 or below. It is possible that enterotoxin was produced but either inactivated or associated with the ingredients of the mayonnaise which rendered it non-extractable.

Several factors may affect enterotoxin synthesis in mayonnaise, both positively and negatively. The lipolytic activity has been reported to limit staphylococcal growth by production of lipoic acids that are toxic for staphylococcal growth and may be inhibitory for the production of enterotoxins *(20)*. Ovoalbumin and conalbumin may have an enhancing effect on enterotoxin production *(7)*, whereas, presence of lysozyme can inhibit growth of staphylococci *(2,15)*. This substance probably was present in minimal concentrations.

Morita and Woodburn *(12)* indicated that the initial pH of mayonnaise of 3.4 was raised to 4.0 when it was added to salads, with staphylococcal counts increasing 10 to 1000 times after 8 h of incubation at 37°C. These results agree with our observation that the pH was increased by 0.6 to 0.7 unit (5.0 to 5.7) when the salad ingredients were added to the mayonnaise. The fact that no organoleptic changes could be observed when counts were above 10^8 CFU/g, enhances the possibility of staphylococcal food poisoning when salads containing enterotoxigenic organisms are mishandled.

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