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Microflora of Maize Prepared as Tortillas

EDWARD CAPPARELLI[1]* AND LEONARDO MATA[2]

Division of Environmental Biology, Institute of Nutrition for Central America and Panama, Guatemala City, Guatemala

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Very little is known of the microflora in tortillas, the major component in the diet of many Guatemalans and other Central Americans. Based in a Guatemalan highland Indian village, this study examined the types and amounts of bacteria, yeasts, and molds in tortillas and in their maize precursors. Coliforms, *Bacillus cereus*, two species of *Staphylococcus*, and many types of yeast were the main contaminants, but low concentrations of alpha-hemolytic *Streptococcus*, facultative *Clostridium*, and other bacterial types were also found. When tortillas were first cooked, the bacterial counts dropped to 1,000 or fewer organisms per g, a safe level for consumption. Under village conditions, bacterial counts regained precooking levels (about 10^8 organisms/g) within 24 h and rose even higher after 48 h. Reheating caused very little change; hence, bacterial levels remained very high in old tortillas kept for later consumption. A search for the sources of contamination showed that most came from water used in preparation and from the soiled hands of women preparing the tortillas. As an attempt to correct certain nutritional needs of the population, the Institute of Nutrition for Central America and Panama initiated a tortilla fortification project in the Guatemalan village. The bacterial counts in fortified tortillas did not differ significantly from those in ordinary tortillas. Furthermore, the level of contamination was constant among tortillas of different sizes and among tortillas made from different types of maize.

Tortillas are flat, cooked pancakes made from lime-treated corn flour (*Zea mays*) which constitute as much as 60 to 80% of the diet of rural Guatemalan Indian populations and are a common dietary staple throughout Central America. Because of the relatively poor quality of corn protein, the Institute of Nutrition for Central America and Panama devised a plan for fortifying tortillas with a mixture of soy flour, lysine, iron, thiamine, riboflavin, niacinamide, and vitamin A (11).

The importance of tortillas as a food product, the high incidence of bacterial gastrointestinal diseases among Guatemalan Indians (5), and the lack of information concerning this food's microbiological content led to this study. The microbiological implications of fortification were examined by comparing differences in bacterial growth between fortified and unfortified tortillas. Studies were made for tortillas of different sizes and thicknesses and for those made with different strains of maize.

The work was done in the Mayan Indian village of Santa María Cauqué at an altitude of 6,200 feet (ca. 1,889.8 m) in the Guatemalan highlands. During the period of the study environmental temperatures ranged from 10 to 28 C, with an average rainfall of 3.5 mm/day (range, 0 to 28 mm/day; this rainy season extends from June to October). Indian women prepared all the tortillas in their houses; many village households supplied the samples, which were collected only up to 48 h after preparation because tortillas are not kept longer under ordinary circumstances. Tortillas are usually consumed within several hours after preparation; older tortillas are eaten only on trips.

In an attempt to find the sources of bacteria in tortillas, we examined the maize precursors: dry maize, nixtamal (maize that has been cooked with limestone for 50 min and then soaked in water for 14 h [2]), masa (a wet, pasty flour that results from the grinding of nixtamal at the mill), and the water used in preparation. The masa is rolled and patted into a flat pancake and then cooked for 4 or 5 min on a comal, a large, flat, hardened clay plate placed over an open fire. Reheating is also done on the comal, but for a shorter time interval (1 to 2 min).

Although data were obtained in Santa María

[1] Present address: Department of Nutrition and Food Science, 16-325, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

[2] Present address: Director, UNISAN, School of Medicine, University of Costa Rica, San Pedro, Costa Rica.

Cauqué, conditions in other highland villages are almost identical, and results can be safely generalized to cover all such highland villages.

MATERIALS AND METHODS

Samples. Samples, made in individual homes, were randomly selected from among the tortillas and maize sources being prepared for the family's daily consumption. The tortillas were left towel-wrapped in a basket in the same house where they were prepared, until time of culturing (24 or 48 h after preparation). The same women who had made the tortillas also reheated them for later stages of study.

Samples of masa and the water used to moisten it were obtained during tortilla preparation. Maize was obtained the previous night so that it would be from the same batch used in the other samples; nixtamal was obtained at the mill just before being ground into masa. All samples were cultured 15 to 30 min after receipt, with special care taken to process fresh and reheated tortillas immediately.

Dilutions. Tortillas were brought directly to the laboratory at the village health clinic. A 10-g portion of tortilla pieces was ground in a mortar with sterilized sand and placed in a bottle containing 90 ml of charcoal water (tap water that had previously been adsorbed with charcoal, filtered, and sterilized) (14). This 10^{-1} dilution was mixed and then agitated for 5 min; progressive dilutions to 10^{-9} were prepared in charcoal water as needed (5).

Samples for maize, nixtamal, and masa were prepared as above; kernels were ground in a Waring blender when necessary.

The original sample of water was used as 10^{0} dilution, with further dilutions to 10^{-6} prepared.

Media used. Table 1 shows the media and types of incubation used. Total bacterial count was determined by using Trypticase soy agar (supplied by Baltimore Biological Labs, Cockeysville, Md., as were GasPak generators and all other media, except for *cereus*-selective agar, which came from Merck & Co., Inc., Rahway, N.J.), pour-plate method; Tergitol-7 agar with 0.004% triphenyl-tetrazolium chloride, *Salmonella-Shigella* agar, and brilliant green agar were used to select for coliforms. One gram of minced tortilla was also placed in tetrathionate Selenite-F Enrichment broths and restreaked on *Salmonella-Shigella* and brilliant green agars after 24 h of incubation to select for pathogenic coliforms. Mannitol salt agar was used to select for staphylococci; *cereus*-selective agar with 10% egg yolk emulsion in physiological saline (1:1) solution was the medium used to select for *Bacillus*. Schaedler base medium (14) was modified by adding 1% Trypticase soy broth instead of Trypticase and was used in anaerobic culturing (10). Sabouraud dextrose agar and Mycosel agar were used for yeasts and molds.

Media inoculation and incubation. In inoculating plates from various dilutions, we used a 0.01-ml calibrated platinum loop and placed four dilutions on each plate. The plates for anaerobic culture were inoculated with 1.0 and 0.1 ml of the 10^{-1} dilution by the spread-plate method. Anaerobic plating was done both before and after boiling the 10^{-1} dilution for 30 min. Anaerobiosis was achieved by means of GasPak disposable generators (3). Table 1 lists the incubation periods and temperatures.

Enumeration and identification of bacteria. Enumeration was made directly from the plates; representative colonies from each plate were Gram stained (Kopeloff method) and further identified through biochemical tests (1, 6, 7, 9, 12, 13). All colonies found in anaerobic culture were subcultured aerobically at 37 C for 24 h; almost all were found to be facultative.

RESULTS

Microflora of tortillas. Coliform levels of 10^{3} to 10^{7} organisms/g were obtained in 24- to 48-h-old tortillas; *Alcaligenes faecalis*, *Klebsiella* sp., and *Escherichia coli* were the most common of those encountered (Fig. 1), indicating a high level of fecal contamination during tortilla preparation. These same species were encountered in the water at levels of 10^{4} to 10^{8} organisms/ml. Cooking the masa killed most coliforms, but a sufficient number survived to return the concentrations to precooking levels within 24 to 48 h of storage at room temperature (Fig. 2).

TABLE 1. *Media and conditions of incubation for microbiological examination of tortillas*

Agar[a]	Microorganisms	Type of incubation	Period of incubation (h)	Growth range ($\log_{10}$)
TS	Total bacterial count	Aerobic (37 C)	24	3–9
T_7T, SS, BG	Coliforms	Aerobic (37 C)	24	<3–7
MS	*Staphylococci, micrococci, bacilli*	Aerobic (37 C)	24	3–8
SE	*Bacilli, staphylococci*	Aerobic (37 C)	24	3–9
BC-I	*Clostridia, lactobacilli, bifidobacteria, staphylococci, streptococci, sarcinae*	Anaerobic (37 C)	48	0–3
S, M	Yeasts, molds	Aerobic (22 C)	7 days	<3–9

[a] TS, Trypticase soy agar; T_7T, Tergitol-7; agar with 0.004% triphenyl-tetrazolium chloride; SS, *Salmonella-Shigella* agar; BG, brilliant green agar; MS, mannitol salt agar; SE, *cereus*-selective agar; BC-I, modified Schaedler base medium; S, Sabouraud dextrose agar; M, Mycosel agar.

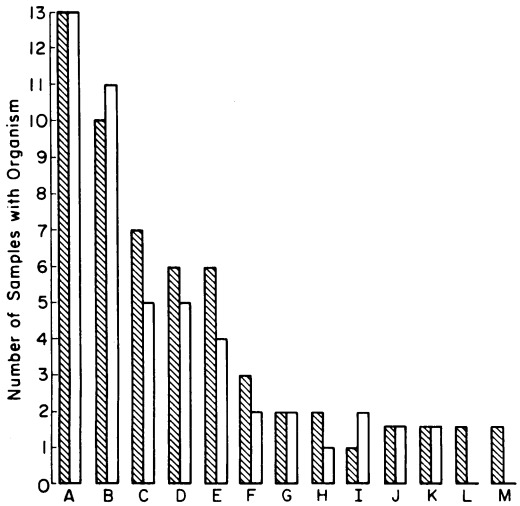

FIG. 1. *Identification of coliforms in tortillas. Fortified tortillas are represented by the striped bar; unfortified tortillas are represented by the white bar. This figure includes coliforms obtained both directly and by enrichment. (A) Total samples with coliforms; (B) Alcaligenes faecalis; (C) Escherichia coli; (D) Klebsiella pneumoniae; (E) Enterobacter hafniae; (F) Pictobacterium; (G) Enterobacter cloacae; (H) Serratia sp.; (I) Citrobacter freundii; (J) Proteus mirabilis; (K) Enterobacter liquefaciens; (L) Enterobacter aerogenes; (M) Klebsiella ozaenae.*

Neither *Shigella* nor *Salmonella* were observed, even with tetrathionate and Selenite-F Enrichment; however, this finding does not rule out tortillas as a possible vector for pathogenic coliforms. Such bacteria would probably be much overgrown by other coliforms. The high fecal contamination rates make tortillas a prime candidate for further study in this area. Furthermore, the high *E. coli* counts suggest the probable presence of enteropathogenic *E. coli*.

Staphylococci. Staphylococci levels in tortillas reached 10^7 to 10^8 organisms/g (Fig. 2), with equal numbers of *Staphylococcus aureus* and *S. epidermidis* found both in tortillas and in their maize sources. The high counts of *S. aureus* in the older tortillas could have significant health implications, because certain strains may produce a heat-stable enterotoxin in foods and also because of the possibility of staphylococcal infection.

Bacilli. *Bacillus cereus*, *B. macerans*, *B. megaterium*, *B. polymyxa*, and *B. subtilis* were found in the maize precursors, with no one species predominating. Most species of *Bacillus* were killed during cooking, leaving almost exclusively *B. cereus*. Certain strains of *B. cereus*, in high concentrations, can cause food poisoning by producing an enterotoxin (8). Hence, the high *B. cereus* levels observed in tortillas after 24 or 48 h of storage (up to 10^9 organisms/g) may make their consumption hazardous (Fig. 2). *B. subtilis* and *B. megaterium* are also occasionally observed in tortillas.

Clostridia. All clostridia encountered in anaerobic culture were found to be facultative. In tortillas, they appeared in low concentrations (10^1 to 10^2 organisms/g). Further identification was not pursued because facultative clostridia are nonpathogenic.

Streptococci. Streptococci were encountered only in anaerobic culture and never in concentrations greater than 10^2 organisms/g. The streptococci present were almost exclusively alpha-hemolytic, so further identification was not pursued.

Other anaerobes. A variety of other species were encountered, including *Sarcina*, *Lactobacillus*, *Coccobacillus*, and *Micrococcus*, always in very low dilutions and almost always facultative. Because these species are not pathogenic to man, they were not further identified.

Yeasts. Both tortillas and maize sources supported a great quantity and variety of yeasts. Because yeast identification is both difficult and complex, only precursory examination was done, although further work in this area would be of great interest. Examinations revealed species of a red-pigmented *Rhodotorula*, *Candida*, *Trichosporon*, *Geotrichum*, *Torulopsis*, *Saccharomyces*, *Pytirosporum*, and others.

Molds. Molds were found in very high concentrations in tortilla precursors, but were almost completely absent in tortillas, even up to 4 days after preparation. Colony morphology showed species of *Penicillium*, *Aspergillus*, *Neurospora*, and *Rhizopus* in the maize precursors. No further identification was carried out because these species were not present in any of the tortillas. However, the possibility of mycotoxin production by *Aspergillus* and *Penicillium* during the growth of maize should be investigated. Some mycotoxins are thought to be carcinogenic after repeated consumption (4). Unless they are inactivated by the limestone treatment of maize kernels (2, 15), their presence could have serious health implications.

Microfloral population of tortilla precursors during and after preparation. During early stages of tortilla preparation, bacterial levels rose rapidly, with most initial bacterial contamination resulting from the water used in preparation (Fig. 3) and probably from the hands of the women preparing the tortillas.

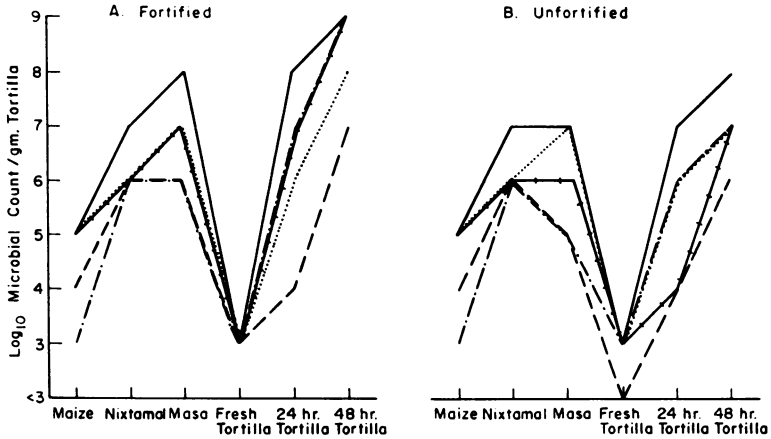

FIG. 2. *Microfloral levels in maize during preparation and storage of tortillas. Symbols:* (——), *total counts;* (-----), *coliforms;* (·····), *Staphylococcus sp.;* (-·-·-), *Bacillus sp.;* (—+—+), *yeasts. A slight difference was observed in flora levels between fortified and unfortified tortillas.*

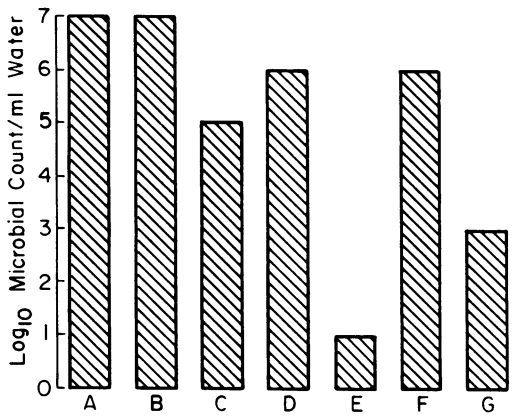

FIG. 3. *Microflora in water used in tortilla preparation. (A) Total count; (B) coliforms; (C) Staphylococcus sp.; (D) Bacillus sp.; (E) Clostridium sp.; (F) yeasts; (G) molds.*

Most bacteria were killed by cooking (Fig. 2). Enough survived, however, to return bacterial concentrations to precooking levels after 24 h and to even higher levels after 48 h. Yeast contamination was found in maize and throughout preparation. Molds did not reappear after preparation of tortillas, indicating that cooking effectively killed them. After 7 to 10 days, molds grew again on tortillas because of air contamination during storage.

Fortified tortillas supported slightly greater aerobic growth than unfortified tortillas, both after 24 and 48 h, but the high levels present make small variations relatively insignificant. A 2-logarithm range in *B. cereus* levels between fortified and unfortified tortillas was the most important disparity; the several-logarithm difference in nonpathogenic yeasts had little effect on the safety of tortillas. Therefore, the microbiological difference between fortified and unfortified tortillas is minimal with respect to the safe consumption of either kind.

Fresh tortillas are safe for consumption, but stored tortillas have sufficiently high bacterial concentrations within 24 h to make them dangerous to health. Most significant in tortillas more than several hours old are the extremely high levels of *S. aureus*, *B. cereus*, and coliform bacteria.

Tortillas of various sizes and from several different types of maize showed no significant difference in bacterial counts or species isolated.

Effects of reheating tortillas. In 24-h-old tortillas, reheating dropped the bacterial count 2 to 3 logarithms, but the counts nevertheless remained very high (Fig. 4). After 48 h, the reheating decrease was slightly less, leaving extremely high bacterial counts (10^6 to 10^7 organisms/g). This indicated that the usual 1- to 2-min reheating time for the older tortillas did not render the tortillas safe for consumption. Possible existence of enterotoxins from *B. cereus* and *S. aureus* further increased the danger. Because such enterotoxins are often heat stable and pathogenic, a study of their existence in tortillas 48 h old or more would be worthwhile.

Toasting or longer periods of reheating would possibly lower bacterial concentrations to safe levels. This is currently unfeasible, however, because additional heating would alter taste and texture and also increase fuel consumption.

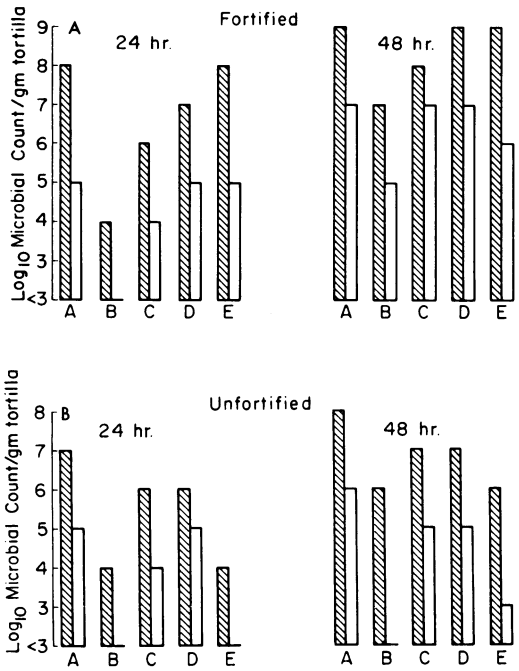

FIG. 4. *(A & B) Effects of reheating on tortilla microflora. Cold tortillas are represented by the striped bar and reheated tortillas by the white bar. Ages of tortillas and fortification status are shown in the figure. (A) Total count; (B) coliforms; (C) Staphylococcus sp.; (D) Bacillus cereus; (E) yeasts.*

DISCUSSION

Tortillas offer a very rich medium for bacterial growth. With the Indians' relative disregard for sanitary procedures, plus the high levels of bacterial and fecal contamination found in the water used during tortilla preparation, a great amount of contamination occurs during the preparation process. Although cooking destroys many of these bacteria, a sufficient number survive to bring bacterial concentrations up to precooking levels within 24 to 48 h. Because of the possible production of enterotoxins and because of the possible contamination by other pathogens, the Indians should avoid consuming older tortillas that have been stored unrefrigerated.

The results indicate that sanitation and the use of comparatively clean water should be stressed to those living in such villages. Some of the water samples from tortilla preparation arrived in our laboratory clouded with masa and containing dead flies. Water used in tortilla preparation should be changed daily instead of being used for an extended period. Adopting such simple sanitation procedures would do much to lower contamination in tortillas that are not consumed quickly after preparation, especially in tortillas prepared for trips.

This study involved 46 women in one highland village where conditions were similar to those of other indigenous villages. At lower elevations in Central America, ambient temperatures are much higher, and the bacterial growth can be assumed to be even more rapid. Hence, the need for better sanitary methods in the preparation of tortillas must affect populations throughout Central America.

ACKNOWLEDGMENTS

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