Microbiology of Corn and Dry Milled Corn Products[1]

R. J. BOTHAST, R. F. ROGERS, and C. W. HESSELTINE, Agricultural Research Service, U.S. Department of Agriculture, Northern Regional Research Laboratory, Peoria, Illinois 61604

ABSTRACT

A microbiological survey was conducted on corn, grits, and flour from three mills in the Kansas-Nebraska-Oklahoma area. Duplicate samples were taken 1 week apart in November, February, May, and August. The frequency of mold infection for whole-corn kernels ranged from 55 to 77% and was highest in February. Species of *Fusarium* and *Penicillium* occurred in approximately 50% of the kernels examined. In addition, *Aspergillus flavus,* other species of *Aspergillus, Nigrospora, Alternaria, Helminthosporium, Trichoderma, Chaetomium,* and mucoraceous fungi were identified. Counts per gram of sample were determined for total aerobic bacteria, psychrotrophic bacteria, total coliforms, fecal streptococci, total thermophilic aerobic spores, flat-sour spores, total molds, *A. flavus,* and actinomycetes. Microbial counts were usually lowest in grits, followed by whole corn, and then flour. All samples were negative for salmonellae and only a few contained low levels of fecal coliforms, *Clostridium perfringens* and coagulase-positive staphylococci.

Corn enters the U.S. consumer's diet in the form of grits, meal, and flour from the dry miller. These milled corn products go primarily into such foods as: breakfast cereals, snacks, malt beverages, pancakes, mush, muffins, corn bread, baby foods, bakery products, and meat products. The combined per capita consumption of these milled fractions has increased from 10.5 lb. in 1961 to 11.6 lb. in 1972, while per capita consumption of corn in breakfast cereals has increased 50% during the past 20 years (1).

Current publicity (2) on food-borne illnesses and intoxications has intensified interest in microbial quality and safety of food products and ingredients.

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Abnormally high microbial populations of any type of organisms are indicative of poor raw material or poor methods of handling. A survey on the numbers and kinds of microorganisms present in whole corn and milled corn products was undertaken to answer questions about microbial quality and safety in the dry-milling industry. An exact interpretation of specific numbers can be made only in light of full information regarding the product under study and its intended use.

This survey also is a part of continuing studies at the Northern Regional Research Laboratory on the microbial ecology of cereal products.

MATERIALS AND METHODS

The American Corn Millers Federation provided whole corn, grits, and flour from three mills in the Kansas-Nebraska-Oklahoma area. Duplicate samples were taken 1 week apart in November, February, May, and August. Samples were collected and coded as described in our 1970–71 microbial survey (3). The methods primarily followed were those outlined by the American Public Health Association (4); all media were purchased from Baltimore Biological Laboratories.[2] After thoroughly mixing each sample, 11 g. was aseptically transferred to a 6-oz. rubber-stoppered dilution bottle containing 99 ml. of sterile 0.1% peptone and approximately 10 g. of sterile sand. From this primary dilution (1:10), serial dilutions (without sand) were prepared. Analysis of variance was conducted according to Snedecor and Cochran (5) on the $\log_{10}$ of the actual counts.

Total Aerobic Bacteria

From appropriate dilutions, 1.0 and 0.1 ml. were pipetted in duplicate into sterile disposable petri dishes to which Standards Methods Agar containing 100 p.p.m. cycloheximide was added. Plates were incubated at 28°C. and counted after 3 days.

Psychrotrophic Bacteria

Counts of bacteria able to grow at refrigerator temperatures were determined in a similar manner to total counts, except that the plates were incubated at 5°C. and counted after 14 days.

Total Coliforms

A 1-ml. aliquot of the primary dilution (1:10) was transferred into each of 10 petri plates and poured with violet red bile (VRB) agar. Solidified plates were overlaid with 5 ml. of VRB agar. Plates were incubated at 35°C. and typical colonies (dark red, 0.5 mm. or more in diameter) were counted after 24 hr.

Fecal Coliforms

Two typical VRB colonies were transferred from each plate into tubes of phenol red-lactose broths. Tubes of broth were incubated at 35°C. for 24 hr. Two drops (0.1 ml.) from each positive tube (acid and gas) were transferred into EC broth fermentation tubes, which were incubated at 45.5°C. for 24 hr. EC tubes displaying gas were considered positive for fecal coliforms.

[2]The mention of firm names or trade products does not imply that they are recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

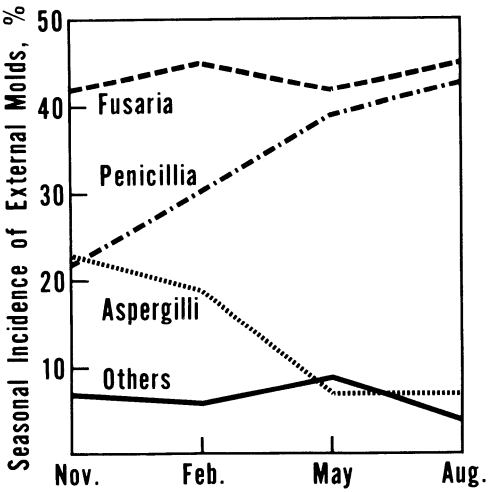

Fig. 1. Relationship between external molds averaged over mill and fraction for each month.

Fecal Streptococci

A 1-ml. aliquot of the 1:10 dilution was transferred into each of 10 petri plates, and the plates were poured with KF streptococcal agar. Plates were incubated at 35°C. for 48 hr., and all red or pink colonies were counted.

Coagulase-Positive Staphylococci

Ten grams of each sample was transferred into 250 ml. of cooked meat medium containing NaCl. The medium was prepared by adding 31.25 g. (12.5%) of cooked meat and 25.0 g. (10.0%) of NaCl. The cooked meat medium was incubated at 37°C. for 24 hr., and 0.1 ml. of the medium was spread on each of two plates of Vogel and Johnson (VJ) agar. VJ plates were incubated at 37°C. and examined after 24 and 48 hr. Two or more representative colonies which reduced tellurite were transferred to brain-heart infusion agar slants and incubated at 37°C. for 24 hr. Slants were scraped and emulsified in 0.3 ml. of rabbit coagulase plasma, incubated in a 37°C. water bath for 4 hr., and examined for coagulation.

Salmonella

Thirty grams of each sample was transferred into 100 ml. of tetrathionate broth and incubated at 43°C. After both 24 and 48 hr., sample was streaked on brilliant green agar plates containing 80 mg. sodium sulfadiazine per liter, bismuth sulfite agar, and Hektoen enteric agar. These plates were incubated for 24 hr. at 37°C. Two colonies from each plate suspected of being positive were picked and inoculated on triple sugar-iron (TSI) agar slants. Those cultures yielding positive reactions for salmonellae on TSI were typed against *Salmonella* O polyvalent antisera.

Clostridium perfringens

The enrichment procedures of Lillard (6) were modified. Five grams of each

TABLE I. MICROBIAL CONTENT[1] OF CORN, GRITS, AND FLOUR

Corn Sample	Total Aerobic Count	Psychrotrophic Count	Total Coliform Count	Fecal Coliforms	Fecal Streptococci Count	Coagulase-Positive Staphylococci
Whole kernels						
November	4,200 a[2]	370	44	Neg	16	Neg
February	20,000 b	1,600	16	Pos (1/6)	63	Neg
May	2,000 a	130	7	Neg	3	Neg
August	1,900 a	82	2	Neg	1	Neg
Geometric mean	4,300 A	330 A	10 A		9 A	
Grits						
November	2,900 ab	38	10	Neg	2	Neg
February	1,400 b	45	1	Neg	1	Neg
May	7,200 a	360	3	Neg	4	Neg
August	370 c	41	0	Neg	1	Neg
Geometric mean	1,900 B	91 B	2 B		2 B	
Flour						
November	16,000 a	350	93	Neg	88	Neg
February	13,000 a	880	39	Neg	140	Neg
May	16,000 a	1,300	33	Pos (1/6)	170	Pos (1/6)
August	9,100 a	150	6	Neg	87	Neg
Geometric mean	13,000 C	520 A	29 C		120 C	

[1]Each value is the geometric mean of six samples (two from each of three mills) expressed on a count/g. basis.

[2]Numbers with no letter in common are significantly different ($P<0.05$).

Salmonella	*Clostridium perfringens*	Thermophilic Spores: Total aerobic spore count	Thermophilic Spores: Flat-sour spore count	Total Mold Count	*Aspergillus flavus* Count	Aerobic Actinomycete Count
Neg	Pos (2/4)	2	1	5,700	29	33
Neg	Pos (2/6)	3	1	19,000	10	57
Neg	Neg	4	3	16,000	9	22
Neg	Neg	2	1	4,800	9	30
		2 A	1 A	9,700 A	12 A	33 A
Neg	Neg	10	5	460	11	8
Neg	Neg	4	2	280	6	5
Neg	Neg	14	8	490	6	10
Neg	Neg	6	5	580	8	3
		8 B	4 B	440 B	8 A	6 B
Neg	Neg	54	18	4,100	36	49
Neg	Neg	42	18	5,000	41	29
Neg	Neg	42	35	1,800	44	86
Neg	Neg	27	24	2,700	23	37
		40 C	23 C	3,200 C	35 B	46 A

sample was transferred into 40 ml. of freshly boiled and cooled thioglycollate broth in 25 mm. × 200 mm. tubes. These tubes were then heated at 80°C. for 15 min. to shock spores and reduce competitive growth. Further selectivity for *C. perfringens* was obtained by incubating the tubes at 37°C. for 18 hr. Serial dilutions were then plated and overlaid on SPS agar and incubated anaerobically (Gaspak) for 24 hr. at 37°C. Typical black colonies (usually two from each plate) were transferred into tubes of thioglycollate broth. The presence of *C. perfringens* was confirmed on the basis of gram-positive bacilli, which are nonmotile, reduce nitrate to nitrite, and are indol-negative.

Thermophilic Spores (Total Aerobic and Flat Sour)

Into 100 ml. of melted and tempered dextrose tryptone (DT) agar was transferred 20 ml. of the 1:10 dilution from each sample. The agar mixture was shaken and then heated 25 min. in an Arnold steamer. After heating, the mixture was cooled to 45°C. and poured equally into five petri plates. When the plates had hardened, they were overlaid with DT agar to prevent spreading. The plates were incubated at 55°C. and read at 24 and 48 hr. All colonies present signify the total thermophilic aerobic spore count, whereas those that were round, 2-3 mm. diameter, and usually surrounded by a yellow halo, made up the flat-sour spore count.

Total Mold Count

From appropriate dilutions, both 1.0 and 0.1 ml. were pipetted in duplicate into sterile disposable petri dishes to which was added yeast extract agar (0.4% yeast extract, 0.4% dextrose, 1.0% malt extract, and 1.5% agar) containing 30 p.p.m. tetracycline. Plates were incubated at 28°C. and counted after 3 days. After 5 days, the predominate external molds were identified based on cultural and morphological characteristics.

TABLE II. SEASONAL DISTRIBUTION OF MOLDS INFECTING SURFACE-STERILIZED WHOLE CORN (%)

Mold	November 1971	February 1972	May 1972	August 1972
Kernels infected	75	77	62	55
Fusarium[1]	31.5[2]	39.3	29.0	15.0
Penicillium	20.0	26.0	16.3	20.0
Aspergillus flavus	4.5	5.0	6.0	5.0
Aspergillus niger	5.5	3.7	7.0	3.0
Other *Aspergillus* spp.	8.0	6.0	6.7	7.7
Nigrospora	6.5	2.3	2.3	1.0
Alternaria	1.0	1.3	1.0	0.3
Helminthosporium maydis	0.5	0.7	0.7	0.3
Trichoderma	0.5	0.7		
Mucor		0.3		0.3
Rhizopus		1.0	0.7	0.7
Absidia				0.3
Chaetomium			0.3	0.7
Other	2.0	0.3		0.7

[1]Primarily *F. moniliforme*.
[2]For each date, 300 kernels were examined. Values are the percentage of kernels infected with a particular mold.

Aspergillus flavus Count

Aspergillus flavus and closely related species in each sample were enumerated on a newly developed *Aspergillus* differential medium (ADM, 1.5% tryptone, 1.0% yeast extract, 0.05% ferric citrate, and 1.5% agar) containing 30 p.p.m. tetracycline (7). The procedures outlined for total molds were followed, except that only yellow-orange reverse pigmented colonies were counted.

Aerobic Actinomycetes

This population was estimated by counting hard, leathery colonies appearing on the same plates prepared for the enumeration of total aerobic bacteria after the plates had remained at 28°C. for an additional 11 days, giving a total incubation of 14 days. After 2 weeks, the sporulating actinomycete colonies are easily distinguished from the dried, degenerate bacterial colonies.

Frequency and Kind of Mold Infection

The percentage of mold-infected kernels was determined on all samples of whole corn. From each sample were selected 50 whole kernels which were surface-sterilized with 1% sodium hypochlorite (NaOCl) for 1 min. and washed twice with sterile water. The kernels were plated on malt-extract agar (3.0% malt extract and 1.5% agar)—five kernels to a plate—and incubated at 28° C. for 7 days. On the basis of cultural and morphological characteristics, the infecting or internal fungi were identified.

RESULTS

The microbial content of whole corn, corn grits, and corn flour over the four sampling months is presented in Table I. Although geometric means (averages based on $\log_{10}$ of the actual counts) are given, it should be emphasized that microbial enumeration varies considerably. Statistical analyses of the data showed that total aerobic bacteria counts were significantly higher in February for whole corn, significantly lower in August for grits, and consistently the same for flour. Analyses for fraction differences agreed with last year's survey (3) in that grits contained significantly fewer bacteria (1,900 per g.) than corn (4,300 per g.) or flour (13,000 per g.) and flour counts were significantly greater than the other two. These same fraction differences also applied to counts for psychrotrophic bacteria, coliforms, fecal streptococci, *A. flavus,* and actinomycetes.

Like the bacterial data, total mold counts correlated with last year's survey (3) and were significantly lower in grits (440 per g.) than in corn (9,700 per g.) and flour (3,200 per g.). However, unlike bacteria counts, mold counts in flour were significantly lower than in the whole corn.

An analysis of correlation between the organisms enumerated showed that total aerobic bacteria counts significantly correlated only with psychrotrophic ($r = 0.53$) and actinomycete ($r = 0.36$) counts. Psychrotrophic counts correlated ($r = 0.46$) with total mold counts, and as expected, total thermophilic aerobic spore counts correlated ($r = 0.98$) with flat-sour spore counts. Otherwise, there were no significant correlations.

Low levels of fecal coliforms were detected in one lot of whole corn and in one flour sample. Coagulase-positive staphylococci were found in one sample of

flour while four whole corn samples contained low levels of *C. perfringens*. All samples were negative for salmonellae.

Tentative identification of each mold colony counted on dilution plates indicated that species of *Fusarium, Penicillium,* and *Aspergillus* made up most of the external mold population. Figure 1 depicts the relationship between these major genera averaged over mills and fraction for each month. As expected from the literature (8,9) and last year's survey (3), penicillia increased with storage more than the other fungi. In contrast, fusaria remained more or less constant, and aspergilli decreased. Perhaps penicillia overgrew aspergilli judging from the obvious inverse relationship demonstrated.

The frequency of mold-infected kernels of unmilled corn ranged from 55 to 77% over the four sampling months and reached a high in February (Table II). As previously shown (3), species of *Fusarium* (29%) and *Penicillium* (21%) were the dominant molds. In addition, a significant proportion of the corn kernels was infected with *A. flavus* (5%) and other species of *Aspergillus* (12%). Also *Nigrospora, Alternaria, Helminthosporium, Trichoderma, Chaetomium,* and mucoraceus fungi were identified.

DISCUSSION

All corn and other cereal grains are exposed to insects and microorganisms as they grow and ripen in the field. Many microorganisms in the soil are blown by the wind, and some of these fungi and bacteria come in contact with the growing plant. During harvest, the grain is mechanically damaged and exposed to additional dust and microorganisms. Whole corn naturally contains a few thousand to millions of bacteria and mold propagules per gram.

Usually no microbial problems occur with corn and its milled products because they are kept dry. However, for the manufacturers of modern convenience, refrigerated, and specialty foods in which milled corn products are ingredients, high levels of microorganisms in grits and flour could create a problem. Some of these foods are moist and provide excellent media for the growth of microorganisms if the foods are mishandled. Contamination with psychrotrophic organisms in refrigerated foods increases the problem because these organisms are able to grow despite proper cold storage. To ensure a reasonable shelf-life and safety in modern foods, all ingredients must have a low overall microbial population and must be free of health hazards; e.g., mycotoxins, coagulase-positive staphylococci, *Salmonella,* and other intestinal pathogens.

In line with health hazards, the point needs to be made that the presence of fecal coliforms and fecal streptococci on corn usually cannot be interpreted as indicative of direct fecal contamination or insanitation. However, the presence of large numbers of these types of organisms raises a warning flag that the conditions which brought about the contamination could easily give rise to spoilage or loss of quality, or create a health hazard.

Doty (10) has recommended bacterial counts below 5,000 per g. for flours used in convenience foods. Powers et al. (11) have listed the microbiological requirements for dehydrated space foods as follows: Total aerobic plate count—not greater than 10,000 per g.; fecal coliforms—negative in 1 g.; fecal streptococci—not greater than 20 per g.; coagulase-positive

staphylococci—negative in 5 g.; *Salmonella*—negative in 10 g. These recommendations are intended only as guidelines since there are no official microbiological standards and since there certainly are questions about how applicable microbiological requirements for dehydrated space foods are to milled corn products. Nevertheless, on the basis of these guidelines, data in Table I and in last year's survey indicate that corn grits are of high microbial quality and present no immediate health hazard. On the other hand, corn flour appears to be borderline with respect to total counts, thermophilic spores, and fecal streptococci. Probably flour of the type sampled in this survey would not be used in modern convenience and specialty foods, but flour milled from grits would be substituted. Perhaps some of the techniques reported by Vojnovich et al. (12) could be applied to improve the microbial quality of flour.

Bacteria and mold counts tended to be highest in February, normally a colder month than May, August, or November. This increase in February is contrary to the 1970–71 survey (3) where counts were similar for the four sampling months. However, when considering moisture migration and condensation, which is common during winter storage of corn, along with the fact that various species of *Penicillium* grow rather well at cold temperatures (13), these higher counts in February are not surprising.

During the past decade, it has been emphasized that molds not only cause spoilage but sometimes produce toxins in grains if environmental conditions are conducive to their growth. *A. flavus,* in particular, has been of concern because it can produce aflatoxin. This survey and last year's survey indicate that the level of *A. flavus* in corn entering dry-milling channels is rather low. These levels would not indicate an immediate aflatoxin hazard. However, good handling and storage practices are a necessity if growth of *A. flavus* and possible toxin production are to be prevented. The need to avoid mold growth in corn is further accentuated by the predominance of fusaria and the increasing levels of penicillia found in this survey, since certain species of these genera can also produce toxins.

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