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Review

Role of Microbiological Guidelines in the Production and Commercial Use of Milled Cereal Grains: A Practical Approach for the 21st Century

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ABSTRACT

A contemporary survey of the microbiological profile of five milled cereal grains—wheat, corn, oats, whole wheat, and durum—was conducted largely from 2003 to 2005, with routine laboratory data obtained by North American dry-milling operations. When compared to data reported in the previous century, the contemporary data showed similar or reduced quantitative counts for indicator tests (e.g., total aerobes, yeasts, molds, coliforms, and *Escherichia coli*) as well as a substantially lower incidence of salmonellae. The implementation of modern management systems for the control of food quality and safety, i.e., good agricultural practices, good manufacturing practices, and the hazard analysis critical control point system, together with the excellent microbiological profiles, has eliminated the need for microbiological specifications and lot acceptance criteria for milled cereal grains. Instead, microbiological monitoring guidelines, such as the periodic testing of aerobic plate counts and mold counts, can be used to verify compliance with the requirements of food quality and food safety management systems.

The domestication of cereal grains about 100 centuries ago fueled the gradual rise of human civilizations. As the emergence of our modern civilization was accelerated by advancements in science and technology, cereal grains and their milled products remained the most important agricultural commodities in the world *(24).*

It is only in the past two centuries that we have come to understand the microbiological causes of foodborne illness and food spoilage, enabling the development of control measures to avoid these hazards. Principal early examples of the application of microbiological control measures include regulations for the pasteurization of milk and the chlorination of drinking water *(5, 31).* Each of these regulations includes microbiological testing as a means of verifying that the respective control processes have been adequately applied.

Witnessing the public health success of the regulated pasteurization and chlorination processes, the modern food industry, which emerged after 1945, began to require similar microbiological specifications and testing for many of its raw materials and finished products. Commonly required quantitative indicator tests included aerobic plate counts (APCs) and yeast, mold, coliform, and *Escherichia coli* counts. Qualitative tests for the presence of salmonellae were the most commonly required pathogen test. Quasi-official protocols for developing specifications and testing plans provided support for these efforts *(14, 23).* The utility of these industry requirements has gradually faded because of improved production controls and food safety management systems in the food industry and because of greatly advanced testing technology. Despite these advances, rather widespread demands for microbiological testing persist in the food industry, demands that are sometimes reinforced by the regulatory and consumer advocacy communities. The dry-milling industry has also been affected by these developments in the greater food industry, even though its products are very close to being raw agricultural commodities. Therefore, this report was undertaken to examine the microbiological profiles of five milled cereal grains and to consider the proper application of microbiological guidelines for their production and use.

LITERATURE REVIEW

Because our contemporary data have been collected only from North American mills, this review of the literature is limited to North American data in order to permit more relevant comparisons between historical and contemporary data. Our extensive literature searches have revealed relatively few North American reports on the microbiological profiles of milled cereal grains.

A 1947 investigation by Milner et al. *(21)* showed that wheat would not support mold growth when stored at 30°C for 20 days if its moisture content was 14.5% or lower. At wheat moisture levels above 14.5%, predominantly osmo-

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TABLE 1. *Log reduction of microbial counts during milling of wheat*

Material	Reference	*n*	Aerobic plate count/g	Fungi/g[a]
Wheat	*13*	53	5.29	3.62
Flour	*13*	53	3.94	3.39
		Reduction	1.35	0.23
Wheat	*27*	54	4.78	2.66
Flour	*27*	54	3.52	2.78
		Reduction	1.26	0

[a] Combined yeast and mold counts.

philic molds, such as *Aspergillus glaucus,* were able to grow.

Reports from 1968 and 1978 showed that the dry-milling process reduced the APCs of wheat by more than 90%, while the fungal counts were relatively unchanged (Table 1). A relatively comprehensive microbiological profile of wheat flour milled in 1989 (Table 2), together with *Salmonella* incidence data (Table 3), exemplifies the types of microbiological assays performed on milled cereal grains in the latter part of the 20th century.

Numerous researchers have evaluated processes for the reduction of microbial counts in milled cereal grains. Such reductions are not easy because it is considerably more difficult to kill microbes with dry heat than it is with moist heat. Furthermore, the process must not significantly degrade product quality or functionality. Despite these difficulties, many of the experimental processes were demonstrated to be effective in reducing microbial populations.

Hesseltine *(13)* tried two approaches for the reduction of APCs in wheat flour: heating the wheat before milling and heating the flour after milling. Wheat at a moisture level of 15% was dry heated at 60°C for 1 h. The flour milled from the heated wheat had an APC that was 2.26 log lower (2.43 versus 4.69) than the flour milled from the unheated wheat. Wheat flour stored at a combination of temperatures and times (28°C for 105 days, 37°C for 24 days, or 46°C for 7 days) had APC values that averaged 1.3 log lower (3.7 versus 5.0) than those of the unheated flour. Wheat flours at water activity values of 0.40 to 0.60 (freshly milled wheat flour has a water activity of 0.60 to 0.65, a value that gradually decreases during storage) were stored at relatively high temperatures (69 to 77°C) and tested over several hours. The *D*-value (time to reduce a population by 1 log, or 90%) was determined to be about 1 h

TABLE 2. *Log microbiological profile of wheat flour milled in 1989*[a]

Assay	*n*	Geometric mean	SD
Aerobic plate count	1,354	4.17	0.52
Yeasts	1,648	2.12	0.40
Molds	1,682	2.90	0.52
Coliform, MPN[b]	1,477	1.20	0.59

[a] Values are expressed in counts per gram *(26).*

[b] Most-probable-number procedure.

TABLE 3. *Incidence of* Salmonella *in wheat flour*

Yr	*n*	Positive		Reference
		No.	%	
1989	3,040	40	1.32	*26*
1984–1991	1,170	4	0.34	*28*
Total	4,210	44	1.05	

over this temperature range. The *z*-value (change in temperature necessary to change the *D*-value by 1 log) was established as 30°C *(2).*

Corn flours at 10 to 15% moisture were inoculated with a variety of salmonellae and stored at 49°C. After 24 h of storage, the salmonellae populations had decreased by approximately 3 log *(35).* Corn flour at 12% moisture showed a 2-log APC decrease when stored at 65°C for 1 day, 57°C for 2 days, or 46°C for 5.3 days *(36).* Blends of corn flour, soy flour, and nonfat dried milk were inoculated with 3 log of *Salmonella* Anatum per g of blends of corn flour, soy flour, and nonfat dried milk. While an indeterminate number of the salmonellae died from the osmotic shock of rapid drying, all inoculated samples were *Salmonella* positive before heating. All samples were found to be *Salmonella* negative after heating at 60°C for 1 day, 54°C for 1.9 days, 49°C for 4 days, or 43°C for 9.3 days *(3).*

Microwave treatment of 22.7-kg (50-lb) bags of blends of corn flour, soy four, and nonfat dried milk produced a 2- to 5-log reduction of *Salmonella* Senftenberg because of the extended period necessary to cool the palletized bags (Table 4). The nonlinear response of the cooling time resulted from different pallet patterns being used for various treatments *(4).* An attempt to reduce the microbial count in rye flour by the use of pulsed electric fields produced a minor (0.6-log) reduction in the APC *(17).*

CONTEMPORARY MICROBIOLOGICAL PROFILE OF MILLED CEREAL GRAINS

Contemporary microbiological data for milled cereal grains were obtained from North American Millers' Association member companies, which represent a substantial portion of the North American dry-milling capacity (Table 5). Data were collected from the routine quality assurance records of five types of milled cereal grains from 2003

TABLE 4. *Effect of microwave heating of 22.7-kg bags of corn-soy-milk blend in the reduction of* Salmonella *Senftenberg*

Time[a]	Temp[b]	Cooling[c]	Log reduction
3.9	57	10	1.96
6.5	61	23	3.25
7.0	67	11	3.29
9.5	78	60	4.68
10.0	82	11	4.70

[a] Time of microwave application (in minutes).

[b] Internal temperature (in degrees Celsius) after microwave application.

[c] Time (in hours) to cool product to 43°C.

TABLE 5. *Origin of contemporary microbiological data for North American milled cereal grains*

Milled cereal grains	No.		% industry capacity[a]
	Samples	Mills	
Wheat	6,598	98	68
Corn	3,758	8	72
Oats	2,275	4	49
Whole wheat	435	16	—
Durum	418	5	36

[a] Compiled from Duensing et al. *(10)*, Milling and Baking News *(20)*, and NAMA member companies. Current whole wheat industry capacity is unknown.

TABLE 6. *Microbiological profile of North American wheat flour for the period 2003 through 2005*[a]

Assay	*n*	Geometric mean	SD	Maximum
Aerobic plate count	6,598	3.79	0.70	6.99
Yeasts	6,573	1.31	0.74	5.86
Molds	6,869	2.44	0.63	5.86
Coliform—MPN	3,688	1.63	0.89	3.73
Coliform—Petrifilm	2,467	2.65	0.78	4.86
E. coli—MPN	3,735	0.23	0.29	2.56
E. coli—Petrifilm	2,921	0.74	0.38	2.99

[a] Values are expressed as log counts per gram.

through 2005, except for the milled oat data, which were collected from 2001 through 2005. As available, each mill submitted data for the following microbiological tests. (i) APC and yeast and mold count data were submitted, as determined by standard quantitative plating procedures. The yeast and mold counts were tabulated and analyzed separately, as the latter are commonly used to monitor microbiological guidelines in the dry-milling industry. (ii) Coliform and *E. coli* count data were submitted, as determined either by most-probable-number (MPN) procedures or by direct plating on Petrifilm dry rehydratable film. (iii) *Salmonella* data were submitted, as determined by cultural, immunoassay, or PCR procedures. A tabulation of sample size and specific detection procedures is presented in Table 12.

All microbiological procedures were taken from the *Compendium of Methods for the Microbiological Examination of Foods* or the *Bacteriological Analytical Manual (8, 30).* All data were sent to a central office, blinded to protect the confidentiality of the individual mills, and forwarded to the Microbiology Working Group for analysis. The data for each microbiological assay and each type of milled grain were collated but were not sorted by season, year, location, or individual varieties of a particular grain. All data, except for *Salmonella* data, were converted to log counts per gram for the calculation of geometric means and standard deviations and the plotting of histograms to show the distribution of the data.

The milling industry receives occasional customer requests for specifications or testing related to microorganisms other than those listed above. These requests are almost always resolved without resorting to specification development or product testing. Therefore, current data on these microorganisms in milled cereal grains are essentially nonexistent. *E. coli* O157:H7 and *Listeria monocytogenes* have not been implicated with public health issues related to milled cereal grains or cereal products. Each of these is typically associated with meat or dairy products. Several surveys of wheat flour revealed a very low incidence (0.03%) of *Staphylococcus aureus,* as well as a low incidence of *Enterococcus* spp. *(16, 26).* There has been a minimal level of interest in testing for enterococci as indicators of fecal contamination; however, the food industry in general and the dry-milling industry in particular have relied on coliform and *E. coli* tests to fulfill this purpose. ''Rope'' spoilage caused by strains of *Bacillus subtilis* was an occasional problem in the early baking industry. It was gradually recognized that inadequate cleaning and sanitation procedures of the bakery mix equipment, not the minimal presence of rope spores in milled cereal grains, caused this type of spoilage. One survey of wheat flour reported that rope spores were present at a level of about 10 spores per g *(11).*

A major part of our report entails the analysis of a considerable store of contemporary data collected by the North American dry-milling industry. Since the term flour is generally used synonymously with wheat flour, the dry-milling industry uses the term milled cereal grain to describe the products obtained from the dry milling of each cereal grain. This report is based on the analysis of extensive data for dry-milled wheat, corn, and oats and on the analysis of fewer amounts of data for dry-milled whole wheat and durum.

The term good manufacturing practices (GMPs) has been used in this document instead of the term good hygienic practices (GHPs), as it is more readily recognized by North American readers. The term GHP is widely used outside North America. GMPs and GHPs describe essentially similar practices that are important in the prerequisite programs used to support the hazard analysis critical control point (HACCP) system of food safety management *(6).*

RESULTS

The current microbiological profile of wheat flour (Table 6) is rather close to the profiles reported earlier (Tables 1 and 2). Adjusted for sample sizes, the current APC geometric mean of 3.79 (6,200/g) is slightly lower than the geometric mean of the earlier reports, 4.14 (14,000/g). In comparing Tables 2 and 6, the current profiles for yeast and mold counts are similarly somewhat lower than the earlier data. The current coliform-MPN counts are slightly higher than those in earlier reports. Comparisons of the coliform and *E. coli* Petrifilm test results to those in earlier reports are not possible because Petrifilm dry rehydratable films were not available for use in the earlier reports.

The remaining data may represent the first comprehensive reports of the microbiological profiles of North American dry-milled corn (Table 7), oats (Table 8), whole wheat (Table 9), and durum (Table 10). The yeast and mold counts

TABLE 7. *Microbiological profile of North American dry-milled corn products for the period 2003 through 2005*[a]

Assay	*n*	Geometric mean	SD	Maximum
Aerobic plate count	3,758	2.76	0.83	5.95
Yeasts	1,772	1.49	0.73	4.93
Molds	1,810	2.64	0.91	6.04
Coliform—MPN	18	0.70	0.76	2.30
Coliform—Petrifilm	3,456	1.12	0.68	4.87
E. coli—MPN	18	0	0	0
E. coli—Petrifilm	3,722	0.10	0.25	2.30

[a] Values are expressed as log counts per gram.

TABLE 9. *Microbiological profile of North American whole wheat flour for the period 2003 through 2005*[a]

Assay	*n*	Geometric mean	SD	Maximum
Aerobic plate count	435	4.41	1.15	6.00
Yeasts	438	1.79	0.93	4.82
Molds	438	2.58	0.81	5.79
Coliform—MPN	295	1.98	0.95	4.41
Coliform—Petrifilm	110	3.64	0.62	4.78
E. coli—MPN	410	0.16	0.27	2.49
E. coli—Petrifilm	135	0.84	0.42	1.99

[a] Values are expressed as log counts per gram.

are very similar for all five milled cereal grains. The mold counts are rather consistently about 1 log higher than the yeast counts. For the remaining quantitative assays, the corn and oat flour results were lower than the results obtained for wheat flour, while the whole wheat and durum flour results were somewhat higher than those obtained for wheat flour.

Collectively, the present microbiological results are quite similar to the earlier results, except for *Salmonella* incidence. The current *Salmonella* incidence in wheat flour, 0.14% (Table 11), is significantly lower than the earlier reported incidence of 1.05% (Table 3), as nearly identical numbers of samples were analyzed during each time period. None of the milled cereal grains, other than wheat flour, yielded positive results in the *Salmonella* assays. Therefore, Table 12 is useful only to show the methods used and the sample sizes analyzed. While five of the six *Salmonella*-positive wheat flour samples were detected by PCR methodology, we cannot directly compare the performance of the various methodologies because multiple testing methods were not performed on each sample.

Each category of milled cereal grain also yielded very low *E. coli* counts. Many of these were below the limit of detection, and all of the geometric means were substantially below 1.0. The distributions of the many thousands of data points are presented in Figures 1 through 5. It is in these histograms that the number of results at or below the lower limits of detection can be observed.

DISCUSSION

Improved sanitation in grain handling and milling. As documented above, the present microbiological profiles of milled cereal grains are somewhat ''better,'' in terms of lower numbers, than the earlier profiles summarized in Tables 1 and 2. Interestingly, in his report of almost 40 years ago *(13),* C. W. Hesseltine observed, ''In general, counts from many wheat and flour samples indicate numbers lower than those reported in the older literature. Lower flour counts, it would seem, reflect the better sanitation that is being practiced by the milling industry.''

To be sure, the dry-milling industry has continued to improve sanitation in its operations with the implementation of GMPs, such as better cleaning of the grains before milling, more frequent and improved cleaning of milling and transportation equipment, and improved bird control. Implementation of good agricultural practices in the production of cereal grains has undoubtedly also contributed to the seemingly steady reduction in microbial counts noted over the past century or so *(25).* To a large extent, cereal crops today are mostly segregated from animal production activities. Farmers also have more sophisticated harvesting, storage, and transportation equipment to protect the grains from infestation and other forms of contamination. The modern global food industry, including the dry-milling industry, has initiated substantial improvements in product quality and safety by the use of better management procedures. Chief among these is the HACCP system of food safety that was pioneered in the food industry about 40 years ago *(6, 22).* Even though the dry-milling industry

TABLE 8. *Microbiological profile of North American milled oat products for the period 2001 through 2005*[a]

Assay	*n*	Geometric mean	SD	Maximum
Total aerobic count	2,275	2.38	1.19	7.28
Yeasts	201	1.91	0.98	4.18
Molds	201	1.68	0.65	4.43
Coliforms—MPN	816	0.54	0.69	4.04
Coliforms—Petrifilm	1,599	0.78	0.32	3.20
E. coli—MPN	1,142	0.003	0.043	0.60
E. coli—Petrifilm	1,722	0.70	0.02	1.18

[a] Values are expressed as log counts per gram.

TABLE 10. *Microbiological profile of North American durum flour for the period 2003 through 2005*[a]

Assay	*n*	Geometric mean	SD	Maximum
Aerobic plate count	418	4.60	0.75	5.80
Yeasts	340	1.27	0.68	3.95
Molds	339	2.29	0.60	4.18
Coliform—MPN	282	1.72	1.24	4.52
Coliform—Petrifilm	152	3.55	0.82	5.30
E. coli—MPN	268	0.18	0.29	2.66
E. coli—Petrifilm	227	0.82	0.33	1.30

[a] Values are expressed as log counts per gram.

TABLE 11. *Incidence of* Salmonella *in milled cereal grains*

Milled grain	*n*	Positive samples	
		No.	%
Wheat	4,358	6	0.14
Corn	1,772	0	0
Oat	714	0	0
Whole wheat	286	0	0
Durum	180	0	0

TABLE 12. Salmonella *incidence in milled cereal grains; methods and sample sizes*

Method	Sample size (g)	Type of milled cereal grain (no. positive/no. tested)				
		Wheat	Corn	Oat	Whole wheat	Durum
Cultural	25	1/811	0/569	0/73	0/164	0/63
Enzyme	25	0/638		0/641	0/29	
Immunoassay	100	0/556			0/93	
	375	0/257				0/117
PCR	25	5/2,096	0/18			
IsoGrid[a]	50		0/1,076			
	150		0/37			
1-2 test[b]	50		0/72			
Total		6/4,358	0/1,772	0/714	0/286	0/180

[a] Hydrophobic grid membrane filters.
[b] Selective motility and immunodiffusion method.

does not have available some of the definitive critical control points and processes that are available in other segments of the food processing industry, the HACCP system and its supporting prerequisite programs, such as GMPs, as used in the dry-milling industry, have undoubtedly helped reduce the microbiological profiles of milled cereal grains.

While it may be interesting to speculate how much additional reduction in the microbiological profiles of milled cereal grains might be achieved in the future, it might be better to ask, in the interests of public health and food safety, ''is a greater reduction in microbial counts of milled cereal grains necessary?'' Modern agricultural and milling operations handle many millions of tons of cereal grains each year. While not documented in this report, it is accurate to claim that the microbiological profile of milled cereal grains is rather similar to that of some of the more highly processed foods that are subjected to more rigorous process treatments, including cooking. There is no public health or food safety benefit to be derived from further reductions in the microbiological profile of milled cereal grains. Of course, the dry-milling industry will maintain and improve its sanitation procedures, but such improvements need not be monitored or mandated by specifications or regulations with the expressed intent to achieve further reductions in microbial populations. It is the conclusion of this report that most microbiological specifications and testing requirements for milled cereal grains are unnecessary.

Definitions and defect action levels (DALs). In considering the potential need for specifications or regulatory actions regarding the microbial content of milled cereal grains, it is important to examine the position of milled cereal grains in the continuum of raw and processed foods. According to the definitions in the Federal Food Drug and Cosmetic Act *(32),* a raw agricultural commodity is ''Any food in its raw or natural state, including all fruits that are washed, colored, or otherwise treated in their unpeeled natural form prior to marketing.'' A processed food is defined as ''Any food other than a raw agricultural commodity that has been subject to processing, such as canning, cooking, freezing, dehydration, or *milling*'' (italics used for emphasis).

Even though milled cereal grains are included in the definition of processed foods because of the dry-milling process, they are only one small step removed from the definition of a raw agricultural commodity. Milled cereal grains are not processed in the same manner in which, for example, raw milk is processed by pasteurization in order to reduce its microbiological load and eliminate pathogenic microorganisms that might be expected to be present. Processes such as cooking and canning, as included in the definition above, kill all of the vegetative microorganisms originally present in the raw foods. The canning process typically applies a more lethal heat treatment than cooking processes, resulting in the destruction of most bacterial spores. In contrast, the dry-milling process has no feature that actively destroys microorganisms. The microbiota of raw wheat is reduced by more than 1 log (Table 1) by aspiration and bran removal. Beyond these preliminary steps, the dry-milling process is not able to provide further reductions of the microflora, unless a special heating step is applied either before or after the milling process. Thus, the types of microorganisms originally present on cereal grains, including the relatively rare occurrence of pathogenic microorganisms, can be expected to be present, albeit in substantially reduced numbers, in the milled cereal grains.

The U.S. Food and Drug Administration *(29)* has developed DALs for 116 raw commodities and processed foods to limit ''natural or unavoidable defects in foods for human use that present no health hazard.'' The presence of small amounts of insect or rodent contamination is permitted in wheat, wheat flour, and corn meal according to the DALs that have been established for these foods (Table 13). Insects and rodents can be carriers of pathogenic microorganisms. The presence of insects, insect fragments, rodent hairs, or fecal excreta in milled cereal grains could explain the rare occurrence of pathogens in these products (Table 11). The significance of insect and rodent defects in cereal grains and milled cereal grains is judged to be ''aesthetic,'' a term that is defined in the DALs as ''offensive to the senses.'' Therefore, it can be concluded from these DALs that the presence of minute amounts of insect or rodent contamination in cereal grains and milled cereal grains does not present a public health risk under normal conditions of production, transportation, and preparation for consump-

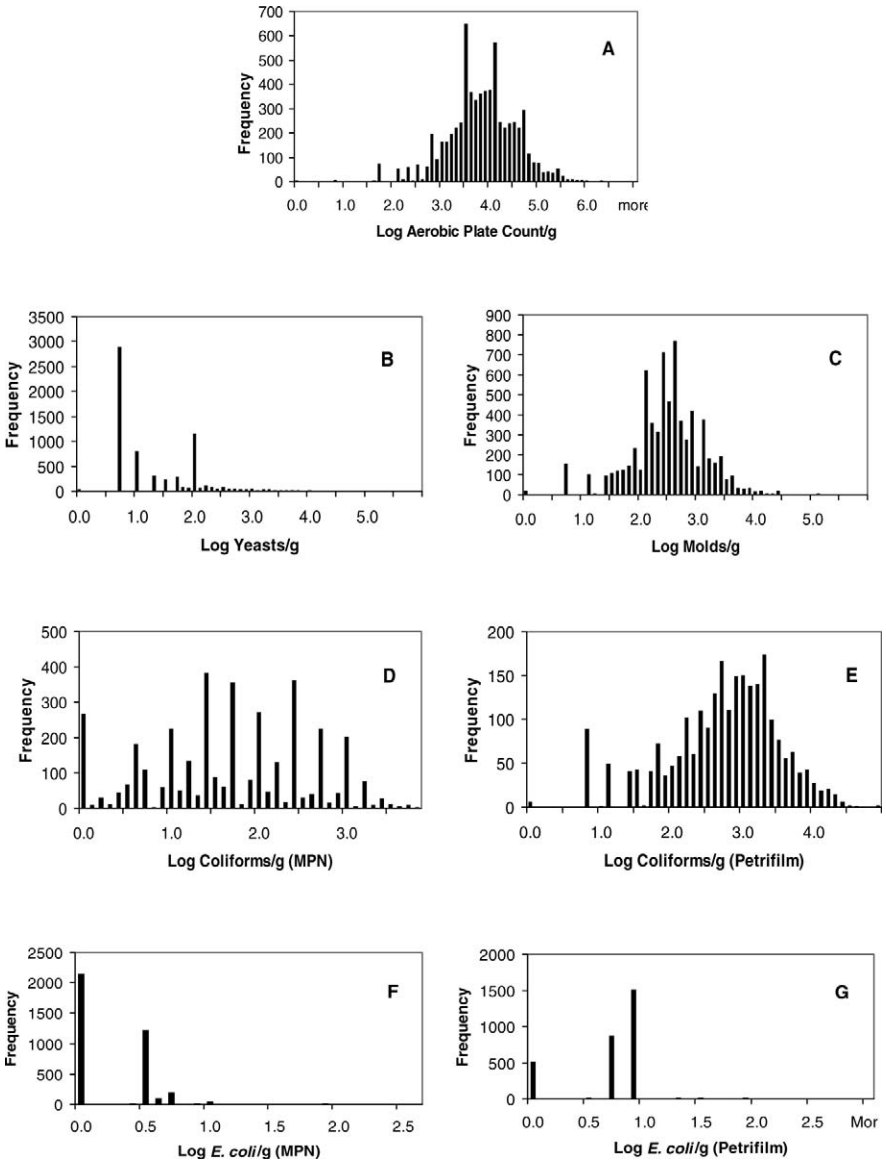

FIGURE 1. *Distribution of microbiological counts in North American wheat flour, 2003 through 2005; aerobic plate count (A), yeasts (B), molds (C), coliforms—MPN (D), coliforms—Petrifilm (E),* E. coli*—MPN (F), and* E. coli*—Petrifilm (G).*

tion, because milled cereal grains have a long history of safe use.

Microbiological kill steps in milled cereal grains. In addition to their low microbiological loads and the rare presence of pathogenic microbes, there is an even more obvious reason for the microbiological safety of milled cereal grains. Almost all milled cereal grains are baked, fried, or cooked before consumption. In commercial bakeries, food service operations, and consumer kitchens, milled cereal grains receive heat treatments that are lethal to vegetative microorganisms, including the occasional cell of a pathogenic microorganism that could be introduced by insect or rodent contamination.

There are relatively few historical uses of milled cereal grains that do not involve baking, frying, or cooking before consumption. Typically involving relatively small quantities of milled cereal grains, these uses include, for example, addition to dehydrated infant foods, seasoning mixes, and raw cookie dough used in ice cream. To additionally ensure pathogen reduction and food safety, some millers and their customers have developed heat treatments for such specialty uses of milled cereal grains. Much of the early research for the development of these processes has been described in the ''Literature Review'' *(2–4, 13, 17, 35, 36).* The specialty heat treatment processes are used today for ''niche'' markets. Such treatments are not necessary for the vast preponderance, millions of tons, of milled cereal grains that are normally baked, fried, or cooked before consumption.

A small outbreak of salmonellosis in 2005 was associated with cake batter ice cream, in which the cake batter was not heated before being added to the ice cream, even though the cake batter mix was labeled with baking instructions to be followed before consumption *(33).* Because cake batter mixes can contain *Salmonella*-sensitive ingredients—for example, dried egg or dairy products—it was suspected that the cake batter mix was the source of the *Salmonella* contamination in this outbreak. The U.S. Food and Drug Administration issued a bulletin *(34)* to the retail and food service industries advising ''that incorporating an ingredient that is intended to be cooked into a ready-to-eat food that will not be cooked or otherwise treated to eliminate

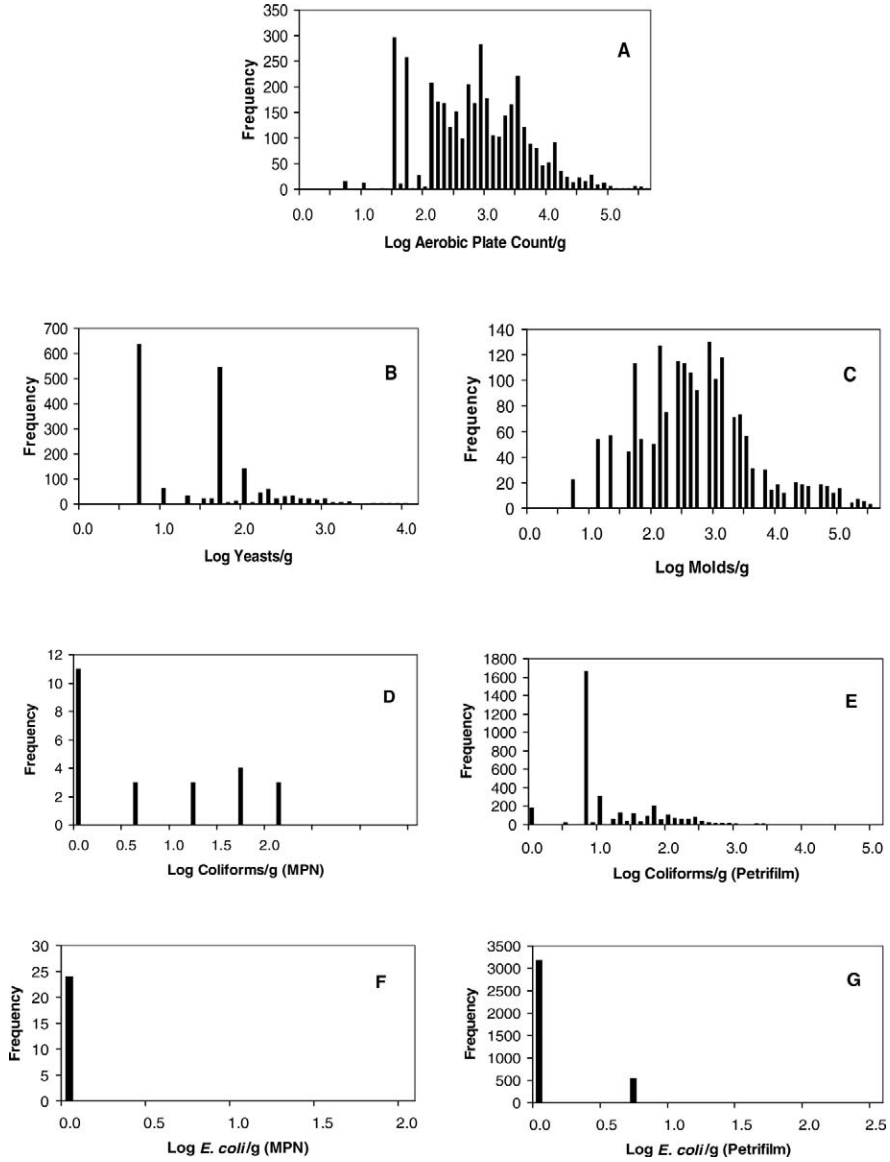

FIGURE 2. *Distribution of microbiological counts in North American dry-milled corn products, 2003 through 2005; aerobic plate count (A), yeasts (B), molds (C), coliforms—MPN (D), coliforms—Petrifilm (E),* E. coli*—MPN (F), and* E. coli*—Petrifilm (G).*

microorganisms of public health concern can pose a serious food safety risk.''

Coliform and *E. coli* methods. Two methods for the quantification of coliforms and *E. coli,* MPN and Petrifilm, were used by the milling operations that submitted data for this report. As summarized in Table 14, in comparison to the MPN method, the Petrifilm method yields somewhat higher counts with considerably reduced variability. These methods represent two extremes of technology in use in modern food microbiology laboratories. The MPN method is complex, tedious, expensive, and time-consuming. As many as 9 days of culture transfers and incubation may be required for the identification of *E. coli.* Furthermore, the MPN method is a semiquantitative method that yields an imprecise estimate of coliform and *E. coli* populations. In contrast, the Petrifilm method is relatively simple, inexpensive, and rapid. Coliform and *E. coli* counts can be determined quantitatively on Petrifilm within 24 h, with an accuracy typical of that obtained with standard plating procedures. Because of its inherent cost, incubation times, and inaccuracy, the continued use of the MPN method for the detection of coliforms and *E. coli* is no longer necessary.

The future of coliform testing and requirements. As described in the introduction to this report, the early use of microbiological specifications for pasteurized dairy products *(31)* and treated drinking water *(5)* was gradually extended to the use of microbiological specifications for many food ingredients and products. The coliform test was the principal microbiological specification with early significance for public health protection in dairy products and drinking water. Raw milk could be contaminated with bovine feces. Sources of drinking water could be contaminated with human sewage. Since coliform bacteria are generally present in high numbers in feces, a demonstration of their reduction or elimination from dairy products or drinking water indicated that these treated products would be relatively safe for consumption.

While the use of coliform specifications with other food products may have had some validity for foods of animal origin, because these raw materials could have been

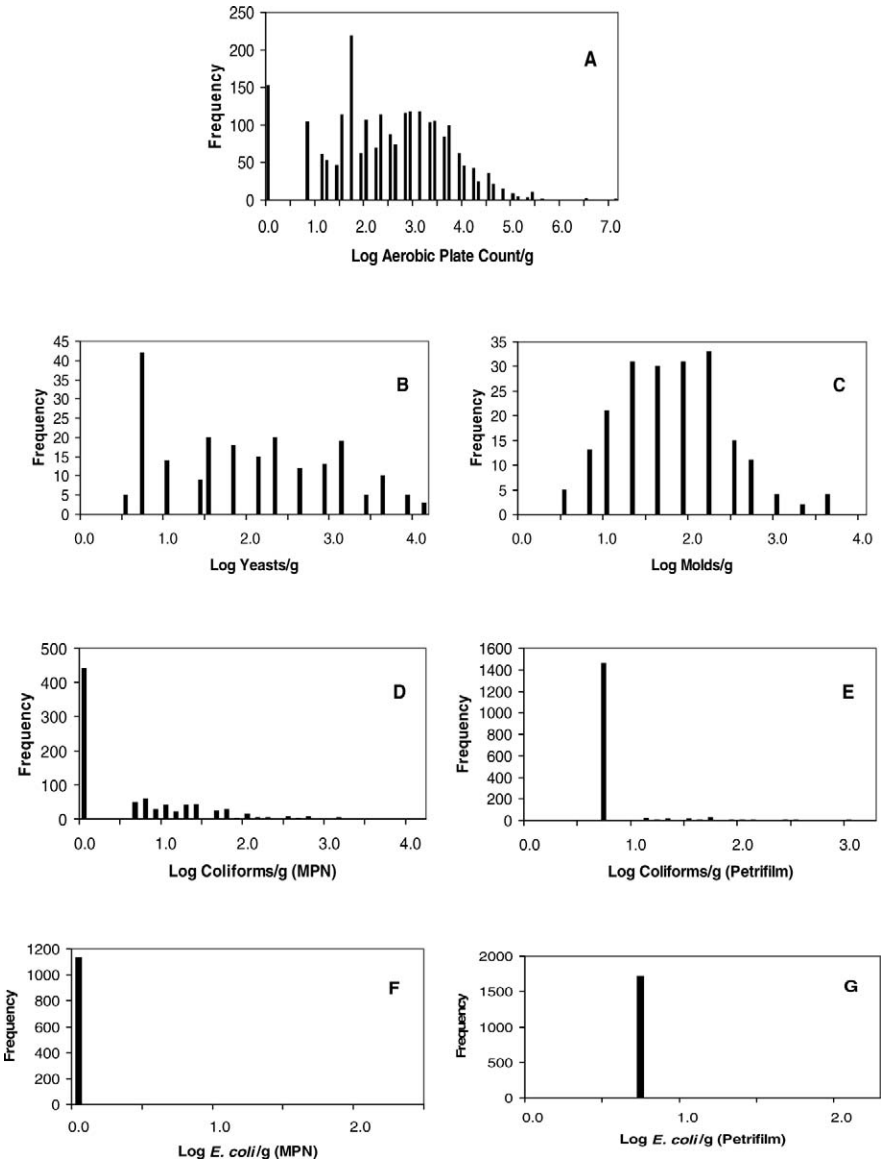

FIGURE 3. *Distribution of microbiological counts in North American dry-milled oat products, 2001 through 2005; aerobic plate count (A), yeasts (B), molds (C), coliforms—MPN (D), coliforms—Petrifilm (E),* E. coli*—MPN (F), and* E. coli*—Petrifilm (G).*

contaminated with feces, they have very little validity as indicators of fecal contamination in foods of plant origin. A number of coliform bacteria, as well as other members of the family *Enterobacteriaceae,* grow predominantly on green plants and in environmental niches, rather than in the intestines of animals *(7, 15, 18, 28).* Hence, their presence in most processed foods should not be used to indicate fecal contamination. Whole and milled cereal grains best exemplify this conclusion. Coliform bacteria can grow to high numbers on cereal crops. Some of these will unavoidably be carried into the milled cereal grains, where their presence has no public health significance. Nonetheless, because of tradition and customer requests, many dry millers still test their products for coliforms, as demonstrated by the large numbers of coliform tests reported in Tables 6 through 10.

For all practical purposes, the use of coliform tests could be eliminated. In fact, such a change will likely be facilitated by events external to the milling industry. There is a mounting effort within the public health and food microbiology professions to eliminate the use of coliform and fecal coliform designations and tests. Because some of these microorganisms grow well in many environmental niches other than the animal colon, including green plants, the continued use of coliform specifications as an indicator of fecal contamination creates confusion and wastes resources *(7, 9, 19).*

Difficulties in the application of microbiological specifications or guidelines for milled cereal grains. There are a number of significant obstacles that have made it difficult to apply microbiological specifications to milled cereal grains and their derived products. These include poor specifications, variations introduced in laboratory testing, nonrandom distribution of microorganisms in milled cereal grains, difficulty in detecting low-incidence defects, and growing awareness in the food industry that such specifications are impractical and unnecessary and constitute a poor use of resources.

Ideally, the supplier and purchaser should jointly develop microbiological specifications and guidelines for food ingredients and products. This process is sometimes poorly

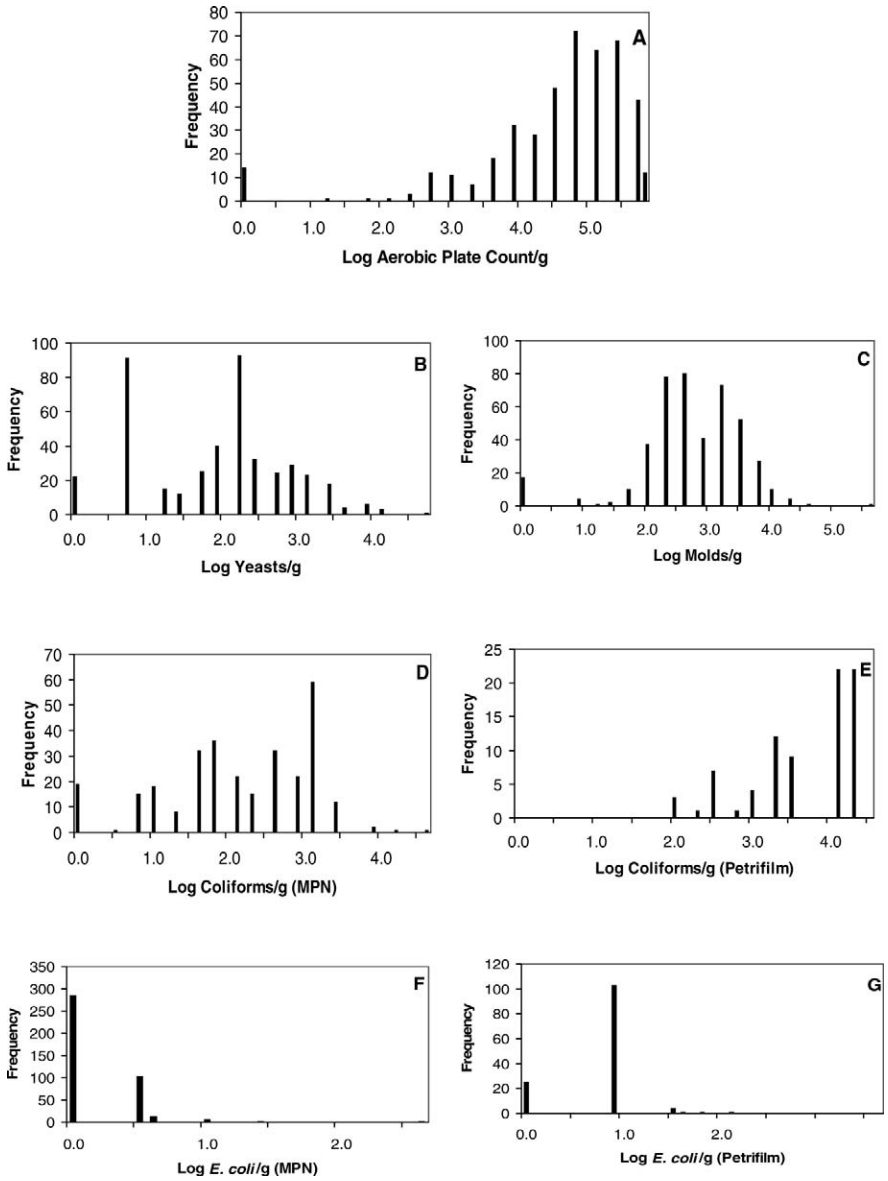

FIGURE 4. *Distribution of microbiological counts in North American whole wheat flour, 2003 through 2005; aerobic plate count (A), yeasts (B), molds (C), coliforms—MPN (D), coliforms—Petrifilm (E),* E. coli*—MPN (F), and* E. coli*—Petrifilm (G).*

done, resulting in disruptions of trade and wasted resources. According to a landmark U.S. government report, a microbiological criterion should include the following items *(23)*: (i) a statement describing the identity of the food or food ingredient; (ii) a statement of the contaminant of concern, i.e., the microorganism or group of microorganisms or its toxin or other agent; (iii) the analytical method to be used for the detection, enumeration, or quantification of the contaminant of concern; (iv) the sampling plan; and (v) the microbiological limits considered appropriate to the food and commensurate with the sampling plan used.

In the experience of this report's writers, microbiological specifications are usually lacking adequate descriptions of the sampling plans and the analytical methods to be used. Moreover, the remaining elements of an adequate microbiological specification have usually been purchaser-driven, sometimes forcing a supplier to accept unreasonable or unwarranted specifications in order to protect its commercial interests. This practice can lead to commercial disruptions, up to and including product recalls that waste time and money both for purchasers and suppliers.

Quantitative testing methods are subject to a number of variable influences that can produce highly disparate results in samples that have essentially identical microbiological profiles, thereby further increasing the difficulties encountered in the application of microbiological specifications. Some of the variation can be attributed to differences in technician experience and skills in the dilution and plating of samples and the counting and reporting of results. In performing APCs on raw milk samples, the variation in replicate counts by a single technician (repeatability) was 7.7%. The variation in replicate counts obtained by multiple technicians (reproducibility) was 18.2% *(12)*. These data are the basis of the often-stated axiom that the variability in microbiological counts attributable to technician inputs is roughly ±20%.

A myriad of additional factors related to the collection, storage, and preparation of samples; the nonrandom distribution and variable physiological state of the microorganisms in the samples; the testing method used; and the quality of the test reagents can significantly affect an individual test result *(8, 14)*. The nonrandom distribution of micro-

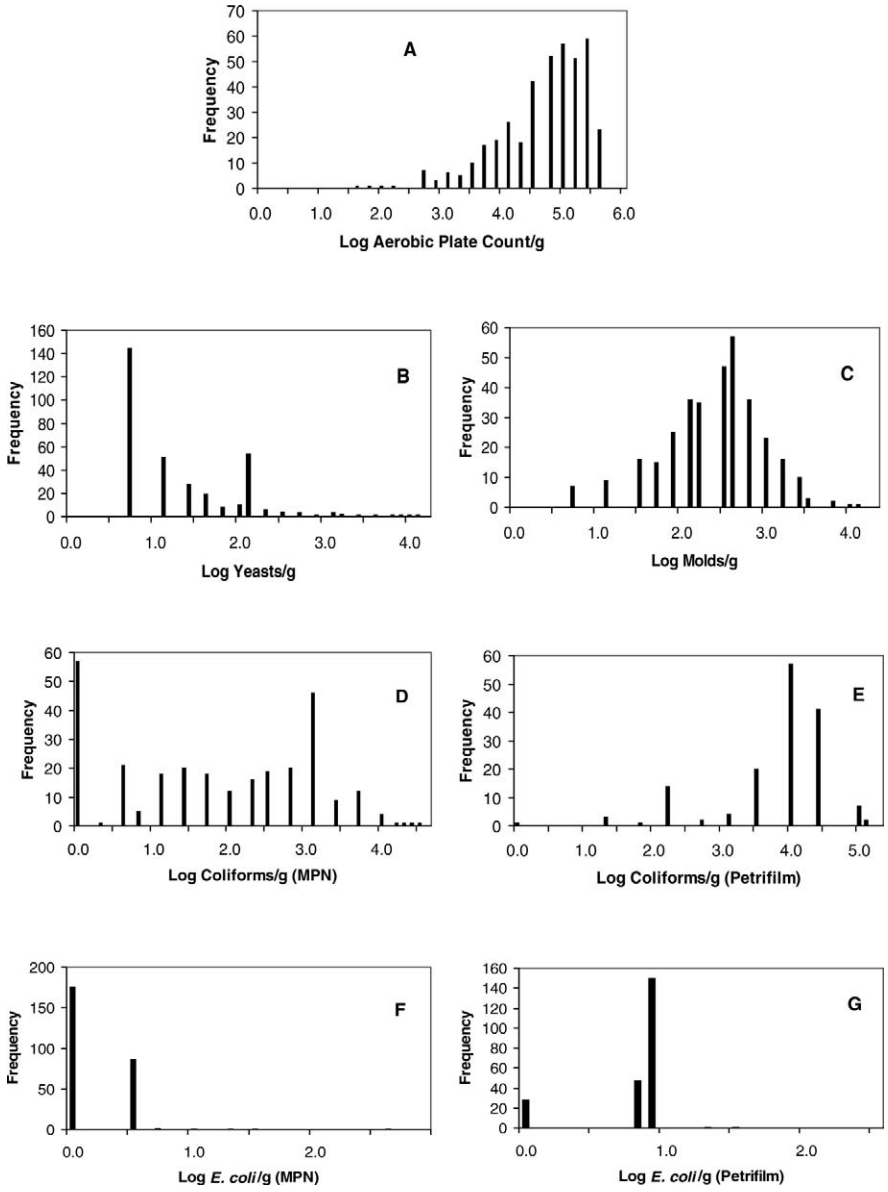

FIGURE 5. *Distribution of microbiological counts in North American durum flour, 2003 through 2005; aerobic plate count (A), yeasts (B), molds (C), coliforms—MPN (D), coliforms—Petrifilm (E),* E. coli*—MPN (F), and* E. coli*—Petrifilm (G).*

organisms in a sample is a particularly important consideration in the analysis of dry materials, such as milled cereal grains *(14).* Agitated liquids, such as fluid milk, can have a nearly random, or homogeneous, distribution of microorganisms. In contrast, dry materials typically have a nonrandom distribution in which ''hot spots'' of microorganisms can occur because of small areas of condensation or settling and stratification of the product during transportation. It is the collective experience of the food microbiology community that the total variability of results obtained in nonselective plating procedures, such as APCs, can be as much as ±0.5 log. The total variability of results obtained in selective plating procedures, such as coliforms and *E. coli,* can be as much as ±1.0 log, while that of MPN procedures can be as much as ±1.5 log *(1).*

Because of the variability in technician skills, testing methods, and sample composition, lot acceptance criteria on food ingredients and products can be difficult to manage. For example, a material subject to an APC specification of 50,000/g would likely be rejected if the laboratory test yielded a result of 75,000/g. Yet, given the variability in laboratory procedures, samples that had an actual APC of 50,000/g could yield results in the approximate range of 17,000 to 150,000/g (±0.5 log). If the same samples had an actual coliform count of 500/g, the results could be expected to fall into an approximate range of 50 to 5,000/g (±1.0 log). If MPN procedures were used, the range of counts would be even greater.

A particular issue with qualitative testing is the great difficulty in detecting contaminants, e.g. salmonellae, which occur at a low incidence. If a lot of food were contaminated at a level such that 1% of the analytical samples contained salmonellae, 300 samples would need to be tested to detect one positive sample, at the 95% confidence level. If the salmonellae incidence were 0.1% (similar to the wheat flour results in Table 11), 3,000 samples would need to be tested to detect one positive sample at the same confidence level *(14).* Clearly, such sampling plans are impractical and can never be put into use. These particular statistics demonstrate the inability of product testing to ensure food safety, a fact

TABLE 13. *Food defect action levels (DALs) for grain and milled cereal grains (29)*

Food	Defect	DALs
Corn meal	Insects	Average of one or more whole insects (or equivalent) per 50 g
	Insect filth	Average of 25 or more insect fragments per 25 g
	Rodent filth	Average of one or more rodent hairs per 25 g
		OR Average of one or more rodent excreta fragments per 50 g; significance: aesthetic
Wheat	Insect damage	Average of 32 or more insect-damaged kernels per 100 g
	Rodent filth	Average of 9 mg (or more) of rodent excreta pellets or pellet fragments per kg; significance: aesthetic
Wheat flour	Insect filth	Average of 75 or more insect fragments per 50 g
	Rodent filth	Average of one or more rodent hairs per 50 g; significance: aesthetic

that contributed to the development and evolution of the HACCP system of food safety.

The application of microbiological specifications for milled cereal grains is impractical and unnecessary. The U.S. government report cited above *(23)* offers the following advice: ''Cereal grains and their milled products have seldom been implicated as vehicles of foodborne disease and thus there does not appear to be a need for bacterial standards. Because grains, flour, grits, and related items are essentially raw agricultural products, and there is little opportunity for microbial growth during their processing, the microbiology of these foods usually does not correlate well with manufacturing practices. Guidelines for these foods, therefore, have little application.'' The use of such guidelines or specifications will waste the time as well as the physical and financial resources of the dry-milling industry and its customers. Developing microbiological specifications, collecting samples, performing laboratory analyses, and fulfilling numerous administrative requirements accumulate tangible and intangible costs. Holding materials until cleared by laboratory testing consumes time and can logistically derail the nearly universal just-in-time and first-in, first-out production and distribution systems that are widely used in the food industry. Product rejections further upset supply chain logistics with costly delays. In our estimation, the dry-milling industry is unnecessarily spending millions of dollars annually in order to comply with impractical microbiological specifications and lot acceptance criteria.

Use of microbiological monitoring guidelines for milled cereal grains. Progressive millers and customers, because of the above considerations, have gradually moved away from microbiological specifications and lot acceptance criteria for milled cereal grains. Rather, microbiological monitoring guidelines are used to verify the sanitary condition of the milling and transportation operations. Monitoring tests are performed periodically (weekly to monthly) at each mill. Monitoring samples are collected in the dry-milling operations in or near the processing equipment. The monitoring guidelines and samples are not linked to specific lots or shipments of whole or milled cereal grains—that is, they are not used as the basis for accept or reject decisions. It is important that this distinction between monitoring guidelines and microbiological specifications be understood and maintained. Monitoring results and corrective actions are typically shared with customers, a practice that maintains customer confidence while replacing the use of microbiological specifications and lot acceptance criteria.

TABLE 14. *Comparison of the MPN and Petrifilm methods for the enumeration of coliform bacteria and* E. coli *in milled cereal grains*

Method	Assay	*n*	Geometric mean	SD
MPN	Coliform	5,099	1.48	0.88
Petrifilm	Coliform	7,784	1.62	0.64
MPN	*E. coli*	5,573	0.18	0.24
Petrifilm	*E. coli*	8,727	0.46	0.25

The APCs and mold counts are most commonly used as monitoring tests. While the microbiological load of raw cereal grains varies considerably because of environmental factors in the field, additional microbial growth will not occur in milled cereal products unless water is unintentionally added by condensation or system leaks. Increased mold counts are the best microbiological indicator of such moisture contamination, as molds can grow at water activity values far lower than those necessary to support bacterial growth. Mold counts are used instead of yeast counts because they are generally 1 log higher than yeast counts (Tables 6 through 10) and are, therefore, a more practical indicator of microbial growth potential. Coliform and *E. coli* counts are not useful as monitoring indicators, as they can grow only at high water activity values, and *E. coli* is present in numbers too low to be a useful quantitative indicator. More importantly, the utility of coliform and *E. coli* tests in milled cereal grains is rapidly diminishing for the reasons discussed above.

Examples of monitoring guidelines that could be used are APC values less than 100,000/g and mold counts less than 5,000/g. The actual monitoring guidelines may be different from these, based on mutual agreement between the purchaser and supplier. Should the monitoring guidelines be exceeded, additional samples would be tested, and system surveillance and corrective actions would be taken to identify and eliminate sites of moisture contamination and to install more effective equipment and procedures when necessary. In our experience, load-out equipment can collect condensation, particularly in cold weather. If not eliminated, the resulting product accumulations will support mold growth. Higher mold counts or APCs in these sites serve as an early warning signal for potentially increased

microbiological counts in the system. Detection and elimination of product accumulations with excessive moisture are more effective means by which to ensure and maintain the microbiological quality of milled cereal grains than is lot-specific testing for conformance to microbiological specifications.

CONCLUSIONS

Excellent and improving microbiological quality and safety of milled cereal grains. Milled cereal grains have a long history of being safe for consumption. This outstanding record is attributable to the excellent sanitary quality of the milled products and to the baking, frying, or cooking of the vast preponderance of milled cereal grains before consumption. Our survey documents an extremely low incidence of salmonellae and excellent microbiological profiles in the five surveyed products. The mean counts of the quantitative indicator microorganisms have been trending downward over the past 50 years or so because of improved quality and food safety management practices in the dry-milling industry. Any lingering concern about the microbiological safety of milled cereal grains should be allayed by these results.

Diminished utility for microbiological specifications. Along with the entire food industry, dry millers have learned that microbiological specifications and lot acceptance criteria are inadequate to ensure food safety. Product testing cannot be used to manage the hazard of low-incidence pathogen contamination. The quantitative tests that have been used to indicate inadequate sanitation or a potential public health hazard bear little or no relationship to the possibilities of contamination or foodborne illness. Milled cereal grains are used in relatively few and limited product applications in which they are not heated before consumption. In these particular applications, specialized processes can be used to heat the milled cereal grains before being used in the product.

Microbiological monitoring guidelines. The diminished utility of microbiological specifications and the impracticality of most quantitative indicator tests, e.g., the coliform test, do not eliminate the necessity of microbiological monitoring to verify compliance with the requirements of good agricultural practices, GMPs, and HACCP systems. The use of the APC and mold count guidelines in a periodic monitoring system can ensure the effective maintenance of the microbiological quality of milled cereal grains. The continued use of microbiological specifications and lot acceptance criteria, with their proven inability to ensure food safety, constitutes a waste of resources both for the dry-milling industry and for its customers. Such a misuse of resources is a strategic technical and commercial issue that can be resolved by the replacement of microbiological specifications and lot acceptance criteria with preventive control measures and periodic monitoring of microbiological guidelines.

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