Food Safety Information Papers

CORN REFINERS ASSOCIATION, INC.

MICROBIAL ASSESSMENT

QUESTION*: What processes and procedures are in place to minimize the risk that food ingredients from the corn wet milling industry will contain pathogenic microorganisms that might pose a significant health risk in food products?*

Executive Summary

The Corn Refiners Association, Inc. (CRA) member companies manufacture a variety of dry and liquid food ingredients. Good Manufacturing Practices (GMP), the Hazard Analysis and Critical Control Point (HACCP) system of food safety management, and quality management systems in CRA member company plants ensure wholesome and safe food products. With regard to the potential for contamination by microorganisms:

- The corn wet milling and subsequent production processes are enclosed systems. These enclosed systems preclude handling and help prevent microbial contamination.
- The corn wet milling process (high temperature) and intrinsic properties of the resulting liquid and dry products [low pH, low water activity (a_w)] create an inhospitable environment for microorganisms.
- Experimental data demonstrate that liquid and dry corn syrups, sweeteners, starches, and acidulants are not only inhibitory to the growth of bacteria (bacteriostatic), but also have the ability to kill undesirable microorganisms (bactericidal), should any be present, during processing, transportation and storage.
- CRA member companies have established qualitative and quantitative analytical procedures for detecting microorganisms in finished products. However, because the distribution of microorganisms may be heterogeneous in nature, no sampling plan can ensure the absolute absence of pathogenic microorganisms. Therefore, product testing is only a component of a larger food safety program, including GMPs, HACCP, and other quality management systems, to minimize the risk of microorganisms contaminating corn wet-milled products.

In total, the corn wet-milling process, intrinsic nature of the products, and quality management systems implemented at CRA member company plants, indicate that it is very unlikely that corn wet-milled products will contain pathogenic microorganisms that might pose a significant health risk. Importantly, there has not been a documented illness outbreak involving pathogenic microorganisms in corn wet-milled liquid and dry products.

1. Introduction

According to the Centers for Disease Control (CDC), foodborne pathogens cause an estimated 9.4 million illnesses in the U.S. per year.[1] Many foodborne pathogens can be found in the feces of animals, as well as in water, soil, or any material exposed to animal feces. Thus, it is reasonable to assume that crops growing in the field can be exposed to pathogens in this manner. Another possible route of exposure is that as food is processed, undesirable organisms can be introduced by poor manufacturing or sanitation practices (including poor employee hygiene). It is important to note, however, that corn, with its protective husk, is well protected from microbial contamination in the field. In addition, enclosed systems and prerequisite plans in corn wet milling, e.g., Good Manufacturing Practices (GMPs), and supporting Hazard Analysis and Critical Control Point (HACCP) systems, are recognized as very effective means of reducing the risk of microbial contamination during processing. Finally, the inherent nature of the corn wet milling process and resulting ingredients further reduces the risk of microbial contamination, as described further below.

It is not surprising, therefore, that a thorough review of government websites, scientific literature, and government/regulatory reports did not identify a single documented foodborne illness outbreak involving pathogenic microorganisms in corn wet-milled products.[2] Specifically, websites hosted by CDC and the U.S. Food and Drug Administration (FDA) were searched for any information pertaining to liquid or dry corn wet-milled products. Particular attention was focused on CDC's Foodborne Outbreak Online Database (FOOD), as well as FDA's Coordinated Outbreak Response and Evaluation (CORE), and recall, market withdrawal, and safety alert system. Relatively little information was found from any of these sources that related specifically to the corn wet-milled products, and none of the information indicated that any of these products were responsible for foodborne outbreaks or product recalls. Thus, there is an absence of historical evidence associating these products with concerns of pathological microorganisms and foodborne illness.

2. Conditions Affecting the Survival or Growth of Microorganisms

In any given environment, a particular microorganism may grow, be killed, or simply survive. Control measures that kill bacteria are known as bacteriocidal, whereas controls that do not kill the bacteria but inhibit growth are known as bacteriostatic. When considering whether a microorganism will survive or grow in any particular environment, three aspects need to be evaluated:

- Environmental or extrinsic factors
- Intrinsic factors
- Characteristics of particular microorganisms of interest

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[1] Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauze, R.V., Widdowson, M-A., Roy, S.L., Jone, J.L., Griffin, P.M. 2011. Foodborne illness acquired in the United States—Major Pathogens. Emerging Infectious Diseases, 17(1):7-15. January.

[2] ToxStrategies, Inc. 2013. Corn Refiners Association, Inc. (CRA), Task 1: Data gathering and evaluation of information. Prepared on behalf of the Corn Refiners Association, Inc. March.

Each of these is discussed below.

Environmental or Extrinsic Factors

The two most important environmental factors influencing microbial survival and growth are temperature and level of oxygen.

Like larger animals, microorganisms have a discrete **temperature** range in which they flourish, and can be classified by the optimum temperatures at which they grow. Most human and animal pathogens belong to the group mesophiles ("middle loving"); their optimum growth range is 20-45°C.[3] Examples of pathogens in this group are *Escherichia coli (E. coli)*, *Salmonella*, *Clostridium botulinum* and *Staphylococcus aureus*. Thermophiles ("hot loving") are microorganisms that grow optimally at high temperatures, 40-70°C, whereas bacteria with cold optimal growth temperatures (0-10°C) that do not grow well at mesophilic temperatures are known as psychophiles. A fourth category is bacteria that grow slowly at temperatures less than 15°C, but prefer growing at warmer temperatures, which are known as psychotrophs.[4] Minimum, optimum and maximum temperature ranges for these groups of microorganisms are summarized in Table 1.

Table 1: Cardinal temperatures for microbial growth[5]

	Temperature (°C)		
Group	**Minimum**	**Optimum**	**Maximum**
Thermophiles	40-45	55-75	60-90
Mesophiles	5-15	30-40	35-47
Psychrophiles (obligate psychrophiles)	-5 to +5	12-15	15-20
Psychrotrophs (facultative psychrophiles)	-5 to +5	25-30	30-35

Microorganisms can also be classified by their **oxygen** requirement for growth. Microorganisms that require oxygen are strict aerobes; without oxygen these organisms may survive, but not grow. Vacuum packaging of foods or hermetically sealing containers, e.g., metal cans, will prevent their growth. Some bacteria and most molds are strict aerobes. Facultative anaerobes comprise the largest group of food-related microorganisms; they are capable of growing whether or not oxygen is present. S*almonella* and *E. coli* are facultative anaerobes. Strict anaerobes cannot grow in the presence of oxygen. These organisms grow in the absence of oxygen, e.g., in well-sealed containers or cans. *Clostridium botulinum*, the microorganism responsible for botulism, is one example of a strict anaerobe.

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3 US Food and Drug Administration (FDA). 2012. *Bad Bug Book*, *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, Second Edition.* Washington, DC. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm.

4 Ibid.

5 International Commission on Microbiological Specifications for Foods (ICMSF). 1980. Microbial Ecology of Foods, Volume 1, Factors Affecting Life and Death of Microorganisms. New York: Academic Press.

Intrinsic Factors

The inherent properties of a food product that influence its susceptibility or resistance to microbial growth and survivability are termed "intrinsic factors." The most important of these intrinsic factors are water activity, osmotic pressure, and pH.

Water activity (a_w) is a measure of the state or condition of water in foods (e.g., availability for microbial growth). Water activity values theoretically range from 1.0 (pure water) to 0 (absolutely no water available). The water activity of a food product depends upon the amount and nature of solutes present. Controlling water activity is one of the oldest and most effective methods for preventing the growth of microorganisms in foods. This is usually accomplished by drying foods (getting rid of water) or by adding solutes such as sugars or salts (to bind the water so it is not available for the microorganism).[6,7]

Osmotic pressure is a measure of the force that must be exerted to prevent water from flowing from one side of a membrane to the other. Solutions with a high osmotic pressure have a strong tendency to adsorb water. If a microbe is placed in a solution with a high osmotic pressure, water will flow out of the microbial cell and into the solution, an effect known as plasmolysis. Conversely, if a microbe is placed in a solution with low osmotic pressure, water will flow into the microbial cell. Cytolysis occurs when sufficient water flows into a cell such that the cell bursts. In either case, microbial cells may die as a result of the osmotic pressure of the environment.

pH is the measure of acidity or alkalinity of a food product. All microorganisms have a specific pH range over which they grow and survive. Bacteria tend to prefer a neutral environment, typically pH 5-7. Yeasts and molds prefer an acidic environment, typically pH 2-5. The growth of microorganisms is influenced by both the initial pH and the buffering capacity of the food. The buffering capacity is the resistance of a food to changes in pH. The higher the buffering capacity, the more the food product resists changes in pH. Foods with low buffering capacity, such as a corn syrup, cannot support as much microbial growth as a food with a high buffering capacity, such as milk.

In combination, these intrinsic factors can prevent or significantly limit a microorganism's ability to survive or grow in a particular food product; however, these factors would not necessarily be relied upon as the sole basis for ensuring product safety.

Characteristics of Particular Microorganisms of Interest

There are four groups of microorganisms that are of concern for food safety: bacteria, molds, viruses, and protozoans. Molds are a concern only because they may produce mycotoxins.[8] In

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6 Ibid.

7 Troller, J.A. 1983. Effect of Low Moisture Environments on the Microbial Stability of Food. In: Economic Microbiology Volume 8, Food Microbiology. Rose, A.H., ed. New York: Academic Press.

8 Corn Refiners Association (CRA). 2011. Food Safety Information Paper, Micotoxins. Prepared by WHITE *Technical Research* GROUP. March. http://www.corn.org/wp-content/uploads/2009/12/mycotoxins.pdf.

contrast to bacteria and molds, viruses and protozoa cannot grow in foods. Control of viruses and protozoa in foods relies on exclusion and inactivation.

Some Gram-positive bacteria, such as *Bacillus* and *Clostridium* species, are capable of producing spores, a very stable, dormant stage of the bacteria. These are not known to be an issue with corn wet-milled products.

Table 2 lists the temperature, pH and water activity ranges that can promote the growth of common pathogenic microorganisms, provided the required nutrients are also present.

Table 2: Ranges of conditions favoring pathogen growth[9].

Pathogen	Temperature (°C)	pH	Water Activity
Bacillus cereus	4 – 55	5.0 – 8.8	≥0.93
Clostridium perfringens	12 – 50	5.5 – 9.0	0.93 – 0.97
Escherichia coli	7 – 46	4.4 – 9.0	≥0.95
Listeria monocytogenes	-0.4 – 45	4.4 – 9.4	≥0.92
Salmonella	5.2 – 46[a]	3.8 – 9.5	0.94 – >0.99
Staphylococcus aureus	10 – 48[b] (7 – 48)[c]	4.5 – 9.6[b] (4 – 10)[c]	0.87 – >0.99[b,d] (0.83 – >0.99)[c,e]

[a]Most serotypes fail to grow at < 7°C
[b]Toxin production
[c]Growth
[d]Aerobic (anaerobic 0.90 – >0.99)
[e]Aerobic (anaerobic 0.92 – >0.99)

As a general rule, pathogenic microorganisms grow most favorably at temperatures below 50°C, within a pH range of 4.5 – 9.5 and at water activity values above 0.86. However, *Salmonella* is an exception to this rule because *Salmonella spp.* have been shown to survive in low-moisture foods and environments [e.g., low-moisture (a_w = 0.176), high-fat (31.8%) confectionary[10], peanut butter with an a_w that did not exceed 0.33[11]]. Importantly, these foods have relatively neutral pH values, in contrast to the lower pH values of most of the corn wet-milled products discussed in the next section.

3. Properties of Corn Wet-Milled Products That Prevent or Inhibit Growth and Survival of Microorganisms

Liquid Products

Liquid corn syrups and sweeteners typically have water activity, osmotic pressure, and pH values that do not permit microorganisms to survive or grow. For liquids, water activity and osmotic pressure are interrelated: solutions with low water activity have high osmotic pressures. Any microbe in syrups experiences a water activity that is too low to support growth and an environment that actually pulls water out of the cell, resulting in death. In fact, the use of high

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9 International Commission on Microbiological Specifications for Foods (ICMSF). 1996. Microorganisms in Foods 5. Characteristics of Microbial Pathogens. London; Blackie Academic & Professional.

10 Kotzekidou, P. 1998. Microbial stability and fate of *Salmonella* Enteriditis in halva, a low-moisture confection. J. Food Protect. 61:181-185.

11 Burnett, S. L., E. R. Gehm, W. R. Weissinger, and L. R. Beuchat. 2000. Survival of *Salmonella* in peanut butter and peanut butter spread. J. Appl. Microbiol. 89:472-477.

concentrations of solutes, such as sucrose, fructose, and corn syrup, is one of the oldest known food preservation methods.[12]

Table 3 details typical water activity and pH of several liquid corn wet milling products. When compared to the optimal water activity and pH needs of common pathogens (Table 2), one can see that corn syrups and sweeteners are an inhospitable environment for growth or survival of pathogenic microorganisms.

Table 3: Syrup processing and product properties deterring pathogen growth and survival.[13]

Product	Processing Temperature (°C)[14]	% solids	pH	Water Activity
42% HFCS	>60 ↓	71	3.9	0.76
55% HFCS		77	4.4	0.66
25 DE Corn Syrup		78	3.7	0.74
36 DE Corn Syrup		80	5.0	0.70
63 DE Corn Syrup		81	5.2	0.59
65% High Maltose Syrup		81	5.0	0.69

Niroomand and co-workers[15,16] inoculated corn and sucrose ingredients with a broad spectrum of bacterial pathogens and followed viability over time. The researchers attempted to inoculate so as to deliver approximately 10^4 to 10^5 cells of each microorganism per gram of product. **This inoculation level was intended to represent a worst-case scenario of intentional contamination or improper handling.** Pathogens tested included *Salmonella*, *Listeria*, *Staphylococcus*, and *E. coli*. Their data supported the following conclusions:

- The number of microorganisms fell below the detection limit in less than three days when syrups and sweeteners were stored at normal holding temperatures (32-46°C).
- When products were stored at the lower temperature limit reached during transportation (27°C), a reduction in the number of microorganisms was still observed, but occurred at a slower rate.
- The fastest rates of reduction were observed in 42 and 55% HFCS.

As noted above, the experiments of Niroomand *et al.* were conducted to mimic a worst-case scenario (high inoculum, optimal situations for growth). Their results suggest that **incidental contamination** of corn syrups and sweeteners with undesirable microorganisms does not present a public health hazard. The authors attribute this finding to final product pH, water activity and

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12 Gomez, R. and A. Herrero. 1983. Chemical Preservation of Foods (Chapter 3). In: Economic Microbiology, Vol. 8, Food Microbiology, Rose, A.H. ed. London: Academic Press.

13 Niroomand, F., W.H. Sperber, V.J. Lewandowski and L.J. Hobbs. 1998. Fate of bacterial pathogens and indicator organisms in liquid sweeteners. *J. Food Protection*. 61(3): 295-299.

14 At a minimum, processing temperatures will reach >60˚C; however, depending on the specific product or step in the process, temperatures above 60˚C will vary considerably.

15 Niroomand, F., W.H. Sperber, V.J. Lewandowski and L.J. Hobbs. 1998. Fate of bacterial pathogens and indicator organisms in liquid sweeteners. *J. Food Protection*. 61(3): 295-299.

16 Lewandowski, V.J. 1998. Fate of microorganisms in corn milling products. *Bev Tech 98*, Savannah GA, March 30 – April 1.

osmotic pressure and even suggest that “because the liquid sweeteners cause the destruction of vegetative pathogens, these ingredients can be considered to be free of microbiological hazards.” **Thus, when considering the hazard potential of environmental pathogens in liquid corn syrup and sweeteners, the likelihood of occurrence is extremely low**.

Dry Products

CRA member companies make a variety of dry products for the food industry, including acidulants, corn syrup solids, dextrose and starches. Table 4 provides typical pH and water activity for these products. As shown, corn wet-milled dry products are also characterized by extremely low water activity—even lower than corn wet-milled liquid products (see Table 3). This exceedingly low water activity, in combination with processing temperatures above 60°C, low product moisture and acidic final product pH values, indicates that corn wet-milled dry products are an inhospitable environment for pathogenic microorganisms.

Table 4: Dry product processing and properties deterring pathogen growth and survival.[17]

Product	Processing Temperature (°C)[18]	pH	Water Activity
Citric Acid	>60 ↓	1.8	0.15
Sodium/Potassium Citrate		8.5-8.8	0.12-0.13
Corn Syrup Solids		4.4-5.5	0.14-0.17
Dextrose		4.3	0.12
Food Starches		4.6-8.7	0.28-0.46

Niroomand, Sperber, Lewandowski and Hobbs extended their work with liquid sweeteners to study the fate over time of microbial pathogens inoculated into dry sweeteners, starches and citrates.[19,20] In this case, the researchers targeted inoculating the samples to approximately 10^5 to 10^6 cells of each microorganism per gram of product, **which again was intended to represent a worst-case scenario of intentional contamination or improper handling**. Not surprisingly, they observed that dry products also exhibit bacteriostatic and bacteriocidal properties against *Staphylococcus*, *Salmonella* and *E. coli*. For citric acid, the number of organisms fell below the detection limit within minutes, which the authors attributed to the low pH. For the remaining dry products, the rate of decline was slower than for citric acid and the liquid products, with some organisms completely dying off by the end of the observation period (12 months) in some products, while other organisms declined substantially, but did not die off completely, in other products. Although the authors concluded that there is need for controls to prevent post-process bacterial contamination of corn wet-milled dry products such as citrates, starches, and dry sweeteners, it is important to note that this study utilized a very high inoculum level (10^5 to10^6 cells per gram) as compared to what might be more typical for these products (1 to 10 cells per gram). **Thus, the hazard potential of environmental pathogens in citrates, starches and dry sweeteners, the likelihood of occurrence is very low.**

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[17] Cargill, Inc. 1999. Fate of Microorganisms in Dry-Wet Corn Milling Products. Unpublished data. September 28.
[18] At a minimum, processing temperatures will reach >60˚C; however, depending on the specific product or step in the process, temperatures above 60˚C will vary considerably.
[19] Cargill, Inc. 1999. Fate of Microorganisms in Dry-Wet Corn Milling Products. Unpublished data. September 28.
[20] Lewandowski, V.J. 1998. Fate of microorganisms in corn milling products. *Bev Tech 98*, Savannah GA, March 30 – April 1.

4. Pathogen Management by the Corn Wet Milling Industry

GMP, HACCP and Quality Management Systems

CRA member companies have implemented quality management systems to ensure the safety of their products. These systems are comprehensive and encompass a number of prerequisite programs such as design and process control, GMPs, sanitation, pest control and personal hygiene. These programs are often coupled with the implementation of a HACCP program. Through the use of hazard analysis, significant food safety hazards are identified and critical control points in the manufacturing process are established as necessary. These points are then controlled and routinely monitored to minimize or eliminate all identified hazards. Other process control points, although not considered "critical" under HACCP, are also routinely monitored to ensure product safety. These programs, in combination with the inherent properties of the products themselves (low water activity and low pH), minimize the risk of microbial contamination in corn wet-milled products.

Product Sampling and Testing

CRA member companies have established qualitative and quantitative analytical procedures for detecting microorganisms in finished products. The 2007 Edition of *CRA Microbiological Methods of the Member Companies* contains the following microbiological methods:[21]

- Mesophilic Aerobic Bacteria
- Mesophilic Yeasts and Mold
- Osmophilic Yeasts, Mold and Bacteria
- Coliform Group of Bacteria
- *Salmonella* species
- Thermophilic Spore-forming Bacteria
- Coagulase Positive *Staphylococci*
- Anaerobic Bacteria
- *Pseudomonas* species
- *Bacillus cereus* count
- Mesophilic Aerobic Spore-formers
- Rapid Microbiological Methods.

These analytical procedures for detecting microorganisms in finished products, as well as rigorous product specifications, assist in the control of microbial pathogens in corn wet-milled products.

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[21] Corn Refiners Association, Inc, *CRA Microbiological Methods of the Member Companies,* 2007 Edition. http://www.corn.org/publications/industry-resources/microbiological-methods/microbiological-methods-toc/.

5. Conclusions

The presence of foodborne pathogens is a matter of significant public health concern. There are no governmental guidelines or specifications for microorganisms in corn wet-milled products; therefore, the corn refining industry has taken a leadership role in conducting research, establishing testing protocols, specifications, GMPs, HACCP, and other Quality Management Systems to control microbial pathogens in corn wet-milled products. These measures, in addition to the intrinsic nature of the products, indicate that it is very unlikely that corn wet-milled products will contain pathogenic microorganisms that might pose a significant health risk. The member companies of the CRA are justifiably proud of the fact that no corn wet-milled product has ever been implicated in an outbreak of illness involving foodborne pathogens. They will continue to work diligently to maintain that record.

Food Safety Information Papers

CORN REFINERS ASSOCIATION, INC

MICROBIAL ASSESSMENT ADDENDUM 1 — *BACILLUS CEREUS*

1. Introduction

In 2013, the Corn Refiners Association, Inc. (CRA), issued a white paper reporting a microbial assessment of food ingredients from the corn wet milling industry. The white paper, entitled "Microbial Assessment", reviewed the processes and procedures that are in place to minimize the risk of pathogenic microorganisms in these food ingredients. This addendum serves to update the "Microbial Assessment", focusing on *Bacillus cereus* (*B. cereus*), and seeks to address questions regarding the ability of *B. cereus* to survive corn-processing conditions and its potential to grow out in consumer foods that contain ingredients from the corn wet milling industry.

A literature review was performed to identify scientific literature and government reports relevant to *B. cereus*, with a particular interest in corn products. The review was specifically focused on literature evaluating the conditions required for *B. cereus* to grow or sporulate, survive during food processing, and germinate as spoilage organisms under formulated food conditions that are common in corn ingredients and products. A primary search of the U.S. Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), European Food Safety Authority (EFSA), and World Health Organization (WHO) websites was performed to identify relevant regulatory opinions and authoritative reports. Search syntax specific to *B. cereus* and corn products was also generated[1] and used to search both public and proprietary literature databases (i.e., PubMed, Google Scholar, ToxPlanet, Embase). Of the approximately 300 titles and abstracts reviewed, 30 were retained for full-text review to determine relevance. Of these 30, five are included in this addendum, because they pertained to the levels of *B. cereus* on corn and in processed corn products for human consumption. Hand-searching of the references from the 2013 "Microbial Assessment" and relevant government reports was also performed to ensure a comprehensive literature review. While an extensive literature base exists for *B. cereus*, the amount of literature evaluating *B. cereus* in corn processing and corn products is limited. The most abundant subject of the relevant literature is related to the intentional application of microbials, including *B. cereus*, to plants for insecticidal/pesticidal purposes, and in some cases, the endophytic (co-) occurrence of the microorganisms. Literature evaluating the occurrence of *B. cereus* in rice and dairy products is also plentiful. Overall, very few resources in the primary literature or on government websites were identified to characterize the levels of *B. cereus* in corn, or that would indicate its continued presence in consumer products containing corn or corn products.

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[1] Database-specific search strings were created using various combinations of the following key words: *Bacillus cereus*, corn, maize, *Zea mays,* cereal, consumer, contamination, growth, processing, occurrence, and spoilage.

2. Genus/species taxonomy

Bacillus cereus is a Gram-positive, endospore-forming, facultative anaerobe that is ubiquitous and commonly found in soil, vegetation, and foodstuffs (FDA 2012; EFSA 2005). It is included in the *Bacillus cereus sensu lato* group, a subdivision of the *Bacillus* genus, consisting of eight formally recognized species: *Bacillus cereus sensu stricto, Bacillus thuringiensis, Bacillus anthracis, Bacillus weihenstephanensis, Bacillus mycoides, Bacillus cytotoxicus, Bacillus toyonensis,* and *Bacillus pseudomycoides* (EFSA, 2005, 2016). While EFSA (2016) reports that these species can be distinguished by phenotypic and genotypic features, the distinction between *B. cereus* and *B. thuringiensis* is not as clear, and the actual contribution of each species to the incidence of foodborne illnesses is not currently known.

3. Optimal growth conditions

Spores of *B. cereus* are present in most categories of food; however, they generally exist in numbers too low to cause foodborne illness (EFSA, 2016). The ability of these naturally occurring spores to survive under the extreme conditions of processing, active growth, and proliferation of *B. cereus* is characterized by the presence of 10^6 cells/g of food vehicle, which is recognized as a hazard to health (FDA, 2012). While a risk exists due to consumption of foods with high initial contamination, multiplication of *B. cereus* after temperature abuse (i.e., improper cooling) is generally more common (EFSA, 2016). In some circumstances, processes that are assumed to prevent microbial growth (e.g., pasteurization) may allow *B. cereus* to proliferate in the absence of prevention measures such as rapid cooling (EFSA, 2016). Optimal growth temperatures for *B. cereus* vary in the literature, with most sources citing temperatures within the range of 28°C to 40°C (FDA, 2012; EFSA, 2005, 2016; FSANZ, 2010). Similarly, minimum and maximum growth temperatures are generally reported to be near 4°C and 55°C, respectively (FDA ,2012; EFSA, 2005, 2016; FSANZ, 2010). Although some strains are able to multiply at low temperatures (4–10°C), emetic *B. cereus* strains (i.e., strains that cause vomiting) are presumably unable to grow. Additionally, strains that produce the toxin cereulide cannot do so below 10°C, or in the absence of oxygen. The generation times of these particular strains are also considerably longer. Growth can occur in pH ranges from 4.9 to 10.0, and a pH <4.5 is considered sufficient to inhibit growth of *B. cereus* (FDA, 2012; EFSA, 2005; FSANZ, 2010). Optimal water activity (a_w) for *B. cereus* is >0.93. **Table 1** depicts optimal conditions as described in literature published by regulatory agencies.

Table 1. Optimum and range growth conditions of *B. cereus* as described in FSANZ (2010), FDA (2012), EFSA (2005, 2016).

	Bacterial Growth		Emetic Toxin Production[a]		Diarrheal Toxin Production[a]	
	Optimum	Range	Optimum	Range	Optimum	Range
Temperature (°C)	28–40	4–55	12–15	10–37	32	10–43
pH	6.0–7.0	4.9–10.0	-	-	8.0	5.5–10
Water activity	-	0.93–0.99	-	-	-	-

[a] As described further below, some strains of *B. cereus* cause vomiting, while others cause diarrhea.

As stated in the 2013 "Microbial Assessment," the low water activity (≤0.76), acidic final pH values (≤5.2), and high processing temperatures (>60°C) indicate that corn wet-milled products (dry and liquid) are inhospitable for the growth of *B. cereus*.

4. Levels in corn products

In studies from outside the United States,[2] *B. cereus* has been reported to be naturally present on corn. For example, *B. cereus* (Strains FM-4 and AG 13) and other *Bacillus* species have been identified in a screen of endophytic bacteria found on Brazilian sweet corn (Figueiredo et al., 2009). Similarly, *B. cereus* has also been identified as an endophytic species in Chinese maize cultivars (Gao et al., 2004; abstract only). Furthermore, in an analysis of bacteria isolated from minimally processed vegetables from supermarkets in Botswana, *B. cereus* represented 7.7% of the microorganisms on corn (Manani et al., 2006). According to the authors, the presence of *B. cereus* (along with other pathogenic organisms) identified on vegetables may have significant public health implications, particularly for high-risk, immunocompromised individuals (Manani et al., 2006).

Three studies were relevant to examining *B. cereus* in processed corn products. Only two studies were identified that reported the presence of *B. cereus*, both of which examined the presence in food products from small indigenous communities. Numerous *Bacilli* species, including *B. cereus*, were found in maize product precursors (e.g., dry maize, masa, nixtamal), during a study of the microflora present in corn tortillas produced in a Guatemalan highland Indian village. Although most of the organisms were killed during the heating process (temperature not specified), *B. cereus* levels remained high, at up to 10^9 cells/gram, after 24 or 48 hours of room-temperature storage. Although reheating the tortillas for 1–2 minutes reduced the *B. cereus* bacterial counts, potentially dangerous levels of bacteria were still present, and heat-stable enterotoxins could remain (Capparelli and Mata, 1975). *B. cereus* has also been identified in a fermented beverage that contains corn produced by the Tapirape Amerindians (Almeida et al., 2007; abstract only). Finally, in a study that sampled 30 commercial cereal products for the presence of *B. cereus*, two ready-to-eat corn-flake cereal samples were evaluated (Lesley et al., 2013). Neither sample was contaminated with *B. cereus*.

In summary, *B. cereus* may exist as an endophytic species on corn that remains present after harvesting. Except for reports from small indigenous communities, no literature was identified to support the presence of *B. cereus* in manufactured corn products.

5. Prevalence as a pathogen

B. cereus has been recognized as a human pathogen involved in food-related infections and is considered an important potential food contaminant due to its ubiquitous nature and ability to survive processing and to grow out during improper storage of prepared foods (EFSA, 2016). Two kinds of foodborne illness are due to *B. cereus.* The first, identified as emetic (vomiting) intoxication, occurs due to the ingestion of growing cells in foods that produce the toxin

[2] No studies were found for the United States.

cereulide. Cereulide is acid- and heat-stable, leaving it activated once it is formed during food processing (EFSA, 2016). The second, a diarrheal infection, is a result of enterotoxins that are produced in the small intestine (EFSA, 2005). Three different enterotoxins (i.e., non-hemolytic enterotoxin (*nhe*), hemolysin BL (*hbl*), and cytotoxin K (*cytK*)) have been linked to the diarrheal type of *B. cereus* food poisoning (EFSA, 2016). While genes encoding *nhe* are found in most *B. cereus* strains, those for *hbl* and *cytK* are found in approximately 30%–70% of *B. cereus strains.* Unlike cereulide, the enterotoxins are susceptible to heat during food processing and enzymatic digestion in the stomach. Thus, enterotoxins may be destroyed during high-heat processing or digestion, but temperature abuse resulting in sporulation may allow for the outgrowth of spores in the small intestine. This outgrowth is considered a qualifying component of the diarrheal type.

Transmission of *B. cereus* food poisoning is caused by the consumption of large numbers of the bacteria or spores (10^5 to 10^8 cells/g of the food vehicle), which is generally a result of poor food handling or storage, or temperature abuse after cooking (EFSA, 2005; FSANZ, 2010). Outbreaks associated with lower numbers in foods (10^3 to 10^4 cells/g of the food vehicle) have also been reported (EFSA, 2005). Incidence data for *B. cereus* food poisoning is limited, and cases are likely under-reported due to the typically mild symptoms and their short duration (FDA, 2012; FSANZ, 2010). Further, because strains of *B. cereus* and *B. thuringienses* are difficult to distinguish from one another, they are typically not differentiated in routine clinical diagnostics or food microbiology (EFSA, 2016). Because of this, it is difficult to understand the impact of each species on foodborne illness.

While almost any food can be contaminated by *B. cereus*, a majority of the outbreaks evaluated by EFSA (2005) were linked to foods prepared by restaurants and catering establishments, likely due to improper refrigeration and temperature abuse. In the United States, *B. cereus* was confirmed in two outbreaks and suspected in six others, accounting for 1% of outbreaks identified by the Foodborne Disease Outbreak Surveillance System in 2015 (CDC, 2017), the most recent year for which data are available. Outbreak-associated illness for *B. cereus* was reported in 25 confirmed cases and 28 suspected cases, with no hospitalizations, and this accounted for 0.3% of all outbreak-associated illnesses reported in 2015. Similar to EFSA's conclusions linking *B. cereus* foodborne illness in the EU to restaurants or catering establishments, all confirmed and suspected outbreaks in the U.S. in 2015 were associated with a catering/banquet facility or a restaurant (CDC, 2017). These cases were also linked to improper refrigeration and temperature abuse. Notably, none of the 2015 U.S. outbreaks were associated with corn or consumer products containing corn or corn products.

6. Conclusions

B. cereus has been recognized as a human pathogen involved in food-related infections, primarily occurring due to improper food preparation and storage methods. However, evidence that *B. cereus* exists as a pathogen in processed food containing corn is limited to case reports of indigenous populations. These reports are from populations where the diligent specifications, Good Manufacturing Practices (GMPs), Hazard Analysis Critical Control Point (HACCP) systems, and other Quality Management Systems of the corn refining industry do not exist. This, in combination with the intrinsic nature of these food ingredients and the lack of reports of associated food illness outbreaks, suggests that the potential for *B. cereus* to pose a significant health risk in corn wet-milled products is very low.

7. References

Almeida EG, Rachid CC, Schwan RF. Microbial population present in fermented beverage 'cauim' produced by Brazilian Amerindians. Int J Food Microbiol. 2007 Nov 30;120(1-2):146-51.

Capparelli E, Mata L. Microflora of maize prepared as tortillas. Appl Microbiol. 1975 Jun;29(6):802-6.

Centers for Disease Control and Prevention (CDC). Surveillance for Foodborne Disease Outbreaks, United States, 2015, Annual Report. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2017.

European Food Safety Authority (EFSA). Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. The EFSA Journal. 2005;175:1-48.

European Food Safety Authority (EFSA). Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. EFSA Panel on Biological Hazards (BIOHAZ). The EFSA Journal. 2016;14(7):4524.

Figueiredo JE, Gomes EA, Guimarães CT, de Paula Lana UG, Teixeira MA, Lima GV, Bressan W. Molecular analysis of endophytic bacteria from the genus Bacillus isolated from tropical maize (Zea mays L.). Braz J Microbiol. 2009 Jul;40(3):522-34.

Food Standards Australia New Zealand (FSANZ). *Bacillus cereus*. 2010. Available at: https://www.foodstandards.gov.au/publications/Documents/Bacillus%20cereus.pdf

Gao Z, Zhuang J, Chen J, Liu X, and Tang S. [Population of entophytic bacteria in maize roots and its dynamic analysis], *abstract only*. Ying Yong Sheng Tai Xue Bau. 2004;15(8):1344-8.

Lesley MB, Velnetti L, Yousr AN, Kasing A, Samuel L. Presence of *Bacillus cereus* s.1. from ready-to-eat cereals (RTE) products in Sarawak. Int Food Res J. 2013;20(2):1031-4.

Manani TA, Collison EK, Mpuchane S. Microflora of minimally processed frozen vegetables sold in Gaborone, Botswana. J Food Prot. 2006 Nov;69(11):2581-6.

Ombui JN, Kagiko MM, Arimi SM. Foodborne diseases in Kenya. East Afr Med J. 2001 Jan;78(1):40-4

U.S. Food and Drug Administration (FDA). Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins. Second Edition. 2012. Available at: https://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf