



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Bacterial Pathogens in Seed Powder and Plant-Based Protein Powder - April 1, 2016 to March 31, 2018

Food microbiology - Targeted surveys - Final report



Summary

The consumption of seed derived products such as seed powder and plant-based protein powder have gained popularity in North America. Seed powders are derived from milled seeds with or without other ingredients. Plant-based protein powders generally contain protein-rich seed powders and other plant-derived ingredients. Their rise in popularity is attributable to the perceived health benefits of omega-3 fatty acids, proteins and fibers contained in these tiny seeds (chia, flax, alfalfa, hemp, etc.). It is also believed that ground seeds offer more health benefits than intact seeds as they are easier for the body to digest. Unfortunately, seed derived products can become contaminated with bacterial pathogens that were initially present in or on seeds. In addition, seed powders and protein powders are low-moisture foods and bacterial pathogens such as *Salmonella* and *Bacillus cereus* (*B. cereus*) can survive for extended periods of time in these low-moisture products. Given that many seed derived products are consumed “as is,” the presence of bacterial pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians, seed derived products (seed powders, plant-based protein powders and dried sprouted seeds/powders) were selected for targeted surveys. The purpose of these surveys was to generate baseline information on the occurrence of pathogenic bacteria of concern in seed derived products on the Canadian market. Results of seed powder and plant-based protein powder testing are reported herein. Results of dried sprouted seeds/powders testing are addressed in a separate report to be published at a later date.

Over the course of this study (April 1, 2016 to March 31, 2018), samples of seed powder (583), and plant-based protein powder (245) were collected from retail locations in 11 cities across Canada. The samples were tested for generic *Escherichia coli* (*E. coli*) and the following bacterial pathogens of concern: *Salmonella* species (spp.), *B. cereus*, *Clostridium perfringens* (*C. perfringens*) and *Staphylococcus aureus* (*S. aureus*). Generic *E. coli* is an indicator of the overall sanitation conditions throughout the food chain from production to the point of sale.

In this study, over 99% of the seed powder and 95% of the plant-based protein powder samples were assessed as satisfactory. *Salmonella* spp. and *S. aureus* (>100 Colony Forming Unit (CFU)/gram (g) in seed powder or >25 CFU/g in plant-based protein powder) were not found in any samples (0/828). Presumptive *B. cereus* was found in 0.5% (3/583) of the seed powder samples at elevated levels ($10^3 < x \leq 10^4$ CFU/g) and in 4.1% (10/245) of the plant-based protein powder samples at elevated levels ($10^2 < x \leq 10^4$ CFU/g). *C. perfringens* was found in 0.8% (2/245) of the plant-based protein powder samples at elevated levels ($10^2 < x \leq 10^3$ CFU/g). A high level (> 10^3 Most Probable Number (MPN)/g) of generic *E. coli* was found in one (0.2%, 1/583) seed powder sample.

In seed powder and plant-based protein powder products, the presence of low levels of *C. perfringens* (≤ 100 CFU/g), *S. aureus* (≤ 100 CFU/g in seed powder, ≤ 25 CFU/g in plant-based protein powder), presumptive *B. cereus* (≤ 1000 CFU/g in seed powder, ≤ 100 CFU/g in plant-based protein powder), or generic *E. coli* (≤ 10 MPN/g in seed powder, ≤ 1.8 MPN/g in plant-based protein powder) is tolerated as they are often found in the environment. Samples where elevated levels of *C. perfringens* ($10^2 < x \leq 10^3$ CFU/g) or presumptive *B. cereus* ($10^3 < x \leq 10^4$ CFU/g in seed powder or $10^2 < x \leq 10^4$ CFU/g in plant-based protein powder) are found indicate that the products may have been produced under unsanitary conditions. Samples where high levels of generic *E. coli* ($>10^3$ MPN/g) are found indicate inadequate sanitation controls during processing and at the processing facility.

The Canadian Food Inspection Agency (CFIA) conducted appropriate follow-up activities and additional sampling. In the case of the sample found to have a high level ($>10^3$ MPN/g) of generic *E. coli*, further sampling and testing was conducted with satisfactory results, and the facility enhanced its product testing program.

Overall, our survey results show that most seed powder and plant-based protein powder products available for sale at retail in Canada have been produced under sanitary conditions and are safe for consumption. However, our results do indicate that a loss of sanitation controls along the food production chain can occur. Consequently, as with all foods, safe handling practices are recommended for producers, retailers and consumers.

What are targeted surveys?

Targeted surveys are used by the CFIA to focus its surveillance activities on areas of highest health risk. The information gained from these surveys provides support for the allocation and prioritization of the Agency's activities to areas of greater concern. Originally started as a project under the Food Safety Action Plan (FSAP), targeted surveys have been embedded in the CFIA's regular surveillance activities since 2013. Targeted surveys are a valuable tool for generating information on certain hazards in foods, identifying and characterizing new and emerging hazards, informing trend analysis, prompting and refining health risk assessments, highlighting potential contamination issues, as well as assessing and promoting compliance with Canadian regulations.

Food safety is a shared responsibility. The CFIA works with federal, provincial, territorial and municipal governments and provides regulatory oversight of the food industry to promote safe handling of foods throughout the food production chain. The food industry and retail sectors in Canada are responsible for the food they produce and sell, while individual consumers are responsible for the safe handling of the food they have in their possession.

Why did we conduct this survey?

The consumption of seeds such as chia, flax, alfalfa and hemp has become increasingly popular in North America in recent years. These tiny seeds are rich sources of omega-3 fatty acids, proteins and fibers, all which are claimed to have many health benefits. Seeds can be eaten either whole or ground, with ground seeds believed to offer more benefits than whole seeds as they are easier for the body to digest. Plant-based protein powders generally contain protein-rich seed powders and other plant-derived ingredients, such as alfalfa and hemp seeds derived plant-based protein powders. Therefore, seed powder and plant-based protein powder products have gained popularity in the Canadian market.

Unfortunately, seeds are agricultural products that can become contaminated with bacterial pathogens during primary production¹. Some seed derived products such as seed powders are cold-processed and are not subjected to a heat treatment aimed at the inactivation of bacterial pathogens. Consequently, seed derived products can become contaminated with bacterial pathogens that were initially present in or on seeds². In addition, seed powders and protein powders are low-moisture foods and bacterial pathogens such as *Salmonella* and *Bacillus cereus* (*B. cereus*) can survive for extended periods of time in these low-moisture products. Given that many seed powder and plant-based protein powder products are consumed "as is," the presence of bacterial pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians, various seed derived products (seed powders, plant-based protein powders and dried sprouted seeds/powders) were selected for targeted surveys. The purpose of these surveys was to generate baseline information on the occurrence of pathogenic bacteria of concern (*Salmonella* species (spp.), *B. cereus*, *Clostridium perfringens* (*C. perfringens*) and *Staphylococcus aureus* (*S. aureus*)), as well as generic *Escherichia coli* (*E. coli*) in seed derived products. Generic *E. coli* is an indicator of the overall sanitation conditions throughout the food chain from production to the point of sale. Results of seed powder and plant-based protein powder testing are reported herein. Results of dried sprouted seed product testing are addressed in a separate report to be published at a later date.

What did we sample?

A sample consisted of a single or multiple unit(s) (individual consumer-size package(s) from a single lot) with a total weight of at least 200 g. All samples were collected from national and local/regional retail stores located in 11 major cities across Canada. These cities encompassed four geographical areas:

- Atlantic (Halifax and Saint John)
- Quebec (Quebec City, Montreal)
- Ontario (Toronto, Ottawa)
- West (Vancouver, Kelowna, Calgary, Saskatoon and Winnipeg)

The number of samples collected from these cities was in proportion to the relative population of the respective areas.

For this study, samples of seed powders were collected between April 1, 2016 and March 31, 2018 and samples of plant-based protein powder were collected between April 1, 2017 and March 31, 2018.

What analytical methods were used and how were samples assessed?

Samples were analyzed using analytical methods published in Health Canada's *Compendium of Analytical Methods for the Microbiological Analysis of Foods*³ (Tables 1 and 2). Health Canada's guidelines⁴ for the presence of pathogenic bacteria and generic *E. coli* in powdered protein were applied in the assessment of plant-based protein powder results (Table 2).

Table 1 - Analytical methods and assessment criteria for bacteria in seed powder

Analysis	Method identification number ^a	Satisfactory	Investigative	Unsatisfactory
<i>Salmonella</i> spp.	MFHPB-20 MFLP-38 MFLP-29	Absent in 25 g	Not Applicable (N/A)	Present in 25 g
<i>Clostridium perfringens</i>	MFHHPB-23	$\leq 10^2$ CFU/g	$10^2 < x \leq 10^3$ CFU/g	$> 10^3$ CFU/g
<i>Bacillus cereus</i>	MFLP-42	$\leq 10^3$ CFU/g	$10^3 < x \leq 10^4$ CFU/g	$> 10^4$ CFU/g
<i>Staphylococcus aureus</i>	MFHPB-21	$\leq 10^2$ CFU/g	$10^2 < x \leq 10^4$ CFU/g	$> 10^4$ CFU/g
Generic <i>E. coli</i>	MFHPB-19	≤ 10 MPN/g	$10 < x \leq 10^3$ MPN/g	$> 10^3$ MPN/g

^a The methods used were the published versions at the time of analysis

Table 2 - Analytical methods and assessment criteria for bacteria in plant-based protein powder

Analysis	Method identification number ^a	Satisfactory	Investigative	Unsatisfactory
<i>Salmonella</i> spp.	MFHPB-20 MFLP-38 MFLP-29	Absent in 25 g	N/A	Present in 25 g
<i>Clostridium perfringens</i>	MFHHPB-23	$\leq 10^2$ CFU/g	$10^2 < x \leq 10^3$ CFU/g	$> 10^3$ CFU/g
<i>Bacillus cereus</i>	MFLP-42	$\leq 10^2$ CFU/g	$10^2 < x \leq 10^4$ CFU/g	$> 10^4$ CFU/g
<i>Staphylococcus aureus</i>	MFHPB-21	≤ 25 CFU/g	$25 < x \leq 10^2$ CFU/g	$> 10^2$ CFU/g
Generic <i>E. coli</i>	MFHPB-19	≤ 1.8 MPN/g	$1.8 < x \leq 10$ MPN/g	> 10 MPN/g

^a The methods used were the published versions at the time of analysis

At the time of writing this report, no assessment guidelines had been established in Canada for the presence of indicator organisms or pathogenic bacteria in seed derived products. As *Salmonella* spp. are considered pathogenic to humans their presence was considered to be a violation of the *Food and Drugs Act* (FDA) Section 4(1)^{a5} and therefore in the absence of assessment guidelines was assessed by the CFIA as unsatisfactory (See Tables 1 and 2).

B. cereus, *C. perfringens* and *S. aureus* are commonly found in the environment and are bacteria that can produce protein toxins in contaminated food or in the intestines of infected humans, which can cause foodborne illness. Elevated levels of these bacteria (See Tables 1 and 2) indicate that the food may have been produced under unsanitary conditions. Therefore, an investigative assessment which may result in further follow-up actions is associated with elevated levels of the bacteria. As the results are based on the analysis of one unit (n=1), further sampling might be required to verify the levels of the bacteria of the lot. The presence of high levels of these bacteria (See Tables 1 and 2) is indicative of potential high enough levels of the bacterial toxins to cause foodborne illnesses. Therefore, samples with high levels of *B. cereus*, *C. perfringens* and *S. aureus* are assessed as unsatisfactory indicating that follow-up activities are warranted. The *B. cereus* method used in this survey is unable to discriminate *B. cereus* from other closely related organisms and therefore results are considered presumptive for *B. cereus*.

Unlike harmful bacterial pathogens such as *Salmonella*, generic *E. coli* is commonly found in the intestines of humans and most strains are harmless. It is considered to be an indicator organism and levels of generic *E. coli* found in a food product are used to assess the overall sanitation conditions throughout the food chain from production to the point of sale. An investigative assessment is associated with elevated levels of generic *E. coli* (See Tables 1 and 2), which may result in further follow-up actions. As the results are based on the analysis of one unit (n=1), further sampling might be required to verify the levels of generic *E. coli* of the lot. An unsatisfactory assessment is associated with high levels of generic *E. coli* (See Tables 1 and 2) as it may indicate a breakdown in good manufacturing practices (sanitation practices), and therefore possibly warranting the initiation of follow-up activities.

What were the survey results?

A total of 828 samples including seed powders (583) and plant-based protein powders (245) were tested for bacterial pathogens of concern (*Salmonella spp.*, *B. cereus*, *C. perfringens* and *S. aureus*), as well as generic *E. coli*. Sample assessment results can be found in Table 3.

Table 3 - Assessment results of bacterial analysis in seed powder and plant-based protein powder samples

Analysis group	Analysis	Unsatisfactory (% of total samples)	Investigative (% of total samples)	Satisfactory (% of total samples)	Number of samples tested
Seed powder (non-sprouted)	<i>Salmonella</i> spp.	0	N/A	579	583
	<i>C. perfringens</i>	0	0		
	<i>B. cereus</i>	0	3		
	<i>S. aureus</i>	0	0		
	Generic <i>E. coli</i>	1	0		
Subtotal		1 (0.2)	3 (0.5)	579 (99.3)	583 (100)
Plant-based protein powder	<i>Salmonella</i> spp.	0	N/A	233	245
	<i>C. perfringens</i>	0	2		
	<i>B. cereus</i>	0	10		
	<i>S. aureus</i>	0	0		
	Generic <i>E. coli</i>	0	0		
Subtotal		0	12 (4.9)	233 (95.1)	245 (100)
Total		1 (0.1)	15 (1.8)	812 (98.1)	828 (100)

Of the seed powder (583) samples tested (Table 3), *Salmonella* spp. was not found in any samples. *C. perfringens* and *S. aureus* levels were found to be below 100 Colony Forming Unit (CFU)/g in all samples. Presumptive *B. cereus* was found in three (0.5%, 3/583) seed powder samples at elevated levels ($10^3 < x \leq 10^4$ CFU/g). A high level ($> 10^3$ Most Probable Number (MPN)/g) of generic *E. coli* was found in one (0.2%, 1/583) seed powder sample.

Of the plant-based protein powder (245) samples tested (Table 3), *Salmonella* spp. was not found in any samples. *S. aureus* and generic *E. coli* levels were found to be below 25 CFU/g and 1.8 MPN/g, respectively in all protein powder samples tested. *C. perfringens* was found in two (0.8%, 2/245) protein powder samples at elevated levels ($10^2 < x \leq 10^3$ CFU/g) and presumptive *B. cereus* was found in ten (4.1%, 10/245) protein powder samples at elevated levels ($10^2 < x \leq 10^4$ CFU/g).

Of the 583 seed powder samples tested (Table 4), 28.6% were domestic and 55.6% were imported (from more than 11 different countries/regions). The country where the product was processed could not be determined for 15.8% of the samples. Of the 245 plant-based protein powder samples tested (Table 5), a large proportion (58%) of the samples was domestic, 11% were imported from the US and 31% were of unknown origin. In terms of production practice, 49.1% (286/583) of the seed powder samples and 18.0% (44/245) of the plant-based protein powder samples were organically produced.

Table 4 - Product origin and production practice of seed powder samples

Product origin	Total number of samples (%)	Organic	Conventional
Domestic	167 (28.6)	133	34
Imported	324 (55.6)	137	187 (1 ^a)
Bolivia	1	0	1
Ecuador	2	1	1
Italy	1	0	1
Japan	1	0	1
Mexico	1	1	0
Netherlands	1	1	0
Paraguay	2	1	1
Peru	5	4	1
Spain	3	1	2
Taiwan	19	1	18
United States	80	37	43
Multiple countries	75	70	5
Unknown	133	20	113 (1 ^a)
Unknown	92 (15.8)	16	76 (3 ^b)
Total	583 (100)	286 (49.1)	297 (50.9)

^a generic *E. coli* ($> 10^3$ MPN/g)

^b presumptive *B. cereus* ($10^3 < x \leq 10^4$ CFU/g)

Table 5 - Product origin and production practice of plant-based protein powder samples

Product origin	Total number of samples (%)	Organic	Conventional
Domestic	142 (58.0)	29 (1 ^a)	113 (4 ^a)
Imported	27 (11.0)	14	13 (1 ^a)
United States	16	6	10 (1 ^a)
Unknown	11	8	3
Unknown	76 (31.0)	1	75 (4 ^a) (2 ^b)
Total	245 (100)	44 (18.0)	201 (82.0)

^a presumptive *B. cereus* ($10^2 < x \leq 10^4$ CFU/g)

^b *C. perfringens* ($10^2 < x \leq 10^3$ CFU/g)

Seed types of seed powder and plant-based protein powder samples are detailed in Tables 6 and 7.

Table 6 – Seed type(s) of seed powder samples

Seed type(s)	Number of samples tested
Chia	162 (3 ^b)
Flax	246 (1 ^a)
Hemp	12
Pumpkin	1
Sesame	10
Almond	86
Chest nut	1
Cocoa nut	9
Hazelnut	6
Peanut	10
Pecan	15
Tiger nut	2
Black bean	5
Soybean	2
Quinoa	11
Alfalfa, pumpkin, sunflower	1
Chia, flax	1
Mixed seeds	3
Total	583

^a generic *E. coli* (> 10³ MPN/g)

^b presumptive *B. cereus* (10³ < x ≤ 10⁴ CFU/g)

Table 7 – Seed type(s) of plant-based protein powder samples

Seed type(s)	Number of samples tested
Alfalfa, chia, flax	1
Alfalfa, chia, hemp, pumpkin	9
Alfalfa, flax	8
Alfalfa, flax, hemp	1
Alfalfa, hemp	112 (5 ^a , 1 ^b)
Alfalfa, pumpkin	4
Alfalfa, pumpkin, sunflower	3 (1 ^b)
Chia, flax, hemp	8 (1 ^a)
Chia, flax, pumpkin	2
Chia, flax, pumpkin, sunflower	2
Chia, hemp	8
Chia, pumpkin	2
Flax, hemp	13
Flax, hemp, pumpkin	1
Hemp	38 (4 ^a)
Pumpkin	8
Soy bean	3
Other (rice)	22
Total	245

^a presumptive *B. cereus* ($10^2 < x \leq 10^4$ CFU/g)

^b *C. perfringens* ($10^2 < x \leq 10^3$ CFU/g)

What do the survey results mean?

In this study, over 99% of the seed powder and 95% of the plant-based protein powder samples were assessed as satisfactory. *Salmonella* spp. and *S. aureus* (>25 CFU/g in protein powder, >100 CFU/g in seed powder) were not found in any samples (0/828).

B. cereus, a common bacterial pathogen in low-moisture foods, was found in 0.5% (3/583, 95% CI: 0.18-1.50%) of the seed powder samples at elevated levels ($10^3 < x \leq 10^4$ CFU/g) and in 4.1% (10/245, 95% CI: 2.23-7.35%) of the plant-based protein powder samples at elevated levels ($10^2 < x \leq 10^4$ CFU/g). *C. perfringens* was found in 0.8% (2/245, 95% CI: 0.22-2.93%) of the plant-based protein powder samples at elevated levels ($10^2 < x \leq 10^3$ CFU/g). Samples where elevated levels of *C. perfringens* and presumptive *B. cereus* were found indicate that the food may have been produced under unsanitary conditions. A high level (> 10^3 MPN/g) of generic *E. coli* was found in 0.2% (1/583, 95% CI: 0.03-0.96%) of the seed powder samples.

The CFIA conducted appropriate follow-up activities such as facility inspections and additional sampling. In the case of the sample found to have a high level ($>10^3$ MPN/g) of generic *E. coli*, further sampling and testing was conducted with satisfactory results, and the facility enhanced its product testing program.

From our survey results, the overall prevalence of *Salmonella* (0%, 0/828, 95% CI: 0-0.46%) and generic *E. coli* (>100 MPN/g) (0.1%, 1/828, 95% CI: 0.02-0.68%) identified in this study is lower than the prevalence reported in a study conducted in the UK market. The UK study² investigated the prevalence of *Salmonella* and generic *E. coli* in whole edible seeds (alfalfa, flax, sesame, hemp, poppy, pumpkin, sunflower, melon, and mixed seeds) collected from retail locations in the UK between 2007 and 2008. The UK study found *Salmonella* and generic *E. coli* (>100 CFU/g) in 0.6% (23/3735, 95% CI: 0.41-0.92%) and 1.5% (55/3735, 1.13-1.91%) of the whole seed samples², respectively. In this survey, presumptive *B. cereus* was found in 1.6% (13/828, 95% CI: 0.92-2.67%) of the seed derived powder products at elevated levels ($10^3 < x \leq 10^4$ CFU/g for seed powder, $10^2 < x \leq 10^4$ CFU/g for plant-based protein powder). A high prevalence of *B. cereus* in raw seeds was reported from two US studies. One US study investigated *B. cereus* contamination in seeds (alfalfa, mung bean, wheat, and mixed seeds) sold for sprouting and found *B. cereus* (>1 CFU/g) in all seeds (100%, 98/98) and over 57% (56/98) at levels >3 CFU/g and 13% (13/98) at levels >100 CFU/g⁶. Another US study investigated *B. cereus* in seeds ordered on line and found *B. cereus* (>1 CFU/g) in 95% (57/60) of the seed samples (single or mixed, alfalfa, broccoli, lentil, mung bean and radish seeds)⁷.

Overall, our survey results indicate that most seed powders and plant-based protein powders sampled appear to have been produced under sanitary conditions. However, our results do indicate that a loss of sanitation controls along the production chain can occur. Consequently, as with all foods, safe handling practices are recommended for producers, retailers and consumers.

References

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