

Bacterial Pathogens in Dried Sprouted Seed Products - November 1, 2014 to March 31, 2018

Food microbiology - Targeted surveys - Final report





Summary

The consumption of dried sprouted seed products has only recently gained popularity in North America. Dried sprouted seeds and powders are derived from partially sprouted seeds which are then dried and in the case of powders, milled. Sprouted seed products are believed to have additional nutritive value as compared to non-sprouted seeds. Unfortunately, dried sprouted chia seed powder was associated with a salmonellosis outbreak in Canada and the US in 2014. There have also been several recalls of dried sprouted seed products due to *Salmonella* contamination over the past several years resulting from enhanced surveillance activities of food safety authorities. Bacterial pathogens initially present in or on seeds can multiply during the partial sprouting process and survive the low drying temperatures. Bacteria, such as *Salmonella* and *Bacillus cereus* (*B. cereus*) can then further survive for extended periods of time in these low-moisture products. Given that many dried sprouted seeds and powders are promoted to be eaten "as is," the presence of bacterial pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians dried sprouted seeds and powders along with non-sprouted seed powders and plant-based protein powders were selected for targeted surveys. The purpose of this survey was to generate baseline information on the occurrence of pathogenic bacteria of concern in dried sprouted seed products on the Canadian market. Results of dried sprouted seed products testing are reported herein. Results of non-sprouted seed powder and plant-based protein powder testing are addressed in a separate report.

Over the course of this study (November 1, 2014 to March 31, 2018), a total of 1936 samples of dried sprouted seed products were collected from retail locations in 11 cities across Canada. Samples were tested for one of three groups of bacterial pathogens of concern: (i) Verotoxigenic *Escherichia coli* O157:H7 (*E. coli* O157:H7) and *Salmonella* species (spp.), (ii) *E. coli* O157:H7, non-O157 Verotoxigenic *E. coli* (non-O157 VTEC) and *Salmonella* spp., and (iii) *Salmonella* spp., *B. cereus*, *Clostridium perfringens* (*C. perfringens*) and *Staphylococcus aureus* (*S. aureus*). Groups i and iii were also tested for generic *E. coli* which is an indicator of the overall sanitation conditions throughout the food chain from production to the point of sale.

In this study, over 97% of dried sprouted seed products were assessed as satisfactory. *E. coli* O157:H7 was not found in any samples (0/1350 samples in groups i and ii). *S. aureus* (>100 colony-forming unit (CFU)/gram (g)) and *C. perfringens* (>100 CFU/g) were not found in any samples (0/586 samples in group iii). *Salmonella* spp. was found in five samples (0.26%, 5/1936 samples in groups i, ii and iii). Non-O157 VTEC was found in four samples (0.39%, 4/1028 samples in group ii), however their serotypes were not part of the group of non-O157 VTEC (O26, O103, O111, O117, O121, and O145) known to cause the majority of non-O157 VTEC

infections in Canada. Presumptive *B. cereus* was found in 31 samples (5.3%, 31/586 samples in group iii) at elevated levels ($10^3 < x \le 10^4$ CFU/g) and in 12 samples (2.0%, 12/586 samples in group iii) at high levels (> 10^4 CFU/g). Elevated ($10^2 < x \le 10^3$ most probable number (MPN)/gram (g)) and high (> 10^3 MPN/g) levels of generic *E. coli* were found in four (0.44%, 4/908 samples in groups i and iii) and two (0.22%, 2/908 samples in groups i and iii) samples, respectively.

In dried sprouted seed products, the presence of low levels of *C. perfringens* (\leq 100 CFU/g), *S. aureus* (\leq 100 CFU/g), presumptive *B. cereus* (\leq 10³ CFU/g), or generic *E. coli* (\leq 100 MPN/g) is tolerated as they are often found in the environment. Samples containing high levels of *C. perfringens* (>10³ CFU/g), *S. aureus* (>10⁴ CFU/g) or presumptive *B. cereus* (>10⁴ CFU/g), are considered to have been produced under unsanitary conditions. Samples where high levels of generic *E. coli* (>10³ 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 such as facility inspections and additional sampling. All Salmonella contaminated samples were identified in the early survey years (2014 and 2015) of the multiple-year survey period and resulted in product recalls. No direct product actions were taken in cases where high levels of presumptive *B. cereus* were identified as the analytical methodology is unable to discriminate *B. cereus* from other closely related organisms. In some cases where high levels of generic *E. coli* were found, corrective actions were implemented by the processing facilities. There were no known reported illnesses associated with consumption of the *Salmonella*, *B. cereus*, and non-O157 VTEC contaminated dried sprouted seed products.

Overall, our survey results indicate that most of the dried sprouted seed products sampled appear to have been produced under sanitary conditions. However, contamination by bacterial pathogens such as *Salmonella* can occur occasionally, and a loss of sanitation controls along the food production chain can occur as well. 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 dried sprouted chia and flax, as well as other dried sprouted seeds (grains, nuts and beans) has only recently gained popularity in North America. Sprouted seeds are believed to have additional nutritive value compared to non-sprouted seeds, as the sprouting process results in the production of compounds such as antioxidants and vitamins. Unfortunately, dried sprouted chia powder was associated with a salmonellosis outbreak in Canada and the US in June 2014¹⁻³, which was the first outbreak linked to dried sprouted seed products. There have also been numerous recalls of dried sprouted seed products due to Salmonella contamination since June 2014 resulting from enhanced surveillance activities of food safety authorities. Food safety investigations² revealed that the chia powder was derived from partially sprouted, dried and milled chia seeds that did not receive a heat treatment aimed at the inactivation of bacterial pathogens. Bacterial pathogens initially present in or on seeds can multiply to high levels during the partial sprouting process, and can survive the low drying temperatures that are aimed at preserving nutrients of the partially sprouted seeds⁴. In addition, dried sprouted seeds and powders are low-moisture foods and bacterial pathogens such as Salmonella and B. cereus can survive in these low-moisture products for extended periods of time. Given that many dried sprouted seeds and powders are promoted to be eaten "as is," the presence of bacterial pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians, dried sprouted seeds and powders along with non-sprouted seed powders and plant-based protein powders were selected for targeted surveys. The purpose of this survey was to generate baseline information on the occurrence of generic *E. coli* and pathogenic bacteria of concern (*E. coli* O157:H7, *Salmonella* spp., non-O157 VTEC, *B. cereus*, *C. perfringens* and *S. aureus*), in dried sprouted seed products on the Canadian market. Generic *E. coli* is an indicator of the overall sanitation conditions throughout the food chain from production to the point of sale. Results of dried sprouted seed testing are reported herein. Results of non-sprouted seed powder and plant-based protein powder testing are addressed in a <u>separate report</u>.

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, dried sprouted seed products (whole, powder, and processed products) were sampled between November 1, 2014 and March 31, 2018. As these products are low-moisture foods with a long shelf life (6 months to 24 months) and a relatively low consumption rate, only a small number of samples were collected in each survey year to avoid repeated sampling of the same product from the same lot. Samples were collected over several years to obtain a statistically representative number of samples. Sample collection and testing information can be found in table 1.

Table 1- Sample collection and testing of dried sprouted seed products

Analysis group	Survey year(s)	Bacterial analyses	Total number of samples collected and analyzed
Group i	2014-2015	Salmonella spp. E. coli O157:H7 Generic E. coli	322
Group ii	2014-2015 2015-2016 2016-2017 2017-2018	Salmonella spp. E. coli O157:H7 Non-O157 VTEC	1028
Group iii	2016-2017	Salmonella spp. B. cereus C. perfringens S. aureus Generic E. coli	586
Total	1936		

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*⁵ (table 2).

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 dried sprouted seeds. As *Salmonella* spp. and *E. coli* O157:H7 are considered pathogenic to humans their presence was considered to be a violation of the *Food and Drugs Act* (FDA) Section 4(1)a⁶ and therefore in the absence of assessment guidelines was assessed by the CFIA as unsatisfactory (see table 2). The detection of non-O157 VTEC was assessed as investigative, indicating that further follow-up actions may be warranted depending upon the serotype identified. All non-O157 VTEC have the potential to cause human illnesses, however at this time there is no established virulence profile for pathogenic non-O157 VTEC and consequently unless previously associated with human illness it is difficult to establish its human health significance. Of the non-O157 VTEC serotypes identified to date, the following have been identified as causing the majority of non-O157 VTEC infections in Canada (O26, O103, O111, O117, O121, and O145)⁷.

Table 2 - Analytical methods and assessment criteria for bacteria in dried sprouted seed products

Bacterial analysis	Method identification number ^a	Satisfactory Investigative		Unsatisfactory	
Salmonella spp.	MFHPB-20 MFLP-38 MFLP-29	Absent in 25 g	Not Applicable (N/A)	Present in 25 g	
E. coli O157:H7	MFLP-30 MFLP-80 MFHPB-10 MFLP-52	Absent in 25 g	N/A	Present in 25 g	
Non-O157 VTEC	MFLP-52	Absent in 25 g	Present in 25 g	N/A	
B. cereus	MFLP-42	≤ 10 ³ CFU/g	10 ³ < x ≤ 10 ⁴ CFU/g	> 10 ⁴ CFU/g	
C. perfringens	MFHPB-23	≤ 10 ² CFU/g	10 ² < x ≤ 10 ³ CFU/g	> 10 ³ CFU/g	
S. aureus	MFHPB-21	≤ 10 ² CFU/g	10 ² < x ≤ 10 ⁴ CFU/g	> 10 ⁴ CFU/g	
Generic <i>E. coli</i>	MFHPB-19	≤ 10 ² MPN/g	10 ² < x ≤ 10 ³ MPN/g	> 10 ³ MPN/g	

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

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 table 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 table 2) is indicative of the potential of 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, E. coli O157:H7), 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 (100 < $x \le 100$)

1000 MPN/g) (see table 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* (> 1000 MPN/g) (see table 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 1936 samples of dried sprouted seed products (whole, powder, and processed products) were tested for one of the three groups (table 1) of bacterial pathogens of concern. Group i and iii samples were also tested for generic *E. coli*. Assessment results can be found in table 3.

Table 3 - Assessment results of bacterial analyses in dried sprouted seed products

Analysis group	Bacterial analysis	Unsatisfactory (% of total)	Investigative (% of total)	Satisfactory (% of total)	Number of samples tested
	Salmonella spp.	2	N/A		
Group i	E. coli O157:H7	0	N/A	318	322
	Generic <i>E. coli</i>	1	1		
	Salmonella spp.	3	N/A		
Group ii	E. coli O157:H7	0	N/A	1021	1028
	Non-O157 VTEC	N/A	4		
	Salmonella spp.	0	N/A		16
	B. cereus	12	31		586
Group iii	C. perfringens	0	0	539	
	S. aureus	0	0		
	Generic E. coli	1	3		
Subtotal		19 (1.0)	39 (2.0)	1878 (97.0)	1936

E. coli O157:H7 was not found in any samples (0/1350 samples in groups i and ii). *S. aureus* and *C. perfringens* were found to be below 100 CFU/g in all samples (586 samples in group iii). *Salmonella* spp. was found in five samples (0.26%, 5/1986 samples in groups i, ii and iii). Non-O157 VTEC was found in four samples (0.39%, 4/1028 samples in group ii). The non-O157 VTEC serotypes identified did not belong to the group of non-O157 VTEC (O26, O103, O111, O117, O121, and O145)⁷ known to cause the majority of non-O157 VTEC infections in Canada. Presumptive *B. cereus* was found in 31 samples (5.3%, 31/586 samples in group iii) at elevated levels $(10^3 < x \le 10^4 \text{ CFU/g})$ and in 12 samples (2.0%, 12/586 samples in group iii) at high levels $(> 10^4 \text{ CFU/g})$. Elevated $(10^2 < x \le 10^3 \text{ MPN/g})$ and high $(> 10^3 \text{ MPN/g})$ levels of generic *E. coli*

were found in four samples (0.44%, 4/908 samples in groups i and iii) and two samples (0.22%, 2/908 samples in groups i and iii), respectively.

Of the 1963 dried sprouted seed products, 55.1% of the samples were domestic, 37.9% were imported from four countries/regions and 7.0% were of unknown origin (table 4). In terms of production practice, 56.6% were organically produced and 43.4% were conventionally produced (table 4).

Table 4 – Product origin and production practice of dried sprouted seed samples

Product origin	Total number of samples tested (% of total)	Organic	Conventional	
Domestic	1067 (55.1)	855(2 ^a)	212 (1 ^a)	
Imported	734 (37.9)	203	531	
Bolivia	75	48	27	
Paraguay	2	2	0	
Taiwan	6	0	6	
United States	584	146 (2 ^a)	438	
Imported (unknown country)	67	7	60	
Unknown	135 (7.0)	38	97	
Total	1936 (100)	1096 (56.6)	840 (43.4)	

a Number of samples found to be contaminated with Salmonella spp.

Product types and seed types of dried sprouted seed products are detailed in table 5.

Table 5 - Dried sprouted seed products by product type and seed type

Table 5 - Dried Sprouted Seed products by product type and Seed type						
Seed type	Whole seed	Powdered seed	Trail mix and cereal	Total number of samples (% of total)		
Other Seeds	515	876	39	1430 (73.9)		
Chia	194	206 (1 ^a)	14	414		
Flax	9	509 (1 ^a)	1	519		
Chia, flax	1	95	0	96		
Chia, flax, hemp	11	42	0	53		
Pumpkin	64	0	3	67		
Sunflower	75 (1 ^a)	0	5	80		
Pumpkin, sunflower	1	1	16 (1 ^a)	18		
Watermelon	10	0	0	10		
Quinoa	149	2	0	151		
Sesame	0	6	0	6		
Vegetable (clover, broccoli, radish)	1	15	0	16		
Grains	65	88	91	244 (12.6)		
Ancient grains	0	3	1	4		
Buckwheat	18	3	21	42		
Brown rice	39	39	11	89		
Blonde rice	1	0	0	1		
Maize	0	0	5	5		
Oat	2	0	2	4		
Spelt	1	0	2	3		
Mixed grains, nuts,	4	43	49 (1 ^a)	96		
Legumes	120	10	0	130 (6.7)		
Adzuki bean	6	0	0	6		
Chick pea	9	0	0	9		
Lentil	90	0	0	90		
Mung bean	15	0	0	15		
Pea	0	10	0	10		
Nuts	49	1	82	132 (6.8)		
Almond	31	0	32	63		
Peanut	0	1	0	1		
Walnut	2	0	5	7		
Mixed nuts and seeds	16	0	45	61		
Total a The number of samples co	749	975	212	1936 (100)		

^a The number of samples contaminated with Salmonella spp.

Further details (product type/seed type) of each unsatisfactory and investigative sample are provided in table 6.

Table 6 - Product type/seed type of unsatisfactory and investigative samples

Table 0 - Froduct type/seed type of unsatisfactory and investigative samples							
Product / seed type	Unsatisfactory Salmonella (present in 25g)	Unsatisfactory B. cereus (> 10 ⁴ CFU/g)	Unsatisfactory Generic E. coli (>10 ³ MPN/g)	Investigative Non-O157-VTEC (present in 25g)	Investigative B. cereus $(10^3 < x \le 10^4)$	Investigative Generic E. coli (10 ² < x ≤ 10 ³ MPN/g)	Number of samples tested per product type
Chia powder	1	0	0	0	4	0	206
Flax powder	1	2	1	3	14	3	509
Chia, flax and hemp powder	0	2	0	0	0	0	42
Chia and flax powder	0	0	0	0	3	0	95
Whole chia	0	8	0	0	7	0	194
Whole sunflower seeds	1	0	1	0	0	0	75
Whole pumpkin seed	0	0	0	0	2	0	64
Sunflower & pumpkin seeds (trail mix)	1	0	0	0	0	0	16
Buckwheat, sunflower seeds, quinoa and almond (trail mix)	1	0	0	0	0	0	49
Whole quinoa	0	0	0	1	0	0	149
Whole lentils	0	0	0	0	0	1	90
Brown rice powder	0	0	0	0	1	0	39
Total	5	12	2	4	31	4	1528

What do the survey results mean?

In this study, over 97% of the dried sprouted seed products tested were assessed as satisfactory. Bacterial pathogen *E. coli* O157:H7 was not found in any samples (0/1350). *S. aureus* and *C. perfringens*, which are bacterial pathogens commonly found in the environment were found to be below 100 CFU/g in all samples (586).

Salmonella spp., a common bacterial pathogen associated with foodborne illnesses, was found in 0.26% (5/1936) of the samples. All Salmonella contaminated samples resulted in product recalls. All Salmonella positive samples were identified in the early survey years (2014 and 2015) of the multiple-year survey period. Non-O157 VTEC was found in 0.39% of the samples (4/1028). As the non-O157 serotypes identified did not belong to the group of non-O157 VTEC (O26, O103, O111, O117, O121, and O145) known to cause the majority of non-O157 VTEC infections in Canada⁷, no direct product actions were taken. B. cereus, a common bacterial pathogen in low-moisture foods, was found in 5.3 % (31/586) and 2.0% (12/586) of the samples at elevated levels ($10^3 < x \le 10^4$ CFU/g) and at high levels (> 10^4 CFU/g), respectively. No direct product actions were taken due to the limitations of the methodology, which is unable to discriminate B. cereus from other closely related organisms and therefore the results are considered presumptive. In some cases, where elevated (100 < x ≤ 1000 MPN/g) and high (> 10³ MPN/g) levels of generic *E. coli* were found (in 0.44% and 0.22% of the samples), corrective actions were implemented by the processing facilities. The CFIA conducted appropriate followup activities such as facility inspections and additional sampling. There were no known reported illnesses associated with consumption of the Salmonella, B. cereus, and non-O157 VTEC contaminated dried sprouted seed products.

Very few studies are available that investigated the microbiological safety and quality of dried sprouted seed products as these product types are relatively new. A recent study⁴ tested for *Salmonella* growth and survival during the production of dried partially sprouted seeds and found that chia, pumpkin and sunflower seeds were capable of supporting growth of *Salmonella* during the soaking/germination process at ambient temperatures. This study also examined the effectiveness of different drying temperatures at the reduction of *Salmonella*. The study found that drying at ambient temperatures (25°C) did not result in any reduction of *Salmonella* in partially sprouted chia seeds and only resulted in the partial reduction (~3 log CFU/g) of *Salmonella* in partially sprouted pumpkin and sunflower seeds⁴.

Overall, our survey results indicate that most dried sprouted seed products sampled appear to have been produced under sanitary conditions. However, our results do indicate that contamination by bacterial pathogens such as *Salmonella* can occur occasionally, and a loss of sanitation controls along the food production chain can occur as well. Consequently, as with all foods, safe handling practices are recommended for producers, retailers and consumers.

References

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