Food Microbiology – Targeted Surveys FINAL REPORT





Summary

Nuts and nut butters are popular foods in Canada and around the world. However, foodborne illness outbreaks of salmonellosis associated with nuts and nut butters have been reported worldwide including in Canada. Nuts are low-moisture foods of agricultural origin that can become contaminated by bacterial pathogens during primary production, harvest, processing and storage. Once nuts are contaminated, bacterial pathogens such as *Salmonella* can survive for long periods of time due to the low-moisture content of the nuts. Nut butters are low-moisture, high fat foods that can be contaminated by contaminated nuts or by cross-contamination during processing and/or storage. In addition, standard thermal treatments are often ineffective at eliminating *Salmonella* from highly contaminated nut butter products due to its ability to develop heat resistance in low-moisture, high-fat environments. Therefore, the microbial safety of nuts and nut butters remains a concern.

Considering the factors mentioned above and their relevance to Canadians, in-shell nuts, shelled nuts and nut butters were selected for targeted surveys. The purpose of the survey was to generate baseline information on the occurrence of bacterial pathogens on the surface of inshell nuts and in shelled nuts and nut butters on the Canadian market. Over the course of this study (April 1, 2012 to March 31, 2015), a total of 2400 in-shell nut samples, 2612 shelled nut samples, and 1142 nut butter samples were collected from retail locations in 11 cities across Canada and were tested for bacterial pathogens of concern: *Salmonella*, *Escherichia coli* (*E. coli*) O157:H7 and *Listeria monocytogenes* (*L. monocytogenes*) (nut butters only), as well as generic *E. coli*. Generic *E. coli* is an indicator of the overall sanitation conditions throughout the food chain from production to the point of sale.

All shelled nuts (100%), nut butters (100%) and over 99.8% of in-shell nuts were found to be free of specific pathogenic bacteria tested for. *E. coli* O157:H7, *L. monocytogenes* and generic *E. coli* (> 100 colony forming unit (CFU)/g) were not found in any samples tested. However, *Salmonella* was found in five in-shell nut samples (5/2400, 0.21%) including two walnut samples (2/792, 0.25%) and three hazelnut samples (3/696, 0.43%). The Canadian Food Inspection Agency (CFIA) conducted appropriate follow-up activities including food safety investigations related to the *Salmonella* contaminated products. All five affected products were recalled from the Canadian market. No reported illnesses were associated with any of the *Salmonella* contaminated products.

The results indicate that almost all of the nut and nut butter samples appear to have been produced under Good Agricultural Practices and Good Manufacturing Practices. Sporadically, *Salmonella* contamination on in-shell nuts can occur.

What Are Targeted Surveys?

Targeted surveys are used by the Canadian Food Inspection Agency (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?

In recent years, it has increasingly been recognized that many foodborne pathogens, such as *Salmonella* and *E. coli* O157:H7 can cause foodborne illnesses when present in low-moisture foods, such as nuts and nut butters. Foodborne illness outbreaks of salmonellosis associated with a variety of nuts (peanuts¹, almonds², pistachios³) and nut butters (peanut butter^{4, 5}) have been reported worldwide. In 2011, foodborne illness outbreaks of *E. coli* O157:H7 associated with in-shell hazelnuts⁶ and potentially linked to in-shell walnuts⁷ have been reported in the United States (U.S.) ⁶ and Canada⁷, respectively. Of these nut associated outbreaks, five occurred in Canada (peanuts in 2001, 2002, almonds in 2001, 2005, and walnuts in 2011). In addition, the use of contaminated nut butter as an ingredient, can result in downstream contamination in nut butter derived products (e.g., peanut butter associated outbreak in 2009 resulted in one of the largest food recalls in the U.S. history⁵).

Both nuts and nut butters can become contaminated by bacterial pathogens at various points along the food chain. Tree nuts and ground nuts (i.e., peanuts) are agricultural products that can be contaminated by bacterial pathogens during primary production, harvest and storage. Once contaminated, bacterial pathogens, such as *Salmonella* can survive for extended periods in nuts as they are low-moisture foods. *Salmonella* has been found on a variety of raw in-shell nuts, such as walnuts⁹, pecans¹⁰, and almonds¹¹. *Salmonella* has also been found in processed

nuts such as ready-to-eat shelled nuts and in-shell processed nuts (roasted, salted)¹². Processed nuts can become contaminated by bacterial pathogens via cross-contamination during processing and/or after thermal treatment. The nut roasting process which uses low roasting temperatures may not be sufficient to eliminate bacterial pathogens from contaminated nuts. Nut butters can also become contaminated due to poor sanitation practices and poor hygiene conditions during processing and/or storage ^{5,13}. Standard thermal treatments have been found to be insufficient at eliminating *Salmonella* from highly contaminated nut butter products¹⁴, as *Salmonella* can develop heat resistance in a low-moisture, high-fat food environment¹⁵.

Given the above information and in response to the nut associated *E. coli* O157:H7 outbreak in Canada⁷, in-shell and shelled nuts and nut butters were selected for targeted surveys over three fiscal years (2012/13-2014/15). All samples were analyzed for *Salmonella*, *E. coli* O157:H7 and generic *E. coli*. Nut butter samples were additionally tested for *L. monocytogenes*. This report details results of the entire survey period of April 1, 2012 to March 31, 2015.

What Did We Sample?

For this survey, a sample consisted of a single unit (e.g. individual consumer-size package(s)) from a single lot. All samples were collected from national retail chains and/or local/regional grocery 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), and the 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. Shelled nuts and nut butters were sampled in equal proportions (25%:25%:25%:25%) in each quarter during the three fiscal years spanning from April 1, 2012 to March 31, 2015. In-shell nuts were collected in accordance with their seasonal availability in a ratio of 20%:10%: 50%: 20% from quarter one (April 1 to June 30) to quarter four (January 1 to March 31), respectively, during the three fiscal years from April 1, 2012 to March 31, 2015. All samples were randomly collected including domestic, imported, organic and conventional products.

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*¹⁶.

The assessment criteria used in this survey (Table 1) are based on the principles of the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods*¹⁷ and associated methods published in Health Canada's *Compendium of Analytical Methods*¹⁶.

Table 1 Analytical Methods and Assessment Criteria for Bacteria on In-shell Nuts and in Shelled Nuts and Nut Butter Samples

Destavial Analysis	Method Identification Number*	Assessment Criteria			
Bacterial Analysis		Satisfactory	Investigative	Unsatisfactory	
Salmonella	MFHPB-20	Absent in 25g	Not Applicable	Present in 25g	
E. coli O157:H7/NM	MFLP-80 MFHPB-10	Absent in 25g	NA	Present in 25g	
Listeria monocytogenes**	MFLP-28 MFHPB-30 MFLP-74	Absent in 25g	≤100 CFU/g	> 100 CFU/g	
Generic E. coli	MFHPB-19 or MFHPB-34	≤ 100 MPN/g or CFU/g	100 < x ≤ 1000 MPN/g or CFU/g	> 1000 MPN/g or MPN/g	

^{*} The methods used were the published versions at the time of analysis.

No assessment guidelines had been established in Canada for *Salmonella, E coli* O157:H7 on/in nuts or in nut butter¹⁷ at the time of writing this report. In the absence of assessment guidelines, the presence of these pathogens on/in nuts and in nut butter was considered to be a violation of the *Food and Drugs Act* (FDA) Section 4(1)a¹⁸ and was therefore assessed by the CFIA as unsatisfactory.

Unlike harmful bacterial pathogens (e.g., Salmonella, L. monocytogenes), 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 \leq 1000 colony forming unit (CFU)/g, or most probable number (MPN)/g), which may result in further follow-up actions. As the results are based on the analysis of one unit (n=1), further sampling is required to verify the levels of generic E. coli of the lot. An unsatisfactory

^{**} The assessment criteria for *Listeria monocytogenes* are based on Health Canada's Policy on *Listeria monocytogenes* in Ready-to-Eat (RTE) foods¹⁹. Nut butters are considered a category 2B product (Aw < 0.92).

assessment is associated with high levels of generic *E. coli* (> 1000 CFU/g, or MPN/g) as it may indicate a breakdown in Good Agricultural Practices and/or Manufacturing Practices (sanitation practices), and therefore possibly warranting the initiation of follow-up activities such as the improvement of sanitation conditions along the food chain.

What Were the Survey Results?

A total of 5012 samples of nuts including in-shell nuts (2400) and shelled nuts (2612) were analysed for *Salmonella*, *E. coli* O157:H7 and generic *E. coli*. Nut butter samples (1142) were tested for *Salmonella*, *E. coli* O157:H7, *L. monocytogenes* and generic *E. coli*. All samples of shelled nuts (100%) and nut butters (100%), and most samples of in-shell nuts (99.8%) were assessed as satisfactory. *E. coli* O157:H7 and *L. monocytogenes* were not detected on/in any samples tested. Levels of generic *E. coli* were all below 100 MPN or CFU/g in all samples tested. *Salmonella* was found in 0.2% (5/2400) of the in-shell nut samples tested and were assessed as unsatisfactory (Table 2).

Table 2 Assessment Results of Nuts and Nut Butter Samples

Pro	oduct Type	Number of Samples	Satisfactory Assessment (% of product type)	Unsatisfactory Assessment Salmonella (% of product type)
Nuts	In-shell nuts	2400	2395 (99.8%)	5 (0.2%)
Nuts	Shelled nuts	2612	2612 (100.0%)	0
N	lut butters	1142	1142 (100.0%)	0
	Total	6154	6149	5

More than eleven varieties of nuts were collected including walnuts (33.2%), hazelnuts (31.2%), pistachios (10.0%), almonds (8.1%), and peanuts (6.8%) (Table 3). The *Salmonella* contaminated samples were found to be two in-shell walnut samples (0.25%, 2/792) and three in-shell hazelnut samples (0.43%, 3/696) (Table 3).

Table 3 Nut Sample Distribution by Product Type

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Product Type	Total Number of Samples (% in total)	Number of In-shell Nut Samples	Number of Shelled Nut Samples	Number of Positive Samples (% by type)
Walnut	1666 (33.2%)	792*	874	2 (2/792, 0.25%) In-shell walnut
Hazelnut	1566 (31.2%)	696*	870	3 (3/696, 0.43%) In-shell hazelnut
Pistachio	503 (10.0%)	481	22	0
Almond	405 (8.1%)	86	319	0
Peanut	339 (6.8%)	233	106	0
Cashew	201 (4.0%)	0	201	0
Pecan	126 (2.5%)	40	86	0
Brazil nut	74 (1.5%)	7	67	0
Pine nut	43 (0.9%)	0	43	0
Chest nut	35 (0.7%)	30	5	0
Macadamia	5 (0.1%)	0	5	0
Mixed nuts	49 (1.0%)	35	14	0
Total	5012 (100%)	2400	2612	5

^{*}Salmonella positive samples were found in this product type.

There were fewer varieties of nut butter sample types and they were mainly comprised of peanut butter (46.3%), almond butter (28.4%), hazelnut butter (13.6%), and cashew butter (7.2%) (Table 4).

Table 4 Nut Butter Sample Distribution by Product Type

Nut butter type	Number of Samples	% of Samples
Almond	300	26.3
Cashew	82	7.2
Chestnut	4	0.4
Hazelnut	155	13.6
Macadamia	16	1.4
Peanut	529	46.3
Pecan	5	0.4
Walnut	4	0.4
Mixed nuts	47	4.1
Total	1142	100

A large portion (48.1%) of the nut samples (in-shell and shelled nuts) were imported from more than 17 countries, of which 39.0% were imported from the U.S. Domestic nuts only accounted for 5.3% of the nut samples and 46.6% of the nut samples were of unknown origin (Table 5). The five *Salmonella* contaminated samples were imported nuts from the U.S.

Different from the nut samples with respect to country of origin, a considerable portion (63.5%) of the nut butter samples were domestically produced. Approximately 26.7% of the nut butter samples were imported from more than ten countries, and 9.8% of the samples were of unknown origin (Table 5).

Table 5 Sample Distribution by Country of Origin

Country of Origin	Nuts (In-shell & Shelled)		Nut Butters	
	Number of Samples	% of Total	Number of Samples	% of Total
Canada	266	5.3	725	63.5
Imported	2411	48.1	305	26.7
Belgium	0	0	18	1.6
Brazil	6	0.1	0	0
China	37	0.7	0	0
France	0	0	2	0.2
Germany	0	0	12	1.1
Indonesia	11	0.2	0	0
Italy	31	0.6	12	1.1
Netherland	0	0	8	0.7
Philippine	0	0	3	0.3
Turkey	106	2.1	0	0
U.S.	1955	39.0	208	18.2
Vietnam	9	0.2	0	0
Other	25*	0.5	3**	0.3
Imported unknown country	231	4.6	39	3.4
Unknown	2335	46.6	112	9.8
Total	5012	100	1142	100

^{*} Sample numbers were combined from ten countries, as each country accounted for less than 0.1% of total number of samples.

^{**} Sample numbers were combined from three countries, as each country accounted for less than 0.1% of total number of samples.

What Do the Survey Results Mean?

In this survey, all samples of shelled nuts (100%), nut butters (100%), and over 99.8% of inshell nut samples were determined to be free of pathogenic microorganisms tested for. *E. coli* O157:H7 and *L. monocytogenes* were not detected in any of the samples tested. Generic *E. coli* was below 100 CFU/g or MPN /g in all samples. Generic *E. coli* is an indicator of the overall sanitation conditions throughout the food chain from primary production to the point of sale.

However, *Salmonella*, a common bacterial pathogen associated with foodborne illnesses, was found in 0.21% (5/2400) of the in-shell nut samples tested. All five *Salmonella* contaminated samples were found during the first fiscal year (2012/13) of the three fiscal year survey. The CFIA conducted appropriate follow-up activities for all *Salmonella* contaminated samples. All affected products were recalled from the Canadian market. No reported illnesses were associated with the *Salmonella* contaminated nut products.

From our survey results, *Salmonella* was found in 0.43% (95% CI: 0.15-1.26%) of in-shell hazelnuts and 0.25% (95% CI: 0.07-0.92%) of in-shell walnuts. The prevalence of *Salmonella* in this survey is comparable to the results reported in studies conducted in the U.S. and the United Kingdom (UK) (Appendix). The prevalence of *Salmonella* has been reported to be in a range of 0 - 2.2% in five varieties of nut samples that were collected at U.S. nut processing facilities in multi-year surveys conducted between 2006-2013 20 . The prevalence of *Salmonella* was reported to be 0 - 4.2% in six types of retail nut samples (n=3656) that were collected at U.S. retail locations between 2014-2015 21 . The presence of *Salmonella* was also reported in an average of 0.1% of ready-to-eat nuts (n=2886) sold at retail in the UK during the winter season of 2008/09 12 .

Overall, our survey results suggest that almost all raw and processed in-shell and shelled nuts and nut butters on the Canadian market are safe for consumption. Sporadically, raw in-shell nuts can become contaminated by *Salmonella*.

Appendix

Comparison of Prevalence of Salmonella in Various Nut Surveys

Sampling Site (Survey Year)	Nuts Type (Number of Samples)	Prevalence of Salmonella (%)
Retail (Canada)	In-shell & shelled nuts (5012)	0 - 0.43% (per type)
(current study)	In-Shell hazelnuts (792)	0.43% (95% CI, 0.15-1.26)
(2012-2014)	In-shell walnuts (696)	0.25% (95% CI, 0.07-0.92)
	Shelled hazelnuts (870)	0
	Shelled walnuts (874)	0
	In-shell and Shelled Almonds (405)	0
	In-shell and Shelled Pistachios	0
	(503)	
Processing	In-shell & shelled nuts (22127)	0 - 2.2% (per type)
facilities (U.S.)	In-shell Almond (455)	0.9-2.2% (per year) (2006-2007)
(2006-2013) ^{9,20}	In-shell pecan (4641)	0.5-1.4% (per year) (2010-2012)
	Hulled pistachios (3966)	0.4-2.2% (per year) (2010-2012)
	In-shell walnuts (2903)	0 – 2.2% (per year) (2011-2013)
	Raw shelled peanuts (10162)	0.1-1.4% (per year) (2009-2011)
Retail (U.S.)	In-shell and shelled nuts (3656)	0 - 4.2% (per type)
(2014-2015) ²¹	Pecans	0
	In-shell hazelnuts	0
	Shelled hazelnuts	0.35% (95% CI, 0.04-1.2)
	Walnuts	1.20% (95% CI, 0.53-2.4)
	Cashew	0.55% (95% CI, 0.15-1.4)
	Pine nuts	0.48% (95% CI, 0.1-1.4)
	Macadamia	4.20% (95% CI, 2.4-6.9)
Retail (UK)	RTE nuts (2886)	0 - 0.9% (per type)
$(2008-2009)^{12}$	Almond (359)	0
	Hazels (195)	0
	Cashews (459)	0
	Walnuts (441)	0
	Shelled brazil nuts (469)	0.4%
	Mixed nuts (329)	0.9%

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