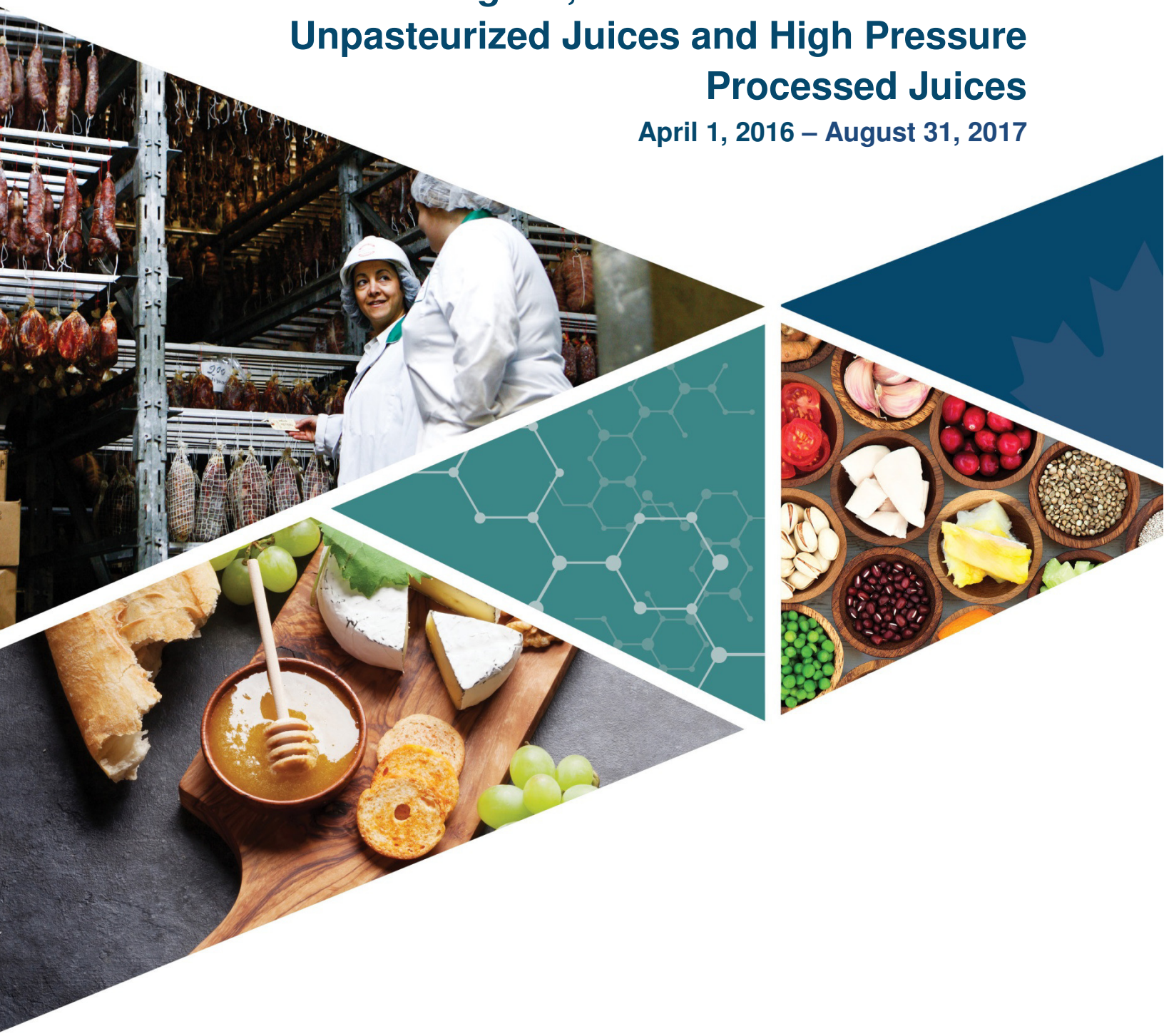
Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Microbiology – Targeted Surveys

FINAL REPORT

Bacterial Pathogens, Viruses and Parasites in Unpasteurized Juices and High Pressure Processed Juices

April 1, 2016 – August 31, 2017



Canada

Summary

The consumption of unpasteurised “raw” fruit and vegetable juices has become increasingly popular in recent years and are consumed by Canadians. Consumers view their consumption as a convenient way of increasing their intake of fresh fruits and vegetables and feel that “raw” juices are more nutritious than pasteurized juices which have undergone a heat treatment to inactivate microbial pathogens. A variety of unpasteurized “raw” juices are available on the Canadian market, such as unpasteurized apple ciders/juices, freshly squeezed/pressed juices, and cold-pressed juices (juice extracted using a hydraulic press). Unfortunately, unpasteurized juices have been previously associated with numerous outbreaks of foodborne illnesses worldwide including Canada. Given that juices are directly consumed, the presence of microbial pathogens creates a potential risk for foodborne illnesses.

A popular alternative to unpasteurized and pasteurized juices are high-pressure processed (HPP) juices. High pressure processing is a process that uses thousands of pounds of hydrostatic pressure to treat cold-pressed “raw” juices to inactivate microbial pathogens at relatively low temperatures (e.g., 18°C) as compared to traditional pasteurization (e.g., 71ºC). Therefore, HPP juices undergo minimal changes in freshness and nutritional content compared to pasteurized juices, while having an improved microbiological safety and longer shelf life compared to cold-pressed “raw” juices and other types of unpasteurized “raw” juices.

Considering the factors mentioned above and their relevance to Canadians, both unpasteurized “raw” juices and HPP juices were selected for this study, which is comprised of three targeted surveys. The purpose of the study was to generate information on the occurrence of pathogenic bacteria, viruses and parasites of concern in unpasteurized and HPP juices on the Canadian market.

Over the course of this study (April 1, 2016 – August 31, 2017), a total of 1828 samples, including 612 unpasteurized and 1216 HPP juice samples were collected from retail locations in 11 cities across Canada. Samples from survey *i*, including unpasteurized juice samples (382) and HPP juice samples (751) were tested for bacterial pathogens of concern (Verotoxigenic *Escherichia coli* O157:H7 (*E. coli* O157:H7), *Salmonella* species (spp.), *Shigella*) and generic *Escherichia coli* (*E. coli*) which is an indicator of the overall sanitation conditions throughout the food production chain. Samples from survey *ii*, including unpasteurized juice samples (218) and HPP juice samples (465) were tested for enteric viruses of concern (Hepatitis A virus (HAV) and Norovirus (NoV) (Genotype I and II (GI and GII)). Samples from survey *iii*, due to analytical methodology limitations were comprised solely of unpasteurized apple juice/cider samples, and tested for enteric parasites of concern (*Cyclospora cayetanensis* (*C. cayetanensis*), *Cryptosporidium species* (*spp.*), *Toxoplasma gondii* (*T. gondii*) and *Giardia* spp.

Bacterial pathogens (*E.coli* O157:H7, *Salmonella* spp., *Shigella*), enteric viruses (HAV, Nov (GI, GII) and parasites (*C. cayetanensis*, *Cryptosporidium spp.*, *T. gondii*, *Giardia spp.*) were not found in any samples. An elevated level of generic *E. coli* (100 < x ≤ 1000 Most Probable Number (MPN)/mL) was found in one juice sample (< 0.1%, 1/1133), which was a cold-pressed unpasteurized juice sample and in response, the Canadian Food Inspection Agency (CFIA) conducted appropriate follow-up activities.

Overall, our study results indicate that almost all of the unpasteurized and HPP juices sampled appear to have been produced under Good Agricultural Practices and Good Manufacturing Practices. One sample in our survey was found to have an elevated level of generic *E. coli,* which may indicate a loss of sanitation controls along the juice production chain. Consequently, safe handling practices are recommended for producers, retailers and consumers.

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 Canadian Food Inspection Agency 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 unpasteurized "raw" fruit and vegetable juices has become increasingly popular in recent years and are consumed by Canadians. Consumers view their consumption as a fast and convenient way of increasing their intake of fresh fruits and vegetables and feel that "raw" juices are more nutritious than pasteurized juices which have undergone a heat treatment to inactivate microbial pathogens. A variety of unpasteurized "raw" juices are available on the Canadian market, such as unpasteurized apple ciders/juices, freshly squeezed/pressed juices, and cold-pressed juices (juice extracted using a hydraulic press). Unfortunately, unpasteurized apple ciders/juices have been associated with numerous outbreaks of foodborne illnesses worldwide including Canada[1]. Given that juices are directly consumed, the presence of microbial pathogens creates a potential risk for foodborne illnesses.

A popular alternative to unpasteurized and pasteurized juices are high-pressure processed (HPP) juices. HPP juices are produced through a two-step process. First, juices are extracted from fruits and vegetables using a hydraulic press to produce cold-pressed "raw" juices. The cold-pressed "raw" juice then undergoes a high–pressure processing step that uses thousands of pounds of hydrostatic pressure to inactivate microbial pathogens and reduce the overall microbial load at relatively low temperatures (e.g., 18ºC) as compared to traditional

pasteurization (e.g., 71ºC). Therefore, HPP juices undergo minimal changes in freshness and nutritional content compared to pasteurized juices, while having an improved microbiological safety and longer shelf life compared to cold-pressed "raw" juices and other unpasteurized "raw" juices.

HPP juices are a relatively new product on the Canadian market. At the start of the survey period (April 1, 2016), HPP juices were regulated as novel foods[2], thereby requiring manufacturers to notify Health Canada (HC) of their intent to sell the products. During the survey period, on December 22, 2016, HC released a statement[3] that HPP products were no longer considered a novel food as enough information and data had been gathered on the safe use of commercial high pressure processing. Regardless, manufacturers of HPP foods remain responsible for ensuring compliance with the *Food and Drugs Act* and *Regulations*[4].

Considering the factors mentioned above and their relevance to Canadians, both unpasteurized juices and HPP juices were selected for this study, which is comprised of three targeted surveys. The purpose of the study was to generate information on the occurrence of (*i*) pathogenic bacteria of concern (survey *i*) Verotoxigenic *Escherichia coli* O157:H7 (*E. coli* O157:H7), *Salmonella* species (spp.), *Shigella* and generic *Escherichia coli* (*E. coli*); (*ii*) enteric viruses of concern (survey *ii*) Hepatitis A virus (HAV) and Norovirus (NoV) (Genotype I and II (GI and GII)); and (*iii*) enteric parasites of concern (survey *iii*) *Cyclospora cayetanensis* (*C. cayetanensis*), *Cryptosporidium species* (*spp.*), *Toxoplasma gnodii* (*T. gondii*) and *Giardia* spp.) in unpasteurized and/or HPP juices on the Canadian market.

What Did We Sample?

For these surveys, a sample consisted of a single unit (e.g., individual consumer-size bottle(s)) with a total volume of at least 200mL. 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), 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.

Sampling of certain types of unpasteurized juices, such as freshly squeezed/pressed juices and unpasteurized apple juices/ciders, was focused in the fall months (September and October) to align with the apple cider production season, while sampling of cold-pressed unpasteurized juices and HPP juices (cold-pressed juices subjected to high pressure processing) was conducted throughout the remainder of the year during the period of April 1, 2016 and August 31, 2017.

What Analytical Methods Were Used and How Were Samples Assessed?

Samples from survey *i*, were analyzed for bacterial pathogens and generic *E. coli* using methods published in Health Canada's *Compendium of Analytical Methods for the Microbiological Analysis of Foods*[5]. See Table 1 for the specific methods used and associated assessment criteria

The assessment criteria for *E. coli* O157:H7 and generic *E. coli* used in this survey are based on the principles of Health Canada's *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods*[9]. No microbiological guidelines had been established in Canada for the presence of *Salmonella* spp., or *Shigella* in juices at the time of writing this report. As *Salmonella* and *Shigella* are considered pathogenic to humans their presence was considered to be a violation of the *Food and Drugs Act* (FDA) Section 4(1)a[4] and therefore in the absence of assessment guidelines was assessed by the CFIA as unsatisfactory (Table 1).

Unlike harmful bacteria (e.g. *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 which may result in further follow-up actions is associated with elevated levels of generic *E. coli* ($100 < x \leq 1000$ Colony Forming Units (CFU) or Most Probable Number (MPN)/mL). 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 assessment is associated with high levels of generic *E. coli* (> 1000 CFU or MPN/mL) as it may indicate a breakdown in Good Agricultural Practices and/or Good Manufacturing Practices, and therefore possibly warranting the initiation of follow-up activities to determine the source of the contamination and improve sanitation conditions along the food chain.

Table 1 - Analytical Methods and Assessment Criteria for Bacteria in Juice Samples (Survey *i*)

Analysis	Method Identification Number*	Assessment Criteria		
		Satisfactory	Investigative	Unsatisfactory
Salmonella spp.	MFHPB-20 MFLP-38 MFLP-29	Absent in 25 mL	Not Applicable (N/A)	Present in 25 mL
Shigella	MFLP-25 MFLP-26	Absent in 25 mL	N/A	Present in 25 mL
E. coli O157:H7	MFLP-30 MFLP-80 MFLP-52	Absent in 25 mL	N/A	Present in 25 mL
Generic *E. coli*	MFHPB-19 MFHPB-27	$\leq$ 100 CFU or MPN/mL	100 < x $\leq$ 1000 CFU or MPN/mL	> 1000 CFU or MPN/mL

* The methods used were the published versions at the time of analysis

Samples from survey *ii* were analysed for viruses using CFIA internally developed methods that detect the presence of ribonucleic acid (RNA) of Hepatitis A virus (HAV) and Norovirus (NoV) (Genotype I and II (GI, GII)). Samples from survey *iii* were analyzed for parasites using CFIA internally developed methods that detect the presence of deoxyribonucleic acid (DNA) of *C. cayetanensis*, *Cryptosporidium spp*, *T. gondii*[6] and *Giardia spp.*[7, 8] See Table 2 for the specific methods used and associated assessment criteria.

Table 2 - Analytical Methods and Assessment Criteria for Virus and Parasite in Juice Samples (Surveys *ii* and *iii*)

Analysis	Method Identification Number*	Assessment Criteria	
		Satisfactory	Investigative
Hepatitis A	CFIA-VAD-02	Not detected	Detected
Norovirus (GI, GII)	CFIA-CRNVA-05 RT-PCR	Not detected	Detected
C. cayetanensis	qPCR assay, melting curve analysis, & sequencing[6]	Not detected	Detected
Cryptosporidium spp.		Not detected	Detected
T. gondii		Not detected	Detected
Giardia spp.	Nested PCR[7, 8]	Not detected	Detected

At the time of writing this report, no assessment guidelines had been established in Canada or internationally for viruses or parasites in juices. In addition, the analytical methods used to analyse the samples detect viral RNA or parasite DNA and cannot discriminate between living and dead viruses or parasites. Consequently, the detection of viral RNA or parasite DNA was assessed as "investigative" and required further consideration to determine appropriate follow-up actions (Table 2).

What Were The Survey Results?

Samples from survey *i* including HPP (751), cold-pressed (234), and freshly squeezed/pressed (148) samples were tested for bacterial pathogens of concern (*E. coli* O157:H7, *Salmonella* spp., *Shigella*) and generic *E. coli,* which is an indicator of the overall sanitation conditions throughout the food production chain (Table 3). Bacterial pathogens (*E. coli* O157:H7, *Salmonella* spp., *Shigella*) were not found in any samples. An elevated level of generic *E. coli* ($100 < x \leq 1000$ MPN/mL) was found in one sample (< 0.1%, 1/1133), which was a cold-pressed unpasteurized juice sample (Table 3).

Table 3 - Assessment Results of Bacterial Analysis in Juice Samples

Juice type	Analysis	Number of Samples Tested	Assessment Results		
			Satisfactory	Investigative	Unsatisfactory
HPP	*Salmonella* spp.	751	751	N/A	0
	Shigella			N/A	
	E. coli O157:H7			N/A	
	Generic *E. coli*			0	
Cold-pressed (unpasteurized)	*Salmonella* spp.	234	233	N/A	0
	Shigella			N/A	
	E. coli O157:H7			N/A	
	Generic *E. coli*			1	
Freshly Squeezed/Pressed (unpasteurized)	*Salmonella* spp.	148	148	N/A	0
	Shigella			N/A	
	E. coli O157:H7			N/A	
	Generic *E. coli*			0	
Total		**1133**	**1132**	**1**	**0**

Samples from survey *ii* including HPP (465), cold-pressed (39) and freshly squeezed/pressed (179) samples were tested for enteric viruses of concern (HAV and NoV (GI and GII). Due to methodology limitations, samples from survey *iii* were comprised of only 12 unpasteurized apple juice/cider samples and were tested for enteric parasites of concern (*C. cayetanensis*,

Cryptosporidium spp., *T. gondii* and *Giardia spp.*). Viruses (HAV, NoV (GI, GII) and parasites (*C. cayetanensis*, *Cryptosporidium spp.*, *T. gondii*, *Giardia spp.*) were not detected in any samples (Tables 4 and 5).

Table 4 - Assessment Results of Viral Analysis in Juice Samples

Juice type	Analysis	Number of Samples Tested	Assessment Results	
			Satisfactory	Investigative
HPP	HAV	465	465	0
	NoV (GI)			
	NoV (GII)			
Cold-pressed (Unpasteurized)	HAV	39	39	0
	NoV (GI)			
	NoV (GII)			
Freshly Squeezed/Pressed (Unpasteurized)	HAV	179	179	0
	NoV (GI)			
	NoV (GII)			
Total		**683**	**683**	**0**

Table 5 - Assessment Results of Parasite Analysis in Unpasteurized Apple Juice/Cider Samples

Juice type	Analysis	Number of Samples Tested	Assessment Results	
			Satisfactory	Investigative
Unpasteurized, Apple Juice/Cider	*C. cayetanensis*	12	12	0
	Cryptosporidium spp.			
	T. gondii			
	Giardia spp.			

A total of 1828 samples, including 612 unpasteurized juice samples (273 cold-pressed and 339 freshly squeezed/pressed) and 1216 HPP juice samples were collected for various testing purposes as described above (Tables 3, 4, and 5).

Of the 1216 HPP juice samples, a majority (67%) of the samples were domestic and 33% were imported (Table 6). Similarly, of the 273 cold-pressed unpasteurized samples, a majority (86%) were domestic, none were imported and 14% were of unknown country of origin. One of the domestic cold-pressed unpasteurized juice samples was found to have an elevated level ($100 < x \leq 1000$ MPN/mL) of generic *E. coli* (Table 6). Of the 339 freshly squeezed/pressed unpasteurized samples, a large portion (60%) were of unknown country of origin, 38% were domestic and 2% were imported (Table 6).

Table 6 - Sample Distribution by Country of Origin

Product Origin	Number of HPP Juice Samples (%)	Unpasteurized Juices	
		Number of Cold-pressed Juice Samples (%)	Number of Freshly Squeezed/Pressed Juice Samples (%)
Domestic	814 (67)	235 (86)*	128 (38)
Import	402 (33)	0 (0)	6 (1) (2)
United States	*382*	*0*	*5*
Imported (Unknown Country)	*20*	*0*	*1*
Unknown	0 (0)	38 (14)	205 (60)
Total	**1216**	**273**	**339**

* One sample found to have an elevated level (100<x≤1000 MPN/mL) of generic *E. coli*

A variety of product types of HPP juices, cold-pressed juices and freshly squeezed/pressed juices were sampled and are detailed in Table 7. One cold-pressed fruit and vegetable juice was found to have an elevated level (100 < x ≤ 1000 MPN/mL) of generic *E.coli.*

Table 7 - Sample Distribution by Product Type

Product Type	Number of HPP Juice Samples (%)	Unpasteurized Juices	
		Number of Cold-pressed Juice Samples (%)	Number of Freshly Squeezed/Pressed Juice Samples (%)
Fruit Juice	369 (30)	67 (25)	265 (78)
Apple Juice/Cider	*2*	*0*	*24*
Blueberry	*4*	*1*	*0*
Coconut	*1*	*0*	*0*
Cranberry	*5*	*0*	*1*
Grapefruit	*0*	*5*	*20*
Lemon	*4*	*2*	*4*
Mixed	*218*	*49*	*110*
Orange	*96*	*9*	*100*
Pineapple	*6*	*0*	*2*
Pomegranate	*2*	*0*	*0*
Sea Buckthorn	*1*	*0*	*0*
Strawberry	*0*	*0*	*2*
Watermelon	*30*	*1*	*2*
Vegetable Juice	25 (2)	5 (2)	6 (2)
Beet	*0*	*0*	*1*
Carrot	*3*	*0*	*3*
Cucumber	*0*	*1*	*0*
Mixed	*22*	*4*	*1*
Spinach	*0*	*0*	*1*
Fruit & Vegetable Juice	822 (68)	201 (73)*	68 (20)
Total	**1216**	**273**	**339**

* One sample found to have an elevated level (100 < x ≤ 1000 MPN/mL) of generic *E. coli*

What Do The Survey Results Mean?

In this study, all (100%) of the unpasteurized juice and HPP juice samples analyzed were free of bacterial pathogens (*Salmonella* spp., *Shigella,* and *E. coli* O157:H7), viruses (HAV, NoV (GI and GII)) and parasites (*C.cayetanensis*, *Cryptosporidium spp.*, *T. gondii*, and *Giardia* spp) tested for. An elevated level of generic *E. coli* (100<x≤1000 MPN/mL) was found in one juice sample (<0.1%, 1/1133), which was a cold-pressed unpasteurized juice sample and in response, the CFIA conducted appropriate follow-up activities.

Similar studies for bacterial pathogens in unpasteurized juices were conducted in the UK, Ireland and Mexico. Similar to our survey results, the studies conducted in the UK and Ireland did not find bacterial pathogens in the juice samples analysed. The 2002 UK study investigated

the microbiological quality and safety of unpasteurized fruit and vegetable juices collected from retail locations in the UK in 2002 and found that no presence of *E. coli* O157:H7, *Salmonella* and generic *E. coli* (>100 CFU/mL) in any of the samples[10]. The survey from Ireland tested for bacterial pathogens and generic *E. coli* in unpasteurized fruit and vegetable juices and smoothies collected from retail and service sectors across Ireland in 2007 and found no presence of *E. coli* O157:H7 (436), *Salmonella* (811) in any of the samples tested for. Generic *E. coli* at levels exceeding 1000 CFU/mL and between 100-1000 CFU/mL were found in 0.2% (2/811) and 0.4% (3/811) of the samples respectively[11]. Contrary to our survey results and the UK and Irish studies, a survey conducted for the presence of *Salmonella* and *Shigella* in freshly squeezed orange juices from retail and street booths in Guadalajara, Mexico found a high prevalence of *Salmonella* and *Shigella* in both retail and street vended juice samples. The prevalence of *Salmonella* and *Shigella* in retail juice samples was found to be 3.9% (2/51) for both pathogens while their prevalence in street vended juice samples was found to be 14.3% (7/49) and 6.1% (3/49) respectively. Additionally, generic *E. coli* at levels exceeding 1000 CFU/mL were found in 39% (39/100) of all the juice samples tested[12].

As with all unpasteurized juices, cold–pressed juices are not exposed to heat or high pressure processing treatments to inactivate microbial pathogens. Consequently, the microbial safety of cold-pressed juices depends on effective sanitation controls of the inputs (raw intact fresh fruits and vegetables), the processing equipment and the processing environment. Studies suggest that an effective HPP step to treat cold-pressed "raw" juices is a pressure of 87,000 psi (pounds per square inch) for 2-9 minutes and results in the same level of reduction in the microbial population as pasteurization[13]. HPP juices are a relatively new product and therefore no reports detailing retail survey results were available for comparison with our study at the time of writing this report.

Overall, our study results indicate that almost all of the unpasteurized and HPP juices sampled appear to have been produced under Good Agricultural Practices and Good Manufacturing Practices. One sample in our survey was found to have an elevated level of generic *E. coli,* which may indicate a loss of sanitation controls along the juice production chain. Consequently, safe handling practices are recommended for producers, retailers and consumers. Unpasteurized juices do not undergo a heat or HPP treatment and therefore prevention of contamination relies solely on Good Agricultural Practices and/or Good Manufacturing Practices during production and processing, from farm to fork. The declaration on product labels of "unpasteurized" is important to allow consumers, especially high-risk population groups (children, elderly and immunocompromised people) to make informed choices[1].

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