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Qualitative Microbiological Risk Assessment of Unpasteurized Fruit Juice and Cider

Regular Paper

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Abstract Until recent decades, unpasteurized fruit juice and cider have been considered non-hazardous with respect to microbiological pathogens due to their acidic nature. However, in light of the many global foodborne illness outbreaks associated with these products, it is apparent that certain bacterial, viral and parasitic pathogens can survive these acidic conditions and remain infectious. Specifically, outbreaks of human illness have been attributed to infection with *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., *Cryptosporidium* spp., *Trypanosoma cruzi*, and hepatitis A, and have been associated with the consumption of apple juice or cider, orange juice and various other types of unpasteurized juices.

The most likely mechanisms by which juice, and the fruit it is processed from, becomes contaminated with pathogenic microorganisms are through direct contact with animal or human faeces, or indirect contact with contaminated water, soil, processing equipment, or infected food handlers.

This risk assessment reviews foodborne outbreaks linked to unpasteurized fruit juice and cider, and evaluates the evidence for effectiveness of measures to control pathogens in these products.

Keywords Unpasteurized, Fruit Juice, Cider, Pathogens, Foodborne Illness, Risk Assessment

1. Introduction

Advances in horticultural, processing, preservation, packaging, shipping, and marketing technologies have made it possible for consumers to be supplied with high-quality fresh fruits year round. Some operations however, have added a dimension of increased risk of human illness associated with pathogenic bacteria, viruses, and parasites [1]. Outbreaks of human illness have been associated with consumption of raw fruits or their unpasteurized products [2]; [3]. Pathogens such as *Listeria monocytogenes*, *Clostridium botulinum*, and *Bacillus cereus* on fresh fruit, can be expected since such flora naturally occurs in the soil [2]. However, contaminated water and raw or improperly composted manure are more likely to contaminate fresh fruit with *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., parasites and viruses [2]. Direct contact with livestock and other animals, or their faeces, can also result in the contamination of fruits with various pathogens. This is of particular concern with respect to drop fruit. Human handling and contact surfaces/equipment represent other potential sources of contamination throughout the preparation of fruit for consumption. This includes all phases: growing, harvesting, packing, processing, and shipping, as well as handling by food workers and consumers. Traditional sanitizing methods such as chlorine treatment, can reduce populations of most pathogens, but does not eliminate them [4].

Until recent decades, it was generally accepted that high acid fruit juices (pH 3.0-4.0) could not support survival and growth of microbial pathogens. However, a number of outbreaks of human illness that occurred during the 1990s were associated with the consumption of unpasteurized fruit juices. Although growth is unlikely at low pH, it is well documented that pathogenic microorganisms may survive in fruit juices, become adapted to the acid environment, and cause outbreaks of foodborne illnesses [5]. Antimicrobial activities related to organic acids such as malic and citric acid have been reported [6]. They are commonly found in fruit juices or they may be added to low-acid foods as a preservation agent [3]. In addition, refrigerated storage can considerably extend the survival of the pathogens in juices. At warmer temperatures, such as room temperature, *E. coli* O157:H7 and *Salmonella* populations will be reduced rapidly, compared to those in refrigerated acid foods [7]; [8]; [9]; [10]; [11]. This should be kept in mind when making decisions regarding the storage temperatures.

With respect to fresh fruit juices, pasteurization is very effective in reducing the number of viable pathogens so they are unlikely to cause illness [8]. However a considerable amount of fresh fruit juice is purchased and consumed in an unpasteurized state and is, therefore, of concern with respect to foodborne illness. The high-acid tolerances of some pathogens add to this concern since juice acidity was once thought to be a major inhibitory barrier.

Between 1974 and 2012, numerous illness outbreaks associated with unpasteurized fruit juice and cider have been reported worldwide, involving approximately 2,527 cases (Table 1). These were caused by *E. coli* O157:H7, *Salmonella* spp., *Shigella* spp., *Cryptosporidium* spp., *Trypanosoma* cruzi, and hepatitis A. Ten of these outbreaks were associated with orange juice, 17 implicated apple juice or cider, and 5 involved other types of fruit juice, such as watermelon, sugarcane, açaí, and guava juice.

In addition to the more commonly associated pathogens mentioned above, another emerging issue is that of orally-acquired Chagas' disease in South America, which has been associated with the consumption of a variety of unpasteurized juices contaminated with the parasite *Trypanosoma cruzi*[12]. The availability in Canada of the unpasteurized juices associated with these South American outbreaks (e.g., açaí juice, sugarcane juice,guava juice) is not clear, so while the risk to Canadians is likely very low, it cannot yet be accurately estimated.

Year	Pathogen	No. of cases	Vehicle	Location	Comments	Reference
1974	*Salmonella* Typhimurium	296	Apple cider	New Jersey, USA	Manure used as fertilizer; drop apples	[13]
1980	Most likely *E. coli* O157:H7	14	Apple cider	Toronto, Ontario, Canada	Not reported	[14]
1991	*E. coli* O157:H7	23	Apple cider	Massachusetts, USA	Drop apples; no washing; cattle raised	[15]
1992	Enterotoxigenic *E. coli*	6	Orange juice	India	Roadside vendors selling fresh squeezed juice	[16]
1993	*Cryptosporidium spp.*	160	Apple cider	Maine, USA	Drop apples	[17]
1993	*Salmonella* spp.	18	Watermelon juice	Florida, USA	Home-made watermelon juice	[18]
1995	*S.*Hartford, Gaminara and Rubislaw	63	Orange juice	Florida theme park, USA	Local processing plant producing for a large Florida theme park; inadequately sanitized processing equipment; unclean facility	[19]; [20]; [21]
1995	*Shigella flexneri*	14	Orange juice	South Africa	Contamination of the hands of staff squeezing the oranges to make juice.	[22]

Year	Pathogen	No. of cases	Vehicle	Location	Comments	Reference
1996	*E. coli* O157:H7	14	Apple cider	Connecticut, USA	Drop apples	[23]
1996	*E. coli* O157:H7	6	Apple cider	Washington State, USA	Made for local church event from local orchard; apples were washed in a chlorine solution	[18]
1996	*C. parvum*	20 confirmed, 11 suspected	Apple cider	New York, USA	Drop apples; orchard adjacent to dairy farm.	[24]
1996	*E. coli* O157:H7	70	Apple juice	Western USA; British Columbia, Canada	Drop apples; improper use of sanitizers; deer and cattle in close proximity; distribution through fresh juices, shakers and energy bars.	[25]
1997	*E. coli* O157:H7	6	Apple cider	Indiana State, USA	All cases visited a local apple orchard and cider pressing operation	[26]
1998	*E. coli* O157:H7	14	Apple cider	Southwestern Ontario, Canada	Total of 4 trees; some in a cattle pasture; drop apples used; apples not washed; distribution to family and friends from 2 local farms	[27]
1999	*S.* Typhimurium	500	Orange juice	South Australia	Oranges were source of contamination	[18]
1999	*S.* Muenchen	200	Orange juice	14 states in USA and 2 provinces in Canada (British Columbia and Alberta)	Juice distributed as both frozen and liquid; for commercial use in restaurants and hotels; products include 'smoothies'; detected in samples taken from blenders and dispensers.	[18]
1999	*S.* Anatum	4	Orange juice	Sarasota County, Florida, USA	Contamination most likely occurred during the manufacturing process	[28]
1999	*E. coli* O157:H7	7	Apple cider	Tulsa, Oklahoma, USA	Contamination most likely occurred at apple orchard or cider pressing operation	[29]
1999	*S.* Typhimurium	16	Mamey frozen puree	Florida, USA	Import from Guatemala and Honduras	[30]
2000	*Salmonella* spp.	14	Orange juice	Colorado, California, Nevada, USA	Unpasteurized citrus products produced by a juice company in California	[31]
2003	*C. parvum*	144	Apple cider	Ohio, USA	Ozone treatment was insufficient to inactivate pathogens	[32]
2004	*E. coli* O111 and *C. parvum*	213	Apple cider	New York, USA	Retail establishment	[33]
2004	Hepatitis A	351	Orange juice	Egypt	Juice contaminated during manufacturing	[34]
2005	*E. coli* O157:H7	4	Apple cider	Ontario, Canada	Juice produced and sold at a small local retail outlet	[35]

Year	Pathogen	No. of cases	Vehicle	Location	Comments	Reference
2005	*Trypanosoma cruzi*	25	Sugarcane juice	Brazil	Juice sold at a roadside kiosk; infected triatomine bugs and opossums were found in and around the kiosk.	[12]
2005	*T. cruzi*	27	Açaí juice	Brazil	All cases consumed juice from a single sales outlet.	[12]
2005	*S.* Typhimurium and Saintpaul	157	Orange juice	Multistate, USA	'Fresh-squeezed' orange juice; outbreak was identified in 24 states.	[36]
2007	*T. cruzi*	103	Guava juice	Venezuela	Outbreak occurred at a school in Caracas; juice may have become contaminated with triatomine bugs during overnight storage outside.	[37]
2007	*E. coli* O157:H7	9	Apple cider	Massachusetts, USA	Not reported	[38]
2008	*S.* Panama	15	Orange juices	The Netherlands	The causative *Salmonella* strain was able to survive under low pH conditions, such as those in the human stomach.	[39]
2008	*E. coli* O157:H7	7	Apple cider	Iowa, USA	Fair, festival; cider purchased from a temporary booth.	[38]
2010	*E. coli* O157:H7	7	Apple cider	Maryland, USA	Retail establishment	[40]

Table 1. Food-borne outbreaks traced to unpasteurized fruit juice and cider (1974 – 2012)

2. Juice Processing

Unpasteurized juice is the unfermented liquid (usually clarified) obtained from the pressing of properly prepared, sound, clean, mature fruit. It can be frozen to allow sale at a later date. Juice concentrated by a heat treatment is a different product (pasteurized juice) and is not covered by this assessment. Unpasteurized cider is the unfermented, unclarified, untreated liquid obtained from the pressing of properly prepared, sound, clean, mature fruit. It includes sweet or soft cider, as well as frozen cider. Hard cider (fermented) is a different product. Apple cider is apple juice that has not undergone a filtration process to remove coarse particles of pulp sediment.

There are differences in the handling of each type of fruit intended for juice. Overall, unpasteurized juice or cider manufacturing includes several processing steps, such as receiving, storing, washing, grinding and extraction, separation/centrifugation, blending of ingredients, and storage. The first processing step is a receiving protocol which includes fruit inspection and grading. After fruits are received and hand-sorted on a conveyer belt, they are mechanically scrubbed and washed with a sanitizing solution, rinsed with water, and ground into a pulp that is consistency of sauce. There are many ways to extract juice depending on the type of fruit and they include squeezing, pressing, grinding, etc. A hydraulic press squeezes/pressed the pulp to extract juice, which flows into refrigerated thanks. The pressing operation can range from manual to mechanical with complete automated system common in the juice industry. A simple example of a flow chart for juice processing can be seen in Figure 1.

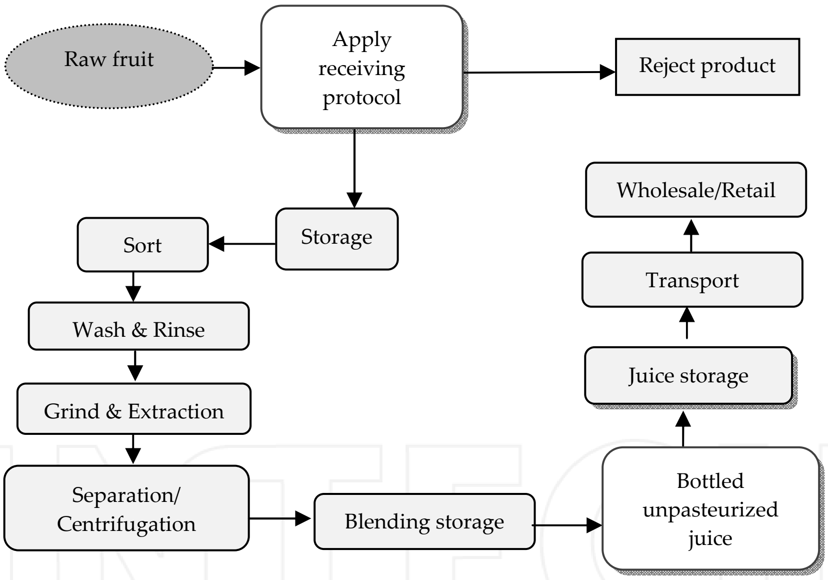

Figure 1. Juice processing chart

3. Purpose of Risk Assessment

This qualitative microbiological risk assessment follows the Codex Principles and Guidelines for the Conduct of Microbiological Risk Assessments. It includes the following qualitative information:

(a) Hazard Identification - identifies the hazards of concern associated with unpasteurized juices and cider;
(b) Hazard Characterization- provides a qualitative or quantitative description of the severity and duration of adverse effects that may result from ingestion of a pathogenic microorganism or its toxin in unpasteurized juice or cider.
(c) Exposure Assessment - considers the likelihood of acquiring food-borne disease through consumption of unpasteurized juice or cider in Canada;
(d) Risk Characterization - shows the likelihood of illness, if the identified hazards are ingested.

These steps were used to determine information gaps with respect to contamination of juice and cider and risk to consumers in Canada. They can also provide scientific support for risk management decisions regarding unpasteurized juice and cider.

4. Scope

This document is a qualitative risk assessment of the microbiological hazards associated with unpasteurized orange juice and unpasteurized apple juice or cider. It is recognized that risks exist for other types of unpasteurized juices, since they cover a wide array from the acidic to the near neutral varieties (e.g., melons). However, the aim of the present document is to reflect mainly upon these two unpasteurized juices that have become concerns in Canada, due to their links with human illness.

An initial literature search was conducted in 2011 and updated in December 2012 and May 2013, with no restriction on the date of publication. Relevant studies were identified in the databases (i.e., Medline, Scopus, and Agricola) using a combination of the following key words: unpasteurized, juice, fruit, apple, cider, orange, outbreak, illness. An internet search was also conducted using the search engines, employing the same terms used in the electronic databases. In addition to published peer-reviewed studies, relevant studies published as conference proceedings and scientific reports were also searched.

5. The Risk Assessment

5.1 Hazard Identification

5.1.1 Escherichia coli

Escherichia coli classes considered of most concern with respect to unpasteurized juices, especially apple cider, belong to enterohemorrhagic *E. coli* (EHEC) (e.g., the *E. coli* O157:H7 serotype), and enterotoxigenic *E. coli* (ETEC). These cause distinct syndromes of diarrheal disease in humans [41].

Animals are the primary asymptomatic reservoir for *E. coli* O157:H7. Cattle, in particular, serve as important reservoirs for the organism [24]. Drop apples can become contaminated by coming into contact with manure from infected animals. In an outbreak of *E. coli* O157:H7 infections in Massachusetts, USA in 1991, a cider press operator also raised cattle, which grazed in a field adjacent to the mill [15]. The presence of animals near a cider mill can result in manure inadvertently contacting apples, equipment, or workers' hands. In addition, apples can become contaminated if transported or stored in areas that contain manure, or if rinsed with contaminated water. In another outbreak in Ontario, Canada in 1998, apple cider was most likely contaminated by the use of apples collected from the ground [27]. In this case, a farmer kept his heifers in the orchard, but only until late July (i.e., a few weeks before apple harvest). However, studies have shown that *E. coli* can survive in soil for > 20 weeks [42]. Run-off from the nearby sheep pasture on a nearby farm could also have been a source of contamination. Another consideration is that *E. coli* O157:H7 is acid-tolerant and can survive at low pH for up to 4 weeks. Therefore, transmission is possible through contaminated apple cider or juice, which has typical pH values of between 3 and 4. In addition to the food borne route, the transmission of *E. coli* O157:H7 through contaminated water supplies, the person-to-person route, and direct animal-to-human transmission, have all been documented [43]; [44]; [45].

ETEC is not considered a serious food-borne disease hazard in countries having high sanitary standards and practices. Contamination of water by human sewage can, however, lead to contamination of foods. Infected food handlers may also contaminate foods [41].

Epidemiology

The Canadian Public Health Agency reports, published in 2009 and 2010 , established that at least 85% of the pathogenic *E. coli* isolates from human cases were of serovar O157, while less than 15% were non-O157[46]; [47]. Non-O157 *E. coli* infections are likely being under-reported in Canada due to inadequate laboratory surveillance. A decrease in verotoxigenic *E. coli* cases was noticed between 2004 and 2009 [46]. In Canada, the incidence of pathogenic *E. coli* shows a distinct seasonal trend, with increased rates in the summer and fall. Although most provincial rates were stable between 2000 and 2004, a slight decrease was noticed in the Eastern provinces, including Ontario [48]. Of the three most frequently reported notifiable enteric pathogens, *E. coli* is third following *Campylobacter* spp. and *Salmonella* spp. [48]. Nevertheless, pathogenic *E. coli* cases showed the highest hospitalization rate, while *Salmonella* infections resulted in the largest number of deaths overall [48].

Of the reported cases in Canada, there is a strong indication that foods of animal origin are an important source of *E. coli* O157:H7 infections. Household settings represent the largest number of reported outbreaks, while community settings resulted in higher outbreak related case counts. Several outbreaks occurred in non-residential institutions including daycare settings [48].

About 10% of patients with hemorrhagic colitis develop hemolytic uremic syndrome (HUS), characterized by acute renal failure, hemolytic anemia and thrombocytopenia. The disease can lead to permanent loss of kidney function. On average, 2-7% of HUS cases are fatal, but the mortality rate in the elderly can be as high as 50%. All people are believed to be susceptible to hemorrhagic colitis, but the young children, elderly and immunocompromised are more sensitive [44]; [49].

Infants in underdeveloped countries, and travelers to these regions, are most at risk of infection with ETEC. At least 25 foodborne outbreaks of ETEC have been documented in the U.S. between 1998 and 2011 [38], but none of them appear to be linked to juice consumption.

Outbreaks specific to E. coli in Unpasteurized Juice and Cider

There were 12 apple juice-associated outbreaks of *E. coli* O157:H7 in North America between 1974 and 2012 (Table 1). Outbreaks occurred in Massachusetts in 1991 (23 cases); Connecticut in 1996 (14 cases); Washington State (6 cases); and Western U.S. and Canada (70 cases in total) [25]. The fifth outbreak occurred in southwestern Ontario, Canada in 1998. This outbreak resulted in 14 confirmed cases implicating non-commercial custom-pressed unpasteurized cider produced from drop apples gathered in an orchard where cattle grazed [27]. A sixth outbreak occurred in October 1999 in Tulsa, Oklahoma [29].

In Faridpur, India, in 1992, 6 people were hospitalized with severe diarrhea after drinking unpasteurized orange juice contaminated with ETEC [16]. This outbreak was associated with juice at four roadside stands. The contamination of juice in this outbreak did not arise from conditions comparable to those found in North American outbreaks. Contamination of juices in the latter have been generally traced back to larger scale pressing operations, that may be within proximity of, or on farms, whereas the outbreak in India was likely associated with a garbage pile which was located near the juice stand.

5.1.2 Salmonella spp.

All known *Salmonella* serotypes are potentially pathogenic to humans. All age groups are susceptible to *Salmonella* spp., but symptoms are more severe in infants, the elderly, and the immunocompromised [49].

Salmonella spp. are commonly found in the intestinal tract of humans and animals, particularly poultry and swine. Humans, and other mammals, birds, reptiles, amphibians, and insects, are all carriers through either their natural surface flora or faeces. *Salmonella* spp. are often transmitted by the faecal-oral route (i.e., person-to-person), but they can also remain viable for several years in soil or water. Therefore, poor adherence to sanitary precautions in fruit-growing operations or juice extraction facilities can also result in the transmission of this pathogen. Because fruit may be harvested after being dropped to the ground, its exterior may become contaminated with *Salmonella* spp. in the field from soil, surface water used for irrigation, or raw manure used as fertilizer. Contamination could continue into processing facilities where infected food handlers, or animal carriers could potentially contaminate fruit and processing equipment [19].

Epidemiology

The Canadian Integrated Surveillance Report, published in 2009 [48], established that the incidence of salmonellosis cases show a moderate summertime association throughout Canada. From 1995 to 2004, there was an overall decline in the national rate of *Salmonella* infections. Of the three most frequently reported notifiable enteric pathogens, *Salmonella* spp. is second to *Campylobacter* spp. [48]. *Salmonella* outbreaks and case clusters accounted for the largest proportion, and the largest number, of outbreak-related cases over the five-year period as compared to *E. coli*, *Campylobacter* spp., and *Shigella* spp. [48]. In 2004, *Salmonella* cases accounted for approximately 10 out of every 1,000 enteric hospitalizations in Canada. Case-fatality rates were 19.2 per 1,000 hospitalized with illness [48]. The C-EnterNet Program of the Public Health Agency of Canada reported that the annual incidence rate of salmonellosis for 2008 in Canada was 18.2 per 100,000 [50]. In addition, it has been estimated that 1.4 million illnesses and 400 deaths due to *Salmonella* spp. occur annually in the United States [36].

In Canada, household settings represented the largest number of reported *Salmonella* outbreaks and case clusters, while community settings resulted in higher related case counts [48]. The mortality rate of typhoid fever, due to *S. typhi*, is 10%, compared to less than 1% for other forms of salmonellosis. *S.* Dublin has a 15% mortality rate in the elderly and *S.* Enteritidis has demonstrated a 3.6% mortality rate in hospital/nursing home outbreaks, with the elderly being particularly affected [49]. Reactive arthritis (Reiter's syndrome) may occur as a sequela in about 2% of culture-proven cases. Septic arthritis, subsequent or coincident with septicemia, may also occur and can be difficult to treat.

Outbreaks specific to Salmonella spp. in Unpasteurized Juice

Salmonella spp. has been implicated in juice and cider outbreaks (Table 1). In 1974, fresh pressed unpasteurized apple cider, suspected of being contaminated with manure, was implicated in an outbreak of *S.* Typhimurium [13]. In 1995, 63 illnesses in Florida [20] were documented and were thought to be related to the consumption of unpasteurized orange juice which was found to contain *S.* Hartford, *S.* Gaminara, and *S.* Rubislaw. This outbreak of diarrheal illness affected at least 62 visitors to a large tourist theme park in Orlando [19]. In this particular outbreak, the same serovars were isolated from unopened bottles of orange juice, unwashed fruit surfaces, and amphibians near the manufacturing plant [21]. The orange juice implicated in this outbreak was produced in a Florida citrus-processing facility. In the spring of 1999, a food poisoning incident in South Australia affected approximately 500 people and was associated with unpasteurized orange juice contaminated with *S.* Typhimurium [18]. In June 1999, over 200 cases of *S.* Muenchen were detected in 14 states (including Washington, Arizona, California and Texas) in the United States and 2 provinces (19 cases in British Columbia and 4 in Alberta) in Canada. Epidemiologic investigations linked this outbreak to unpasteurized fresh squeezed orange juice [51]; [52]. A multistate U.S. outbreak occurred in 2005 and affected 157 people; the outbreak was associated with unpasteurized orange juice contaminated with *S.* Typhimurium and Saintpaul [36]. In the spring of 2008, 15 *S.* Panama cases were reported in The Netherlands [39]. This outbreak of gastroenteritis was related to the consumption of fresh unpasteurized orange juices.

5.1.3 Cryptosporidium spp.

Cryptosporidium is a single-celled protozoan and an obligate intracellular parasite. There are numerous species and genotypes of *Cryptosporidium* found in a large number of different hosts, including humans, worldwide. The infectious stage of the organism, known as oocysts, are 4-5 micrometers in diameter and are shed with the hosts faeces [53]. *Cryptosporidium* spp. oocysts are more resistant than bacteria to most chemical disinfectants such as chlorine, but are susceptible to drying. Currently, no data are available to support oocyst reduction from washing fruits and vegetables [53].

A relatively small number of oocysts can cause illness characterized by watery diarrhea, abdominal pain, nausea, vomiting, fever and other symptoms. These symptoms are self-limiting and generally last 1-2 weeks [54]. Severe and chronic symptoms of cryptosporidiosis are reported among immunocompromised individuals, and are potentially life-threatening.

Transmission of cryptosporidiosis is facilitated by the ability of oocysts to survive for weeks to months in the environment. Routes of transmission include ingestion of oocysts in drinking or recreational water, direct person-to-person (e.g., daycares, institutionalized settings), and zoonoses, particularly through direct contact with cattle and other livestock. Foodborne outbreaks have also been reported [55]. Fresh produce, in particular, may become contaminated pre-harvest (e.g., contaminated irrigation/washing water, infected farm workers, use of manure as fertilizer, contaminated equipment), or post-harvest (e.g., packaging, storage, transport, food-handlers and consumers) [49]; [53]; [56].

Epidemiology

The relative frequency of the disease in the North American population is reported to be approximately 2%. Serological surveys indicate that 80% of the population has been exposed to *Cryptosporidium* [49]. The very young and elderly may be at a higher risk of disease as a result of *Cryptosporidium* infection [57]. Recently, it was estimated that 8% of domestically acquired cases of cryptosporidiosis in the U.S. are foodborne [58].

Outbreaks specific to Cryptosporidium spp. in Unpasteurized Juice

An outbreak of cryptosporidiosis causing 160 primary cases at a local fair in Maine in 1993 was associated with the consumption of cider fresh-pressed from apples picked from an orchard floor contaminated with cattle manure (Table 1). A calf from the same farm that supplied the apples was found to be infected with *Cryptosporidium* spp. [17]. Another outbreak of cryptosporidiosis resulting from consumption of unpasteurized apple cider affected 31 persons in New York in October, 1996 [24]. A third outbreak associated with apple cider in 2003 involved 144 confirmed and probable cases that drank ozonated apple cider in Ohio [32]. In 2004, a fourth outbreak of cryptosporidiosis associated with unpasteurized apple cider was reported in New York [33]. Of the 213 illnesses associated with this outbreak, it was not clear how many could be attributed to *Cryptosporidium* spp. and how many to *E. coli* O111, both of which were detected in the cider and in clinical and environmental samples.

5.1.4 Other Pathogens

There have been other gastroenteritis outbreaks of associated with unpasteurized juice. For example, hepatitis A virus, *Shigella* spp. and *T. cruzi* have been shown to survive in unpasteurized juices including orange juice, and to cause human illness associated with consumption of juice [22]; [34]; [49].

Although, to our knowledge *L. monocytogenes* has not been implicated in any cases of juice-borne illnesses, it has been isolated in unpasteurized apple juice and in an apple-raspberry juice blend in the United States, in the fall of 1996. Analyzed retail juices yielded two unpasteurized samples positive for *L. monocytogenes*: an apple juice and an apple-raspberry blend, with pH values of 3.78 and 3.75, respectively. Three *L. monocytogenes* isolates were characterized [59]. A study carried out to evaluate the microbiological quality of orange juice obtained from squeezing machines in foodservice establishments in Spain revealed that 0.5% examined lots were positive for *Salmonella* spp. and 1% for the presence of *S. aureus* [60].

Many of the unattributed outbreaks associated with unpasteurized juices have been linked to unsanitary handling practices at the point of sale, mainly at the restaurant level [61].

5.1.5 Hazard Identification Conclusion

Outbreak and food testing data demonstrate that unpasteurized fruit juices can contain various pathogens, including *E. coli* O157:H7, ETEC, *Salmonella* spp., *Shigella* spp., staphylococci, *L. monocytogenes*, *K. pneumoniae*, *Cryptosporidium* spp., *Trypanosoma cruzi*, and hepatitis A virus. ETEC and hepatitis A have been mainly associated with unpasteurized orange juice outbreaks due to poor sanitation practices at point of sale. Although not associated with outbreaks in apple or orange juices, the presence of *L. monocytogenes*, staphylococci and *K. pneumoniae*, can be possible indicators of poor manufacturing practices since these organisms can survive in juices depending upon juice acidity and temperature. Raw fruit can be a significant source of juice contamination. *E. coli* O157:H7 and *Salmonella* spp. are considered to be the microbial hazards of immediate concern in this risk assessment as they have been the only pathogens to date that have resulted in human illness in Canada associated with the consumption of unpasteurized apple juice or cider, and orange juice (Table 1). However, the parasitic pathogen, *Cryptosporidium* spp., will also be considered in light of illness outbreaks in the U.S.A.

5.2 Hazard Characterization

5.2.1 Dose Response

The minimum number of *E. coli* O157:H7 cells required to cause illness from unpasteurized juice or cider is unknown. The dose response for *E. coli* O157:H7 in other food-borne outbreaks is considered to be low (10 to 1000 cells) [1]; [62]; [49].

The dose response of *Salmonella* spp. may be as few as 1-20 cells depending upon age and health of host, bacterial strains differences as well as the chemical composition of the food. Such low doses are generally associated with foods containing high percentages of fat, such as cheddar cheese and chocolate [63]. Higher infectious doses such as 10^4 cells and 10^5 cells, have been associated with imitation ice-cream and eggnog, respectively [49]; [64]; [63]. An estimation of infectious dose from consuming unpasteurized juice or cider however, is unknown.

In experimentally infected volunteers, as few as 9 *Cryptosporidium* spp. oocysts have been shown to cause infection in healthy adults [65]. However, the infectious dose in apple cider and other fruit juices is unknown.

5.2.2 Symptoms and Pathogenicity: Severity of Illness

E. coli O157:H7 has been implicated in several unpasteurized juice-related outbreaks and is the most common serotype of enterohemorrhagic *E. coli* (EHEC). EHEC causes illness through infection and intoxication. Three principal syndromes have been linked to EHEC infection. Severe bloody diarrhea, fever, headache, and general malaise, lasting from a few days to a few weeks, describe the first syndrome - hemorrhagic colitis. The incubation period may range from 3 to 9 days and duration of illness may range from 2 to 9 days. Another major syndrome, hemolytic uremic syndrome (HUS), is the leading cause of acute kidney failure in young children. Up to 15% of hemorrhagic colitis victims may develop HUS. The third syndrome is similar to HUS, but also involves fever and brain damage. It is called thrombotic thrombocytopenic purpura (TTP) and occurs infrequently, but the mortality rate can be as high as 50% in the elderly [62]; [49]; [66]. Illness, as a result of ingestion of *E. coli* O157:H7, may therefore be fatal for young children, the elderly and the immunocompromised.

The *Salmonella*-associated illness that is most often associated with unpasteurized juice outbreaks is gastroenteritis caused by nontyphoid strains of *Salmonella* spp. The severity of nontyphoid *Salmonella* infection (known as salmonellosis), varies with the number of bacteria ingested and the susceptibility of the individual. The incubation period ranges from 8 to 72 hours [67]; [63]. Headaches and chills may appear before the full onset of symptoms. The principal symptoms are nausea, vomiting, abdominal pain, dehydration and non-bloody diarrhea that can appear suddenly. Duration of the illness is usually from 1- 4 days and is self-limiting. Individuals usually feel better within 5 - 7 days. About 50% of individuals will continue to excrete *Salmonella* 2 - 4 weeks after remission of the illness, and about 10 - 20% will do so after 4 - 8 weeks. In extremely rare cases, individuals carry and shed *Salmonella* for up to 6 months after initial infection [66]; [67]; [49].

Cryptosporidium spp. typically infect cells lining the small intestine, but extraintestinal infections have been reported in the stomach, biliary and pancreatic sites, and in the respiratory tract. Symptoms of cryptosporidiosis range from mild to severe depending upon the site of infection and the nutritional and immune status of the host [Chalmers and Davies, 2010]. This disease is self-limiting in immunocompetent patients, in which the most common symptoms are watery diarrhea, abdominal pain, nausea or vomiting, fever, malaise and weight loss. Symptoms generally last for about 1-2 weeks, although chronic illness is not uncommon in these patients. Children under 2 years of age are the most frequently and severely affected. Chronic and life-threatening symptoms can occur in immunocompromised patients, and chronic intestinal cryptosporidiosis is listed as an AIDS-defining illness.

5.2.3 Susceptibility Factors

Individuals can acquire *E. coli* O157:H7 infection, salmonellosis, or cryptosporidiosis at any age. Young children and the elderly, as well as the immunocompromised are most susceptible to severe and chronic infections. Complications can arise for children under 5 years old [68].

5.2.4 Conclusions on the Hazard Characterization

Though the incidence of contamination in unpasteurized juices is unknown, the general population, particularly young children, the elderly, and immunocompromised individuals can become ill, if exposed to unpasteurized apple juice /cider or orange juice that is contaminated with pathogens of concern. Illness can occur in the general population however, the susceptible population is more likely to experience severe cases of illness. Epidemiological data have shown that illness can be far-reaching in the general population though volumes of either unpasteurized apple juice/cider or orange juice on the Canadian market appears to be small, compared to pasteurized juice volumes.

5.3 Exposure Assessment

The exposure assessment serves to determine the occurrence of the identified health hazards in the products of concern. *E. coli* O157:H7 [7] and *Salmonella* spp. [63]; [69] have been found to survive for extended periods of time and to tolerate pH values below 4.0 such as are found in fruit juice and cider. Although the experimental exposure of *Cryptosporidium* spp. oocysts to low pH in juices and other beverages has produced contradictory results regarding their survival [70]; [56], illness outbreaks in the U.S. have been associated with *Cryptosporidium* spp. in apple cider.

5.3.1 Potential Modes of Contamination

Contamination of fruit can occur anywhere in the growing, harvesting, cleaning and transportation chain from orchard to processor. Water used in orchards for diluting pesticides, irrigation, and washing apples represents a possible source of contamination. Fruits are raw agricultural commodities and can also be exposed to contamination from animals, birds, insects, and from domestic and agricultural waste. The harvesting and use of drop fruit can increase the risk of contamination. The contact surfaces of equipment used in harvesting, storage, and packing of the fruit may also be contaminated with rodent or animal manure. Other possible sources of contamination include workers harvesting and handling the fruit, and the conditions under which it is stored and shipped.

In addition, juice processing establishments may be sources of contamination. Pathogens introduced into a facility via contaminated fruit could persist if proper sanitation standards are not followed. Information on typical levels of *E. coli*, *Salmonella* spp., *Cryptosporidium* spp. and other pathogens on fruit destined for juice are lacking. Producers of unpasteurized juice should consider that any fruit entering a facility might carry pathogens. Other potential sources of juice contamination are water, insects, contaminated equipment, and poor worker hygiene practices.

5.3.2 Likelihood of Growth

The ability of *E. coli* O157:H7 or *Salmonella* spp. to survive on fruit surfaces during juicing and storage raises concerns about the way fruit and juice are handled. Contamination of the interior can occur through surface bruises, cuts or orifices. Because handling is unavoidable, the extent of microbial attachment will depend on the sanitation conditions in the manufacturing environment that reduce microbial build-up. However, the likelihood of actual growth upon intact fruit surfaces (peels) is minimal, provided procedures to process fruits after harvesting are immediate or storage conditions are adequate. For example, the decision to use drop-fruits (apples or oranges, for example) carry the risk of contamination by *Salmonella* spp., *E. coli* and other pathogens, directly from raw or improperly composted manure, contaminated irrigation water, soil or contact with animals and insects. Therefore, drop-fruits tend to be processed immediately, and before any surface bacterial growth can occur [71]; [72].

Damaged fruit surfaces have sometimes been shown to protect attached bacteria from washing and sanitizing operations. For example, the stem-scar area of 'Valencia' oranges can contain bacterial loads that can be difficult to remove. This is due to the roughness of this area where organisms could be shielded by entrapped air, debris and plant surface structures [73]. In addition, bruised and punctured surfaces of fruit can permit the entry and growth of bacteria. The blossom end of whole apples can allow the uptake of bacterial pathogens from wash waters into the outer core regions inside the fruit. However, research has shown that wash water is less likely to be drawn into the core area if the apple or orange is colder than the wash water [74]; [72].

There is little information available on the survival of *Cryptosporidium* spp. oocysts specifically on fruits. A recent study [75] found that *C. parvum* oocysts attached to apples can remain viable and possibly infectious during prolonged storage (i.e., 6 weeks of cold storage). It is generally considered that fruits such as berries with moist, irregular surfaces likely afford some protection to contaminating parasite cysts or oocysts from dessication. An important distinction to be made between *Cryptosporidium* spp. and bacterial pathogens is that the former does not grow outside the host, so no multiplication will take place on contaminated fruits regardless of the environmental conditions.

Acidic fruit juices were generally believed to be unusual vehicles of transmission for human pathogens. Pathogenic organisms survive rather than grow in such adverse pH conditions. For example, *E. coli* O157:H7 remains viable (without apparent proliferation) for extended periods in refrigerated apple cider (turbid, non-fermented apple juice containing pulp). The pH of apple juice is typically between 3.3 and 4.1 [76]. Research conducted at the University of Tennessee shows that bacteria can survive in apple cider for up to 15 days at pH 4.1 [77]. In fact, *E. coli* O157:H7 was shown to be very resistant to the low pH values of both apple cider and orange juice, when held at either 5 or 25°C. Growth of this pathogen actually occurred in one brand of apple cider with a pH of 3.98 [6]. Zhao *et al.*, (1993) [78] also found that *E. coli* O157:H7 can survive at 8°C for up to 31 days in apple cider (pH 3.1 to 3.7), with no apparent growth. Eleftheriadou *et al.* (1998) [79] reported that *S.* Typhimurium survived in apple juice of pH 3.6 for at least 30 days. *Salmonella* serotypes and *Cryptosporidium* spp. are also resistant to low pH [71]; [24]. Although the average pH level of Florida orange juices is 3.7 (range: 3.4 to 4.0), they have been implicated in *Salmonella* outbreaks. The unpasteurized orange juice that was implicated in the Florida theme park outbreak of 1995 was less acidic than expected - a mean pH of 4.3. The associated salmonellae pathogens were able to survive in detectable numbers up to 27 days at pH 3.5, 46 days at pH 3.8, 60 days at pH 4.1 and 73 days at pH 4.4 [19].

5.3.3 Volumes of Unpasteurized Juice and Cider on the Canadian Market

Over the past number of decades, juice consumption has increased in Canada to the point where in 1997, national figures exceeded 715 million liters [80]. Apples are Canada's number one fruit crop with about 409,000 tonnes grown in 2010 [81]. Based on information supplied through the Canadian Horticultural Council, it is estimated that 70 million liters of apple juice are produced annually in Canada. Based on information from the Canadian Food Inspection Agency (CFIA) and the Food Safety Network, about 4 million liters of unpasteurized juice and cider are sold annually, representing 5.2% of the total retail juice and cider in Canada [80]. The consumption of unpasteurized orange juice in the U.S. (where oranges are a major agricultural crop), is less than 1% of all orange juice [19]. Oranges are not an agricultural crop in Canada. Consumption data on unpasteurized orange juice in Canada is limited; however volumes of either unpasteurized apple juice/cider or orange juice on the Canadian market appear to be small, compared to pasteurized juice volumes. Unpasteurized juices and cider sold in grocery stores, health food stores, and cider mills in Canada are not required to be labeled as unpasteurized or to have a warning label. However, juice producers are encouraged to voluntary label their products as unpasteurized.

5.3.4 Testing of Unpasteurized Juice and Cider in Canada

Samples of unpasteurized juices have been analyzed for coliforms, generic *E. coli* and *E. coli* O157:H7 by the CFIA. CFIA surveys in the fall of 1996 and 1997, reported no detectable contamination with *E. coli* O157:H7 [80]. In a more recent CFIA survey from 2004 to 2009, 175 samples were tested; of those samples, 168 (96%) were classified as satisfactory, while 5 (3%) were unsatisfactory. Of the 5 unsatisfactory samples, 4 were positive for coliforms and 1 for visible mould. The remaining 2 samples gave inconclusive results [82].

5.3.5 Process Controls

Unpasteurized juice producers in Canada are encouraged to use the Code of Practice [83] to minimize the potential for contamination. The latter was developed by a Steering Committee consisting of Health Canada, the CFIA, provincial, industrial, and consumer representatives.

5.3.5.1 Treatment of Fruits

Harvesting and Receiving

Excluding the use of drop fruit would greatly minimize microbiological contamination of unpasteurized juice products, but not eliminate it. Animal grazing (wild and domestic), the use or presence of manure, using contaminated water for irrigation, as well as unclean storage and transport bins are some of the mechanisms through which drop fruit can become contaminated with pathogens [79]; [71]; [84]. There is presently no system in place that could guarantee the exclusion of the use of drop fruit either intentionally or accidentally, that is destined for processing into juice. Despite this, Walderhaug *et al.* (1999) [72] stated that microorganisms can enter fruit from the trees (including oranges and apples) through punctures, wounds and slits during growing and harvesting. Even insects, birds and dust can act as vectors for plant and human pathogens. Therefore, contamination is always possible and there can be no guarantee that only pathogen-free fruits will enter processing operations.

Sanitizing

There are many ways that incoming fruit can be sanitized. Presently, both physical and chemical treatments are being used by the fresh juice industry to reduce microbial populations on fruit surfaces before juice extraction. The following are examples: washing and brushing is frequently used in the apple juice/cider industry [71]; [85][; fruits may be treated with chemical sanitizers in a soak flume [4]; [71]; [73]; hot water immersion at 70°C for 2 min or 80°C for 1 min are applied to oranges [73]; [86]; and combination methods such as roller brush washers that spray water mixed with suitable sanitizers [79] are also used. Several chemicals have been tested for their effectiveness in reducing microbial populations on fresh fruits, including ozone, chlorine dioxide, chlorinated water sprays, and alkaline washing solutions [87]; [88]. It should be noted that *Cryptosporidium* spp. is resistant to most chemical disinfectants [70], and physical disinfection may be preferable [56].

Research focused upon oranges and apples has shown that these fruits can internalize pathogens through their stem or calyx ends during the sanitizing step. However, internalization can be greatly reduced when the immersion bath is warmer than the fruit (especially apples) [74]. Therefore at the present time, the possibility still exists for pathogens to continue onto the juicing stage, despite the wash-process used. The wash-process control cannot be relied upon to totally exclude pathogens.

5.3.5.2 Treatment of Unpasteurized Juices

Preservatives

Chemical preservatives can delay spoilage and can increase the microbiological safety and shelf-life of unpasteurized juices [89]. They tend to be added to freshly pressed juices in holding tanks. Class II preservatives like sodium benzoate and potassium sorbate, as listed in the Canadian Food and Drug Regulations, are allowed up to maximum concentrations of 0.1% (1,000 ppm) and can be found in unpasteurized juices that are sold at retail in Canada.

The effectiveness of sodium benzoate is limited against pathogens. It is most effective at acidic pH values of 2.5 to 4.0 [89], but is relatively ineffective near neutral pH. It is therefore most applicable to acidic juices. A study by Zhao et *al.*, [78] concluded that 0.1% sodium benzoate can reduce the level of *E. coli* O157:H7 in apple cider while suppressing the growth of yeasts and moulds. They found that benzoate was an antimicrobial agent to *E. coli*

O157:H7 at 8°C. It caused a decimal reduction that was greater than 4 $\log_{10}$ CFU/ml of the pathogen within 7 to 10 days in apple cider. Yeasts and mould growth was suppressed at this temperature. The literature reports that sorbic acid could be combined with sulphur dioxide to prevent microbial growth, oxidation and enzymatic spoilage of fruit juices [90]. Taking into account that part of the population is sensitive to sulphites, Health Canada requires that sulfites must be declared on the label when present in prepackaged foods in a total amount of 10 parts per million or more.

Potassium sorbate can also be used as a direct additive in fruit juices, especially in fresh apple juice and cider in Canada. It can inhibit yeasts and moulds, but is less effective against bacteria. The sorbates are most effective at low to medium pH levels with a maximal level of use at about pH 6.5. The study by [78] showed that 0.1% potassium sorbate had minimal effect on *E. coli* O157:H7 populations in apple cider (pH 3.1 to 3.7). Survivors were detected, along with mould growth, for 15 to 20 days at 8°C or 1 to 3 days at 25°C. This was unacceptable and had to be used in combination with sodium benzoate to have any significant antimicrobial effects against the pathogen at this acidic pH. In general, potassium sorbate is combined with small quantities of sulphur dioxide to protect fruit juices against oxidation, enzymatic and bacterial spoilage, particularly lactic acid and acetic acid fermentation [90].

Other Treatments

Unpasteurized orange juices are available both fresh refrigerated and frozen. In the fresh-squeezed orange juice industry, the lower the storage temperature, the more extended the shelf-life of the fresh-squeezed juice [79]. Delayed spoilage occurs because the process is able to slow bacterial growth rates, inactivate some viable pathogens, and slow chemical or enzymatic reaction rates. However, the initial quality of the juice to be frozen is of prime importance, since freezing juice cannot alter or mask pre-existing effects of bad fruit quality and/or unhygienic practices employed during production. Negligible loss of flavour or ascorbic acid occurs with unpasteurized orange juice after freezing, and frozen orange juice has an indefinite shelf-life [79]. However, since some pathogens can survive freezing, this technology cannot be fully relied upon as a process control.

A study was performed by Ulias and Ingham (1999) [76] on unpasteurized apple cider with a combination of inactivation treatments. It was shown that a 5-$\log_{10}$ CFU/ml reduction in *E. coli* O157:H7 can be achieved by a combination of freeze-thawing of cider, combined with warming for 6 hours at 35°C. This potential control process is still at a developmental stage and supporting data will have to be submitted prior to the consideration of this process as an effective control against pathogens of concern. More recently, Ingham *et al*. (2006) [91] demonstrated that the addition of cranberry juice at 15% to unpasteurized apple cider, followed by warm holding (45°C for 2 h) and freeze-thaw steps (-20°C for 24 h, 5°C for 24 h) achieved a ≥5-$\log_{10}$ CFU/ml reduction in numbers of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. A few studies have reported that *C. parvum* oocysts can survive for considerable periods of time when suspended in water which has been frozen at -20°C or warmer [92].

Duan and Zhao (2009) [93] showed that essential oil of lemongrass or cinnamon leaf at 0.2 to 0.3 μL/mL and freeze-thaw treatment alone could cause 5-$\log_{10}$ CFU/mL reductions in the population of *S*. Enteritidis in strawberry juice. However, combined essential oil and freeze-thaw treatment was necessary to obtain the same reduction in the population of *E. coli* O157 in the juice.

In addition, in a study by Raybaudi-Massilia *et al.* (2006) [94], it was shown that a concentration of 2 μL/mL of lemongrass, cinnamon, or geraniol was enough to inactivate *S.* Enteritidis, *E. coli*, and *L. innocua* in apple and pear juices. However, in melon juice, concentrations of 8, 6, and 5 μL/mL of cinnamon, geraniol, and lemongrass were necessary to inactivate the three microorganisms. *E. coli* O157:H7 was reduced by 5-$\log_{10}$ CFU/ mL after 7 days at 4 or 15°C in apple juice supplemented with 10 mM vanillic acid [95]. However, the sensory character of the juice was significantly altered.

The use of ultra-violet light (UV) on raw juices as they are pumped into storage tanks is an emerging non-thermal treatment for pathogen control. There are presently companies in the United States that claim effectiveness of UV light of up to 5-$\log_{10}$ CFU/mL reductions in bacteria using this technology. However, a study with apple cider found that UV light treatment may not be as effective in reducing bacterial numbers (including pathogenic organisms like *E. coli*) [71]. This can be due to very little transmission of the UV light through the cider because of the unit's design and/or the optical density of the cider. A 6-log inactivation of *C. parvum* oocysts was achieved in fresh apple cider exposed to UV light at 14.32 mJ/cm^2 [96].

A number of other treatments are also presently being studied, but cannot be considered as process controls unless Health Canada is provided with substantial supporting data. Thus, manufacturers, packers or importers of unpasteurized fruit juice and cider, who use or incorporate non-thermal process to meet the 5-$\log_{10}$ reduction performance standard, are recommended to consult with Health Canada to determine if a novel food pre-market notification may be required according to the *Food and Drug Regulations*. Research is being done on the following, among others: (a) 'e-beam' technology. It is intended that this technology will be tested against such organisms as *E. coli, Salmonella* and *Cryptosporidium* and will not be limited to one type of juice [97]. It was shown to successfully reduce the levels of generic *E. coli* in juice. (b) High voltage pulsed electric field (PEF) treatment. This appears to be lethal against microorganisms including *E. coli* and *E. coli* O157:H7 in apple juice. Average treatment temperatures are kept at about 25°C and there appear to be no thermal effects that compromise flavor or nutritive value [98]; [99] showed that *E. coli* and *S.* Enteritidis populations in melon and watermelon juices were more sensitive to PEF treatment than *L. monocytogenes*. (c) High Pressure Processing (HPP) has been found to successfully inactivate spoilage microorganisms such as yeasts, their ascospores, moulds and most bacteria typically associated with citrus and apple juices [100]; [101]. A pressure-resistant strain of *E. coli* O157:H7 was found to be more susceptible under acidic conditions after HPP, using orange juice [102]. HPP was also found to inactivate *C. parvum* oocysts suspended in apple juice and orange juice by at least 3.4 $\log_{10}$ after 30 s of treatment [103]. No infectivity was detected in samples exposed to ≥ 60 s of treatment.

Kniel *et al.* (2003) [104] reported that the addition of 0.025% hydrogen peroxide to apple cider, orange juice, and grape juice resulted in a > 5-log reduction of *C. parvum* infectivity. These authors also found that the addition of malic, citric, and tartaric acids to juices and cider inhibited the infectivity of this parasite.

A 5-$\log_{10}$ CFU/ mL reduction was achieved for *E. coli* O157:H7 and *L. monocytogenes* in apple cider after washing fruit with copper ion water and sodium hypochlorite, followed by juicing and sonication at 44 to 48 kHz [105].

5.3.6 Conclusions of the Exposure Assessment

Fruit can become contaminated with pathogens during growth, harvesting, transportation and storage as well as during processing, packaging and distributing. If this occurs, the pathogens of concern can survive in the acidic juice for various times depending upon juice pH and temperature. If contaminated fruits are used, there is a greater chance that the final product will be contaminated with pathogens such as *E. coli* O157:H7 and *Salmonella* spp. Juicing and further processing methods that are presently practiced, do not guarantee the absence of pathogens, should the raw juices be contaminated. The probability in Canada, of unpasteurized juices becoming contaminated with the pathogens identified, is unknown. The level of contamination that might be expected is also unknown, but appears to be low based on current data from sample testing programs (section 5.3.4), and the low incidence of outbreaks from epidemiological data (Table 1). The likelihood of consumption of contaminated unpasteurized juice or cider produced in Canada is also considered to be low based on available data.

5.4 Risk Characterization

5.4.1 Introduction

This risk characterization combines the information from previous sections to determine the risk of illness from consumption of unpasteurized apple juice or cider and orange juice.

5.4.2 Illness

The relative exposure of the general population to unpasteurized juice and cider in Canada is considered low, since the majority of juices available on the market are pasteurized. Despite this, the severity of illness associated with unpasteurized juice outbreaks has been high, resulting in hospitalization and death in susceptible groups. The dose response of the pathogens of concern is unknown in unpasteurized apple and orange juices. Infections that result are generally self-limiting in healthy adults but young children, the elderly and individuals with weakened immune systems are at risk of developing serious complications should they become ill.

5.4.3 Recent Canadian Outbreaks

Of the three Canadian juice-related outbreaks recorded in the 1990's, two involved imported unpasteurized apple juice (1996) and imported unpasteurized orange juice (1999), respectively. The other outbreak (1998) involved locally produced apple cider by a custom presser. In 2005, an outbreak was linked to unpasteurized apple cider produced and sold at a small local retail outlet (Table 1).

5.4.4 Testing Methodology

The methods for the detection of *E. coli* O157:H7, *Salmonella* spp. and other bacterial pathogens in apple juice/cider or other unpasteurized juices are well established. They have proven to be very effective in linking case illnesses from outbreaks to unpasteurized juices. With some outbreaks, additional sub-typing laboratory procedures (pulsed-field gel electrophoresis) that compared DNA isolated from juice products and the related human case isolates, resulted in confirmation of the source of the outbreak. There are currently no standard methods available for the detection of *Cryptosporidium* spp. oocysts in foods, including fruit juices or cider, although the parasite has been detected in apple cider following outbreaks using both microscopy and polymerase chain reaction (PCR). Using a combination of sucrose flotation to concentrate oocysts, and direct immunofluorescence assay for detection, Deng and Cliver (2000) [106] were able to detect as few as 100 *C. parvum* oocysts inoculated into 100 ml of apple juice. The use of immunomagnetic capture further increased the sensitivity of this assay.

5.4.5 Discussion and Conclusions

Unpasteurized juice and cider presents a unique food safety problem because of the following:

1. Raw produce (e.g., apples, oranges, etc.) can be contaminated with microbial pathogens.

   Apples and oranges can become contaminated: i) by coming into contact with soil, ii) from manure of infected animals on orchards, iii) if transported or stored in areas that contain manure, and iv) if rinsed with contaminated water. The use of drop apples to make apple cider may still be practiced, and oranges may be harvested after being dropped to the ground, but this practice has been greatly diminished. The CFIA Code of Practice states that drop fruits should not be used in the production of unpasteurized juices. The Code has been implemented by larger producers, while small producers are often less aware of the risks. Bacterial internalization is very common in dropped or damaged apples and oranges, which further imply that dropped or damaged fruit should not be used for the production of unpasteurized juices[84]; [107]. Contamination could continue into the processing facilities where small animals and insects could enter poorly sealed processing facilities and contaminate processing equipment [19]. Washing procedures have been shown to cause some fruits to internalize pathogens, thereby contributing to the problem at the juicing stage. However, adherence to good manufacturing practices, including hand washing, cleaning and sanitizing equipment can help to reduce the risk of contamination.

2. Microbial pathogens can survive the food processing treatment.

   Juicing facilities involve pressing of the fruit that may potentially contaminate juice contact-surfaces and equipment after processing of batches. When contaminated fruit is used to manufacture juice or cider, the prevailing conditions during processing can allow survival of microorganisms including *E. coli* O157:H7, *Salmonella* spp., and *Cryptosporidium* spp. For the unpasteurized fresh-pressed market, absence of thermal treatment would allow the potential for the survival of pathogens if present. Although fruits and pressed juices may be refrigerated, most pathogens of concern are able to survive.

3. Foodborne pathogens can survive in the product.

   The acidic nature of unpasteurized fruit juices is not lethal for *E. coli* O157:H7, *Salmonella* spp. or *Cryptosporidium* spp. The absence of an effective treatment such as heat means that spoilage bacteria and pathogens will continue to survive for extended periods in low pH refrigerated fruit juices. If preservatives are added to apple cider and juice containing *E. coli* O157:H7, they may not inactivate all the cells that are present.

4. Unpasteurized juice is consumed without a treatment to destroy pathogenic microrganisms.

   Unpasteurized juice and cider is marketed as mainly fresh-pressed or extracted. Consumers traditionally consume these juices without any treatment, since they are viewed as more natural and nutritious beverages. The juices are popular among the health conscious, and orange, apple juice and cider can be obtained year-round in groceries or at foodservice establishments. Therefore, if juices are contaminated and consumed, the probability of illness exists, especially in young children, the elderly and the immunocompromised.

The probability in Canada of unpasteurized juices or cider becoming contaminated with the identified pathogens of concern is unknown. It is recognized that good agricultural and manufacturing practices reduce the possibility that the juice or cider will be contaminated. Currently other technologies (e.g., UV, PEF, HPP - refer to section 5.3.5.2) are being explored as means to provide safe juices to consumers.

The volumes of either unpasteurized apple juice/cider or orange juice currently on the Canadian market are small as compared to pasteurized juice volumes. Though the relative exposure of the Canadian population to unpasteurized juice and cider is low, unpasteurized juices tend to be viewed as fresh, healthy and nutritious, and can be served to young children, the elderly and general consumers. In addition, since unpasteurized juice and cider sold in Canada are not required to be labeled as unpasteurized or to have a warning label, to the general consumer they are often indistinguishable from pasteurized products. Epidemiological data shows that if illness does occur due to consumption of contaminated unpasteurized juices or cider, it can be severe. The percentage of the Canadian population that consumes these products is considered to be low and the incidence of illness as a result of consumption is unknown. The level of contamination that might be expected in unpasteurized juice and cider is also unknown, but appears to be low based on current data from sample testing programs (section 5.3.4) and the low number of outbreaks from epidemiological data (Table 1). Resulting infections may be self-limiting in healthy adults. Young children, the elderly and individuals with weakened immune systems are considered to be at greatest risk of developing serious complications should they become ill.

5.4.6 Data Gaps and Research Needs

The risk assessment has been used to identify data gaps and research needs that have importance in terms of public health impact. There are many data gaps that limit the conclusions of the hazard characterization.
The following has been identified:

a) Development of new/improved disinfection and sanitizing methods to reduce or eliminate pathogenic microorganisms from fruits before harvest.
b) Levels of pathogens and pathogen survival on fruits destined for fresh juice or cider.
c) Prevalence of contamination of unpasteurized fruit juices at the retail level in Canada.
d) Unpasteurized juice and cider consumption data in Canada and worldwide.

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