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Microbiological analysis of frozen profiteroles and mini chocolate eclairs implicated in a national salmonellosis outbreak

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ABSTRACT

Between November 2018 and May 2019, Canada experienced a nationwide salmonellosis outbreak linked to the presence of *Salmonella enterica* ser. Enteritidis in frozen profiteroles. Analysis of the implicated food products revealed low levels of *Salmonella* ranging from 0.2 to 0.7 MPN/100g. Water activity and pH of the food samples ranged from 0.9479 to 0.9867 and 4.6–6.8 respectively indicating conditions conducive to bacterial growth. Higher levels of the hygiene indicators *Enterobacteriaceae* and coliforms were associated with *Salmonella* positive samples compared to *Salmonella* negative samples. Investigation of the relationship between storage conditions, temperature, and pathogen levels during thawing revealed that the profiteroles reached temperatures permissive to pathogen growth (≥5 °C) much sooner than pathogen growth was observed and that the composition of the food matrix can influence bacterial levels upon thawing. Collectively these data can be used to inform guidance to minimize the risk of infection from the consumption of contaminated cream-filled frozen desserts.

1. Introduction

Between November 2018 and May 2019, Canada experienced a nationwide salmonellosis outbreak linked to the presence of *Salmonella enterica* ser. Enteritidis in frozen profiteroles. In total, 85 illnesses and 22 hospitalizations were reported from seven provinces. Three deaths were reported but it was not determined whether the *Salmonella* infections contributed to those deaths. Many of the affected individuals reported consuming frozen profiteroles or mini chocolate eclairs in the days preceding their illnesses (Government of Canada, 2019). This is the first recorded instance of frozen profiteroles implicated in a salmonellosis outbreak in Canada.

Profiteroles and eclairs are cream-filled pastries. The basic pastry is made from a high hydration dough consisting of flour, water, eggs and a fat such as butter. The filling can be made from a variety of ingredients that include cream, eggs, and sugar. Other flavourings such as fruit puree, chocolate, and spices may also be added. *Salmonella* can be introduced into the product through any one of these raw ingredients (Smith et al., 2004). Pathogens present in the pastry dough are likely to be inactivated during the baking process, leaving the cream filling as the source of potential infectious agents. The high water activity of the pastry combined with the available nutrients in the filling provide adequate conditions for bacteria to survive and replicate (Bryan, 1976). Analysis of frozen cream puffs that were implicated in a salmonellosis outbreak in Japan in 1998 revealed *Salmonella* levels ranging between 4 and 5 log CFU/g (Hara-Kudo and Takatori, 2011). Since these products are ready to eat upon thawing, there are no opportunities for consumers to reduce bacterial numbers via a lethal process prior to consumption.

The manufacturers of the profiteroles and eclairs implicated in the 2018/2019 Canadian salmonellosis outbreak recommended thawing the product either at room temperature for 30 min or in the refrigerator for 90 min prior to serving. There is no reported data on bacterial behavior during the thawing of non-meat food commodities. Studies examining thawed meats indicate that the time-temperature recommendations given by the manufacturer are adequate to prevent the growth of *Salmonella* during thawing (Marriott et al., 1980; Roberts, 1972). Federal guidelines on the defrosting and storage of foods are aimed at keeping foods out of the 4 °C (40 °F) to 60 °C (140 °F) temperature range in order to prevent bacterial growth. Much of the guidance on safe thawing practices is aimed at meat and meat products that require cooking or reheating after being thawed. Health Canada recommends storing foods at room temperature for no more than 2 h (Government of Canada, 2017). Explicit guidance on the thawing of frozen foods that do not require reheating (such as profiteroles and other desserts) is limited and represents a potential gap in food safety messaging to the public.

The purpose of this investigation was to examine the lots of frozen

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profiteroles and eclairs that were implicated in the 2018/2019 Canadian salmonellosis outbreak for the presence and levels of *Salmonella*. To gain insight on the factors that may have contributed to the survival of *Salmonella* in these products, the water activity, pH, and background microbiota levels were determined. The impact of various thawing procedures on the levels of *Salmonella* in frozen profiteroles was examined in order to assess the safety of the recommended thawing instructions provided on the product packaging and to inform future guidance on the safe thawing of frozen desserts.

2. Materials and methods

2.1. Food samples and storage conditions

Between April and May of 2019, the Canadian Food Inspection Agency shipped samples of recalled frozen profiteroles and mini chocolate eclairs to Health Canada for analysis. Samples were shipped in Styrofoam boxes on ice packs. All samples were at 4 °C or below upon receipt (Table 1). Eight samples were received in their sealed, retail packaging with each sample consisting of 3–10 retail packs sharing the same best before date. One sample consisted of a single retail pack that had been opened previously. In all cases, the retail pack was an opaque, white plastic container with a white snap-on lid. The retail pack ranged from 325 to 645 g and contained 26–48 pieces per pack. Five samples were received as sub-samples and were packed in heavy duty plastic sampling bags. The temperatures of all samples were recorded immediately upon receipt (Table 1). The average mass of individual pastry pieces was 12 g (classic profiteroles), 13 g (flavoured profiteroles), and 14 g (mini chocolate eclair).

Samples were stored in their original packaging in Styrofoam boxes in a −20 °C walk-in freezer until analysis. Unless stated otherwise, samples were analyzed immediately upon removal from the freezer. Experiments conducted under thawing conditions were performed by removing the required amount of profiterole from the freezer, placing them in sterile sampling bags and storing in one of the following conditions: 4 °C for 2 h and 24 h, 10 °C for 6 h and 24 h, and room temperature (RT, 22 °C ± 1 °C) for 30 min–24 h.

Table 1
Sample information and *Salmonella* detection results of frozen profiterole and mini-chocolate eclair samples implicated in a foodborne salmonellosis outbreak.

Sample	Product Type	Packaging	Unit size (g)	Analytical unit (g)	*Salmonella* detected
1	Egg nog profiteroles	Retail, open	1 × 375	5 × 45	Y
2	Egg nog profiteroles	Retail, closed	7 × 375	5 × 100	Y
3	Mini chocolate eclairs	Retail, closed	10 × 365	4 × 74	N
4	Classic profiteroles	Retail, closed	10 × 325	2 × 188	N
5	Tropical fruit profiteroles	Retail, closed	3 × 645	4 × 188	N
6	Mini chocolate eclairs	Retail, closed	8 × 365	2 × 188	N
7	Classic profiteroles	Retail, closed	8 × 325	4 × 74	N
8	Classic profiteroles	Retail, closed	8 × 325	2 × 188	N
9	Classic profiteroles	Retail, closed	1 × 325	1 × 225	N
10	Coconut profiteroles	Sub-sampled	approx. 1 kg	2 × 188	Y
11	Passionfruit profiteroles	Sub-sampled	approx. 1 kg	2 × 188	N
12	Mango profiteroles	Sub-sampled	approx. 1 kg	2 × 188	N
13	Pineapple profiteroles	Sub-sampled	approx. 1 kg	2 × 188	N

[a] This sample was excluded from further analysis due to the elevated temperature upon receipt.

2.2. Physicochemical characterization of food samples

Water activity (a_w) was determined using an AquaLab Series 4 TE a_w meter (Decagon Devices Inc., Pullman, WA) using between 2 and 4 g sample (Health Canada, 2020). For each food type, duplicate measurements were conducted on two independent samples for a sample size of four. pH determinations were carried out in duplicate using the procedures described in MFHPB-03 (Health Canada, 2014) using a pH/ORP combination titration electrode (Orion 8192BNUWP ROSS Ultra pH electrode, Thermo Scientific, Ottawa, ON) and 5–7 g food sample in 2 mL double distilled water. For both sets of measurements, food samples were removed from storage at −20 °C and allowed to thaw at room temperature for 30 min prior to analysis. For measurements of the cream alone, the cream was removed from the frozen puff using a sterile knife and spoon. For measurements of the cream and crust together, a cross section of the frozen puff was obtained using a sterile knife. Reported values are the average ± standard deviation of each independent measurement.

2.3. Enumeration of profiterole microbiota

Background microbiota were enumerated by standard plate count methodology using 25 g portions from each sample and buffered peptone water (BPW) as the diluent as previously described (Tamber et al., 2016). Total aerobes (TA) and psychrophiles (PSY) were recovered from plate count agar (PCA) after respective incubation at 35 °C for 48 h and 18 °C for 5 d. *Enterobacteriaceae* (EB) and total coliforms (CF) were enumerated on 3M Petrifilm *Enterobacteriaceae* Count and Coliform Count plates respectively (Innovation Diagnostics, Blainville, QC). Bacterial counts were determined for each sample in duplicate. Reported results are the combined average ± standard deviation for each product type.

2.4. Salmonella detection and MPN analyses

With the exception of the egg nog profiteroles, all samples were initially tested for the presence of *Salmonella* as described in MFHPB-20 (Health Canada, 2009b). Based on the amount of sample available for analysis, each was divided into analytical units as indicated in Table 1. Buffered peptone water (BPW) was used as the preenrichment broth for all samples. A sub-set of cream-filled profiteroles was preenriched in Brilliant Green water (BGW), and a sub-set of mini-chocolate eclairs was preenriched in skim milk media (SMM) to see if BGW and SMM differed in performance compared to BPW. Secondary enrichment was carried out in Rappaport-Vassiliadis soya and tetrathionate brilliant green broths. Xylose lysine deoxycholate (XLD) and Brilliant Green Sulfa agars were used for selective plating. The identities of presumptive *Salmonella* colonies were confirmed through biochemical (API20E, bioMérieux, St. Laurent, QC), serological (FactorD, Becton Dickinson, Mississauga, ON), and molecular methods (Rahn et al., 1992).

The egg nog profiteroles and other samples testing positive for *Salmonella* were analyzed by a three-dilution, five-tube MPN. Subsamples for MPN analysis were divided into five portions of 50 g each (open egg nog profiteroles) or 100 g (closed egg nog profiteroles, coconut profiteroles). Each portion was placed in a sterile sampling bag to which nine volumes of BPW were added. The profiteroles were pummelled in a stomacher at high power for 2 min (Seward Laboratory Systems, Bohemia, NY). One tenth of the mass was removed from each bag (first dilution) and placed into a second set of bags (second dilution). The third dilution series was prepared by removing one tenth of the mass from the second dilution into fresh sampling bags (Fig. 1). Each dilution series was then subject to secondary enrichment and selective plating as

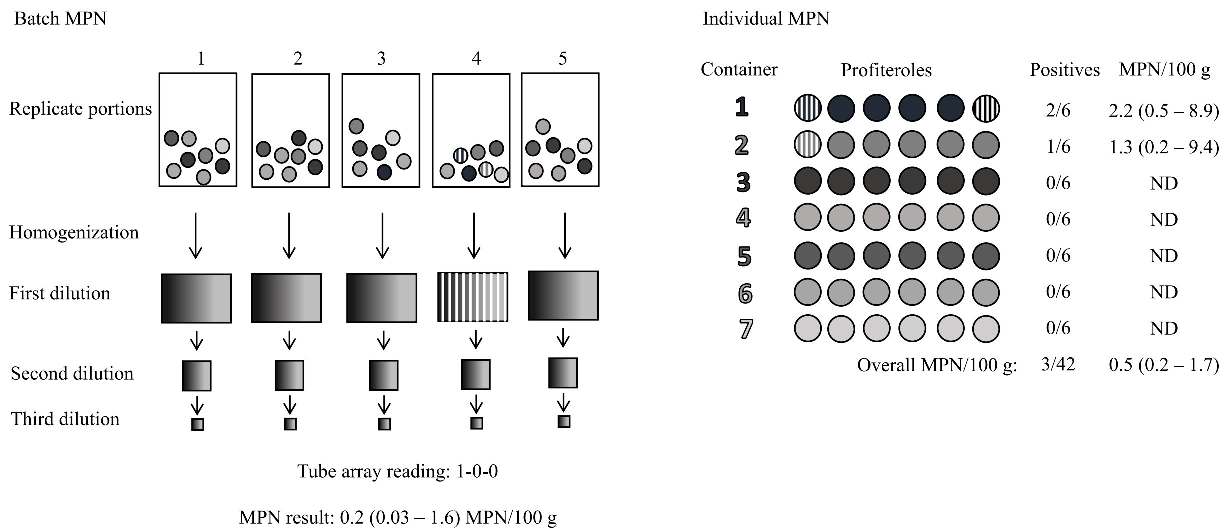

Fig. 1. Batch and individual MPN for the estimation of *Salmonella* levels and distribution pattern in egg nog profiteroles implicated in a salmonellosis outbreak. Two approaches for sample portioning were compared. The food sample consisted of seven closed retail containers. For the batch MPN (left), five replicates were prepared using an equivalent number of profiteroles from each retail container. After homogenization, these replicates were used to create a three dilution, five tube array. Using this method of sample portioning, a tube array reading of 1-0-0 was obtained resulting in an MPN of 0.2 (0.03–1.6) MPN/100 g for the sample. For the individual MPN (right), six profiteroles from each container were analyzed individually for the presence of *Salmonella*. Using this approach, the presence of *Salmonella* was detected in two containers. MPN values for each container are indicated and the overall MPN for the entire sample was 0.5 (0.2–1.7) MPN/100 g. MPN values are given with their lower and upper 95% confidence limits. Vertical lines indicate the analytical units that were positive for *Salmonella*. ND = not detected.

described above. Reported tube patterns of the MPN analyses are the number of tubes at each dilution testing positive for the presence of *Salmonella*.

The sample of closed egg nog profiteroles consisted of multiple retail packs and an equal number of profiteroles was taken from each container to form the first dilution series of the MPN (batch MPN, as described above). To gain a better understanding of how *Salmonella* was distributed within this sample, a second MPN was performed in which six individual profiteroles from each of the seven containers were tested individually for the presence of *Salmonella* (individual MPN, Fig. 1). Most probable numbers for all MPN tube arrays were calculated as described by the US Food and Drug Administration (Garthright and Blodgett, 2003).

2.5. Temperature profiling

Individual classic profiteroles were removed from storage at −20 °C, placed into separate sterile sampling bags and thawed in a 4 °C fridge, 10 °C incubator, or at room temperature (RT) for the specified times. The surface temperatures of the profiteroles were determined by placing an infrared thermometer (InfraPro3, Oakton, Vernon Hills, IL) perpendicular to each profiterole and measuring from a distance of approximately 15 cm away. Interior temperature was measured by placing a digital instant read probe thermometer (ThermoPro, Toronto, ON) into the geometric center of each profiterole. Both thermometers were verified prior to use using a laboratory grade thermometer (Traceable, VWR, Mississauga, ON) that is calibrated every two years. Results reported are the average ± standard deviation of three (no thaw, RT 24 h) and six (all other time-temperature conditions) measurements.

2.6. Thawing experiments

To investigate growth dynamics during thawing, three diverse *S. enterica* ser. Enteritidis strains were chosen to artificially inoculate classic profiteroles. The strains were from the *Salmonella* culture collection housed at Health Canada and consisted of strains 1353 (isolated from frogs legs in 1996, ST74), 1185 (isolated from ground chicken, date unknown, ST11, H_2S^-) and 2019001 (isolated from egg nog profiteroles, this study).

The levels of the three strains in profiteroles were assessed individually as follows. Strains were cultured on tryptic soy agar (TSA) from storage at −80 °C and grown overnight in tryptic soy broth at 37 °C with shaking at 250 RPM for 24 h. A 10^{-4} dilution of each culture in saline resulted in a strain suspension containing 10^5 CFU/mL. One hundred microliters of one suspension was injected into the center of the required number of profiteroles. The inoculated profiteroles were placed in separate sterile sampling bags, re-frozen and stored at −20 °C to allow for acclimatization of the *Salmonella* cells. After four days, profiteroles were removed from frozen storage and thawed at either 4 °C, 10 °C, or room temperature. At the specified times, the level of *Salmonella* in each profiterole was determined by standard plate count using BPW as the diluent and XLD-TSA thin layer agar plates as the plating media. Plates were incubated at 35 °C for 24 and 48 h prior to counting. Experiments were conducted over three trials with three independent cultures for each strain type. Reported data is the aggregate of the three trials using data from all three strains (median ± standard deviation, with an n of at least 9 for each data point). Due to a limited quantity of remaining sample, experiments investigating *Salmonella* levels in thawed flavoured profiteroles were only inoculated with one strain (2019001) and conducted twice with an n of either 6 (classic and passionfruit) or 4 (egg nog).

2.7. Statistical analysis

Differences between pairs of means were compared using the Student's *t*-test as indicated in the text at a significance level of 0.05. Statistical calculations were done using the Data Analysis Tool pack available in Microsoft Excel (2016).

3. Results and discussion

3.1. Detection and enumeration of Salmonella in frozen cream filled pastries

Health Canada's reference method for the detection of *Salmonella* in foods (MFHPB-20) recommends different preenrichment media for different foods. BPW is recommended for most foods, SMM for chocolate and confectionery products, and BGW for many dairy-based foods. Therefore, initial testing sought to determine whether the choice of preenrichment media (BPW vs BGW for profiteroles, BPW vs SMM for mini-chocolate eclairs) could have potential impacts on the detection of *Salmonella* in these food products. No differences with respect to *Salmonella* presence/absence and the types of bacteria recovered on the selective agars were noted with any sample (data not shown).

Three samples tested positive for the presence of *Salmonella*, coconut profiteroles and both samples of egg nog profiteroles (Table 1). MPN analysis of the three samples revealed low numbers of *Salmonella* that ranged from 0.2 MPN/100 g to 0.7 MPN/100 g (Table 2). These concentrations are equivalent to 1 MPN per 143–500 g product or 11 to 38 profiteroles. The values reported here are in agreement with published values of *Salmonella* and other foodborne bacterial pathogens in naturally contaminated foods (Catford et al., 2017; Gill et al., 2019). Analysis of foods implicated in previous salmonellosis outbreaks indicate that levels as low as these can be associated with foodborne illness (Greenwood and Hooper, 1983; Lehmacher et al., 1995; Vought and Tatini, 1998).

To gain more information regarding the distribution of *Salmonella* in the profiteroles, individual eggnog profiteroles from the closed sample were analyzed for the presence of the pathogen. Three out of 42 profiteroles tested positive resulting in an overall *Salmonella* concentration of 0.5 MPN/100g (Fig. 1). This value is similar to the one obtained for this sample using the batch MPN, suggesting that *Salmonella* were distributed heterogeneously in the contaminated production lot, with a few profiteroles contaminated with a small number of *Salmonella* cells. The three positive profiteroles originated from two containers, #1 (two positives) and #2 (one positive) resulting in respective *Salmonella* concentrations of 2.2 MPN/100 g and 1.3 MPN/100 g in each container. This distribution supports our hypothesis of heterogeneous contamination within each production batch or lot. Despite the differences in *Salmonella* concentration within the individual containers, the levels of background microbiota (aerobic colonies, psychrophiles, *Enterobacteriaceae*, coliforms) did not vary (Fig. 1).

3.2. Water activity, pH, and bacterial levels associated with frozen profiteroles and eclairs

Table 3 lists the a_w and pH of the profiterole and eclair samples. At 0.99, the a_w of the egg nog profiteroles was significantly higher than any other pastry type, followed by the coconut, mango, and pineapple flavoured profiteroles (a_w = 0.97). The mini chocolate eclairs, passionfruit, and classic profiteroles had the lowest a_w values of 0.95. These a_w values are permissive for the growth of *Salmonella*, with the expectation that the product types with higher a_w would support the higher growth rates of *Salmonella* (Baylis et al., 2011). It was not possible to obtain accurate measurements of the a_w at the interface of the pastry crust and cream; however measurements of the cream and crust alone did not differ from the values obtained with the pastry cross sections (data not shown).

In general, the pH of the cream filling was lower than that of the whole pastry, but the differences were not significant. The pH of the cream ranged from 3.8 to 6.7 and 4.6–6.8 for the cream and crust together. When analyzed as an aggregate, the pH of the fruit flavoured profiteroles (median pH 4.6 (cream) and 5.4 (whole cross section)) was significantly lower than that of the non-fruit flavoured pastries (median pH 6.6 (cream and whole cross section)). These reported pH values are all able to support the growth of *Salmonella* with the pH values of the non-fruit flavoured pastries (egg nog, classic, eclair) closer to the ideal pH for growth (Baylis et al., 2011).

Table 4 lists the bacterial counts associated with the profiterole and eclair samples. Both the total aerobe and psychrophile counts ranged from 3.9 to 5.0 log CFU/g. The *Enterobacteriaceae* and total coliform

Table 2
Salmonella levels in frozen profiteroles implicated in a foodborne salmonellosis outbreak as determined by batch MPN analysis.

Product Type	Tube pattern	MPN/100g	LCL[a]	UCL[b]
Egg nog profiteroles (open container)	1-0-0	0.5	0.1	3.4
Egg nog profiteroles (closed containers)	1-0-0	0.2	0.03	1.6
Coconut profiteroles	2-0-1	0.7	0.2	2.4

[a] Lower 95% confidence limit.
[b] Upper 95% confidence limit.

Table 3
Water activity and pH of frozen profiteroles and mini chocolate eclairs implicated in a foodborne salmonellosis outbreak.

Product Type	aw (whole)	pH (whole)	pH (cream)
Classic profiteroles	0.9479 ± 0.001	6.8	6.7 ± 0.1
Mini chocolate eclairs	0.9498 ± 0.006	6.6	6.4
Egg nog profiteroles[a]	0.9867 ± 0.005 [b]	6.6	6.5
Coconut profiteroles	0.9691 ± 0.003 [c]	6.2[d]	5.5 ± 0.2 [d]
Mango profiteroles	0.9656 ± 0.001	5.6 ± 0.1 [d]	4.6 ± 0.1 [d]
Passionfruit profiteroles	0.9487 ± 0.001	4.6 [d]	3.9 ± 0.1 [d]
Pineapple profiteroles	0.9669 ± 0.005	5.2 ± 0.1 [d]	4.5 ± 0.1 [d]

[a] Values determined using profiteroles from closed container.
[b] Significantly higher than all other product types tested.
[c] Significantly higher than classic, eclair, and passionfruit profiteroles; significantly lower than egg nog profiteroles.
[d] Significantly lower than the non-fruit flavoured pastries.

Table 4
Enumeration of background microbiota in frozen profiteroles and mini chocolate eclairs implicated in a foodborne salmonellosis outbreak (log CFU/g).

Product Type	Total aerobes	Psychrophiles	Enterobacteriaceae	Total coliforms
Egg nog profiteroles (open container)[a]	5.0	4.2	4.4 [b]	4.2 [b]
Egg nog profiteroles (closed containers)[a]	5.0 ± 0.3	5.0 ± 0.3	3.5 ± 0.2	3.2 ± 0.4
Mini chocolate eclairs	4.9 ± 0.2	4.9 ± 0.2	2.8 ± 0.2	2.8 ± 0.2
Classic profiteroles	3.9 ± 0.4	3.9 ± 0.5	2.7 ± 0.5	2.5 ± 0.6
Tropical fruit profiteroles	4.3 ± 0.2	4.2 ± 0.1	2.5 ± 0.5	2.5 ± 0.2
Coconut profiteroles[a]	4.4	4.7 ± 0.1	3.2	3.2 ± 0.1
Passionfruit profiteroles	4.4	4.6 ± 0.3	2.5 ± 0.1	2.3 ± 0.3
Mango profiteroles	4.1	4.3	2.0	2.2
Pineapple profiteroles	4.1	4.1 ± 0.1	2.0	2.0 ± 0.1
Salmonella positive	4.9 ± 0.3[c,d]	5.0 ± 0.2 [c,d]	3.7 ± 0.5 [c,d]	3.5 ± 0.6 [c,d]
Salmonella negative, all pH	4.3 ± 0.4	4.3 ± 0.5	2.6 ± 0.4	2.5 ± 0.4
Salmonella negative, pH > 6	4.3 ± 0.6	4.3 ± 0.6	2.8 ± 0.4	2.6 ± 0.4

[a] *Salmonella* positive sample.
[b] Significantly higher than all other sample types, $p < 0.05$.
[c] Significantly higher than *Salmonella* negative samples, all pH (mini-chocolate eclairs, classic, tropical, passionfruit, mango, pineapple), $p < 0.05$.
[d] Significantly higher than *Salmonella* negative samples, pH > 6 (mini-chocolate eclairs, classic), $p < 0.05$.

counts ranged from 2.0 to 4.4 and 2.0–4.2 log CFU/g respectively. The *Salmonella* positive samples (coconut, egg nog profiteroles) had significantly higher counts of all bacterial populations compared to the *Salmonella* negative samples, regardless of the pH value. The higher values of the *Salmonella* positive samples were largely driven by the values of the egg nog profiteroles which had the highest levels of total aerobes (both open and closed samples), *Enterobacteriaceae* (open sample) and coliforms (open sample). In terms of microbiological quality, the level of total aerobes would have been deemed marginally acceptable/borderline whereas the levels of *Enterobacteriaceae* and coliforms would have been considered unsatisfactory or unacceptable based on the guidelines for ready to eat foods and bakery products established by the United Kingdom and Canada (Health Canada, 2008; Health Protection Agency, 2009a). These levels of *Enterobacteriaceae* and coliforms are indicative of poor hygiene during production or contamination post-production (Health Canada, 2008, 2009a). The quality of the remaining samples including the coconut profiteroles would have been assessed as acceptable/satisfactory in terms of aerobic colony counts and marginally acceptable/borderline based on *Enterobacteriaceae* and coliform counts. These results would warrant investigation of additional samples to detect contamination, and review of production practices to address potential breaches of hygiene (Health Canada, 2008; Health Protection Agency, 2009a).

3.3. Effect of thawing on Salmonella levels in frozen profiteroles

The manufacturer of the frozen profiterole and mini chocolate eclairs recommended the products be thawed at room temperature for 30 min or at 4 °C for 90 min prior to consumption. Storage of the naturally contaminated egg nog profiteroles from the closed containers at either 22 °C for 30 min or 4 °C for 2 h resulted in the same MPN result as reported in Table 2 (0.2 MPN/100g). Similarly, the levels of background microbiota did not change during these thawing conditions (data not shown).

To investigate the relationship between time, temperature, and *Salmonella* levels in greater detail, artificially inoculated classic profiteroles were thawed under different conditions (Fig. 2). After storage at 4 °C for 2 h the surface temperature of the profiteroles was permissive to the growth of *Salmonella* upon immediate removal from the refrigerator (6.7 ± 0.4 °C) whereas the interior was still frozen (0.6 ± 1.4 °C). No change was observed in the levels of *Salmonella* after this storage period. After additional storage at 4 °C (24 h), both the surface and interior temperature approached 6 °C upon removal from the fridge and a small (0.4 log CFU/g) increase in *Salmonella* levels was observed (Fig. 2A). An increase of this magnitude corresponds to a little more than two times the cell population in the frozen profiteroles. The cause of this increase is not known as *Salmonella* is not reported to grow at refrigerated temperatures. However, it may be due to artefacts of the enumeration procedure. If, for example, the bacteria assumed an aggregative or filamentous state during the acclimatization period at −20 °C, the initial counts of the unthawed profiteroles would have underestimated actual cell concentration (Mattick et al., 2003).

After thawing at 10 °C for 6 h, a condition chosen to simulate temperature abuse, both the surface and interior temperature of the profiterole was 13 °C and there was a slight (0.1 log CFU/g) but insignificant increase in *Salmonella* levels. Overnight storage at 10 °C resulted in a 0.5 log CFU/g increase in *Salmonella* concentration corresponding to a three-fold increase compared to that of the unthawed profiteroles (Fig. 2B). Thawing at room temperature (22 °C) for 30 min raised the surface temperature to 11 ± 2 °C while the interior core remained frozen at −0.1 ± 1 °C. An additional half hour at room temperature resulted in surface and interior temperatures of 17 ± 0.3 °C and 13 ± 0.9 °C, respectively. After 90 min at room temperature, the surface and interior temperature of the profiterole had equilibrated to 20 °C and at this time, a slight (0.5 log CFU/g) increase in *Salmonella* concentration was observed. Additional storage at 22 °C did not change the temperature appreciably but the levels of *Salmonella* were 0.6 and 2.6 log CFU/g higher after a total storage time of 6 and 24 h than in the unthawed condition. These results show that temperatures permissive to *Salmonella* growth (≥5 °C) can be reached on the surface of a frozen food stored at room temperature in half an hour, and an hour for the interior. At these times, the change in *Salmonella* concentration was negligible, and increased slightly after 1.5 and 6 h, culminating in a 400-fold increase after 24 h compared to the unthawed profiteroles. This trajectory suggests a long lag period is required before growth can take place, with longer times required at colder temperatures. Studies on ground beef also showed no or slight changes to pathogen levels upon thawing at ambient temperatures for 8–12 h with significant increases at longer storage times suggesting a recovery period is required after removal from frozen storage (Ingham et al., 2005; Lianou and Koutsoumanis, 2009; Manios and Skandamis, 2015; Marriott et al., 1980).

Variation between studies may be due to methodological differences and differences in the food matrix. Roccato et al. showed a significant increase in *Salmonella* levels in poultry kebabs after an overnight thawing period at 23 °C, whereas no increases were observed in poultry burgers or sausages after a similar thawing period (Roccato et al., 2015). To investigate the effects of the food matrix on the levels of *Salmonella* during thawing, two flavours of artificially inoculated profiterole were compared to the classic. Passionfruit profiteroles were used to compare the effects of pH (3.9 vs 6.7). The ingredient list of this flavour included a number of acidic ingredients (passionfruit puree and seeds, citric acid, fatty acids) and preservatives (potassium sorbate). We hypothesized that the increased acidity of this product would result in lower numbers of *Salmonella* during thawing compared to the classic variety and this was the result that was observed. The passionfruit profiteroles had significantly lower levels of *Salmonella* after incubation at 22 °C for 6 and 24 h, 4 °C for 24 h, and 10 °C for 24 h than the classic profiteroles (Table 5, superscript b).

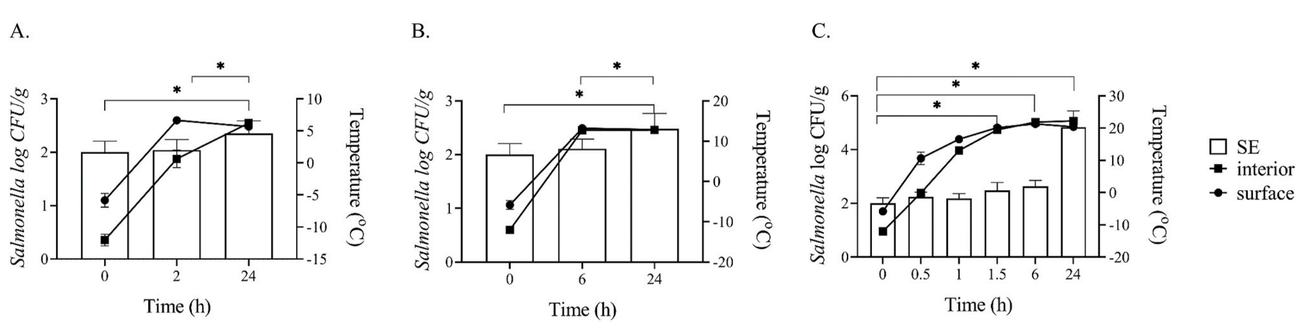

Fig. 2. Temperature profile and *Salmonella* levels in thawed profiteroles. Profiteroles were inoculated with 4 log CFU/g *S. enterica* ser. Enteritidis, frozen, and thawed at the indicated temperatures and times. *Salmonella* was enumerated at the indicated time points by standard plate counting methodology (bars, limit of detection = 1 log CFU/g, n = 9). Surface (circles) and interior (squares) temperatures of uninoculated profiteroles taken from the same container (n = 3–6). Asterisks indicate *Salmonella* levels that are significantly different (P < 0.05).

Table 5
Impact of profiterole filling on inoculated *Salmonella* levels (log CFU/g) during thawing.

Thawing Procedure	Classic	Passionfruit	Egg nog
None (frozen)	2.1 ± 0.2	2.0 ± 0.2	2.2 ± 0.4
22 °C, 90 min	2.2 ± 0.2	2.0 ± 0.3	2.2 ± 0.3
22 °C, 6 h	2.7 ± 0.1[a]	2.0 ± 0.1[b]	2.3 ± 0.2[b]
22 °C, 24 h	5.8 ± 0.2[a]	3.5 ± 0.6[a,b]	5.1 ± 0.2[a,b]
4 °C, 24 h	2.2 ± 0.2	1.8 ± 0.4 [b]	1.9 ± 0.2
10 °C, 24 h	2.2 ± 0.2	1.9 ± 0.4 [b]	2.1 ± 0.1

[a] Significantly different compared to frozen profiterole of the same flavour.
[b] Significantly different compared to classic profiterole at the same time point.

The egg nog profiteroles were also examined to investigate the effect of a_w on the levels of *Salmonella* during thawing. Our hypothesis was that the more favourable a_w of the egg nog profiteroles (0.99) would lead to increased levels of *Salmonella* compared to the classic profiteroles (0.95). However, this hypothesis was not supported. The levels of *Salmonella* in the egg nog profiteroles were equivalent to those of the classic profiterole with the exception of 22 °C for 6 and 24 h, when a lower level of *Salmonella* was observed in the egg nog profiteroles (Table 5, superscript b). The reason for this difference is not known but may be attributed to differences in the composition of the profiterole filling. The egg nog profiterole filling contained eggs, vegetable oil, sugar and water; whereas the classic profiterole filling only contained cream and sugar. Regardless, it appeared that pH had more of an effect on *Salmonella* concentration than a_w and one way to limit bacterial growth in cream filled pastries could be through the addition of acids or other inhibitory ingredients. An increase in *Salmonella* levels after storage at 22 °C for 24 h was observed in all three profiterole types (Table 5, superscript a). Collectively, our results underscore the importance of refrigeration for thawing and short-term storage of frozen cream-filled desserts.

4. Conclusions

In 2019, a national foodborne outbreak linked to the presence of *Salmonella* in frozen profiteroles occurred in Canada. Examination of the food product revealed the presence of low levels of *Salmonella* (0.2–0.7 MPN/100 g) or 1 *Salmonella* bacterium per every 143–500 g serving, equivalent to 11–38 profiteroles). The intrinsic physicochemical properties (a_w and pH) of the food product were conducive to pathogen growth. *Salmonella* positive food samples were associated with higher levels of *Enterobacteriaceae* and coliforms by approximately 1 log unit indicating an unhygienic production process, or a post-production contamination event. The addition of acids or other inhibitory ingredients can serve to minimize pathogen growth in the event of contamination. However, proper storage of frozen foods is necessary to reduce the likelihood of illness. Based on the results presented here, the recommendation would be to thaw frozen cream-filled desserts in the refrigerator, since lower temperatures offer a greater window of time before pathogens can replicate. If thawing at room temperature cannot be avoided, it should be done for short periods, not exceeding 1 h.

Declaration of competing interest

None.

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