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Study of the cross-contamination and survival of *Salmonella* in fresh apples

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ABSTRACT

The present work aimed at studying the cross contamination of apples by *Salmonella* during the processing of commercial fresh apples and its survival capacity on apple at room temperature. For the first study, the typical process of fresh apples was simulated at laboratory scale in which an apple that was artificially contaminated by *Salmonella* at different concentration levels (8, 6 and 5 log cfu/apple) was introduced in one batch and processed including a simulated transport/washing step and drying step using sponges to simulate the porous material used in the industry. Results indicated that at 8 log cfu/apple, 50% fresh apples were contaminated after processing, with all analysed environmental samples being positive for the pathogen, consisting of washing water and sponges. However, at lower inoculum levels (5–6 log cfu/apple) no cross contamination was detected in apples, and only environmental samples showed contamination by *Salmonella* after processing including both water and sponges. Experiments on the survival of *Salmonella* on apple showed that the pathogen was capable to survive for 12 days, only showing a significant drop at the end of the experiment. Finally, two-class attribute sampling plans were assessed as tool to detect *Salmonella* in different contamination scenarios in fresh apple. This analysis indicated that with the highest inoculum level, a total of 16 apples would be needed to reach 95% of detecting *Salmonella* (i.e. lot rejection). In turn, when low levels were assessed (5–6 log cfu/apple), a large number of apples ($n = 1021$) would have to be sampled to obtain the same confidence level (95%). If the environment is sampled (i.e. water and sponges), a lower number of samples would be needed to detect the pathogen. However, the feasibility of environmental sampling has not been assessed from a practical point of view. Overall, the results in this study evidenced that cross contamination by *Salmonella* might occur during processing of fresh apples and subsequently, the pathogen might survive for a noticeable period of time.

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1. Introduction

In recent years, foodborne illness associated with fresh produce has become more common (EFSA, 2013). The main challenges with the food safety of fresh produce are that these products are often grown outside in an uncontrollable environment where they are exposed to contamination from different sources and are often consumed raw and without any forms of risk reducing treatments, such as heat treatment. The contamination of fresh produce with hazardous microorganisms is also heterogeneous, unevenly spread and most likely present in low numbers. This makes it very difficult to detect the presence of foodborne pathogens such as *Salmonella* spp. or others during routine sampling.

Until now, leafy greens and sprouted seeds have caused the most numbers of outbreaks due to many outbreaks associated with leafy greens and large outbreaks (>1000 persons ill) associated with sprouts (EFSA, 2013; Michino et al., 1999; Buchholz et al., 2011). However, other types of fresh produce, such as tomatoes and melons have also caused outbreaks. There is little information about microbial hazards of tree fruits, such as apples, although there have been a few outbreaks of foodborne disease associated with consumption of apple cider. In these cases, *E. coli* O157:H7 and *Cryptosporidium* were the culprits (Millard et al., 1994; Anonymous, 1997; Blackburn et al., 2006). There is sufficient evidence, through experiments, that such products may be contaminated by enteric pathogens and that the pathogens may survive for some time on and in the products, especially minimally processed fruits and also in apple cider (Alegre et al., 2009; Zhao et al., 1993). A study by Abadias et al. (2006) indicated that only a few apple samples from orchards or packhouses harbored *E. coli*, suggesting that contamination is sporadic and consequently will be difficult to detect. In this sense, the development of new real-time, non-destructive online inspection methods could help to improve detection of pathogens in fruits as already demonstrated in previous work (Yang et al., 2012).

Given the low frequency of contamination, and the great lack of data, it is unknown if and how a sporadic contamination of tree fruits could

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be spread during processing and thus further contaminate uncontaminated products. In this sense, it is assumed that the potential for cross-contamination is present if one (or more) units are contaminated. If cross contamination occurs and no lethal or inactivation treatments are applied afterward, sampling plans may become important tools intended to detect the possible contamination (Jongenburger et al., 2011a, 2011b), although their effectiveness will depend on the levels of contamination and their distribution in the final product to be tested. In this study apples were chosen as a model product for tree fruits for investigating the potential for cross contamination and survival of an enteric pathogen such as *Salmonella* spp. during industrial processing and subsequent storage, respectively.

The aim of this work was i) to study the potential for cross contamination of apples simulating industrial processing, ii) to assess the survival *Salmonella* on the apples after a cross contamination event, and iii) based on these data, to assess suitable sampling plans to detect *Salmonella* in apples.

2. Material and methods

For the experiments, fresh, unprocessed Norwegian apples of the variety Summerred (apples grown in Norway) were collected directly at a local packing house. In addition, organically produced apples imported from Italy (Süd-Tirol) of the variety Braeburn were purchased at a local supermarket for the final rounds of the experiments. The apples used presented no injuries (cuts) and were not treated prior to the experiment. The organic apples may have been washed, but had not received any further treatment.

2.1. Preparation of inoculum and contamination of apples

Salmonella Reading (VI 51763), previously isolated from spent irrigation water from sprout production (Robertson et al., 2002), was plated onto Blood agar from glycerol stock kept at −80 °C and incubated at 37 °C overnight. One single colony was transferred to 9 ml of buffered peptone water (BPW) (OXOID, Basingstoke, United Kingdom) and incubated overnight at 37 °C. The culture was serially diluted in BPW and 100 μl of the appropriate dilution was carefully spot-inoculated on the apple surface to obtain the initial contamination on apple. The contaminated apples were dried for 1–2 h in a safety cabinet at room temperature. A total of 3 apples were contaminated in each round for the washing experiments; two were used as inoculation controls, while the third was stored in a plastic bag with 29 uninoculated apples in the refrigerator at 3 ± 2 °C overnight before processing. To quantify *Salmonella* in the inoculum, 100 μl of the appropriate dilutions was plated in parallel on blood agar (bovine blood) and incubated at 37 °C overnight. The cross-contamination experiment was performed at three initial contamination levels corresponding to 5, 6 and 8 log cfu/apple.

For the storage experiment, single apples were drop inoculated and air-dried as described above before further storage. The initial inoculums on the apples corresponded to 6 log cfu/apple.

2.2. Experimental design

2.2.1. Simulation of processing line

A total of 30 apples including one contaminated apple were processed through a simulated apple processing line. A vat with 12 l potable water was used to simulate transport/washing bath and two rounds with 15 apples in each round were left in the bath for approximately 5 min with gentle shaking. After the transport/wash bath the apples were rolled over sponges (to simulate the porous material that are used to dry and drain off water) before they were left to air-dry for 1 h. After drying the apples were packed in zip-lock plastic bags with seven or eight apples in each bag simulating commercial packaging. The apples were stored at 3 ± 2 °C overnight prior to analysis.

2.2.2. Storage experiment

To investigate the survival of *Salmonella* on the surface of apples after a simulated cross contamination event, a storage experiment was carried out at room temperature. A total of 30 apples were used, where 28 were spot inoculated as described above (2.1.1), and two left uninoculated as negative controls. Three apples were analysed immediately as inoculation control, while the remaining 25 inoculated apples were stored in zip-lock bags with five apples in each bag. The negative apples were also stored together. The apples were stored at 22 °C and ~70% RH for a total of 12 days. One bag with five apples was removed and analysed after 1, 2, 5, 6 and 12 days, respectively.

2.3. Bacterial analysis

The apples, sponges and water were analysed for the presence of *Salmonella* using a modified version of NMKL no. 71, 5th ed. 1999 (NMKL, 1999). Briefly, for qualitative analysis in the processing experiment, each apple was analysed separately, adding 225 ml BPW to each apple. The apples were gently rubbed in the BPW. The sponges were distributed into Stomacher bags, 1–2 per bag depending on the size and 225 ml of BPW was added. A volume of 500 ml of the processing water was retrieved from the vat and filtered through a 0.45 μm filter (Millipore S-Pak^{TM} Membrane Filters, Millipore, Billerica, MA, USA) and 100 ml BPW was added to the filter prior to enrichment. Enrichments were incubated 37 °C for 24 ± 3 h. A total of 100 μl of the enrichment cultures was transferred to 10 ml of Rappaport-Vassiliadis-Soya Peptone Broth (OXOID) and incubated for 24 ± 3 h at 41.5 °C, followed by plating on XLD (OXOID) and Brilliance™Salmonella agar (OXOID). The plates were incubated at 37 °C for 24 h and examined for typical and suspicious colonies. The presence of *Salmonella* was confirmed by testing the colonies on Triple Sugar Iron agar (Difco, MD, USA) and Urea agar (Agar base: OXOID, with 40% Urea (Sigma-Aldrich, St. Louis, MO, USA) followed by agglutination with omnivalent *Salmonella* antiserum (Enteroclon Anti-Salmonella A-67, omnivalent, Sifin, Berlin, Germany).

For quantification of *Salmonella* in the storage experiment, each apple was gently rubbed in 100 ml of BPW. Serial dilution series were prepared in BPW and 0.1 ml of the appropriate dilutions was plated on XLD agar. An aliquot of 1 ml of the primary dilution was plated on three plates to achieve a detection limit of 100 cfu/apple. The apple–BPW mix was further incubated and analysed as described above for qualitative detection in case there were less than 100 cfu of *Salmonella* per apple.

2.3.1. Data treatment and mathematical modelling

Experiments were repeated three times in different days in order to capture biological variability. For cross contamination experiments, probability of cross contamination was described as percentage (per one) of positive samples obtained in the different scenarios. Survival data of *Salmonella* spp. on fresh apples were tabulated and standardized to represent $\Delta N = N_0 - Nt$ expressed in log cfu/g with respect to time in days. These standardized data were submitted to regression analysis to fit different mathematical functions describing survival or log decrease along time (Table 1). The regression procedure was performed by the curve fitting tool implemented in the MATLAB 7.7.0 Software (The MathWorks Inc. 2008).

3. Results

3.1. Cross contamination at processing line

Concentration levels transferred to environment and apples were not quantified in the experiment since levels were below the limit of quantification (<100 cfu/apple). Hence, results were expressed as the number or the percentage of positive samples. Results indicated that cross contamination did not take place at low initial inoculum levels,

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