Research Note

Bacteriological Survey of Ready-to-Eat Lettuce, Fresh-Cut Fruit, and Sprouts Collected from the Swiss Market

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ABSTRACT

The consumption of ready-to-eat fresh vegetables has increased significantly in the recent decades. So far, no data are available on the bacteriological burden and the prevalence of foodborne pathogens in ready-to-eat lettuce, fresh-cut fruit, and sprouts on the Swiss market. This study was based on investigations carried out during 2 months of the summer season in 2011. Samples of 142 salads, 64 fresh-cut fruit, and 27 sprouts were included in this study. Escherichia coli, an indicator microorganism for fecal contamination, was only found in 5 lettuce samples, with amounts ranging between 2 and 3 log CFU/g. No Salmonella spp. were detected from any of the 233 samples analyzed in this study, and a low occurrence was found for contamination with L. monocytogenes, Shiga toxin–producing E. coli, enteropathogenic E. coli, and Cronobacter. From the results of the present study, we conclude that even in a country where the use of chlorine solutions to sanitize fruits and vegetables in the fresh-cut industry is not allowed, it is possible to produce ready-to-eat lettuce, fresh-cut fruit, and sprouts with high microbiological standards. Strict maintenance of good practices of hygiene at preharvest, harvest, and postharvest levels is of central importance to ensure both public health protection and product quality.

Fresh produce is promoted as part of a healthy diet, and its consumption has increased over the last years. The International Fresh-cut Produce Association defined fresh-cut products as fruits or vegetables that have been trimmed and/or peeled and/or cut into a 100% usable product that is bagged or prepackaged to offer consumers convenience while still maintaining its freshness.

However, since fresh produce is eaten raw, it can also pose a public health risk. Foodborne pathogens can be introduced onto fresh vegetables at any point during the food production line. At the preharvest level contact with contaminated irrigation water, soil, manure, or fecal matter of wild animals is possible. Foodborne pathogens can bind to plant leaves and/or be internalized via the leaves or the endophytic root system (11, 15). During harvest, asymptomatic human carriers could contaminate the products, and at the postharvest level, the produce could be contaminated by contact with polluted process water, asymptomatic human carriers, or the production environment. Foodborne outbreaks associated with the consumption of fresh produce, fresh-cut fruit, and sprouts have been well documented in the literature (5, 18). Even though Salmonella is the most common cause of disease outbreaks associated with fresh produce, including sprouts, melons, tomatoes, and lettuces (8, 17, 19, 25, 26, 28), there are other pathogens (Listeria monocytogenes, Shiga toxin–producing E. coli [STEC], norovirus) that have been described as relevant microbial hazards (4, 9, 10, 22, 23, 27). A large outbreak of hemolytic-uremic syndrome caused by STEC O104:H4 linked to sprouts occurred recently in Germany (7).

Even though there are some field surveys about the microbiological quality of ready-to-eat fresh produce in the literature (1, 6, 14), the majority of these studies was done in countries where fresh minimally processed produce manufacturers use chlorine-based washing as the decontamination procedure. In Switzerland, and in other countries in the European Union, however, it is not permitted to use chlorine solutions to sanitize fruits and vegetables in the fresh-cut industry (2). The aims of this pilot study were therefore to assess the bacteriological burden of ready-to-eat lettuce, fresh-cut fruit, and sprouts and to determine the occurrence of foodborne pathogens under these production conditions.

MATERIALS AND METHODS

Sampling. This study was based on investigations carried out during 2 months of the summer season in 2011 (July through August). Samples (n = 233) originated from a large Swiss production plant producing fresh-cut and washed, ready-to-eat salads and fresh-cut fruit. This plant supplies products to retail stores all over Switzerland. Since only drinking water can be used for the washing step of the products, and no decontamination process exists, strict basic hygienic measures on the plant level and good agricultural practices are of major importance.

Samples of 142 salads, 64 fresh-cut fruit, and 27 sprouts were included in this study. The samples were taken at the production plant level by the quality management team of the plant and sent to laboratory on the same day.
Examination for total viable count (TVC) and \( E. \) coli was performed in accordance with International Organization for Standardization (ISO) standard 4833:2003 and ISO standard 16649-2:2004, respectively. In brief, \( 10 \) g of each sample was homogenized in \( 100 \) ml of \( 0.85\% \) saline solution. The homogenates and decimal dilutions thereof were plated onto plate count agar (Oxoid AG, Basel, Switzerland) and RAPID \( E. \) coli agar (Bio-Rad Laboratories AG, Reinach, Switzerland) and were incubated for \( 48 \) to \( 72 \) h at \( 30 \)°C and for \( 24 \) h at \( 37 \)°C, respectively. The detection limit was \( 100 \) CFU/g, and colony counts were expressed as log CFU per gram.

Detection of \( S. \) Typhimurium. Examination for \( S. \) Typhimurium spp. was done in accordance with International Organization for Standardization (ISO) standard 4833:2003 and ISO standard 16649-2:2004, respectively. In brief, \( 10 \) g of each sample was preenriched in \( 100 \) ml of buffered peptone water (CM1049, Oxoid AG) for \( 24 \) h at \( 37 \)°C. From the first enrichment, \( 1 \) ml was incubated for \( 24 \) h at \( 37 \)°C in \( 10 \) ml of Kauffmann tetrahionate-novobiocin broth (CM1048, Oxoid AG) supplemented with novobiocin–sodium salt (74675, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) in accordance with the manufacturer’s instructions, and \( 0.1 \) ml was incubated for \( 24 \) h at \( 41.5 \)°C in \( 10 \) ml of Rappaport-Vassiliadis soya peptone broth (CM0866, Oxoid AG). After plating onto mannitol–lysine–crystal violet, brilliant green agar (MLCB; CM0783, Oxoid AG) and xylose lysine deoxycholate agar (CM0469, Oxoid AG), plates were incubated for \( 24 \) h at \( 37 \)°C. Presumptive colonies were confirmed as \( S. \) Typhimurium by biochemical properties with the following tests: oxidation reaction, acid production from mannitol, o-nitrophenyl-\( \beta \)-d-galactopyranoside test, \( H_2S \) and indole production, and proof of urease and lysine decarboxylase.

### TABLE 1. TVC results for 233 ready-to-eat vegetable samples collected from the Swiss market

<table>
<thead>
<tr>
<th>Log CFU/g</th>
<th>Ready-to-eat lettuce ((n = 142))</th>
<th>Fresh-cut fruit ((n = 64))</th>
<th>Sprouts ((n = 27))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3–4</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>4–5</td>
<td>0</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>5–6</td>
<td>20</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>6–7</td>
<td>65</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>7–8</td>
<td>51</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>&gt;8</td>
<td>6</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

The TVCs of the ready-to-eat lettuce, fresh-cut fruit, and sprout samples are summarized in Table 1. Ready-to-eat, raw, precut vegetables, fruits, and sprouted seeds differ from meat and milk products with respect to the composition of their microflora and to their TVCs. No TVC process hygiene criteria are defined in the Commission Regulation No 2073/2005 on microbiological criteria for foodstuffs for this product category (3). The TVCs of the ready-to-eat lettuce in our study were higher compared with other surveys, which are mainly ranging from \( 5 \) to \( 6 \) log CFU/g \((1, 6, 14)\). This could be because in Switzerland the use of chlorine solutions to sanitize fruits and vegetables in the fresh-cut industry is not allowed. \( E. \) coli, an indicator microorganism for fecal contamination, was found in five lettuce samples, with amounts ranging between \( 2 \) to \( 3 \) log CFU/g. This is in accordance with the limit of \( 3 \) log CFU/g for Standardization (ISO) standard 4833:2003 and ISO standard 16649-2:2004, respectively. In brief, \( 10 \) g of each sample was homogenized in \( 100 \) ml of \( 0.85\% \) saline solution. The homogenates and decimal dilutions thereof were plated onto plate count agar (Oxoid AG, Basel, Switzerland) and RAPID \( E. \) coli agar (Bio-Rad Laboratories AG, Reinach, Switzerland) and were incubated for \( 48 \) to \( 72 \) h at \( 30 \)°C and for \( 24 \) h at \( 37 \)°C, respectively. The detection limit was \( 100 \) CFU/g, and colony counts were expressed as log CFU per gram.

Detection of \( S. \) Typhimurium. Examination for \( S. \) Typhimurium spp. was done in accordance with International Organization for Standardization (ISO) standard 4833:2003 and ISO standard 16649-2:2004, respectively. In brief, \( 10 \) g of each sample was preenriched in \( 100 \) ml of buffered peptone water (CM1049, Oxoid AG) for \( 24 \) h at \( 37 \)°C. From the first enrichment, \( 1 \) ml was incubated in \( 100 \) ml of Fraser broth (CM0895, Oxoid AG) in accordance with the manufacturer’s instructions, and \( 0.1 \) ml was incubated for \( 24 \) h at \( 41.5 \)°C in \( 10 \) ml of Rappaport-Vassiliadis soya peptone broth (CM0866, Oxoid AG). After plating onto mannitol–lysine–crystal violet, brilliant green agar (MLCB; CM0783, Oxoid AG) and xylose lysine deoxycholate agar (CM0469, Oxoid AG), plates were incubated for \( 24 \) h at \( 37 \)°C. Presumptive colonies were confirmed as \( S. \) Typhimurium by biochemical properties with the following tests: oxidation reaction, acid production from mannitol, o-nitrophenyl-\( \beta \)-d-galactopyranoside test, \( H_2S \) and indole production, and proof of urease and lysine decarboxylase.

### TABLE 2. Occurrence (number of samples) and characteristics of different foodborne pathogens detected in 233 ready-to-eat vegetable samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Salmonella spp.</th>
<th>L. monocytogenes</th>
<th>Cronobacter spp.</th>
<th>STEC</th>
<th>EPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready-to-eat lettuce</td>
<td>0</td>
<td>5(^a)</td>
<td>2(^b)</td>
<td>1(^c)</td>
<td>11</td>
</tr>
<tr>
<td>Fresh-cut fruits</td>
<td>0</td>
<td>0</td>
<td>2(^b)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sprouts</td>
<td>0</td>
<td>0</td>
<td>1(^b)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) All of serotype 1/2a; detected after a two-step enrichment process; <2 log CFU/g.

\(^b\) C. sakazakii.

\(^c\) eae negative; non-O157.
defined in the Commission Regulation No 2073/2005 for precut, ready-to-eat fruits and vegetables.

No *Salmonella* spp. were detected from any of the 233 samples analyzed in this study, and a low occurrence was found for contamination with *L. monocytogenes*, STEC, *Cronobacter*, and EPEC (Table 2). *Listeria monocytogenes* 1/2a was isolated after a two-step enrichment process in 5 of the 142 lettuce samples. In all of these samples, the contamination level was <2 log CFU/g, which fits with the food safety criteria for *L. monocytogenes* defined in the Commission Regulation (EC) No 2073/2005 for precut, ready-to-eat fruits and vegetables. STEC and EPEC were found in 1 and 11 lettuce samples, respectively. The STEC strain belonged to the non-O157 STEC group and was negative for the *eae* gene. In other surveys from various other regions, including Spain, Norway, and the United Kingdom, in which the authors reported negative results for STEC, they screened only for STEC O157 (1, 13, 20). No other studies reporting EPEC detection are available. Recently, Schmid et al. (21) provided results that plants might be the natural habitat of *Cronobacter* spp. Our study provides even more evidence for this hypothesis since *Cronobacter sakazakii* was found in two ready-to-eat lettuce samples, two fresh-cut fruit samples, and in one sprout sample.

From the results of the present study, we conclude that in a country where the use of chlorine solutions to sanitize fruits and vegetables in the fresh-cut industry is not allowed, it is possible to produce ready-to-eat lettuce, fresh-cut fruit, and sprouts with high microbiological standards. Nevertheless, since fresh produce is eaten raw, a potential public health risk remains. The food safety legislation of the European Union regulates every level of the food chain, and affects all member countries and third parties wishing to export food into the European Union. The onus of compliance is placed on food operators. They must implement compulsory self-checking programs, following the hazard analysis critical control point approach. Strict maintenance of good practices of hygiene at preharvest, harvest, and postharvest levels is of central importance to ensure both public health protection and product quality.

**REFERENCES**


