Research Note

Microbiological Quality of Fresh-Cut Carrots and Process Waters

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ABSTRACT

Fresh vegetables may be contaminated by pathogens in different ways after harvest. Pathogenic microorganisms associated with fresh vegetables can cause severe outbreaks of foodborne disease. We discuss here the results of microbiological analysis of carrot samples, as well as of washing, processing, and wastewater samples. Washed, unpeeled carrots generally contained the highest aerobic plate counts (mean, 5.5 log CFU/g). Escherichia coli was not detected in any carrot or water sample examined. The amounts of coliform bacteria and enterobacteria were higher in carrot samples taken from the first steps in the processing line than in samples taken in later phases of the process. Yersinia pseudotuberculosis was not detected in any of the samples by the cultivation method; however, nonpathogenic Yersinia enterocolitica was detected from most carrot samples and almost all washing water and wastewater samples but only from 2 of 10 process water samples. Using a more-sensitive real-time PCR method, pathogenic Y. enterocolitica was found from several carrot samples, and when these positive samples were cultivated, no pathogenic Y. enterocolitica strains were detected.

In recent years, the fresh-cut vegetable market has grown rapidly, and foodborne-disease epidemics of vegetable origin have become more frequent. Spoilage or pathogenic microorganisms may contaminate vegetables via the seeds or during growth in the field, harvesting or postharvest handling, processing, packaging, storage, or distribution (5–7, 12, 20). During the several processing steps of fresh-cut products, such as trimming, washing, peeling, cutting, and slicing, the vegetables are at risk of contamination by pathogenic bacteria (9). The process of slicing and shredding provides essential growth nutrients for the microbial flora residing on these products (35). An important cause of pathogenic contamination is human, which can potentially contaminate the fresh produce at any stage from farm to fork (35).

Microflora are usually on the surfaces of vegetables, but some microbes can also break into the inner tissues through surface damage, in some cases even in healthy plants (33, 39). Not all risks in fresh vegetable production can be eliminated, and thus, the aim is to minimize the risks (12, 43). Washing raw material before cutting, during fresh-cut processing, and before use is the most effective way of minimizing the risk of the presence of pathogens and of any residue left on the produce from harvesting and handling (28). In addition to washing, refrigeration conditions (1), appropriate production techniques (e.g., the use of sharp cutting tools) (19), and an appropriately short time between processing and packing (44) are of importance when improving or maintaining the hygiene quality of vegetable products. Other techniques leading to improved hygiene are also probably beneficial (11, 14, 27, 30, 34, 37, 44), but their efficacy is limited (29).

Some pathogenic bacteria, such as Listeria monocytogenes and pathogenic biotypes of Yersinia enterocolitica and Yersinia pseudotuberculosis, are able to grow in vegetables stored at low temperatures (6, 24). Y. enterocolitica is the major cause of yersiniosis in humans, although Y. pseudotuberculosis–associated disease is probably underreported (18). Yersiniosis is the third most commonly reported zoonosis in the European Union. The majority of Y. enterocolitica isolates from food and environmental sources are nonpathogenic types. Pigs are the primary reservoir for the human pathogenic types of Y. enterocolitica, mainly biotype 4 (serotype O:3) (13). Yersiniosis is more common in countries with temperate climates than in tropical or subtropical regions (22), and the highest rates in the European Union were observed in Lithuania and Finland (13).

Carrots have been implicated by epidemiologic investigations as a source of yersiniosis infection, although the mechanisms of contamination of fresh produce have not been identified (23). Y. pseudotuberculosis associated with raw carrots caused gastrointestinal symptoms in a Finnish school in 2004 (24) and a widespread outbreak in southern Finland in August to September 2006 (31). Infections of Y. pseudotuberculosis are caused by, e.g., ingestion of the bacteria from raw vegetables or water or direct contact with infected animals (13).

The aim of this study was to improve the processing of fresh-cut vegetables through versatile improvements of the
production chain and, in particular, process hygiene (26). In this article, the results of the microbiological analysis of carrot samples, as well as of washing, processing, and wastewater samples obtained from carrot-processing companies, are discussed.

MATERIALS AND METHODS

Samples. Samples were collected from six carrot-processing plants in Finland. First, the levels of hygiene of the processing plants were evaluated by taking samples from environmental surfaces and indoor air (26). At the same time, samples of vegetable raw materials, finished vegetable products, and washing, processing, and wastewaters were taken.

Microbiological analyses. Microbiological analyses were carried out in two laboratories, in 2009 at MTT Agrifood Research Finland (MTT) and in 2012 at the laboratory of the Water Protection Association of the River Kokemäki, Hämeenlinna (KVVY). Microbiological analyses were carried out as outlined in Table 1. At MTT, 25 g of edible, grated parts of whole carrots were weighed, diluted in 225 ml of one-quarter-strength Ringer solution (Merek, Darmstadt, Germany), homogenized for 1 min at 260 rpm in a Stomacher 400 circulator (Seward Ltd., London, UK), and serially diluted. At both MTT and KVVY, the surface parts of carrots were peeled for Yersinia analyses, and 25 g of carrot peels or 10 ml of water sample was weighed for enrichment. At KVVY, for microbes other than Yersinia, 11 g of peeled carrot was diluted in 99 ml of NaCl-peptone diluent (0.1% tryptone, Oxoid, Basingstoke, UK; 0.85% NaCl, Merck) and homogenized in a Smasher (AES Chemunex, Bruz, France). Serial decimal dilutions were prepared by using the 9-ml Dilucup-MRD (maximum recovery diluent) system (0.85% NaCl, 0.7% peptone; LabRobot Products AB, Stenungsund, Sweden) per well. The growth medium used at MTT came from Difco (Difco, BD, Sparks, MD) and the growth medium used at KVVY from Oxoid if not otherwise indicated.

The presence of Y. enterocolitica was analyzed at MTT according to ISO 10273 with modifications. Samples were enriched in phosphate–sorbitol–bile salt broth or Irgasan–ticarcillin–potassium chloride (ITC) broth (Lab M Ltd., Heywood, Lancashire, UK) and incubated at 22 to 25°C for 5 to 6 days or 3 days, respectively. The peptone sorbitol bile (PSB) broth was mixed with 0.25% KOH and streaked onto cefsulodin-Irgasan-novobiocin (CIN) agar (Lab M Ltd.) or Salmonella–Shigella–deoxycholate calcium chloride (SSDC) agar (Lab M Ltd.), which were incubated at 30°C for 24 or 48 h, respectively. The ITC enrichment was spread directly onto CIN agar and SSDC agar. Suspect colonies were streaked onto plate count agar, and urea-positive and oxidase-negative (Bacteroid oxidase, Merck) colonies were confirmed by the API E20 test (bioMérieux SA, Marcy-l’Étoile, France). Y. enterocolitica biotypes were tested for the hydrolysis of esculin, lipase activity, and acid production from xylose, trehalose, or salicin. PCR testing for the presence of the ail gene and the virulence plasmid of Y. enterocolitica was performed at the Finnish Food Safety Authority (Evira).

Y. pseudotuberculosis was analyzed according to the methods of Bacteriological Analytical Manual Online (42), modified. Samples were enriched in phosphate-mannitol-peptone broth at 4°C for 7 and 14 days. The enrichment was mixed with KOH before being streaked onto CIN agar and Y. pseudotuberculosis selective agar (unpublished, Evira) and incubated at 30°C for 24 h. The inv gene and the virulence plasmid of Y. pseudotuberculosis were determined at Evira.

At KVVY, an in-house real-time (RT) PCR method, modified from ISO 10273, was used. Samples were enriched in tryptone soy broth and yeast extract at 25°C for 24 h for the determination of Y. enterocolitica and Y. pseudotuberculosis by RT-PCR (40, 41). DNA restriction was performed with an Epicentre DNA purification kit (MasterPure Complete DNA Purification kit, Epicentre Biotechnologies, Madison, WI). RT-PCR was run using an Applied Biosystems 7300 real-time PCR system. In the case of a positive result, samples were enriched in PSB and ITC broths and incubated at 25°C for 2 to 3 days or at 25°C for 48 h, correspondingly, and they were cultivated on CIN agar and incubated at 30°C for 24 h and for 48 h if no typical growth was seen earlier. Since no Y. pseudotuberculosis was detected by RT-PCR, there was no need for cultivation of the samples.

RESULTS AND DISCUSSION

In the present study, washed, unpeeled whole carrots contained in general the highest aerobic plate counts (mean, 5.5 log CFU/g). Escherichia coli was not detected in any of
the samples (Table 2). The counts of coliform bacteria and enterobacteria increased during the processing. The counts of yeasts were lowest in the peeled and highest in the grated carrots. The counts of molds were lowest in the processed carrots. The initial total bacterial counts of minimally processed stick carrots have been reported to increase during processing from 4.7 to 7.0 log CFU (15). Milder slicing treatment has been shown to cause less physical damage and reduced microbial loads in fresh-cut carrot slices (3). In grated carrots, the total bacterial counts have been high, around 7 log CFU/g (32) or 6 log CFU/g (4).

Grated carrots contained the highest microorganism counts among vegetables (7.8 log CFU/g of aerobic mesophilic microorganisms, 6.1 log CFU/g of yeasts and molds, and 6.2 log CFU/g of Enterobacteriaceae) in a Spanish study (1). None of the vegetable samples examined in that study were positive for pathogenic E. coli O157:H7 or pathogenic Y. enterocolitica. In our study, the mean aerobic plate counts of the grated carrot samples were lower, 4.8 log CFU/g, as were the numbers of yeasts (3.5 log CFU/g) and molds (<2 log CFU/g). In an earlier study, the counts of yeasts and molds in shredded carrots were approximately 6 log CFU/g (4). In one study in the United Kingdom, the number of Enterobacteriaceae in ready-to-eat vegetables exceeded 10^7 CFU/g at a maximum (34), whereas in the present study, the maximum count of Enterobacteriaceae was lower, 1.4 × 10^4 CFU/g. The maximum number of E. coli organisms in the United Kingdom study was 10^4 to 10^5 CFU/g (35), whereas in the present study, the corresponding number was significantly lower (<10 CFU/g). Pathogenic bacteria, including Salmonella, E. coli O157:H7, and Campylobacter spp., were not isolated in carrots in a large Canadian survey from farmers’ markets (n = 206). E. coli was detected in 4.4% of carrot samples (8). In the present study, all the E. coli values were below the detection limit of 10 CFU/g. However, it is difficult to directly compare different studies, because the vegetable raw materials, their storage and processing, and the test procedures differ considerably from each other.

In this study, the process water samples contained less microbes and coliform bacteria than the washing water samples. E. coli was not detected in any of the process waters (Table 3). Mesophilic bacteria and aerobic plate counts of carrot wash water were measured in the Spanish study 2 h after the beginning of the process (36). The counts were 4.9 log CFU/ml and 0.9 log CFU/ml, respectively. In our study, the heterotrophic plate counts were 4.9 log CFU/ml in the process water, and coliform counts were 3.7 log CFU/100 ml. Contaminated wash water could be a risk for the quality of fresh vegetable products.

In 2009, nonpathogenic Y. enterocolitica strains were detected in several of our water and carrot samples. According to the biochemical tests, the Y. enterocolitica strains were mostly identified as nonpathogenic 1A biotypes (24 of 32 strains). Four strains were either biotype 6 or were Yersinia mollaretii or Yersinia hancockii, which were formerly called Y. enterocolitica biotypes 3A and 3B, respectively (42, 45). The biotypes of four strains were unidentified. None of the strains were pathogenic according to the PCR. In general, if Y. enterocolitica was observed in peeled carrots, it was also observed in the corresponding cut and grated samples.

### Table 2. Microbial groups on the washed carrots

<table>
<thead>
<tr>
<th>Microbe type</th>
<th>Whole (n = 12)</th>
<th>Peeled (n = 12)</th>
<th>Peeled and cut (e.g., cubes, slices) (n = 4)</th>
<th>Peeled and grated (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Aerobic plate count</td>
<td>4.2–7.9</td>
<td>5.5</td>
<td>2.6–6.7</td>
<td>4.7</td>
</tr>
<tr>
<td>E. coli</td>
<td>&lt;1</td>
<td>—</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Coliform bacteria</td>
<td>2.4–5.7</td>
<td>4.2</td>
<td>1.6–3.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>2.3–3.5</td>
<td>3.5</td>
<td>1.6–3.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Yeasts</td>
<td>&lt;2–5.0</td>
<td>—</td>
<td>&lt;2–4.1</td>
<td>—</td>
</tr>
<tr>
<td>Molds</td>
<td>&lt;2–3.3</td>
<td>—</td>
<td>&lt;2–2.5</td>
<td>—</td>
</tr>
</tbody>
</table>

—, not calculated.

### Table 3. Microbial groups in carrot washing waters and processing waters (rinsing and cooling of processed carrots)

<table>
<thead>
<tr>
<th>Microbe type</th>
<th>Wash water (n = 5)</th>
<th>Process water (n = 10)</th>
<th>Unit of measure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Heterotrophic plate</td>
<td>4.8–6.6</td>
<td>5.5</td>
<td>3.7–5.7</td>
</tr>
<tr>
<td>E. coli</td>
<td>&lt;1</td>
<td>—</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Coliform bacteria</td>
<td>3.5–5.5</td>
<td>4.2</td>
<td>2.0–5.5</td>
</tr>
<tr>
<td>Fecal coliform</td>
<td>1–2.2</td>
<td>—</td>
<td>&lt;1–2.2</td>
</tr>
</tbody>
</table>

—, not calculated.
In the study in 2012, some carrot and process water samples were analyzed by RT-PCR. Pathogenic Y. enterocolitica was observed in all washed carrot samples and in almost all peeled carrot samples. However, when the positive RT-PCR samples were cultivated, no pathogenic Y. enterocolitica strains were detected. Water has been relatively widely investigated and revealed to be a significant reservoir for nonpathogenic Y. enterocolitica (2). Nonpathogenic Y. enterocolitica was found in almost all of our samples of wastewater and washing water but in only 2 of the 10 process water samples. If the process environment is favorable for the growth of nonpathogenic Yersinia, there is also a risk of the growth of pathogenic Yersinia.

Y. pseudotuberculosis was not detected in any of our samples. Among the 32 Y. enterocolitica strains isolated, 24 strains (75%) belonged to the nonpathogenic biotype 1A. In France, 27% of 58 samples of grated carrot obtained from eating establishments were contaminated with Yersinia; 7% of the samples contained Y. enterocolitica serotypes potentially pathogenic to humans (10). In a Finnish study, 64% of the Yersinia findings from 462 patients were Y. enterocolitica biotype 1A (38). In a Korean study of ready-to-eat vegetables, 77.8% of Y. enterocolitica findings were biotype 1A (25). Although biotype 1A has been designated as nonpathogenic, there is some evidence for mechanisms of pathogenicity (21). RT-PCR assays have provided better estimations and more-rapid results for the occurrence of pathogenic Y. enterocolitica and Y. pseudotuberculosis in clinical, food, and environmental samples than have culture methods (16, 17, 40, 41). In our study, some washed, peeled, and grated carrot and process water samples were analyzed by the RT-PCR technique and pathogenic Y. enterocolitica isolates were found in most of these carrot samples. RT-PCR–positive samples were then cultivated, but Y. enterocolitica was not found. In some other studies examining the occurrence of pathogenic Y. enterocolitica in foods, the prevalence has been higher by PCR than by the culturing method (2).

In Finland, Yersinia has been observed particularly when carrots have been stored for more than 6 months. During this project, the Finnish authorities clarified instructions concerning the control of Yersinia in products and processing environments in the vegetable processing industry. The results show that only low levels of pathogenic Y. enterocolitica existed in carrots. In order to prevent hygiene and health problems with pathogens as early as possible in the production chain, more information is needed concerning the transmission routes and the infective dose of pathogenic Yersinia for humans, and testing methods and rapid tests should be developed.

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REFERENCES


