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Bacterial quality and safety of packaged fresh leafy vegetables at the retail level in Finland

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ABSTRACT

Consumption of packaged fresh leafy vegetables, which are convenient ready-to-eat products, has increased during the last decade. The number of foodborne outbreaks associated with these products has concurrently increased. In our study, (1) label information, (2) O_2/CO_2 composition, (3) bacterial quality and (4) safety of 100 fresh leafy vegetables at the retail level were studied in Finland during 2013. Bacterial quality was studied using aerobic bacteria (AB) and coliform bacteria (CB) counts, and searching for the presence of *Escherichia coli*, *Listeria* and *Yersinia*. The safety was studied by the presence of *Salmonella*, *ail*-positive *Yersinia*, *stx*-positive *E. coli* (STEC) and *Listeria monocytogenes* using PCR and culturing. Important label information was unavailable on several packages originating from different companies. The packaging date was missing on all packages and the date of durability on 83% of the packages. Storage temperature was declared on 62% of the packages and 73% of the packages contained information about prewashing. The batch/lot number was missing on 29% of the packages. Very low oxygen (O_2) (<1%) and elevated carbon dioxide (CO_2) (2–22%) concentrations were measured in all packages labelled to contain a protective atmosphere. O_2 and CO_2 concentrations varied widely in the rest of the packages. AB and CB counts were high in the leafy vegetable samples varying between 6.2 and 10.6 and 4.2–8.3 log cfu/g, respectively. In most of the samples, the AB and CB counts exceeded 10^8 and 10^6 cfu/g, respectively. A positive correlation was observed between the AB and CB counts. *E. coli* was isolated from 15% of the samples and *Yersinia* from 33%. *L. monocytogenes* was isolated from two samples and *ail*-positive *Y. enterocolitica* in one. Using PCR, STEC was detected in seven samples, and *Salmonella* and *ail*-positive *Y. enterocolitica* in two samples each. The AB and CB mean values of products originating from different companies varied widely. High AB and CB counts and pathogenic bacteria were detected in ready-to-eat products not needing washing before use. Our study shows that the bacterial quality and safety of packaged fresh leafy vegetables is poor and label information on the packages is inadequate. More studies are needed concerning the impact of a protective atmosphere on bacterial growth, and the impact of washing for removing bacteria.

1. Introduction

Packaged salads including fresh leafy vegetables (leafy greens) are widely consumed ready-to-eat food products (EFSA, 2014). They are mostly minimally processed including steps like cutting, washing, dewatering, packaging and storage. Several washing steps are used to cool and clean the leafy vegetables, mainly to eliminate field dirt and debris but also to decrease microbial contamination. Some microorganisms will be removed from the product during washing, but they can also be spread from contaminated parts to non-contaminated ones (Ailes et al., 2008). Processing leafy greens into fresh-cut products may increase the risk of bacterial contamination by breaking the external barrier of the produce (Francis et al., 2012). Using disinfectants to inactivate bacteria may be applicable at this stage (Lynch et al., 2009). Irradiation, which reduces microbial contamination without damaging the texture and colour of the produce, is currently not permitted for use on leafy vegetable in most European countries (EU, 2009). The maximum radiation dose for vegetables is 1 kGy which has shown to be effective for elimination of foodborne pathogens in leafy greens. However, the efficacy is influenced by the atmosphere in which it is packaged (Olaimat and Holley, 2012).

Fresh leafy vegetables are products with a short shelf-life, especially when stored in inappropriate conditions. Storage temperature and humidity are very important factors influencing the quality and safety of leafy greens (Francis et al., 2012). Packaging effectively prevents water loss but due to respiration, vegetables consume oxygen (O_2) and produce carbon dioxide (CO_2), thereby modifying the gas composition within the package. Modified atmosphere packaging (MAP) techniques, including passively- and actively-altered environments, are commonly used for fresh produce (Caleb et al., 2013; Sandhya, 2010). MAP can extend the shelf life of fresh vegetables considerably compared to atmosphere packaging. Packaging is also applied to ease product handling and for hygienic purposes. However, concerns have been raised of

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possible growth of pathogens such as *Salmonella*, *Listeria monocytogenes* and *stx*-positive *Escherichia coli* (STEC) in fresh and fresh-cut produce under MA during prolonged storage especially at abusive temperatures (Caleb et al., 2013; Francis et al., 2012).

The minimum labelling requirements of packaged vegetables in the EU includes clear product type markings including ingredients, country of origin, quality class, net weight, and the company name and address on each package (EU, 2011). However, storage temperature information and best before/use-by dates are also important for consumers. Furthermore, consumers need clear guidance on whether the products require washing before use. Packages should also be labelled with a batch number or with comparable information providing reliable traceability. Irradiated vegetables must be labelled.

The number of reported outbreaks associated with food of non-animal origin including leafy greens has increased (EFSA, 2013). Increased consumption, large-scale production and efficient distribution during the past decade may have contributed to this increase. Outbreaks of *Salmonella*, STEC, enteropathogenic *Yersinia* (*Yersinia enterocolitica* and *Yersinia pseudotuberculosis*) and *L. monocytogenes* have been associated with fresh leafy greens (Callejón et al., 2015; Little and Gillespie, 2008; MacDonald et al., 2011; Marder et al. 2014; Nuorti et al., 2008; Taban and Halkman, 2011). Packaging type and storage temperature may impact the growth of these pathogens due to prolonged storage.

The aim of this work was to study (1) the quality and (2) safety of packaged leafy vegetables from retail shops, (3) the label information on the packages and (4) the packaging's gas composition effect on microbial quality and safety were also studied.

2. Material

2.1. Sampling and gas composition

One hundred packaged fresh leafy green vegetables were bought from retail stores in Finland in 2013 (Table 1). All products were packaged in a bag. The dominant product type was mixed leafy greens (60%) containing frequently arugula, radicchio, mizuna, romaine and spinach. The rest of the products were spinach (19%) and different leafy greens (21%: head lettuce, frisee, arugula, kale and romaine). Most (57/60; 95%) of the products sampled between January and April were imported, while domestic products were mainly (33/40; 83%) sampled in August and September due to low domestic production during the winter season. The imported products mainly originated from Italy and Spain. A maximum of five different packages were tested per week. All packages were from different batches. The gas composition was measured using a gas sensor (Checkpoint, PBI Dansensor, Ringstedt, Denmark) before microbiological studies (Table 1).

2.2. Labelling

Product type describing the product, product ingredients, country of origin, quality class of the vegetable(s), net weight, batch/lot number, company name and address, nutritional information, packaging type (protective atmosphere), packaging date, date of durability/best before date and storage temperature of the package were recorded (Table 2). Information concerning whether the product should be washed before use or whether it was ready to use was additionally checked for.

2.3. Microbiological quality

Up to 100 g of each sample was transferred to a stomacher bag (Seward stomacher 400 classic bags, Sussex, UK) and 200 ml of buffered peptone water (BPW (ISO), LAB M, Kerava, Finland) was added and mixed gently by hand for 30 s.

The numbers of aerobic bacteria (AB) and coliform bacteria (CB) were determined using the drop plating method (DIN, 1984). The sample was further diluted (1:10) in peptone saline water (distilled water with 0.85% NaCl and 0.1% peptone) to 10^{-8}. Plate count agar (PCA, Oxoid, Basingstoke, UK) and Chromocult (Merck, Darmstadt, Germany) plates for AB and coliforms, respectively, were inoculated from tenfold dilutions in duplicate. The PCA and Chromocult plates were incubated at 30 °C for 24–48 h and 37 °C for 24 h, respectively.

To detect *E. coli*, 100 μl of the samples mixed with BPW (1:3) was plated on Chromocult and violet-red-bile (VRB, Oxoid) plates, which were incubated at 42 °C for 24 h. Furthermore, the most probable number (MPN) of *E. coli* was determined using the Colilert® test (IDEXX Laboratories, Westbrook, Maine) according to manufacturer's instruction, to increase the sample volume. The samples mixed with BPW were diluted tenfold and cultured in separate Colilert trays to detect the numbers of viable *E. coli* between 1 and 2419 in 100 ml. Coliform counts were also possible to measure using the same test.

2.4. Microbiological safety

The presence of *Salmonella*, *Yersinia* and *Listeria* were studied by culturing. First, these pathogens were cultured directly on a selective agar plate: 100 μl of the sample mixed with BPW was inoculated on a xylose-lysine-deoxylate (XLD, LAB M) agar for *Salmonella*, Oxford agar (LAB M) for *Listeria* and cefsulodin-irgasan-novobiocin (CIN, LAB M) agar for *Yersinia* isolation. *Salmonella* isolation continued by inoculating 100 μl of the overnight (18–20 h) enrichment (in BPW at 30 °C) on the modified semisolid Rappaport-Vassiliadis (MSRV, LAB M) agar. After 24-h incubation at 42 °C, any spreading growth (if present) found on the MSRV plate was further plated on an XLD plate, which was incubated for 24 h at 37 °C. *Listeria* was isolated by inoculating 100 μl of the overnight enrichment into 10 ml of Fraser broth (LAB M) and after 48-h incubation, 10 μl was inoculated on an Oxford agar (LAB M) plate which was incubated at 37 °C for 24–48 h. *Yersinia* was isolated on a CIN agar plate after cold enrichment for 8 d at 4 °C in peptone-mannitol-bile salt (PMB) broth (10 ml of BPW + 90 ml of PMB) and an alkaline treatment (0.5% KOH) of PMB broth for 20 s. CIN plates were incubated at 30 °C for 20–24 h. Typical colonies on XLD and CIN plates were identified using API 20E strips (bioMérieux, Marcy l'Etoile, France), and typical colonies on Oxford plates using API Listeria strips (bioMérieux).

The presence of *Salmonella enterica*, STEC and enteropathogenic *Yersinia* spp. (*ail*-positive *Y. enterocolitica* and *Y. pseudotuberculosis*) were examined using real-time PCR. DNA was extracted from the overnight enrichment of BPW using Chelex®100 resin (Bio-Rad, Hercules, California). Shortly, 100 μl of the overnight enrichment was centrifuged at full speed (13,000 × rpm) for 1 min. The supernatant was removed

Table 1
Origin of packaged fresh leafy vegetables from retail level studied in Finland year 2013.

	Number of packages (%) sampled					Number of packages (%) with					
						O_2: <1% CO_2: 2–22%		O_2: 1–18% CO_2: 2–10%		O_2: >18% CO_2: <2%	
Origin of packages	2013	January–April		August–November							
Domestic	36	3	(8.3)	33	(91.7)	11	(30.6)	15	(41.7)	10	(27.8)
Imported	64[a]	57	(89.1)	7	(10.9)	8	(12.5)	24	(37.5)	32	(50.0)
All	100	60	(60.0)	40	(40.0)	19	(19.0)	39	(39.9)	42	(42.0)

[a] Italy:39, Spain:15, Sweden:8, France:1, Netherlands:1.