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Microbiological Quality of Bagged Cut Spinach and Lettuce Mixes▽

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Analysis of 100 bagged lettuce and spinach samples showed mean total bacterial counts of 7.0 $\log_{10}$ CFU/g and a broad range of <4 to 8.3 $\log_{10}$ CFU/g. Most probable numbers (MPN) of ≥11,000 /g coliforms were found in 55 samples, and generic *Escherichia coli* bacteria were detected in 16 samples, but no *E. coli* count exceeded 10 MPN/g.

Increases in the worldwide consumption of ready-to-eat (RTE) produce have resulted in increases in food-borne illness associated with these products (11). In the United States in 2006, a multistate outbreak of *Escherichia coli* O157:H7 was traced to bagged spinach (1) and, a few months later, another outbreak implicated bagged lettuce in fast-food restaurants, thus raising concerns about the microbiological quality of RTE produce.

The quality of RTE leafy greens has recently been surveyed in the United Kingdom (8, 10), Spain (12), and Brazil (3), but there are no recent data on the microbial quality of bagged leafy greens in the United States. In this study, we tested 100 bags of RTE leafy greens purchased in 2007 from stores in the Washington, DC, metropolitan area for total, coliform, and generic *E. coli* bacterial counts to assess their microbiological quality. Although recent outbreaks had implicated specific brands and products, we opted to do a broad survey rather than to focus on specific produce types or brands. Hence, the 100 samples, many labeled "triple-washed" or "ready to eat," consisted of 45 spinach and 55 lettuce mixes (12 different varieties), including organic products. The samples included five brands, of which 20% were local store brands that may only have regional distribution, but the rest were national brands that are widely available. The samples were tested using the U.S. FDA *Bacteriological Analytical Manual* (2; http://www.cfsan.fda.gov/~ebam/bam-4.html) methods. Briefly, 50 g of the product was blended with 450 ml of buffered peptone water, from which 1:10 serial dilutions were made. Total bacterial counts were done by standard plate count, where 0.1 ml of each dilution was plated in duplicate on Trypticase soy agar (BD Diagnostics, Sparks, MD). The coliform and *E. coli* bacterial counts were done with the *m*ost *p*robable *n*umber (MPN) method with ColiComplete discs (Biocontrol, Bellevue, WA) (AOAC official method 992.30). The discs contain X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), which in coliforms are cleaved by β-galactosidase to yield a blue product. The discs also contain 4-methylumbelliferone-β-D-glucuronide, which in *E. coli* bacteria is cleaved by β-glucuronidase to yield blue fluorescence (365-nm UV). After 48 h at 37°C, a combination of coliform-positive (blue) and *E. coli*-positive (fluorescence) tubes was used to estimate the levels of each indicator from the MPN table.

The mean total count of the samples examined was around 7.0 $\log_{10}$ CFU/g, with a broad range of <4 to 8.3 $\log_{10}$ CFU/g (Table 1). Our data are consistent with the results of a 1998 U.S. study of 52 bagged salads which found a mean total bacterial count of 7.0 $\log_{10}$ CFU/g (4). Similarly, a study from Spain showed that the total bacterial counts for 140 RTE lettuce samples at 16 university restaurants ranged from 3.01 to 7.81 $\log_{10}$ CFU/g (12), while an analysis of 133 RTE leafy salads in Brazil found that 51% had counts that were >6.0 $\log_{10}$ CFU/g (3). Interestingly, we saw a large variation in counts not only among the samples, but also within same-brand products that had identical "use by" dates and were tested on the same day. For example, five such seemingly "identical" romaine and spinach samples had bacterial counts that ranged from 5.3 to 7.0 $\log_{10}$ CFU/g and <4.0 to 7.4 $\log_{10}$ CFU/g, respectively. Also, except for 23 samples that came in plastic tubs, condensation moisture was observed at the bottom of many bags. Since water is essential for microbial growth, condensation moisture in the bags would be expected to promote microbial growth; hence, 80% of the bags were opened and sampled from the bottom. The top-sampled bags had a mean total count of 6.96 $\log_{10}$ CFU/g, with a range of 4.8 to 7.86 $\log_{10}$ CFU/g. In contrast, the bottom-sampled bags had a mean total count of 7.65 $\log_{10}$ CFU/g and a range of <4 to 8.3 $\log_{10}$ CFU/g. Although bottom sampling yielded higher counts, these findings are preliminary, and additional studies, in which the same bags are sampled from both the top and bottom, are needed to fully establish the effects of condensation moisture on bacterial counts.

The coliform counts of the samples we tested ranged from

TABLE 1. Total microbiological counts of 100 bagged spinach and lettuce mixes

Product and type	No. of samples	Mean ($\log_{10}$ CFU/g)	Range ($\log_{10}$ CFU/g)
Spinach			
Conventional	33	7.7	<4.0–8.3
Organic	12	7.2	5.5–8.0
Lettuce mix			
Conventional	39	7.0	4.5–7.9
Organic	16	7.3	5.7–8.0

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TABLE 2. Coliform and *E. coli* bacterial contents of 100 bagged spinach and lettuce mixes

Product and type (no. of samples)	Coliform bacteria (MPN/g)						*E. coli* bacteria	
	<3.0	<3–10^1	10^1–<10^2	10^2–<10^3	10^3–<10^4	>10^4	No. of positive samples	Range (MPN/g)
Spinach								
Conventional (33)	1	1	3	3	4	21	4	3–3.6
Organic (12)	0	0	0	3	3	6	2	3.6–9.2
Lettuce mix								
Conventional (39)	0	1	2	6	10	20	9	3–9.2
Organic (16)	0	0	0	4	4	8	1	3.6

<0.47 to ≥4.0 $\log_{10}$ MPN/g (Table 2), similar to the range of <0.47 to 3.38 $\log_{10}$ MPN/g reported for RTE lettuce in Spain (12). We were unable to obtain a mean coliform count, as 55/100 samples exceeded our counting limit of ≥11,000 MPN/g. Generic *E. coli* bacteria were detected in 10 lettuce and 6 spinach samples (Table 2). The highest *E. coli* level found was 9.2 MPN/g, but 12/16 samples had counts of 3.6 MPN/g or less. Most of the *E. coli*-positive samples (11/16) had total counts of >6.0 $\log_{10}$ CFU/g, and 14/16 samples had coliform counts of ≥11,000 MPN/g. There are no *E. coli* limits for bagged produce in the United States, but guidelines and limits exist in other countries. The Brazilian standard for salads that are minimally processed before consumption has a fecal coliform limit of 100 CFU/g, and analysis of 133 salad samples showed that 73% exceed this limit (3). The guidelines of the United Kingdom Public Health Laboratory Service for RTE foods, including bagged produce, have set *E. coli* count limits of <20 CFU/g as satisfactory, 20 to <100 CFU/g as acceptable, and ≥100 CFU/g as unsatisfactory (9). In two large surveys in the United Kingdom, 3,200 organic and 3,852 conventional RTE salads were tested and it was found that 0.5% exceeded the 100-CFU/g *E. coli* limit and were unsatisfactory (8, 9). Although we found 16% of our samples to have *E. coli* bacteria, none exceeded 10 MPN/g, but whether low *E. coli* counts are prevalent in other bagged leafy greens in the United States remains to be determined.

These surveys show that the microbial flora and content of RTE produce are highly variable and complex. One study (6) looked at the microbiological quality of fresh, uncut produce from production through packing and showed that indicator levels in mustard greens and spinach remained fairly constant but that for cilantro, parsley, and, especially, cantaloupe, the indicator levels actually increased during packing, hence showing that the microbial load can vary by processing but also depends on the produce type. The processing for whole produce is probably distinct from that for cut, bagged produce, so it is uncertain if the high counts or the variations in counts we observed in the bagged leafy greens are due to produce type variations or to microbial growth, if any, during processing. Our finding that there are wide ranges, as well as large variations, in counts among samples and even among seemingly "identical" samples, coupled with the rare observation of a spinach leaf with visible filth (Fig. 1), suggests that differences or inconsistencies in processing parameters may also have an effect on microbial load. Lastly, the finding that bagged leafy greens (3, 4; this study) can have ranges of total (4 to 7 $\log_{10}$ CFU/g) and coliform (1 to 4 $\log_{10}$ MPN/g) bacterial counts similar to those for produce sampled in the field (5, 7) also suggests that, microbiologically, these products may be very difficult to clean and process.

FIG. 1. Photograph of a spinach leaf obtained from a bagged sample.

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