Journal of Food Protection, Vol. 68, No. 6, 2005, Pages 1143–1146

Comparison of the Microflora on Organically and Conventionally Grown Spring Mix from a California Processor

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MS 04-171: Received 27 April 2004/Accepted 27 November 2004

ABSTRACT

Considerable speculation has occurred concerning the potential for higher numbers of foodborne pathogens on organically grown produce compared with produce not grown organically. The microflora composition of spring mix or mesclun, a mixture of multiple salad ingredients, grown either by organic or conventional means was determined. Unwashed or washed spring mix was obtained from a commercial California fresh-cut produce processor who does not use manure in their cultivation practices. Fifty-four samples of each type of product were supplied over a 4-month period. Analysis included enumeration of total mesophiles, psychrotrophs, coliforms, generic *Escherichia coli,* lactic acid bacteria, yeasts, and molds. In addition, spring mix was analyzed for the presence of *Salmonella* and *Listeria monocytogenes.* The mean populations of mesophilic and psychrotrophic bacteria, yeasts, molds, lactic acid bacteria, and coliforms on conventionally grown spring mix were not statistically different ($P > 0.05$) from respective mean populations on organically grown spring mix. The mean population of each microbial group was significantly higher on unwashed spring mix compared with the washed product. Of the 14 samples found to contain *E. coli,* eight were from nonwashed conventional spring mix, one was from washed conventional spring mix, and four were from nonwashed organic spring mix. *Salmonella* and *L. monocytogenes* were not detected in any of the samples analyzed.

In recent years, consumption of vegetable salads has increased significantly because of the health interest and diet trends of consumers. Contamination of fruits and vegetables with pathogenic microorganisms can occur while growing in fields or orchards during harvesting, postharvest handling, processing, or distribution *(13).* Minimally processed, cut, and packaged lettuce is a convenient food popular among consumers *(15).* Bagged salad can be exposed to a range of conditions during growth, harvest, distribution, and processing that could increase the potential for microbial contamination.

Several outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables *(3).* There have been multiple outbreaks of *Salmonella* in recent years involving raw fruits and vegetables *(5).* In 1996, there was an *Escherichia coli* O157:H7 outbreak involving leaf lettuce in two U.S. states, causing 49 people to become ill *(9).*

Demand for organic food has grown steadily since 1990, with sales of organic food growing about 20% per year. Recent industry estimates have reported total organic retail food sales through all outlets of $7.8 billion in 2000, a 20% increase over 1999 sales *(7, 8).* In December 2000, the final regulations for organically grown agricultural products were released by the U.S. Department of Agriculture. This rule lists methods, practices, and substances that can be used in producing and handling crops so they can be labeled organic *(1).* Some U.S. farmers are looking to organic farming systems as a potential means to lower input costs, decrease reliance on nonrenewable resources, capture high-value markets and premium prices, and boost farm income.

Numerous studies have questioned the nutritional value, pesticide use, and consumer acceptability of organic produce versus conventionally grown produce *(2, 11, 12).* However, few data are available to address the question of microbial quality of organically grown produce compared with produce grown by conventional means. Spring mix, also known as mesclun, is a mixture of multiple salad ingredients such as baby red romaine, royal red oak, lollo rossa, red merveille, red perella, red fire, sangria, tango, little gem, green romaine, green perella, sierra, green oak leaf, cocard, brunia, arugula, tatsoi, mizuna, red mustard, green mustard, red chard, beet tops, amaranth, baby spinach, radicchio, and frisee. The purpose of this study was to determine the composition of the microflora of spring mix grown by either organic or conventional cultivation practices.

MATERIALS AND METHODS

Enumeration and identification of microorganisms from conventional and organic spring mix samples. Bagged unwashed and washed conventionally grown and organically grown spring mix samples were received once or twice weekly from April to August from a California grower of conventional and organic produce. The grower aseptically collected the samples with quality control samples from the production line, bagged the samples, placed them in coolers with icepacks, and shipped them overnight to the Department of Food Science and Technology at the University of Georgia. Samples were analyzed microbiologically within 24 h of obtainment for all sampling dates.

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TABLE 1. *Total mean mesophilic and psychrotrophic bacteria, yeast, mold, lactic acid bacteria (LAB), and coliform populations for spring mix grown by conventional and organic cultivation practices*[a]

Type of spring mix	Mesophilic bacteria	Psychrotrophic bacteria	Yeasts	Molds	Coliforms	Hetero-fermentative LAB	Homo-fermentative LAB
Conventional	5.76 A ($n = 106$)	5.84 A ($n = 107$)	5.18 A ($n = 108$)	3.54 A ($n = 108$)	2.75 A ($n = 104$)	5.10 A ($n = 100$)	4.80 A ($n = 100$)
Organic	5.78 A ($n = 107$)	5.85 A ($n = 108$)	5.19 A ($n = 108$)	3.54 A ($n = 108$)	2.97 A ($n = 103$)	5.22 A ($n = 99$)	4.79 A ($n = 99$)

[a] Values for mean populations (log CFU per gram) of conventional and organic spring mix in columns followed by the same letter are not significantly different ($P > 0.05$).

Samples were plated onto several types of media for bacterial enumeration. Twenty-five–gram samples were aseptically weighed into 225 ml of 0.1% buffered peptone water (pH 7.0, Difco, Becton Dickinson, Sparks, Md.) and blended with a lab stomacher (Tekmar model 4000, Cincinnati, Ohio) for 60 s on normal speed. Serial 10-fold dilutions were prepared in 0.1% buffered peptone water. To enumerate total aerobic mesophilic bacteria, duplicate 0.1-ml samples of appropriate dilutions were spread plated onto plate count agar (Difco, Becton Dickinson) plates, which were incubated at 37°C for 48 h before counting (CFU). To enumerate total aerobic psychrotrophic bacteria, another set of plate count agar plates were prepared and incubated at 7°C for 7 days before colonies were counted *(6).* Coliforms and *E. coli* were enumerated by plating duplicate 1.0-ml samples of appropriate dilutions onto 3M Petrifilm *E. coli*/coliform count plates (3M, St. Paul, Minn.). The plates were incubated in stacks of no more than 20 for 24 to 48 h at 37°C before colonies with the appearance of that described by the manufacturer were enumerated. To enumerate yeasts and molds, duplicate 0.1-ml samples of appropriate dilutions were spread plated onto dichloran rose bengal chlortetracycline agar plates (Oxoid, Inc., Ogdensburg, N.Y.), which were incubated upright at 25°C for 3 to 5 days before colonies were counted.

Enrichment procedures were done to detect the possible presence of *Listeria monocytogenes* and *Salmonella.* For *L. monocytogenes* analysis, 1 ml was removed from each stomached sample and placed in a 9-ml tube of *Listeria* enrichment broth (UVM formulation, Oxoid). The tubes were incubated for 24 h at 28°C. *Listeria* selective agar base with *Listeria* selective supplement (Oxford formulation SR 140, Oxoid) was used for isolating and differentiating *L. monocytogenes.* After streaking, these plates were incubated for 48 h at 37°C. Suspect colonies were identified with a Remel Micro-ID *Listeria* system (Remel, Inc., Lenexa, Kans.).

For *Salmonella* preenrichment, 1 ml of each stomached sample was placed in a 9-ml tube of lactose broth and incubated at 37°C. After 24 h, 1 ml of the lactose broth was inoculated into 10-ml tubes of tetrathionate broth base, Hajna (Difco, Becton Dickinson), and selenite cystine broth (Difco, Becton Dickinson) for selective enrichment. These tubes were incubated at 37°C for 24 h. Loop portions (3 mm) from the Selenite cystine and tetrathionate enrichment tubes were streaked for isolation onto duplicate bismuth sulfite agar (Difco, Becton Dickinson) and xylose lysine desoxycholate agar (Difco, Becton Dickinson) plates. The plates were incubated inverted at 37°C for 24 to 48 h before examining for typical *Salmonella* presumptive colonies. Triple sugar iron agar (Difco, Becton Dickinson) and lysine iron agar (Difco, Becton Dickinson) tube slants were used for biochemical screening of presumptive *Salmonella* colonies. These tubes were incubated for 24 h at 37°C *(6).* Any presumptive positive triple sugar iron and lysine iron agar tubes were further analyzed with a Remel Micro-ID for the rapid determination of *Enterobacteriaceae.*

Lactic acid bacteria were enumerated on Petrifilm aerobic count plates (3M, Minneapolis, Minn.) as per the manufacturer's instructions. Heterofermentative and homofermentative lactic acid bacteria can be differentiated under these conditions on the basis of whether gas bubbles are associated with the colonies. Gas is present with the heterofermentative lactic acid bacterial colonies that develop. After removing portions of each sample and handling as described, 41 ml of concentrated (4×) MRS broth was added to each stomached bag. This resulted in half-strength MRS concentration. The stomacher bag was then mixed for 1 min by shaking manually. Serial 10-fold dilutions were prepared in lactobacilli MRS broth (Difco, Becton Dickinson), and duplicate 1.0-ml samples of appropriate dilutions were spread onto Petrifilm aerobic count plates (3M). Healthy colony growth was achieved by incubating Petrifilm plates anaerobically in a BBL GasPak jar (Becton Dickinson, Cockeysville, Md.) with the hydrogen and carbon dioxide anaerobic GasPak system. The entire GasPak jar was placed in the 37°C incubator for 48 h before plates were removed and colonies counted.

Statistical analysis. Data were subjected to an analysis of variance by the SAS General Linear Model procedure with sum of square type III *(14)* with replicates (27 levels), spring mix type (2 levels), and wash type (2 levels). Spring mix and wash types were considered significantly different at $P < 0.05$.

RESULTS

When the microbial populations of conventionally and organically grown spring mix were compared, the total mean populations of mesophilic and psychrotrophic bacteria, yeasts, molds, lactic acid bacteria, and coliforms for conventional spring mix were not statistically different ($P > 0.05$) from the corresponding mean populations for organic spring mix (Table 1). For example, the total mesophilic bacterial counts for conventional and organic spring mix were 5.76 and 5.78 log CFU/g, respectively. This suggests no microbial differences between conventional and organic spring mixes provided by the producer, regardless of whether the product was washed.

Results from experiments in which all unwashed spring mix was compared with all spring mix washed with 5 ppm free chlorine for a variety of microbial parameters are summarized in Table 2. The total mean populations of mesophilic and psychrotrophic bacteria, yeasts, molds, lactic acid bacteria, and coliforms for unwashed spring mix were sig-

TABLE 2. *Total mean mesophilic and psychrotrophic bacteria, yeast, mold, lactic acid bacteria (LAB), and coliform populations for spring mix grown by organic and conventional cultivation practices; washed spring mix was treated with chilled water, 5 ppm free chlorine, and citric acid*[a]

Type of spring mix	Mesophilic bacteria	Psychrotrophic bacteria	Yeasts	Molds	Coliforms	Hetero-fermentative LAB	Homo-fermentative LAB
Unwashed	6.24 A ($n = 107$)	6.25 A ($n = 108$)	5.57 A ($n = 108$)	3.91 A ($n = 108$)	3.17 A ($n = 103$)	5.64 A ($n = 100$)	5.35 A ($n = 100$)
Washed	5.30 B ($n = 106$)	5.43 B ($n = 107$)	4.80 B ($n = 108$)	3.17 B ($n = 108$)	2.55 B ($n = 104$)	4.68 B ($n = 99$)	4.24 B ($n = 99$)

[a] Values for mean populations (log CFU per gram) between conventional and organic spring mix in columns followed by a different letter are significantly different ($P < 0.05$).

nificantly ($P < 0.05$) higher than the corresponding populations for washed spring mix. For example, unwashed spring mix had 6.24 log CFU/g mesophilic bacteria, whereas washed spring mix was lower at 5.30 log CFU/g. A 0.62- to 1.11-log CFU/g reduction between the unwashed and washed spring mix was present for all the microbial parameters measured.

Salmonella and *L. monocytogenes* were not isolated from spring mix grown by either conventional or organic cultivation practices. It should be noted that methods used to isolate these pathogens did not follow U.S. Food and Drug Administration protocols, and sensitivity might have been lessened. Selective plating produced colonies similar in appearance to that typical of *Salmonella* from 16 of the samples, but subsequent biochemical testing showed they were, in fact, not *Salmonella* but *Klebsiella oxytoca, Arizona hinshawii,* and *Citrobacter freundii.* This illustrates the importance of confirming the identification of presumptive isolates. Likewise, presumptive positive *L. monocytogenes* isolates from five samples were not confirmed as *L. monocytogenes* with additional biochemical testing. On the basis of the 3M Petrifilm *E. coli*/coliform count plates, 14 *E. coli* isolates were found at a detection level of fewer than 10. Of these, eight were from nonwashed conventionally grown spring mix, one was from washed conventionally grown product, and four were from nonwashed organically grown spring mix.

DISCUSSION

Numerous studies have questioned the nutritional value, pesticide use, and consumer acceptability of organic versus conventionally grown produce *(2, 11, 12).* Few data are available to address the question of microbial quality of organically grown produce compared with produce grown by conventional means. In the United States, there are no industry standards for microbial population of bagged lettuce *(10).*

In this survey, the product analyzed was from one source. By using only one sample source, conclusions drawn from the sample data could be limited in that they only pertain to the product grown and handled under conditions used by the individual grower and possibly others that use similar methods. However, this limitation is compensated for by the advantages in using a sole source for samples. Surveying the two product types produced by a sole grower provided consistency in the type, quality, and age of the products sampled. In addition, by using one source, the products were grown and handled in relatively consistent manners. These factors eliminated variation that would be encountered if samples were drawn from unknown or multiple sources.

The grower of the supplied spring mix washes both the conventional and organic spring mix three times. The spring mix is first washed in stainless steel wash tanks that contain cold water to remove dirt and lower the temperature of the product. The second and third wash tanks are used to control microbial contamination and to lower the product temperature to 1°C. The wash water is treated with a maximum of 5 ppm of free chlorine and citric acid. The citric acid is added to lower the pH of the water. Lowering and maintaining the pH of the water allows for the use of less chlorine.

The low level of chlorine used would seem inadequate compared with typical levels of 50 to 200 ppm, which are commonly used in washing produce *(4).* However, because there was a statistical difference ($P < 0.05$) between the unwashed and washed produce and no potential pathogens were found on the produce, the system used by this grower appears to be appropriate for achieving a high-quality product.

The grower who provided unwashed and washed conventional and organically grown spring mix for use in this research did not use manure in the growing of their conventional or organic products. In their case, they used the common practice of ''green fertilization,'' which uses cover crops in place of manure to provide needed nutrients. The cover crops that grew produced most of the required soil nitrogen. However, fertilizers were also used for additional nutrients. Their strategy was to have balanced soil nutrition rather than adding large amounts of fertilizers. This resulted in plants with fewer diseases and a longer shelf life, which is important for increased crop yield and decreased production costs.

Organic farming has been one of the fastest growing segments of U.S. agriculture during the 1990s; however, organic farming will face considerable challenges in competing with conventional produce *(2).* Without the use of chemicals, it is both more difficult and more expensive to produce fresh fruit and vegetables that are attractive and free from blemishes, pests, and diseases. Because of the

decreased yields and increased costs of producing organic produce, higher costs are covered by the consumer, which is why organic produce is more expensive than conventionally grown produce.

In this study, there were 54 samples of each of the four types of spring mix for a total of 216 samples. Although the samples received for our research were from only one relatively large grower, it is a respectable grower who processes both organic and conventional produce in the same manner using the common practice of green fertilization. It is difficult to determine the percentage of growers who use green fertilization strategies compared with those using composted manure. Much of the concern over food safety related to organically grown produce focuses on producers who use manure in their cultivation routines. Whether or not the results from this study are indicative of other producers is not possible to decide. However, it is worth noting that for some products on the market, like those grown under green fertilization practices, they may not have the same safety concern issues as those grown with manure.

ACKNOWLEDGMENT

We thank Dr. Glen Ware, College of Agricultural and Environmental Sciences, University of Georgia, for his assistance with the statistical analysis.

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