A Microbial Comparison of Ready-to-Eat (RTE) Conventional and Organic Spinach and Arugula

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As the organic food market continues to produce significant sales revenue, growers and retailers are increasingly interested in investing in this market. Since leafy greens may be prepared without a kill step, maintaining proper food safety standards is vital regardless of the production system. To evaluate the efficacy of growing systems, a general microbial comparison was made between products that were grown and processed either organically or conventionally. Packaged, triple washed spinach and arugula displayed in chill cases were purchased from local retail markets. Packages were chosen with code dates remaining of approximately 1 week, and analyzed for microbial content on the same day. The samples were tested for aerobic mesophilic microorganisms, total coliforms, and Escherichia coli. The results showed that the mean aerobic microorganism count and standard deviation for conventional and organic leafy greens were 6.89 ± 0.13 and 7.27 ± 0.13 log10 CFU/g, respectively; levels that were not significantly different. Additionally, no significant differences were found for percent positive samples for E. coli and total coliforms between each production method. This study investigated the long term trend in these produce items over a multiple month time period to determine general microbial incidence of the two growing and handling systems.

Leafy greens are vegetables that are harvested and consumed for their leaves, most of which are eaten raw. These herbaceous plants encompass a wide range of species varying greatly in size, shape, and flavor such as leaves of the Cruciferae family, which includes bok choy, arugula, and mustard greens, the Chenopodiaceae family, which includes Swiss chard and spinach, and the Compositae family, which includes endive, romaine, and butterhead lettuce. Spinach, a popular leafy green consumed in America, and arugula, which is rising in popularity, are well known for being high in folate, iron, lutein, magnesium, calcium, soluble fiber, ascorbic acid, carotenoids, as well as many antioxidants relative to their low caloric content. These phytochemicals have been found to stimulate the immune system, scavenge for free radicals, reduce inflammation, and are known for their health-related benefits, such as reducing the risk of illnesses like cardiovascular disease and cancer (Herr and Buchler, 2010; Singh et al., 2001). According to Singh et al. (2001), people who have iron and vitamin A deficiencies should regularly consume green leafy vegetables to replenish those lacking nutrients. Some studies suggest organically grown produce have higher ascorbic acid, antioxidants, and phenolic compounds (Arbos et al., 2010; Hallmann and Rembiatkowska, 2012; Rembiatkowska, 2007; Zhao et al., 2006) and contains little to no pesticide residues, nitrates, or nitrites (Ragare et al., 2004; Winter and Davis, 2006; Woese et al., 1997). Accordingly, growers and retailers are showing increased interest in organic production methods and products as US organic sales continue to increase with the current reported value over $21 billion in 2008 (Greene, 2012). Due to the perceived health benefits of leafy greens, the exploration of different cropping system effects may provide useful information to consumers and the industry.

Organic crop production entails ecologically and economically sustainable growing methods. Numerous differences exist both between and within organic and conventional production systems. However, the main differences are seemingly attributed to the organic system’s prohibition of synthetic chemicals and fertilizers and processing methods requiring approved organic sanitizers. With known differences for organic production, the USDA has required third party agents to certify organic growers before they may use the USDA certified organic seal, thus ensuring that standards are met to provide consumer assurance (USDA, 2013; Winter and Davis, 2006). As is common for conventional growers, organic growers are not only interested in profitable production but are also aiming to reduce environmental pollution, increase soil fertility, and to conserve plant biodiversity (IFOAM, 1998; USDA 2013).

According to consumer surveys, common assumptions include the belief that organic produce is more nutritious (Byrne et al., 1992; Huang, 1996; Jolly, 1991) and safer for the lack of chemical residues when compared with their conventional counterparts (Huang, 1996; Wilkins and Hillers, 1994; Zhao et al., 2007). However, after reviewing the literature, the evidence is not substantially supportive of those claims, and may even be contradictory. Several studies show significant differences between organic and conventional produce (Hallmann, 2012; Hallmann and Rembiatkowska, 2012; Kapusta-Duch and Leszczynska, 2013), while some studies find no significant difference (Juroszek et al., 2009; Kapusta-Duch et al., 2013; Pieper and Barrett, 2009). This could be due to the variability among studies that includes but is not limited to seasonal effects, geographical region, level

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of ripeness or maturity, source of fertilizer, days watered prior to harvest, and cultivar (Winter and Davis, 2006; Young et al., 2005). While assessing nutritional quality differences of produce is important, food safety differences also provide vital quality insight in that microbial contamination issues may arise with the organic growing system’s use of nontraditional chemicals, pesticides, and fertilizers. Consumers purchase vegetables for a variety of reasons. Whether they choose organic produce for the belief of higher nutritional quality compared to conventional produce or for the lower chemical residue levels, food safety should not be compromised. With the use of nontraditional fertilizers and chemicals, determining whether the organic system has significant increased risk for microbial contamination should be supported or refuted to provide information to consumers and to the industry. During any point in the food production chain such as production, processing, distribution, or preparation, food may become contaminated (De Roeve, 1998) from numerous sources such as soil, feces, irrigation water, processing equipment, and transportation systems (Beuchat, 2002; Johannesen et al., 2002). Assays to assess indicators of food quality and safety and to reflect proper implementation of food handling procedures can include plate counts of aerobic mesophilic microorganisms and of total and fecal coliforms. Commonly found in water, soil, and vegetation, as well as potentially in feces of warm-blooded animals, coliforms consist of Gram-negative, non-sporing bacteria, facultative anaerobic rod-shaped bacteria that ferment lactose with the production of acid and gas within 48 h when incubated at 35 °C (Feng et al., 2002). Testing of coliforms is used to indicate sanitary quality of water or as a general indicator of a food processing facility’s sanitary conditions. A vital concern for the food safety of leafy greens is the absence of human pathogens, which have been recently found in a variety of leafy green products including organic spinach and spring mix implicated in foodborne illness outbreaks (CDC, 2012). Fecal contamination, which signifies a breech in proper food safety procedures, may be indicated by the presence of *Escherichia coli* because it is native to guts of warm-blooded animals, typically is found in fecal matter and not usually in the environment, and is easily detectable due to its ability to ferment lactose (Caplenas and Kanarak, 1984). However, it is important to note that fecal coliforms are not always found in feces. Thus, the presence of fecal coliforms may indicate either fecal contamination or unsanitary processing (Feng et al., 2002).

Currently, comparison studies of organic and conventional production and handling systems for store-bought, ready-to-eat (RTE) leafy greens and their microbiological characterizations seem to be scarce in the literature, in spite of the numerous studies on other commodities. The objective of this research was to determine if the aerobic mesophilic microorganisms, total coliform, and *E. coli* microbial loads significantly differ in store-bought RTE spinach and arugula that is grown and processed either by conventional or organic methods.

### Materials and Methods

**Leafy greens.** Packaged, triple washed organically grown and conventionally grown spinach and arugula were purchased and processed the same day, once per month from March to May from a local Publix supermarket (Gainesville, FL), and were stored at 4 °C. Three (in April) or five (in March and May) of these bags were removed from cold storage to form one 10-g composite sample, processed, and plated in triplicate. Over the 3-month period, 13 samples were analyzed for the four treatments for a total of 52 samples. Only samples with 1 week ± 1 d out from their best-if-used by date were selected for processing.

**Media.** Media consisted of Butterfield’s phosphate buffered solution (BPB) (pH 7.2, Difco, Becton Dickinson, Sparks, MD), 3M™ Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN), and 3M™ Petrifilm™ *E. coli*/Coliform Count Plates (3M).

**Sample Processing and Enumeration of Aerobic Mesophilic Microorganisms, Coliforms and *E. coli*.** Procedures for sample processing and enumeration of aerobic mesophilic microorganisms were as described by Phillips and Harrison (2005) with a few exceptions. Ten-gram samples were aseptically weighed and blended with buffered solution for a 1:10 dilution. Following serial 10-fold dilutions in BPB solution, 1-mL samples were plated in triplicate, and incubated for 48 h ± 3 h at 35 °C ± 1 °C before enumeration (AOAC 990.12). Coliforms and *E. coli* were enumerated by similar procedures by plating onto respective plates in triplicate and were incubated for 48 h ± 2 h at 35 °C ± 1 °C (AOAC 991.14).

**Statistical Analysis.** After combing the data for each repetition within each treatment (organic or conventional) over the three month period, the data were subjected to a two-way analysis of variance (ANOVA) using JMP Pro 10. The classification variables used were replication, product, and type with log$_{10}$ colony forming units per gram (CFU/g) as the response variable. Post-hoc analysis was performed for aerobic mesophilic microbial counts using a Student’s *t*-test for comparison between all organic and conventional mean values and for percent positive of coliforms and *E. coli* using a Fisher’s exact test. Significant differences were determined at *P* < 0.05.

### Results and Discussion

After analysis comparing the organic (O) and conventional (C) aerobic mesophilic counts, the results showed that the counts were not significantly different as denoted by the same letter in Table 1. These findings are consistent with other microbiological surveys analyzing or comparing organic and conventional leafy greens showing a range of microbial loads from $\sim 4$ log$_{10}$ CFU/g to $7$ log$_{10}$ CFU/g and yielded no differences in comparisons (Abadias et al., 2008; Allen et al., 2013; Oliveira et al., 2010; Phillips and Harrison, 2005).

Similarly, analysis comparing organic and conventional product’s percent positive of total coliforms as well as the analysis for percent positive of *E. coli* were determined to not be significantly different with both *P* values > 0.05 (Table 2). Similar research findings comparing both production and handling system’s co-

<table>
<thead>
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<th>Level</th>
<th>Least squares mean</th>
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<tbody>
<tr>
<td>O</td>
<td>7.27 a</td>
</tr>
<tr>
<td>C</td>
<td>6.89 a</td>
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Table 1. Student’s *t*-test between RTE organic (O) and conventional (C) spinach and arugula.

<table>
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<th>Coliform (%)</th>
<th>E. coli (%)</th>
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<tr>
<td>O (n=26)</td>
<td>C (n=26)</td>
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<tr>
<td>23 (88) a</td>
<td>17 (65) a</td>
</tr>
<tr>
<td>1 (4) a</td>
<td>0 (0) a</td>
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*1Percentage of samples analyzed that were positive for coliforms.*

*2Percentage of samples analyzed that were positive for *E. coli.*
liform and *E. coli* prevalence were in agreement with the results of this study’s findings of no significant differences (Ailes et al., 2008; Allen et al., 2013; Phillips and Harrison, 2005).

Even though there is an increase in organic sales especially for produce, there are still hurdles for the organic production system to overcome when competing against conventionally grown produce (Batt and Giblett, 1999; Greene, 2012). Prohibition of synthetic chemicals is one main cause of the increased production cost of organic produce due to the high cost of non-synthetic pesticides, herbicides, and fungicides. Pests and diseases have the potential to be more devastating to organic compared to conventionally grown crops. The ability to produce comparable nutritious and safe fruits and vegetables of high quality comes at a premium for producers that is passed on to consumers.

This study provides general microbial results comparing conventional and organic RTE leafy greens at the point of consumer purchase at the retail level. The results suggest higher microbial loads for organic products (~0.4 log CFU/g higher aerobic mesophilic microorganisms, 20% higher total coliforms and a single positive sample for *E. coli* indicating unsanitary processing or fecal contamination.) Statistically, organic RTE spinach and arugula appear to have no significant difference of aerobic mesophilic microorganisms, total coliforms, and *E. coli* when compared to conventional RTE spinach and arugula. Due to a slight trend of increased aerobic mesophilic microbial load with a *P* value close to 0.05 and the presence of *E. coli* attributed to the sampled organic products, more rigorous implementation of food safety programs may be needed if not already in effect to reduce risk of contamination at any step of the food production and handling chain. However, further research is necessary to support this claim.

**Literature Cited**


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Rembiałkowska, E. 2007. Quality of plant products from organic agriculture after assembly, Argentina, Germany.


