CHRISTIE ANN PHILLIPS
Comparison of the Microflora on Organically and Conventionally Grown Spring Mix
(Under the Direction of DR. MARK HARRISON)

Due to consumer health interests and diet trends consumption of vegetable salads has grown with sales increasing at 20% per year since 1990. Little data is available comparing the microbial quality of organically grown produce to produce grown by conventional methods. This research determined the microflora composition of conventional and organic spring mix. Spring mix or mesclun, a mixture of multiple salad ingredients, provided by a grower of both conventional and organic produce was evaluated for the presence of total mesophilic and psychrotrophic bacteria, *Escherichia coli* and coliforms, *Listeria monocytogenes*, *Salmonella* spp., yeasts and molds, and lactic acid bacteria. When comparing the size of the populations of each microbial group, washed organic and conventional spring mixes were not significantly different ($P \leq 0.05$). Populations of all the microbial types tested were significantly ($P \leq 0.05$) lower on chlorine washed spring mix than on unwashed spring mix.

INDEX WORDS: Organic, Conventional, Minimally processed vegetables, Salad, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*

COMPARISON OF THE MICROFLORA ON ORGANICALLY AND CONVENTIONALLY GROWN SPRING MIX

by

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CHAPTER 1

INTRODUCTION & LITERATURE REVIEW

In recent years, there has been an increased per capita consumption of fresh and minimally processed vegetables in the United States as well as in other countries. Many people are beginning to understand the health benefits associated with consuming fresh fruits and vegetables on a daily basis. An increase in importation of produce to countries where standards for growing and handling produce may be compromised has resulted in heightened interest in outbreaks of human gastroenteritis that may be attributed to contaminated fresh produce, especially salad vegetables (Beuchat, 1996). Advances in agronomic, processing, preservation, distribution, and marketing technologies allow the produce industry to supply nearly all types of fresh fruits and vegetables for purchase year round. These advanced technologies have also brought an increased risk of human illness associated with a variety of pathogenic microorganisms (Beuchat, 1996).

Contamination of fruits and vegetables with pathogenic microorganisms can occur while growing in fields or orchards, or during harvesting, postharvest handling, processing, and distribution (Beuchat, 1996). Numerous genera of spoilage bacteria, yeasts, and molds, and an occasional pathogen may be present on fresh produce. Several outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables. Bacteria, viruses and parasites that may be present in water used for irrigation or in soil in which produce is grown are capable of causing human disease (Beuchat, 1996). Ready-to-eat salads predominantly contain psychrotrophs such as

Pseudomonas spp. and *Erwinia* spp., in addition to lactic acid bacteria, including *Leuconostoc mesenteroides* (Garcia-Gimeno and Zurera-Cosano, 1997).

Minimally processed vegetables first appeared in the U.S. about 30 years ago and were mostly used for the catering and fast food industries. There is a wide range of minimally processed vegetables (e.g., carrots, types of salads, leek, celery, etc.) but lettuce and chicory are the most highly consumed (Nguyen-the and Carlin, 1994). Minimally processed vegetables may be simply trimmed vegetables (e.g., whole lettuce stripped of its outer leaves) or may consist of trimmed, peeled, sliced/shredded and washed and/or disinfected vegetables (Francis et al., 1999). The main attributes of minimally processed vegetables include the presence of cut surfaces or damaged plant tissues, minimal processing that cannot ensure sterility or microbial stability of the product, active metabolism of the plant tissue and confinement (Nguyen-the and Carlin, 1994). Minimally processed vegetables retain much of their indigenous microflora after processing. A potential safety problem may occur if pathogens form part of this microflora (Francis et al., 1999).

An important cause of losses of unprocessed fruits and vegetables may be the result of postharvest diseases. The presence of cut produce surfaces and high moisture content in the packages increases the risk of microbial spoilage in minimally processed fruits and vegetables. Because most minimally processed fruits and vegetables are consumed without any heat treatment, contamination with foodborne pathogens and their multiplication during storage is also a concern (Nguyen-the and Carlin, 1994).

Minimally processed, cut and packaged lettuce is a convenient food that is popular among consumers (Szabo et al., 2000). Bagged salad may be exposed to a range

of conditions during growth, harvest, distribution and processing which may increase the potential for microbial contamination. U.S. sales of bagged lettuce were over $1 billion in 1996, which was a 10-fold increase in sales since 1991 (Hagenmaier and Baker, 1998).

Spring mix also known, as mesclun is a mixture of multiple salad ingredients. Seasonally a bag of spring mix contains 12-18 items 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.

Farming covers the raising, growing, storing or processing of food (Anonymous, 1997a). Organic farming is a means of food production without the use of synthetic fertilizers or pesticides while conventional methods of farming allow the use of commercial fertilizers and pesticides to produce vegetables and crops. There are many similarities and differences between organic and conventional farming. Both production methods apply practices to reduce the risk of soil erosion, pest problems and effects on the environment. Cultivation use is important in both methods. Greater risk of soil erosion is possible in organic farming, which relies on tillage and cultivation to control weeds. Conventional farmers reduce the risk of soil losses by no till and minimum till methods such as planting the next crop directly into the stubble from the previous crop and using herbicides to control weeds. Conventional farming practices may include combination fungicide/insecticide treatments to protect the seed from soilborne diseases and insects, whereas organic farmers use biological controls and crop rotation. The use of synthetic fertilizers and pesticides as well as genetic engineering of plants is prohibited

in organic production. In order for the crops to be certified organic no synthetic fertilizers and pesticides can be used on certified organic farms for at least three years prior to harvest. Conventional growers implement integrated pest management (IPM) that uses chemical, cultural, biological, and mechanical measures to control weeds and insects.

Many consumers think organic farming means no pesticides are used; however, this is not entirely accurate. Many farmers use pesticides that are naturally occurring (Colberg, 2000). While any pesticide use is discouraged in organic farming systems, a rather wide range of 'biorational' pesticides are permitted for use (Kuepper, 2000). Pesticides permitted for organic farming are grouped into classes: (1) minerals, (2) botanicals, (3) soaps, (4) pheromones, and (5) biologicals. Minerals include sulfur, copper, diatomaceous earth, and clay based materials like Surround®. Botanicals include commercial materials such as rotenone, neem, and pyrethrum, as well as quassia, equisetum, and ryania. Other botanicals like Black-Leaf 40® and strychnine are prohibited in organic production due to their toxicity. There are a number of soap-based products that are effective as insecticides, herbicides, fungicides, and algicides. However, detergent based products are not allowed for crop use in organic farming. Pheromones, usually used to attract species to one another, can be used as a means to confuse and disrupt pests during their mating cycles, or to draw them into traps. Biologicals are one of the fastest growing areas in pesticides developed for organic control of highly problematic pests. *Bacillus thuringiensis* (Bt) formulations can be used for control of lepidopterous pests and Colorado potato beetle (Kuepper, 2000).

Agricultural systems could have been described as ‘organic’ or ‘traditional’ before the introduction of artificial fertilizers in the late 1800s and synthetically compounded pesticides in the 1940s. Since World War II, the majority of farmers use synthetic agricultural inputs to increase productivity and to reduce the impact of pests and diseases (Clarke, 1991). In the market place produce sold by organic or traditional farmers was not differentiated because the industry was small enough for growers and retailers to know each other on a personal basis. Before the 1980s only a small number of retail outlets specialized in organic produce requiring little need for standards.

There is no worldwide acceptable definition for organic agriculture (Clarke, 1991). According to the U.S. Department of Agriculture’s National Organic Program and its interpretation of the Organic Foods Protection Act, the definition for organic production is a production system that is managed in accordance with the Act and regulations to respond to site-specific conditions by integrating cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity (Anonymous, 2000f). For some people organic agriculture can be thought of as a philosophy or way of life (McLaurin, 2001). Organically produced food is preferred by consumers because of the perceived health attributes, and the concern about pesticide residues, the environment (such as soil and water quality and wildlife habitat), and farm worker safety (Greene and Glaser, 2001a).

During the 1990’s organic farming became one of the fastest growing segments of U.S. agriculture (Greene, 2000). In the 1990’s, organic vegetable production in California grew in large part to escalating nationwide demand for organically grown fresh fruits and vegetables. Due to overseas demands, California is exporting organic

vegetables in increasing numbers (Gaskell et al., 2000). Producers, exporters, and retailers are still struggling to meet consumer demand for a wide range of organic products (Greene, 2000). The most recent industry estimates reported total organic retail food sales through all outlets to be $7.8 billion in 2000, a 20% increase over 1999 sales (Greene and Glaser, 2001c). Organic farming has had a deeper impact in the fruit, vegetable, and specialty grain sectors than in other farm sectors. In 1997, about 2 percent of apple, grape, lettuce and carrot acreage was organic. In 1997, California farmers grew nearly half of the organic vegetables, using six private groups for certification. Over 4,000 acres of lettuce, about 2,600 acres of carrots, and nearly 2,000 acres of tomatoes were grown in California in 1997 (Greene, 2000). In excess of $95 million from 1,452 California organic fruit and vegetables farms was produced by farm gate sales in 1995. The number of farms had grown by nearly 60 percent to an estimated 2,300 in 1998 (Gaskell et al., 2000).

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. Organic farming systems rely on ecologically based practices such as cultural and biological pest management, and nearly exclude the use of synthetic chemicals in crop production. The fundamental components and natural processes of ecosystems, such as soil organism activities, nutrient cycling, and species distribution and competition, are used to work directly and indirectly as farm management tools. Planting and harvesting dates are carefully planned and crops are rotated, habitat needs for food and shelter are provided for predators and parasites of crop

pests, and animal and green manure are cycled in organic crop production systems (Greene, 2000).

In the early 1970's, mostly nonprofit, private organizations began developing certification standards as a way to support organic farming and hinder consumer fraud. In the late 1980's, some states began offering organic certification services. The Organic Foods Production Act (OFPA) of 1990 was passed to establish national standards for organically produced commodities. This federal legislation required that all except the smallest organic farmers must be certified by a state or private agency qualified under national standards developed by USDA (Greene, 2000). USDA announced the final national standards for the production, handling, and processing of organically grown agricultural products on December 20, 2000 (Greene and Glaser, 2001; Anonymous, 2000f).

The new labeling requirements apply to raw, fresh products and processed foods that contain organic ingredients. Foods sold, labeled, or represented as organic will have to be produced and processed in accordance with the NOP standards. All farm and processing operations that grow and process organic foods and whose gross agricultural income from organic sales totals $5,000 or more must be certified by USDA accredited certifying agents (Anonymous, 2000c).

The percentage of organic ingredients in a product determines how it is labeled. Food products labeled as "100 percent organic" must contain, excluding water and salt only organically produced ingredients. Foods that are labeled "100 percent organic" and "organic" cannot be produced using excluded methods, sewage sludge, or ionizing radiation. At least 95 percent of the ingredients must be organically produced (except

water and salt) for products to be labeled “organic”. The remaining ingredients must consist of nonagricultural substances approved on the National List of non-organically produced products that are not commercially available in organic form (Anonymous, 2000c).

Processed products labeled “made with organic ingredients” contain at least 70 percent organic ingredients. The principal display panel of the product may list up to three of the organic ingredients or food groups. These processed products also cannot be made using excluded methods, sewage sludge or ionizing radiation. The USDA seal cannot be used anywhere on the package. Processed products that contain less than 70 percent organic ingredients cannot use the term organic anywhere on the principal display panel. They may, however, identify the specific ingredients that are organically produced on the ingredients statement on the information panel (Anonymous, 2000c).

Organic produce costs more due to the intensive labor involved and generally produces lower yields than traditional chemical farming (Scarpa, 1994; Geier, 1998). The goal is more oriented towards ‘optimum’ yields, which may give an average 20% less return. Organic farmers strive towards ‘optimum’ yields rather than maximum yields because maximum means more chemical input and costs, which are not normally paid back. Due to increased production costs and lower yields consumers pay a price premium of up to 30% more for organically grown foods compared to conventional foods (Anonymous, 1997a).

Organic produce will not always look as good as conventionally grown produce due to imperfections such as wormholes, odd shapes or unappealing skin (Applegate, 2001). Conventional farming has raised our expectations as to what fresh, good quality

produce should look like. However, in blind taste tests, consumers generally cannot differentiate between conventionally and organically grown food (Anonymous, 1997a). Many advocates currently market organic produce as pure and healthy. Conventionally grown products are equally safe and nutritious due to strict regulation and guidelines (Anonymous, 1997a).

There are several alternatives for adding fertility to organically grown crops (Anonymous, 1997a). Composted manure use is one popular choice. The cultivation of legumes or other deep-rooting plants (cover crops) may also be used to provide fertilizer to the organic farming system.

Manures are plant and animal waste products that are recycled by returning them to the soil. Manures are used to supply nutrients and organic matter to the plant that cannot be obtained from soil alone. Compared to traditional fertilizers, the concentration of nutrients in manures is very low. One ton of traditional farmyard manure made with straw supplies similar amounts of nutrients to only 50-100 kg of a modern concentrated compound fertilizer. Manures typically contain large amounts of water, as much as 95 percent and farmyard manures contain about 75 percent. Due to the large water content and the low concentration of plant nutrients in the manures large quantities are needed to supply an appreciable part of the nutrient requirements of the plant. The organic matter naturally present in manures is attacked and transformed by microorganisms when returned to the soil. The carbon present is converted to carbon dioxide and makes no long-term contribution to the organic matter content of the soil. Other parts of the organic matter are converted to humus, which remains in the soil. Humus is a black or dark brown, colloidal, very complex organic material, which is a very valuable soil

component. It increases the ability to hold water available to the plant and reduces the leaching of nutrients through its very high cation-exchange capacity (Simpson, 1986).

All manures play a role in long-term soil fertility and the maintenance of humus in the soil. However, very large amounts of manures need to be applied to have substantial long term effects on the organic matter content of the soil. This is necessary due to the very high water content of many manures and the loss of a great deal of the organic matter during decomposition in the soil (Simpson, 1986).

Composting is a biological process in which microorganisms convert organic materials such as manure, sludge, paper, food wastes and leaves into a soil-like material called compost. The composting process produces heat that drives off moisture and destroys pathogens (Rynk et al., 1992).

Microorganisms play a major role in the composting process. A diverse group of microorganisms from bacteria and fungi to actinomycetes helps to keep the composting process from collapsing when conditions change. All three groups of microorganisms that participate in composting have thermophilic and mesophilic species. In composting, bacteria are the most numerous organisms and are generally faster decomposers than other microbes. Fungi are more tolerant of low-moisture and low-pH conditions than bacteria but are less tolerant of low-oxygen environments. They are also better at decomposing woody substances and other decay-resistant materials. Actinomycetes form filaments like fungi but they are technically classified as bacteria. They are primarily aerobic, like fungi and have low tolerance for acidic conditions. In the early stages of composting, bacteria tend to flourish, before the easily degraded materials are consumed. Near the end of composting fungi and actinomycetes become more important, feeding on

the resistant materials that remain. Bacteria will continue to dominate as long as conditions remain favorable for composting (Rynk et al., 1992).

Thermophilic organisms become more active as the compost pile heats. At temperatures above 71^{o}C, nearly all active organisms die, leaving only heat-resistant spores. As the pile cools, sporeformers, thermophilic populations, and then mesophilic organisms recover. As time goes by the pile becomes cool enough to be inhabited by common soil microorganisms, protozoa, worms, mutes, insects and other large organisms that feed upon microorganisms and organic matter. Plant, animal and human pathogens can be found in manures, sewage sludge and crop residues. The elevated temperatures achieved during composting, along with competition and antagonism among the microorganisms considerably reduces the number of pathogens. Some pathogenic organisms may survive and grow in the cooler sections of the pile, but the overall risk is greatly reduced (Rynk et al., 1992).

Postharvest Handling:

Good Agricultural Practices (GAP) need to be developed and formalized to minimize the risk of a variety of hazards or contaminants: chemical, physical (e.g., sand and soil, wood, plastic or metal shards), and biological (e.g., *Salmonella*, *Listeria*). Prior land use, adjacent land use, water source and method of application, fertilizer choice (such as the use of manure), compost management, equipment maintenance, field sanitation, movement of workers between different operations, personal hygiene, domestic animal and wildlife activities all have the potential to adversely impact food safety (Suslow, 2000).

Postharvest handling of produce should include preventive food safety programs, sanitation of equipment and food contact surfaces, and waste water disinfection. Produce handlers at all degrees of production should be concerned about food safety and decay/spoilage control. *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Listeria*, *Cryptosporidium*, Hepatitis A, and *Cyclospora* have been associated with fresh fruits and vegetables. Poor or unsanitary postharvest practices such as nonpotable cooling water and ice have been implicated as causes for several cases of foodborne illness (Suslow, 2000).

The currently recommended means for consumers to reduce microbial contamination on raw fruits and vegetables is washing with tap water. Washing produce in tap water may have some effectiveness in removing soil and other debris however; it should not be relied upon to completely remove microorganisms. Tap water has limited or no effect on killing microorganisms ranging from 10^3 to 10^9 CFU/g on raw and minimally processed produce (Beuchat et al., 1998).

There are several types of treatment known to be partially effective in removing disease-causing organisms from the surface of whole and cut raw fruits and vegetables or from contact surfaces during handling. None of these treatments can be relied upon to totally disinfect produce, especially when used at levels that will not cause deterioration in sensory quality (Beuchat, 1998). Chemical and physical treatments should be considered as methods of disinfection, causing reductions in populations of microorganisms but not always producing pathogen free fruits and vegetables (Beuchat, 1998).

There are various types of disinfectants used to disinfect produce, each with its own success in killing microbial cells. The effectiveness of each disinfectant depends on the nature of the cells as well as the characteristics of fruit and vegetable tissues and juices. The mechanism of action of many disinfectants on microbial cells is poorly understood (Beuchat, 1998).

The proper use of a disinfectant in postharvest wash and cooling water can help prevent both postharvest diseases and foodborne illnesses. According to existing organic standards all forms of chlorine (e.g., liquid sodium hypochlorite, granular calcium hypochlorite, and chlorine dioxide) are controlled materials. The California Certified Organic Farmers (CCOF) has recently modified the threshold of residual chlorine allowable for use. Due to food safety concerns a maximum of 10 ppm residual chlorine measured downstream of the wash step is allowable by CCOF (Suslow, 2000).

Sodium hypochlorite is the only practical chemical sanitizer allowed for washing fruits and vegetables according to Title 21 of the U.S. Code of Federal Regulations (Beuchat et al., 1998). A maximum of 0.2% (2,000 ppm) hypochlorite can be used in wash water; however, this concentration is not typically used as a sanitizer in commercial settings, but is used to assist in the lye peeling of fruits and vegetables. On a commercial scale water containing 50 to 200 ppm of chlorine is commonly used to sanitize whole fruits and vegetables as well as fresh cut produce with a contact time of 1-2 minutes (Beuchat, 1998; Beuchat et al., 1998). The number of viable pathogenic bacteria on produce and contamination of food preparation areas may be minimized by washing with chlorinated water (Beuchat et al., 1998).

Liquid sodium hypochlorite is most commonly used for both organic and conventional operations. The pH of the water must be adjusted to between 6.5 and 7.5 for optimum antimicrobial activity using a minimum concentration of chlorine. Most of the chlorine is in the form of hypochlorous acid (HOCl) at this pH range, which dispenses the highest rate of microbial death and minimizes the release of irritating and potentially hazardous chlorine gas (Cl_2). If the water is too acidic chlorine gas will surpass safe levels (Suslow, 2000). It is very important to maintain, adequate chlorine in wash water; otherwise it can actually lead to increased microbial populations on produce (Nguyen-the and Carlin, 1994). In organic production, products used for pH adjustment must be from a natural source such as citric acid, sodium bicarbonate, or vinegar (Suslow, 2000).

A pH of 6.0-7.5 is most appropriate for effective sanitizing activity without damaging equipment surfaces. Water temperature of 4^{o}C achieves maximum solubility of chlorine. The temperature of the chlorinated water should ideally be at least 10^{o}C higher than that of fruits and vegetables being disinfected. This will minimize the uptake of wash-water through stem tissues and open areas in the skin or leaves (Beuchat, 1998).

Adding 100 ppm of free chlorine to water used to wash lettuce leaves has been reported to reduce populations of aerobic mesophiles by more than 98% as compared to a 93% reduction using tap water alone. The microbial numbers did not decrease significantly when the washing time in chlorine solution increased from 5 to 30 min. However, extended washing in tap water resulted in a reduction comparable to that obtained with chlorinated water (Beuchat et al., 1998). Lettuce treated with 200 ppm chlorine for 10 min had a maximum log reduction of *L. monocytogenes* 1.3 $\log_{10}$ CFU/g at 4^{o}C and 1.7 $\log_{10}$ CFU/g at 22^{o}C (Zhang and Farber, 1996). Populations on shredded

lettuce inoculated with 3.60 $\log_{10}$ *Salmonella baildon* were reduced by less than 1 log when immersed for 40 s in a 20 or 200 ppm free chlorine solution (Weissinger et al., 2000).

Ozone is another option for water disinfection and other postharvest applications. Ozonation is faster acting than permissible concentrations of chlorine. It is a powerful oxidizing treatment and is effective against microbes and foodborne pathogens resistant to chlorine. One disadvantage of ozone is that it must be generated on-site at the time of use and has a very low stability, as short as 20 min. Capital and operating costs are also higher for ozone than for chlorine (Suslow, 2000).

Two other options for sanitation and water disinfection are food-grade hydrogen peroxide (0.5 to 1%) and peroxyacetic acid. Peroxyacetic acid (PAA) has very good performance, compared to chlorine and ozone, in removing and controlling microbial biofilms in dump tanks and flumes. However, the disadvantage of PAA is a higher cost per unit and availability limited to large bulk units (Suslow, 2000). Other possible disinfectants for fruits and vegetables include chlorine dioxide (ClO_2) and trisodium phosphate (TSP) (Beuchat, 1998).

Due to postharvest food safety concerns, the use of incompletely composted animal manure in organic production is prohibited. According to current organic regulations a waiting period of 120 days is necessary between the date the composted manure is applied to the soil and the date a crop intended for human consumption is planted. Composting reduces the potential for inhibition of plant growth that is often associated with the use of raw manure. If properly composted manure is applied directly to growing vegetable crops there is little concern. Composting enables the degradation of

many if not most organic components however, it cannot eliminate heavy metals. Heavy metals become concentrated during composting, which is a concern with sewage sludge (biosolids). In the past biosolids were frequently used in manures, however, they are currently prohibited from use in the new organic regulations due to this reason and the threat of the presence of pathogenic bacteria (Suslow, 2000).

The foundation of successful organic vegetable crop production lies in soil quality or health. Soil functions to sustain crop productivity, maintain environmental quality, and provide for plant, animal, and human health as well as being the main medium for crop growth. Sustaining and improving soil quality over the long term is very important to consider as a primary management goal. Soil quality and soil health describe the soil's ability to perform the important functions necessary for survival of crops (Mitchell et al., 2000).

A primary attribute of soil quality assessment is soil organic matter (SOM) content. SOM influences soil properties such as infiltration rate, bulk density and biological activity. SOM serves as a reservoir for plant macronutrients, specifically nitrogen, and also aids in plant micronutrient nutrition. It is very important in facilitating water and air infiltration into the soil, as well as increasing water retention by the soil, and maintaining soil tilth (Mitchell et al., 2000). Soil tilth is the state of aggregation of the soil (Faught, 1996). However, when large quantities of fresh organic matter, such as that found in animal manure, are added to the soil, the stimulation of plant pathogenic organisms and seed and seedling pests such as cabbage maggots and wireworms can cause serious damage and losses to the crops (Mitchell et al., 2000).

In California, cover cropping (also called green manuring) is widely seen as a valuable part of soil quality management in organic production systems. The terms cover crops and green manure are often used interchangeably. However, according to Kluchinski (1996) and Thurston et al. (1996) theses terms are not identical. A cover crop is grown for the purpose of covering and protecting the soil. A green manure is a plant or crop, which is turned under or incorporated into the soil while green, or soon after flowering, in order to enrich the soil. Before the development of synthetic fertilizers and pesticides, cover and green manure crops were used extensively. Green manuring has been traced back to the days of Cato (234-149 B.C.) (Marciak, 2000), where a legume crop was grown and turned under on poor vineyard land. The recent awareness in sustainable agriculture, intensifying concerns about increasing atmospheric CO_2 levels, global warming, and the potential roles that carbon removal in the soil may have in justifying the green house effect may also result in farmers reconsidering the use of these cover crops (Kluchinski, 1996; Mitchell et al., 2000). In California, there is growing interest in the use of cover crops to store carbon and improve resource efficiencies in organic growing systems (Mitchell et al., 2000).

There are both benefits and disadvantages to using cover and green manure crops (Porter, 1999 and Mitchell et al., 2000). Cover crops and green manure crops can provide a practical and economical means for supplying organic matter to the soil by enhancing soil fertility, preventing soil erosion by water and wind, attracting beneficial insects, spiders, and predatory mites, and reducing nitrate leaching losses to groundwater during periods between crops (Kluchinski, 1996; Mitchell et al., 2000; Porter, 1999). However, cover crops and green manure may critically limit a grower's option for

planting and harvesting alternative main cash crops. The utilization of cover crops may also result in potentially harmful consequences such as soil moisture depletion, temporary immobilization of plant nutrients, increased pest problems and elevated management and associated costs.

Legume green manuring is becoming popular among farmers. Legume green manuring is the most beneficial of all cover crops because legumes fix nitrogen from the atmosphere and convert it into a form that is available to other plants (Marciak, 2000). Legumes such as alfalfa, clovers, field peas and vetch can be planted with small grains. Some other examples of cover crops and green manure crops include ryegrass planted into corn, rye planted into corn at harvest, buckwheat planted into corn at harvest and buckwheat planted before soybeans (Porter, 1999).

Total Mesophilic and Psychrotrophic Bacteria:

Raw vegetables contain microorganisms prior to entering the processing chain. Some processing procedures may increase the number of mesophilic bacteria: shredding and slicing were found to increase counts from 10^3-10^4 to 10^5-10^6 CFU/g for a variety of vegetables and from 10^4 -10^5 to 10^6 CFU/g for lettuce and chicory salads. Mesophilic bacterial counts on plate count agar are highly variable and range from 10^3 to 10^9 CFU/g. Although these counts were quite high, the product quality is often acceptable (Nguyen-the and Carlin, 1994). Mesophilic bacteria counts at the beginning of storage may be a more useful indicator of storage stability than counts when the product is spoiled. The proportion of decayed leaf pieces in samples of shredded chicory salads ranged from 5 to 40% after 10 days of storage at 10^oC; however, mesophilic bacteria counts did not differ significantly among the samples (Nguyen-the and Carlin, 1994).

Minimally processed vegetables are typically stored at refrigerated temperatures that may select for psychrotrophic microorganisms. Initial counts of psychrotrophic bacteria could represent less than 1% of the mesophilic bacteria counts in some samples (Nguyen-the and Carlin, 1994). Garcia-Gimeno and Zurera-Cosano (1997) found initial counts of psychrotrophic bacteria to be 1.07×10^5 CFU/g in salads stored at 4^oC. Marchetti et al. (1992) sampled throughout a year mixed salads for total psychrotrophic counts and found levels ranging from 6.23-8.51 $\log_{10}$/g. Some psychrotrophic bacteria present in minimally processed produce may be pathogenic such as *L. monocytogenes* (Szabo et al., 2000). Temperature abuse of chilled produce during storage will permit more rapid growth of *L. monocytogenes* (Francis et al., 1999).

Lactic Acid Bacteria:

Lactic acid bacteria (LAB) are responsible for the fermentative preservation of many foods, particularly fruits and vegetables (Breidt and Fleming, 1997). LAB species can produce a variety of metabolites, which lower pH, which is inhibitory to competing bacteria, including psychrotrophic pathogens. Lactic acid bacteria are generally much more resistant to low pH than other bacteria. The pH range for lactic acid bacteria is 3.2-9.6 with an optimum pH being in the range of pH 4.0-4.5.

There is little data on vegetable products and the interactions between pathogens and background microflora. Lactic acid bacteria, which are normal microflora present on produce, have antimicrobial effects due to their ability to lower pH, generate hydrogen peroxide, competing for nutrients, and producing antimicrobial compounds such as bacteriocins and antibiotics. Lactic acid bacteria (LAB) are naturally present on vegetables in low numbers. However, they can reach high counts on some ready-to-eat

products, in particular products with modified atmosphere packaging containing high CO_2 concentrations, where they may grow faster than the aerobic spoilage bacteria (Francis et al., 1999).

The numbers of lactic acid bacteria found on samples of minimally processed vegetables differ among authors. Lactic acid bacteria enumerated on MRS under anaerobic conditions had counts reaching 10^9 CFU/g in some cases, but were usually lower than the counts for mesophilic bacteria (Nguyen-the and Carlin, 1994). Garcia-Gimeno and Zurera-Cosano (1997) found counts of 8×10^2 CFU/g for lactic acid bacteria in packaged salads, while Marchetti et al. (1992) found counts of 10^5-10^7 CFU/g of lactic acid bacteria. Lactic acid bacteria isolated from minimally processed produce include heterofermentative LAB such as *Leuconostoc* spp. and *L. mesenteroides*, as well as homofermentative LAB.

Yeast and Molds:

Another method commonly used to indicate food quality is yeast and mold count. When conditions for bacterial growth are less favorable yeasts and molds frequently become predominant on foods (Doyle et al., 1997). Yeast and mold counts are usually less than mesophilic or lactic acid bacteria (Nguyen-the and Carlin, 1994). Yeast and mold counts from sampled minimally processed vegetables ranged from 10^4-10^8 CFU/g. Marchetti et al. (1992) reported 10^4-10^5 CFU/g of yeast in packaged salads.

***Listeria monocytogenes*:**

Listeriae are gram positive, nonsporeforming rod shaped bacteria consisting of six species (Jay, 1996). The genus *Listeria* belongs to the *Clostridium* subbranch collectively with *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Brochothrix*.

Listeria monocytogenes is considered a public health concern because of the severity and the nonenteric nature of the disease (meningitis, septicemia and abortion), a high case-fatality rate (around 20 to 30% of cases), a frequently long incubation time, and a tendency for immunocompromised individuals who have underlying conditions to become infected. Serious forms of listeriosis have an unknown onset time but it may range from a few days to three weeks. Gastrointestinal symptoms probably appear after 12 hours (FDA/CFSAN, 2001). Most healthy persons do not show any symptoms. *Listeria monocytogenes* is different from most other foodborne pathogens because it is ubiquitous, resistant to diverse environmental conditions such as low pH and high NaCl concentrations, and is microaerobic and psychrotrophic (Jay, 1996).

Listeriosis is the illness caused by *L. monocytogenes*. Symptoms of listeriosis are similar to the flu with occasional gastrointestinal illness. Severe infections can cause meningitis, septicemia, encephalitis and intrauterine infections. Most healthy people do not become ill from exposure to *L. monocytogenes*, but there are several groups who are at risk for listeriosis. Pregnant women are 20 times more likely to contract listeriosis than other healthy adults. People who are immunocompromised, including the elderly, cancer and AIDS patients, diabetics, and people suffering kidney disease are also at high risk for listeriosis. A person with AIDS has a 300 times greater chance of contracting listeriosis than a normal healthy person. Susceptible populations have a mortality rate of 20 to 40%. Every year approximately 1800 people contract listeriosis, 425 of which die (Forgey, 1999).

Listeria monocytogenes is commonly found in the environment, being isolated from soil, feces, sewage, silage, manure, water, mud, hay, animal feces, dust, birds,

animals and humans. Because *L. monocytogenes* occurs widely in soil and in the agricultural environment, it is present naturally on many vegetables. Agricultural practices such as irrigation of vegetables with polluted water or fertilization with contaminated manure may lead to contamination with *L. monocytogenes* (Francis et al., 1999). It is important to practice proper silage production techniques and minimize the use of untreated animal waste as fertilizer (Forgey, 1999). *L. monocytogenes* is carried and multiplied by domestic animals and is abundant in soils (Nguyen-the and Carlin, 1994). Minimally processed vegetables and salads may harbor psychrotrophic microorganisms such as *L. monocytogenes*. Soil and environmental samples show that *L. monocytogenes* can survive for several months. Bacteria could definitely be present on crops at harvest time in fields that were contaminated by fertilizer before sowing (Nguyen-the and Carlin, 1994). It may be difficult to eliminate contamination completely. However, eliminating pollution of vegetable fields by waste, water, sewage, or manure would greatly reduce the incidence of *L. monocytogenes*.

The first documented foodborne outbreak of listeriosis occurred in Canada in 1981 and involved 34 perinatal cases and seven adults. The vehicle of transmission was coleslaw with stored cabbage being the source of *L. monocytogenes* (Francis et al., 1999).

***Salmonella*:**

In recent years, fruits and vegetables have received an unsavory reputation as vehicles of human salmonellosis. Consumer demand for fresh produce year round has led to increased global export of fresh and dehydrated fruits and vegetables from countries that enjoy tropical and subtropical climates. The minimum standards for hygiene during production, harvesting and distribution of products in these countries does

not always occur, leading to product contamination. The fertilization of crops with untreated sludge or sewage effluents potentially contaminated with antibiotic resistant *Salmonella* spp., the irrigation of garden plots and fields and the washing of fruits and vegetables with contaminated waters, and the repeated handling of products by local workers are areas that undermine product safety. The bacterial quality and safety of frequently ready-to-eat products could be enhanced by irrigation of fields with treated effluents, washing fruits and vegetables with disinfected waters and education of local workers on the hygienic handling of fresh produce (D'Aoust, 1997).

Salmonella spp. are gram-negative nonsporeforming rods belonging to the *Enterobacteriaceae* family. They are facultative anaerobes, which have an optimum growth temperature of 37^{o}C, a maximum growth of = 54^{o}C and a minimum growth temperature of 2 to 4^{o}C. *Salmonella* spp. can readily adapt to extreme environmental conditions, which is reflected in their growth at a wide temperature range (D'Aoust, 1997).

Salmonellae are widely distributed in nature, with humans and animals being their primary reservoirs. *Salmonella* spp. primarily reside in the intestinal tract of animals such as birds, reptiles, farm animals, humans, and occasionally insects (Jay, 1996).

Several clinical conditions can result from human *Salmonella* infections, including enteric (typhoid) fever, uncomplicated enterocolitis, and systemic infections by non-typhoid microorganisms. Typhoid fever is a serious human disease linked to the typhoid and paratyphoid strains. Symptoms of enteric fever appear after a period incubation ranging from 7 to 28 days and may include diarrhea, headache, and prostration (D'Aoust, 1997).

Human infections with non-typhoid salmonellae result in enterocolitis, which appears 8 to 72 hours after contact with the invasive pathogen (D'Aoust, 1997). Symptoms of *Salmonella* food poisoning include nausea, vomiting, abdominal pain, headache, chills, and diarrhea usually accompanied by prostration, muscular weakness, faintness, moderate fever, restlessness, and drowsiness (Jay, 1996). These symptoms usually persist for 2-3 days if proper treatment of fluid and electrolytes are given (D'Aoust, 1997).

Salmonella spp. are one of the leading cause of foodborne disease in humans (D'Aoust, 1997). There have been multiple outbreaks in recent years involving raw fruits and vegetables. In 1991, there was an outbreak of *Salmonella* Poona in the U.S. and Canada involving cantaloupe, which had 245 illnesses and 2 deaths. Another outbreak of *Salmonella* Poona involving cantaloupe occurred in the U.S. and Canada in 2000. There were 39 illnesses with no deaths. In 1990, *Salmonella* Javiana sickened 176 people throughout the U.S. who consumed contaminated tomatoes. Another outbreak in 1993 in the U.S. sickened 100 people with *Salmonella* Montevideo. In 1998-1999, *Salmonella* Baildon contaminated tomatoes throughout the U.S. and 85 people became ill with 3 people dying (D'Aoust, 2001).

***Escherichia coli*:**

Coliforms are gram-negative asporogeneous rods that ferment lactose within 48 hours. Coliforms are represented by four genera of the family Enterobacteriaceae: *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella* (Jay, 1996). Coliforms are nonpathogenic, gram-negative bacteria that grow well on a large number of media and in many foods. Growth in foods is poor or very slow at 5^{o}C. Growth temperatures as low

as –2°C and as high as 50°C have been reported. Coliforms have been shown to grow at a pH range of 4.4-9.0.

Most market vegetables harbor small numbers of lactose-fermenting, gram-negative rods of the coliform type, but if these products have been harvested and handled properly, the numbers tend to be quite low and do not pose a threat to public health (Jay, 1996). It is often desirable to determine the incidence of *E. coli* in a coliform population since it is more indicative of fecal contamination than the other genera and species noted.

In 1982, *Escherichia coli* O157:H7 was first identified as a foodborne pathogen and is now recognized as a significant cause of foodborne illness (Wright et al., 2000). Each year in the United States an estimated 73,000 cases of infection and 61 deaths occur (Anonymous, 2001e). It is estimated that human *E. coli* infections costs the U.S. between $400 and $900 million annually in medical costs and lost productivity (Jones, 1999).

Cattle are the primary reservoir for *E. coli* serotype O157:H7 with the pathogens able to reside in the gut with little observable effect. However, in the human gut this strain is highly infectious because it has the ability to colonize the intestine and produce lethal toxins. A very low infectious dose with the ingestion of only 10 to 50 cells for full symptoms to develop is possible for establishment of *E. coli* O157 in the human intestine (Jones, 1999).

Hemorrhagic colitis (bloody diarrhea), hemolytic uremic syndrome (HUS), non-bloody diarrhea and thrombotic thrombocytopenic purpura (TTP) are possible clinical symptoms of infection with *E. coli* O157. The most common symptom is hemorrhagic colitis, which typically appears 1 to 5 days after oral ingestion, with most patients recovering after 10 days. In a small percentage of immunocompromised patients (10%)

especially young children, pregnant women and the elderly, the infection can result in life-threatening complications such as HUS and TTP (Jones, 1999). It is possible for people to become infected but not experience any symptoms (Doyle et al., 1997).

E. coli O157:H7 enters the intestines after oral consumption where it attaches firmly to the intestinal mucosa and produces Shiga-like toxins. The walls of the intestines become porous allowing additional toxin to enter the bloodstream and induce HUS. Subsequent damage to the red blood cells results from the toxins, necessitating blood transfusions in more than 70% of HUS cases. Kidney damage occurs resulting in about 50% of HUS patients to suffer acute kidney failure and require dialysis. After the onset of infection, the excretion of *E. coli* O157 in feces of infected patients usually lasts between 60 and 120 days (Jones, 1999).

E. coli O157:H7 is able to remain viable in soil for greater than 4 months and appears to be a highly durable pathogen possessing the capability to adapt easily to environmental stresses. Although most human cases of *E. coli* O157 food poisoning have been related to the consumption of contaminated meat and dairy products, there is also evidence that human infection has occurred through the ingestion of contaminated soil, fruits and vegetables and drinking water (Jones, 1999).

There is a possibility for contamination of raw salad vegetables with *E. coli* O157:H7 during the assembling of ready-to-eat meals, which also include beef or other potential carriers of the organism and in preparation kitchens of food service establishments. There is also the possibility of *E. coli* O157:H7 being present on raw vegetables that were irrigated with contaminated water (Abdul-Raouf et al., 1993). Following irrigation with polluted water *E. coli* survived more than 21 d on lettuce in the

field (Nguyen-the and Carlin, 1994). In 1996, there was an *E. coli* O157:H7 outbreak involving leaf lettuce in two U.S. states causing 49 people to become ill (Guzewich et al., 2001).

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CHAPTER 2

COMPARISON OF THE MICROFLORA ON ORGANICALLY AND CONVENTIONALLY GROWN SPRING MIX[1]

[1]Phillips, C.A. and M.A. Harrison. To be submitted to J. Food Prot.

INTRODUCTION

In recent years, consumption of vegetable salads has increased significantly due to the health interest and diet trends of consumers. Contamination of fruits and vegetables with pathogenic microorganisms can occur while growing in fields or orchards, or during harvesting, postharvest handling, processing, and distribution. Minimally processed, cut and packaged lettuce is a convenient food that is popular among consumers (Szabo et al., 2000). Bagged salad may be exposed to a range of conditions during growth, harvest, distribution and processing which may increase the potential for microbial contamination.

Several outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables (Beuchat, 1996). There have been multiple outbreaks of *Salmonella* spp. in recent years involving raw fruits and vegetables (D'Aoust, 2001). In 1996, there was an *Escherichia coli* O157:H7 outbreak involving leaf lettuce in two U.S. states causing 49 people to become ill (Guzewich et al., 2001).

Demand for organic food has grown steadily since 1990, with sales of organic food growing about 20% per year. The most recent industry estimates has reported total organic retail food sales through all outlets to be $7.8 billion in 2000, a 20% increase over 1999 sales (Greene and Glaser, 2001c). In December 2000, the final regulations for organically grown agricultural products were released. This rule lists methods, practices, and substances that can be used in producing and handling crops so they can be labeled organic (Anonymous, 2000f). 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. There are many similarities and differences between organic and conventional farming.

There have been numerous studies questioning nutritional value, pesticide use and consumer acceptability of organic produce versus conventionally grown produce (Batt and Giblett, 1999; Klonsky, 2000; Kuchler et al., 2000). However, little data is available to address the question of microbial quality of organically grown produce compared to 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 the research was to determine the composition of the microflora of conventional and organic spring mix.

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 24, 2001 to August 16, 2001 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 Styrofoam coolers with icepacks and shipped them overnight to the University of Georgia Department of Food Science and Technology. The majority of samples were analyzed microbiologically within 24 h of obtainment for all sampling dates.

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) and blended using a lab stomacher (Tekmar model 4000; Cincinnati, OH) 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 (PCA; Difco, Detroit, MI) plates, which were incubated at 37^{o}C for 48 h before colony forming units (CFU), were counted. To enumerate total aerobic psychrotrophic bacteria, another set of PCA plates were prepared and incubated at 7^{o}C for 7 d before colonies were counted (Vanderzant et al., 1992). Coliforms and *Escherichia coli* were enumerated by plating duplicate 1.0 ml samples of appropriate dilutions onto 3M Petrifilm ™ *E. coli*/Coliform Count Plates (3M, St. Paul, MN). The plates were incubated in stacks of no more than 20

for 24-48 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 (DRBC) Agar plates (Oxoid; Basingstoke, Hampshire, England), which were incubated upright at 25°C for 3-5 days before colonies were counted.

Enrichment procedures were done to detect the possible presence of *Listeria monocytogenes* and *Salmonella*. One ml was removed from each stomached sample and placed in a 9 ml tube of *Listeria* enrichment broth (LEB) (UVM Formulation) (Oxoid; Basingstoke, Hampshire, England). The tubes were incubated for 24 h at 28°C. *Listeria* Selective Agar base with *Listeria* selective supplement (LSA) (Oxford Formulation SR 140) (Oxoid; Basingstoke, Hampshire, England) was used for isolating and differentiating *L. monocytogenes*. After streaking, these plates were incubated for 48 h at 37°C. Suspect colonies were identified using a Remel Micro-ID® *Listeria* System (Remel Inc., Lenexa, KS).

One ml of each stomached sample was placed in a 9 ml tube of lactose broth and incubated at 37°C for the pre-enrichment for *Salmonella.* After 24 h, 1 ml of the lactose broth was inoculated into 10 ml tubes of tetrathionate (TT) broth base, Hajna (Difco) and selenite cystine (SC) broth (Difco) for selective enrichment. These tubes were incubated at 37°C for 24 h. Three-mm loop portions from the SC and TT enrichments tubes were streaked for isolation onto duplicate bismuth sulfite agar (Difco) and XLD Agar (Difco) plates. The plates were incubated inverted at 37°C for 24-48 h before examining for typical *Salmonella* presumptive colonies. Triple sugar iron (TSI) agar (Difco) and lysine iron agar (LIA) (Difco) tube slants were used for biochemical screening of presumptive

Salmonella colonies. These tubes were incubated for 24 h at 37°C (Vanderzant et al., 1992). Any presumptive positive LIA and TSI tubes were further analyzed using a Remel MICRO-ID for the rapid determination of *Enterobacteriaceae*.

After removing portions of each sample and handling as described above, 41 ml of concentrated (4X) 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 manually shaking. Serial 10-fold dilutions were prepared in lactobacilli MRS broth (Difco) and duplicate 1.0 ml samples of appropriate dilutions were spread onto Petrifilm ™ Aerobic Count Plates (3M), for the enumeration of lactic acid bacteria as per manufacturer's instructions. Healthy colony growth was achieved by incubating Petrifilm plates anaerobically in a BBL ® GasPak ® jar (Becton Dickinson, Cockeysville, MD) using 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 using the General Linear Model procedure with sum of square type III (SAS, 1989 to 1996) 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* values of =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 $\log_{10}$ and 5.78 $\log_{10}$ CFU/g, respectively. This suggests that there are no microbial differences between the conventional and organic spring mix provided by the producer irregardless of whether the product was washed or not.

Results from experiments in which all unwashed spring mix was compared to all spring mix washed with 5 ppm 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 significantly (p=0.05) higher than the corresponding populations for washed spring mix. For example, unwashed spring mix had 6.24 $\log_{10}$ CFU/g mesophilic bacteria while washed spring mix was lower at 5.30 $\log_{10}$ CFU/g. There was a 0.62-1.11 $\log_{10}$ CFU/g reduction between the unwashed and washed spring mix for all the microbial parameters measured.

Salmonella and *L. monocytogenes* were not isolated from either the conventional or the organic spring mix. Selective plating produced colonies typical of *Salmonella* spp. 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 *Listeria monocytogenes* isolates from 5 samples were not confirmed as *L. monocytogenes* with additional biochemical testing. Based on the 3M Petrifilm ™ *E. coli*/Coliform Count Plates 14 *E. coli* isolates were found at a detection level of <10.

TABLE 1. *Total mean mesophilic and psychrotrophic bacteria, yeast, mold, lactic acid bacteria, and coliform populations (log CFU/g) for conventional and organic spring mix* [a].

Type of spring mix	Mesophilic bacteria	Psychrotrophic bacteria	Yeasts	Molds	Coliforms	Heterofermentative lactic acid bacteria	Homofermentative lactic acid bacteria
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)

N= amount of samples tested

[a] Values for mean populations (CFU/g) of conventional and organic spring mix in columns followed by the same letter are not significantly different (P=0.05).

TABLE 2. *Total mean mesophilic and psychrotrophic bacteria, yeast and mold, lactic acid bacteria, and coliform populations (log CFU/g) for organic and conventional unwashed and washed spring mix*. Washed spring mix was treated with chilled water, 5 ppm chlorine and citric acid.

Type of spring mix	Mesophilic bacteria	Psychrotrophic bacteria	Yeasts	Molds	Coliforms	Heterofermentative lactic acid bacteria	Homofermentative lactic acid bacteria
Unwashed	6.24[a]	6.25[a]	5.57[a]	3.91[a]	3.17[a]	5.64[a]	5.35[a]
	(N= 107)	(N= 108)	(N= 108)	(N= 108)	(N= 103)	(N= 100)	(N= 100)
Washed	5.30[b]	5.43[b]	4.80[b]	3.17[b]	2.55[b]	4.68[b]	4.24[b]
	(N= 106)	(N= 107)	(N= 108)	(N= 108)	(N= 104)	(N= 99)	(N= 99)

N= amount of samples tested

[a, b] Values for mean populations (CFU/g) between conventional and organic spring mix in columns followed by a different letter are significantly different (P=0.05).

DISCUSSION

There have been numerous studies questioning nutritional value, pesticide use and consumer acceptability of organic produce versus conventionally grown produce (Batt and Giblett, 1999; Klonsky, 2000; Kuchler et al., 2000). There is little data available to address the question of microbial quality of organically grown produce compared to produce grown by conventional means. In the United States, there are no industry standards for microbial population of bagged lettuce (Hagenmaier et al., 1999).

In using only one sample source, the conclusion drawn from the sample data could be limited in that it only pertains to the product grown and handled under conditions used by the individual grower and possibly others that use similar methods. This limitation is compensated for the advantages in using a sole source for samples. By surveying the two product types produced by a sole grower there was 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 and/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 present and to lower the temperature of the product. The second and third wash tanks are used to control microbial contamination as well as to lower the product temperature to $1^{o}C$. The wash water is treated with a maximum 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 to typical levels of 50-200 ppm, which are commonly used in washing produce (Beuchat et al., 1998). 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, safe product.

The grower who provided unwashed and washed conventional and organically grown spring mix for use in this research does not use any manure in the growing of their conventional or organic products. Cover crops are used rather than manure for covering and protecting the ground. The cover crops grow most of the required nitrogen; however, fertilizers are also used for additional nutrients. Their strategy is to have balanced soil nutrition rather than adding large amounts of fertilizers. This can result in plants with fewer diseases and a longer shelf life, which is very important for increased crop yield and decreased production costs.

The new organic regulations require that manure be composted for 120 days before being applied to the soil. If manure is properly composted then the likelihood of pathogenic bacteria being present is substantially decreased. In the past vegetables have become contaminated with foodborne pathogens through agricultural practices such as fertilization with manure or sewage sludge (Nguyen-the and Carlin, 1994). However, in the new USDA Organic regulations the use of sewage sludge is prohibited (Greene, 2000). The use of untreated manure is also prohibited due to the potential food safety issues. Therefore, the potential for foodborne pathogens to be present in the soil should be significantly less due to improved standards for growing organic produce.

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 (Batt and Giblett, 1999). Without the use of chemicals, it is both more difficult and more expensive to produce fresh fruit and vegetables which are attractive and free from blemishes, pests and diseases. Due to the decreased yield and the increased costs for producing organic produce, higher costs are left for the consumers to cover, which is why organic produce is more expensive than conventionally grown produce.

The size of this survey is quite large which is important when looking at multiple variables. In this survey, 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 grower, it is a respectable grower who processes both organic and conventional produce in the same manner. This research has important implications for the produce industry, particularly the organic industry, because to our knowledge there has not been a similar study published looking at the same microbial parameters as those covered in this study. The results from this research can be used by the industry and consumers to evaluate whether claims are valid concerning microbiological quality and safety of organically and conventionally grown produce.

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APPENDIX A

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CU	1A	1	770000	8000000	2180000	10000	70	1144000		*	
CU	1A	2	1290000	8800000	1890000	10000	80	1188000			
CU	1B	1	1400000	19400000	500000	20000	10	1166000		*	
CU	1B	2	1150000	4800000	610000	10000	10	913000			
OU	2A	1	860000	10600000	1740000	10000	300	2750000		*	
OU	2A	2	1120000	12500000	2020000	10000	300	3740000			
OU	2B	1	7600000	157000000	9500000	600000	7000	1210000		*	
OU	2B	2	7700000	173000000	20000000	200000	6000	1122000			
CW	3A	1	3000	320000	13900	100	10	990		*	
CW	3A	2	5200	300000	18800	100	10	990			
CW	3B	1	142000	980000	287000	1000	20	9900		*	
CW	3B	2	166000	980000	375000	1000	30	25300			
OW	4A	1	169000	3100000	880000	10000	70	11110		*	
OW	4A	2	119000	5800000	830000	10000	60	11880			
OW	4B	1	8000	1460000	325000	1000	10	10340			
OW	4B	2	14400	1670000	284000	1000	10	9460			
OU	5A	1	2900000	3600000	164000	2000	920000	9790000	1980000		ND[f]
OU	5A	2	3000000	2800000	1000	100	750000	10780000	880000		ND
OU	5B	1	790000	860000	58000	23000	260000	69300	4400		ND
OU	5B	2	710000	960000	55000	28000	300000	61600	13200		ND
CU	6A	1	3100000	5500000	460000	10000	2100000	66000	9900	*	ND
CU	6A	2	4600000	5300000	480000	10000	2200000	74800	15400		ND
CU	6B	1	3600000	3900000	790000	10000	630000	47300	13200		ND
CU	6B	2	3000000	3300000	950000	10000	420000	67100	7700		ND

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
OW	7A	1	193000	1890000	910000	360000	12000	4950	110		ND
OW	7A	2	240000	1890000	1030000	10000	18000	4180	110		ND
OW	7B	1	1130000	2030000	86000	1000	330000	429000	44000		ND
OW	7B	2	1130000	1700000	<1000	1000	420000	550000	99000		ND
CW	8A	1	420000	350000	40000	1000	62000	2860	1760	*	ND
CW	8A	2	410000	490000	63000	1000	57000	4400	1100		ND
CW	8B	1	320000	740000	260000	10000	26000	4620	660		ND
CW	8B	2	300000	1050000	410000	10000	32000	5720	440		ND
CU	9A	1	1530000	4300000	2220000	10000	40000	539000	308000		*
CU	9A	2	1910000	3800000	2020000	10000	60000	561000	286000		*
CU	9B	1	1200000	2980000	410000	10000	120000	924000	517000		*
CU	9B	2	1350000	2790000	530000	10000	120000	429000	946000		*
OU	10A	1	4700000	8600000	1340000	20000	40000	253000	363000		*
OU	10A	2	4900000	9700000	1400000	20000	10000	176000	308000		*
OU	10B	1	18700000	36000000	3100000	2500000	900000	407000	33000	*	*
OU	10B	2	24700000	28000000	4000000	100000	600000	506000	121000		*
CW	11A	1	5000000	3600000	580000	10000	10000	330000	66000	*	
CW	11A	2	4400000	4400000	530000	10000	10000	330000	77000		
CW	11B	1	8300000	11300000	2150000	10000	100	253000	66000		*
CW	11B	2	8700000	8000000	2640000	10000	800	187000	220000		*
OW	12A	1	4200000	3000000	490000	10000	5000	297000	33000	*	
OW	12A	2	3600000	3100000	460000	10000	3000	264000	44000		
OW	12B	1	10000000	14600000	3730000	10000	45000	6710000	1540000	*	*
OW	12B	2	9200000	16200000	4250000	10000	47000	8030000	4620000		*

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CU	13A	1	930000	680000	570000	70000	8000	385000	374000	*	*
CU	13A	2	1000000	520000	88000	7000	5000	374000	297000		*
CU	13B	1	1300000	1220000	138000	3000	3000	484000	209000	*	
CU	13B	2	1340000	940000	53000	2000	3000	539000	407000		
OU	14A	1	1820000	1740000	78000	2000	4000	484000	110000	*	
OU	14A	2	1740000	1600000	139000	6000	2000	363000	231000		
OU	14B	1	1490000	1460000	140000	5000	1000	1980000	660000	*	*
OU	14B	2	1490000	1280000	68000	16000	1000	2530000	220000		*
CW	15A	1	380000	620000	880000	10000	500	4400	122100	*	
CW	15A	2	360000	600000	460000	50000	800	7700	60500		
CW	15B	1	630000	920000	59000	1000	3000	286000	99000	*	
CW	15B	2	830000	1000000	55000	2000	4000	110000	264000		
OW	16A	1	520000	1110000	1140000	10000	430	40700	31900		
OW	16A	2	560000	820000	980000	140000	380	36300	31900		
OW	16B	1	213000	212000	102000	1000	10	15400	11000		
OW	16B	2	229000	252000	96000	1000	10	14300	14300		
OU	17A	1	1210000	1760000	245000	2000	2300	132000	561000	*	*
OU	17A	2	1170000	1770000	97000	3000	3200	374000	121000		
OU	17B	1	450000	790000	139000	2000	250	198000	418000	*	*
OU	17B	2	1180000	800000	114000	1000	360	132000	462000		
CU	18A	1	1460000	1680000	490000	10000	770000	121000	517000		*
CU	18A	2	1520000	2040000	400000	10000	720000	198000	495000		*
CU	18B	1	1300000	2300000	820000	10000	2500	66000	1034000	*	*
CU	18B	2	1360000	3300000	390000	10000	3400	286000	836000		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CW	19A	1	1000000	5700000	610000	10000	3000	3850	3850	*	
CW	19A	2	870000	7600000	530000	10000	4000	4730	4290		
CW	19B	1	1450000	2230000	690000	10000	90	9900	23100		
CW	19B	2	1650000	2290000	880000	10000	160	13200	27500		*
OW	20A	1	260000	1210000	201000	1000	50	26400	20900		
OW	20A	2	340000	1580000	73000	1000	100	27500	12100		
OW	20B	1	7600000	9800000	3400000	100000	30	42900	35200		
OW	20B	2	100000	13000000	2600000	100000	40	33000	60500		
CU	21A	1	1040000	880000	66000	10000	40000	561000	198000	*	*
CU	21A	2	1380000	880000	78000	11000	44000	396000	198000		*
CU	21B	1	1960000	1240000	46000	6000	90	29700	16500	*	*
CU	21B	2	1390000	890000	33000	13000	140	25300	19800		*
OU	22A	1	1630000	1750000	490000	40000	4000	44000	16500		*
OU	22A	2	1890000	1890000	480000	60000	9000	39600	23100		
OU	22B	1	1770000	1890000	310000	40000	50000	51700	39600	*	
OU	22B	2	1500000	1710000	230000	40000	100000	36300	41800		
CW	23A	1	89000	360000	450000	10000	20	22000	11000		
CW	23A	2	389000	310000	340000	10000	40	24200	5500		
CW	23B	1	56000	60000	14500	100	200	34100	13200		
CW	23B	2	64000	71000	18200	100	400	25300	15400		
OW	24A	1	25000	60000	7500	100	20	2310	6050		
OW	24A	2	28000	36000	4300	100	10	3300	9350		
OW	24B	1	10000	12700	900	100	100	4290	2420	*	
OW	24B	2	30000	12900	2600	100	80	3850	2640		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CU	25A	1	610000	1600000	54000	1000	110	91300	27500		
CU	25A	2	600000	1540000	73000	1000	50	84700	30800		
CU	25B	1	1390000	1640000	121000	3000	20	50600	23100	*	
CU	25B	2	1360000	1580000	94000	2000	20	45100	18700		
OU	26A	1	360000	750000	113000	3000	2600	308000	99000	*	
OU	26A	2	360000	750000	65000	2000	3000	198000	132000		*
OU	26B	1	910000	650000	160000	1000	11000	40700	13200		
OU	26B	2	480000	700000	150000	1000	12000	33000	13200		
OW	27A	1	64000	72000	33000	1000	50	36300	12100		
OW	27A	2	91000	67000	29000	2000	30	44000	11000		
OW	27B	1	12400	24000	5700	100	20	3080	110		
OW	27B	2	10600	24700	4500	100	10	1980	440		
CW	28A	1	39000	62000	10100	100	10	60500	59400		
CW	28A	2	57000	71000	2100	100	10	92400	50600		
CW	28B	1	350000	250000	142000	1000	180	319000	22000		
CW	28B	2	390000	250000	128000	1000	160	308000	22000		
OU	29A	1	1250000	1830000	330000	10000	100	693000	143000		*
OU	29A	2	1040000	1280000	270000	10000	100	726000	253000		
OU	29B	1	1690000	1830000	85000	1000	5000	506000	319000		
OU	29B	2	2400000	1830000	50000	3000	2000	561000	231000		*
CU	30A	1	13100000	4300000	281000	1000	240	2640000	770000		*
CU	30A	2	4700000	3400000	329000	5000	140	2750000	1320000		
CU	30B	1	1240000	640000	81000	5000	1600	748000	143000		
CU	30B	2	870000	680000	75000	1000	2600	814000	253000		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CW	31A	1	<10000	300000	95000	1000	90	38500	18700		
CW	31A	2	320000	500000	87000	1000	150	35200	14300		
CW	31B	1	1310000	1630000	180000	1000	600	737000	363000		
CW	31B	2	1140000	1880000	263000	1000	1000	605000	506000		
OW	32A	1	225000	400000	71000	6000	20	39600	13200		
OW	32A	2	246000	400000	110000	1000	30	30800	7700		
OW	32B	1	810000	1920000	1000000	300000	120000	51700	3300		
OW	32B	2	1100000	3150000	1400000	400000	200000	46200	2200		
CU	33A	1	840000	580000	260000	40000	70	176000	132000		
CU	33A	2	1040000	450000	280000	10000	60	154000	198000		
CU	33B	1	1020000	359000	900000	10000	240	176000	110000		
CU	33B	2	1720000	329000	480000	10000	240	165000	132000		
OU	34A	1	3600000	147000	2590000	10000	230	352000	99000	*	
OU	34A	2	2700000	136000	3020000	10000	300	495000	33000		
OU	34B	1	570000	303000	108000	3000	20	70400	20900		*
OU	34B	2	340000	422000	130000	1000	50	60500	28600		
CW	35A	1	7300	9700	1600	400	20	9460	2640		
CW	35A	2	9400	11300	900	700	20	9240	2420		
CW	35B	1	20000	10000	100	200	30	2090	880	*	
CW	35B	2	11000	7200	600	100	40	2640	1100		
OW	36A	1	114000	70000	6500	100	1000	2860	2200	*	
OW	36A	2	107000	65200	8900	100	1000	4840	220		
OW	36B	1	13400	12200	5400	200	50	4510	1320	*	
OW	36B	2	12500	11500	5100	100	80	3410	1870		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
OU	37A	1	184000	83000	8000	1100	180	112200	26400	*	
OU	37A	2	163000	55000	5800	1100	260	108900	33000		
OU	37B	1	280000	159000	40000	6000	6500	125400	34100	*	
OU	37B	2	380000	105000	47000	8000	8000	111100	30800		*
CU	38A	1	1300000	128000	850000	30000	90	68200	20900	*	
CU	38A	2	1180000	115000	720000	10000	100	74800	22000		
CU	38B	1	310000	97000	44000	5000	700	44000	2200		*
CU	38B	2	310000	86000	33000	5000	1000	50600	8800		*
OW	39A	1	6800	16100	6100	100	50	5610	1100	*	
OW	39A	2	9700	9600	5000	200	20	5720	1210		
OW	39B	1	4700	6000	71000	6000	160	7480	8470	*	
OW	39B	2	5600	8100	<1000	<1000	160	5390	6160		
CW	40A	1	23000	24800	2200	300	700	4400	3740	*	
CW	40A	2	25000	21100	1300	100	1600	7150	2530		
CW	40B	1	73000	49000	10600	200	11000	77000	16500	*	
CW	40B	2	84000	41000	8800	100	16000	73700	13200		
CU	41A	1	520000	690000	48000	1000	63000	50600	24200		
CU	41A	2	560000	710000	89000	1000	89000	58300	4400		
CU	41B	1	590000	760000	136000	1000	75000	693000	187000	*	
CU	41B	2	680000	810000	107000	1000	62000	682000	154000		
OU	42A	1	520000	500000	89000	2000	130	605000	352000	*	*
OU	42A	2	380000	550000	75000	2000	110	638000	627000		*
OU	42B	1	320000	179000	100000	1000	70	209000	88000	*	*
OU	42B	2	360000	156000	97000	1000	30	308000	44000		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CW	43A	1	25000	11900	4900	100	10	5720	1760	*	
CW	43A	2	31000	16400	4600	100	50	5940	880		
CW	43B	1	390000	360000	154000	1000	50	3410	660		
CW	43B	2	190000	410000	72000	1000	50	4070	550		
OW	44A	1	29000	20600	3700	100	70	5940	1540	*	
OW	44A	2	30000	17000	2100	100	70	5940	1870		
OW	44B	1	60000	59000	44000	1000	90	ND	ND	*	
OW	44B	2	60000	61000	27000	1000	10	ND	ND		
OU	45A	1	1040000	990000	171000	2000	12000	ND	0	*	
OU	45A	2	1090000	810000	300000	1000	16000	130900	0		
OU	45B	1	2800000	3710000	1200000	10000	150000	55000	0	*	*
OU	45B	2	3000000	2070000	1150000	20000	200000	64900	0		*
CU	46A	1	1190000	1410000	234000	5000	70	550000	0	*	
CU	46A	2	1330000	1290000	220000	3000	70	528000	0		
CU	46B	1	680000	770000	109000	7000	50	88000	0	*	
CU	46B	2	740000	830000	72000	6000	30	93500	0		
OW	47A	1	186000	1070000	243000	1000	130	38500	0	*	
OW	47A	2	151000	970000	287000	3000	60	49500	0		
OW	47B	1	78000	110000	21500	100	50	41800	0		
OW	47B	2	42000	140000	19000	100	30	29700	0		
CW	48A	1	2500	19200	2400	100	10	4400	0	*	
CW	48A	2	3400	14200	2000	400	40	3520	0		
CW	48B	1	10000	39000	9500	200	80	11880	0		
CW	48B	2	13900	30000	11000	300	70	13530	0		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CU	49A	1	560000	430000	170000	120000	30	33000	26400	*	
CU	49A	2	910000	320000	140000	90000	10	36300	17600		
CU	49B	1	790000	330000	48000	2000	20	55000	29700		
CU	49B	2	730000	620000	75000	8000	50	37400	47300		
OU	50A	1	1910000	510000	147000	1000	60	297000	209000	*	
OU	50A	2	1870000	450000	125000	2000	90	308000	231000		
OU	50B	1	370000	320000	33000	2000	190	68200	29700	*	
OU	50B	2	310000	270000	0	0	150	75900	30800		
CW	51A	1	48000	67000	2400	500	90	8800	2530	*	
CW	51A	2	120000	54000	2900	700	10	10010	2090		
CW	51B	1	400000	340000	210000	50000	20	1100	4400		
CW	51B	2	350000	600000	330000	150000	20	6600	19800		
OW	52A	1	116000	123000	21000	7000	290	572000	242000	*	
OW	52A	2	163000	172000	26000	8000	300	462000	187000		
OW	52B	1	37600	45000	4400	1200	130	17930	3300	*	
OW	52B	2	26700	32000	10400	600	100	14410	3300		
CU	53A	1	9300000	3800000	890000	10000	ND	2530000	1650000		ND
CU	53A	2	10300000	3400000	990000	10000	ND	2530000	1540000		ND
CU	53B	1	2150000	6100000	670000	10000	ND	4180000	8360000	*	ND
CU	53B	2	2050000	5500000	680000	10000	ND	4620000	8910000		ND
OU	54A	1	1900000	2000000	140000	3000	ND	8580000	1870000	*	ND
OU	54A	2	1600000	2110000	137000	2000	ND	6930000	3740000		ND
OU	54B	1	1010000	1230000	86000	1000	ND	5610000	990000	*	ND
OU	54B	2	1010000	1200000	96000	1000	ND	5830000	770000		ND

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
OW	55A	1	1010000	1150000	11900	100	ND	72600	14300	*	ND
OW	55A	2	1090000	890000	9500	100	ND	74800	11000		ND
OW	55B	1	>100000	750000	3200	100	ND	29700	4400	*	ND
OW	55B	2	>100000	690000	2900	100	ND	34100	7700		ND
CW	56A	1	470000	6900000	30000	1000	ND	86900	15400	*	ND
CW	56A	2	>10000	7900000	30000	1000	ND	83600	22000		ND
CW	56B	1	>100000	990000	6600	100	ND	4950	770	*	ND
CW	56B	2	>100000	960000	4700	100	ND	5500	660		ND
CU	57A	1	16500000	21000000	4200000	100000	76000	8800000	4400000		*
CU	57A	2	21200000	18800000	3300000	100000	80000	7590000	3410000		*
CU	57B	1	6400000	10400000	2140000	10000	35000	3190000	990000		
CU	57B	2	6500000	7700000	1370000	10000	37000	3080000	1320000		*
OU	58A	1	11900000	15200000	1790000	10000	270000	3080000	990000	*	*
OU	58A	2	8800000	18300000	2030000	10000	190000	8470000	990000		*
OU	58B	1	25000000	14700000	6700000	100000	59000	8910000	1540000		*
OU	58B	2	19700000	17400000	4500000	100000	59000	7370000	2310000		*
CW	59A	1	16100000	16700000	9600000	100000	11700	2310000	3190000	*	*
CW	59A	2	ND	20000000	7400000	100000	10600	1870000	3630000		*
CW	59B	1	14600000	13400000	9500000	100000	107000	8690000	1540000	*	*
CW	59B	2	16500000	17400000	14000000	100000	108000	7920000	1650000		*
OW	60A	1	17300000	30000000	11600000	100000	83000	6160000	880000	*	
OW	60A	2	17400000	23300000	16900000	100000	75000	6380000	770000		
OW	60B	1	8800000	12300000	4100000	100000	48000	1760000	1650000	*	
OW	60B	2	10200000	12300000	7200000	100000	38000	2420000	1320000		*

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CU	61A	1	500000	1440000	220000	10000	4400	495000	286000	*	*
CU	61A	2	500000	1370000	310000	10000	4700	616000	418000		*
CU	61B	1	1440000	1710000	690000	10000	920	286000	715000	*	*
CU	61B	2	1790000	2200000	470000	10000	900	517000	726000		*
OU	62A	1	1420000	930000	127000	9000	640	363000	176000	*	
OU	62A	2	720000	890000	174000	2000	700	198000	187000		*
OU	62B	1	2670000	1050000	250000	10000	3600	286000	396000	*	
OU	62B	2	1030000	790000	470000	10000	3300	506000	231000		
CW	63A	1	770000	790000	1590000	10000	1080	308000	99000	*	
CW	63A	2	590000	740000	240000	10000	1010	176000	44000		
CW	63B	1	450000	115000	26400	100	3400	759000	154000		*
CW	63B	2	640000	152000	30200	100	3600	649000	143000		*
OW	64A	1	278000	400000	46000	1000	830	68200	9900		
OW	64A	2	294000	420000	46000	5000	910	74800	23100		
OW	64B	1	97000	67000	7600	100	770	170500	24200		
OW	64B	2	123000	69000	12600	100	510	160600	26400		
CU	65A	1	1270000	1020000	350000	10000	410	56100	99000	*	
CU	65A	2	1260000	930000	420000	10000	490	50600	73700		
CU	65B	1	4900000	7000000	3000000	100000	60	50600	66000		*
CU	65B	2	4300000	6300000	4500000	100000	60	73700	68200		*
OU	66A	1	1780000	1710000	610000	10000	370	60500	36300	*	
OU	66A	2	2310000	1840000	810000	10000	380	55000	57200		
OU	66B	1	910000	520000	106000	1000	250	46200	59400		
OU	66B	2	780000	670000	118000	1000	160	56100	52800		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CW	67A	1	470000	170000	54000	1000	450	451000	286000	*	
CW	67A	2	530000	180000	92000	1000	400	506000	264000		
CW	67B	1	31000	35000	16800	100	40	3190	2090	*	
CW	67B	2	43000	27000	14600	100	30	2860	2200		
OW	68A	1	2190000	4800000	600000	10000	570	88000	24200	*	
OW	68A	2	1810000	3300000	1010000	10000	560	79200	23100		
OW	68B	1	580000	1050000	570000	10000	280	25300	7700	*	*
OW	68B	2	750000	1000000	670000	10000	260	29700	5500		
CU	69A	1	500000	830000	340000	20000	330	132000	506000	*	*
CU	69A	2	360000	750000	410000	50000	310	77000	682000		*
CU	69B	1	470000	600000	510000	30000	300	110000	770000	*	*
CU	69B	2	540000	850000	2450000	10000	290	165000	539000		*
OU	70A	1	1740000	2430000	560000	30000	40	429000	528000	*	
OU	70A	2	2210000	2090000	370000	10000	60	495000	440000		
OU	70B	1	890000	2260000	650000	10000	260	242000	374000	*	
OU	70B	2	1020000	1670000	570000	40000	220	220000	473000		
CW	71A	1	155000	84000	25000	2000	80	9900	4620	*	
CW	71A	2	108000	67000	20000	3000	180	11110	4840		
CW	71B	1	60000	65000	44000	2000	60	3740	1650	*	
CW	71B	2	43000	82000	44000	1000	90	3300	2200		
OW	72A	1	240000	228000	84000	1000	240	49500	28600	*	
OW	72A	2	280000	240000	90000	1000	200	47300	27500		
OW	72B	1	830000	780000	103000	1000	250	264000	165000		
OW	72B	2	610000	680000	47000	8000	480	176000	374000		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CU	73A	1	1140000	2470000	1080000	10000	530	891000	176000	*	
CU	73A	2	1290000	2150000	1060000	10000	500	869000	297000		
CU	73B	1	250000	320000	91000	3000	120	572000	33000	*	
CU	73B	2	280000	17300	ND	ND	100	572000	66000		
OU	74A	1	1020000	580000	79000	3000	440	693000	143000	*	
OU	74A	2	920000	940000	70000	1000	470	781000	242000		
OU	74B	1	840000	830000	281000	1000	1200	715000	121000	*	
OU	74B	2	940000	1220000	406000	2000	1600	638000	154000		
CW	75A	1	31000	128000	80000	1000	3000	99000	11000	*	
CW	75A	2	39000	151000	94000	1000	1900	106700	13200		
CW	75B	1	530000	750000	470000	10000	50	55000	5500		
CW	75B	2	290000	650000	380000	10000	10	53900	7700		
OW	76A	1	42000	82000	65000	3000	4500	38500	1100	*	
OW	76A	2	42000	76000	56000	1000	3900	33000	2200		
OW	76B	1	161000	63000	30000	1000	12000	9020	1320	*	
OW	76B	2	189000	73000	58000	2000	14000	9790	1210		
CU	77A	1	30200000	24800000	7200000	100000	400000	15730000	5390000	*	ND
CU	77A	2	28200000	26000000	7100000	100000	410000	11110000	6050000		ND
CU	77B	1	24600000	32800000	7400000	600000	390000	8580000	5940000	*	ND
CU	77B	2	22700000	28500000	5600000	200000	390000	7480000	4400000		ND
OU	78A	1	27100000	29300000	3300000	600000	480000	6930000	8800000	*	ND
OU	78A	2	22000000	30000000	3100000	400000	340000	8030000	5610000		ND
OU	78B	1	17600000	15400000	4400000	400000	56000	12870000	3190000	*	ND
OU	78B	2	17900000	13700000	3200000	100000	53000	8580000	3740000		ND

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CW	79A	1	4200000	3700000	1720000	10000	2300	9020000	550000	*	ND
CW	79A	2	3300000	3400000	2010000	30000	2900	9020000	1650000		ND
CW	79B	1	19800000	1780000	1040000	40000	1100	693000	66000	*	ND
CW	79B	2	27600000	1520000	1100000	10000	2400	561000	66000		ND
OW	80A	1	2700000	2900000	1900000	40000	3400	495000	121000	*	ND
OW	80A	2	2900000	5800000	2150000	40000	3500	484000	110000		ND
OW	80B	1	790000	540000	450000	20000	3000	1012000	165000	*	ND
OW	80B	2	630000	710000	280000	20000	2500	946000	231000		ND
CU	81A	1	1700000	430000	350000	10000	10	154000	242000	*	
CU	81A	2	1200000	710000	290000	20000	20	55000	209000		
CU	81B	1	7400000	2700000	1880000	10000	10	44000	24200	*	
CU	81B	2	5000000	4600000	1680000	10000	10	31900	22000		
OU	82A	1	1360000	860000	310000	10000	700	57200	31900	*	
OU	82A	2	1540000	850000	320000	20000	100	59400	30800		
OU	82B	1	1890000	3700000	2640000	10000	500	112200	20900	*	
OU	82B	2	2240000	3000000	2180000	10000	700	121000	18700		
CW	83A	1	3400	1900	1500	200	50	2640	2200		
CW	83A	2	3600	1100	1800	100	60	3300	1320		
CW	83B	1	15000	10400	4900	7300	10	3520	2200		
CW	83B	2	16300	7200	4700	100	10	2860	2310		
OW	84A	1	61000	79000	13400	1100	2200	24200	35200	*	
OW	84A	2	62000	63000	14800	100	2500	22000	25300		
OW	84B	1	31000	52000	20000	2000	400	28600	4400		
OW	84B	2	48000	35000	34000	1000	300	17600	15400		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CU	85A	1	1000000	1210000	143000	11000	310	407000	209000	*	
CU	85A	2	>10000	1770000	158000	2000	350	473000	187000		*
CU	85B	1	830000	1320000	156000	3000	230000	517000	143000		*
CU	85B	2	840000	1260000	100000	1000	380000	264000	352000		*
OU	86A	1	1240000	1170000	68000	1000	9000	528000	242000	*	*
OU	86A	2	920000	1080000	130000	1000	9000	583000	198000		*
OU	86B	1	1330000	1900000	580000	10000	50	2420000	1320000		*
OU	86B	2	1400000	1380000	530000	10000	40	3520000	1320000		*
CW	87A	1	43000	17100	5100	200	30	13750	4400	*	
CW	87A	2	38000	16000	ND	ND	30	14630	3960		
CW	87B	1	11000	80000	29000	1000	12000	134200	23100		
CW	87B	2	98000	44000	49000	2000	12000	156200	29700		
OW	88A	1	61000	52000	14900	400	1000	57200	34100	*	
OW	88A	2	68000	52000	13900	200	600	42900	39600		
OW	88B	1	540000	11500	7200	100	1000	18150	3960		
OW	88B	2	360000	9600	11700	300	1000	17380	4950		
CU	89A	1	2040000	1970000	790000	10000	180	1980000	550000	*	*
CU	89A	2	1740000	1870000	720000	10000	330	1760000	1320000		*
CU	89B	1	2230000	2130000	400000	10000	280	1540000	1870000	*	*
CU	89B	2	1540000	2220000	560000	10000	360	1980000	1430000		*
OU	90A	1	4700000	4300000	830000	10000	320	2420000	2530000	*	*
OU	90A	2	4100000	4600000	920000	10000	280	3410000	2640000		*
OU	90B	1	3400000	4900000	1220000	10000	230	3630000	2750000	*	*
OU	90B	2	3700000	5100000	1710000	10000	130	3520000	2640000		*

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CW	91A	1	114000	142000	73000	4000	70	56100	63800	*	
CW	91A	2	162000	189000	67000	1000	70	56100	55000		
CW	91B	1	1650000	1540000	1240000	10000	300	42900	75900	*	
CW	91B	2	1520000	1470000	1150000	10000	500	40700	41800		
OW	92A	1	112000	149000	26000	2000	2000	35200	69300	*	
OW	92A	2	130000	167000	28000	2000	2000	36300	83600		
OW	92B	1	121000	128000	17900	200	100	27500	22000	*	
OW	92B	2	110000	114000	16600	300	100	27500	19800		
CU	93A	1	1240000	2800000	470000	20000	500	539000	396000	*	*
CU	93A	2	1440000	2500000	580000	10000	400	528000	264000		
CU	93B	1	3900000	6200000	1840000	10000	1500	660000	2200000	*	*
CU	93B	2	3700000	4500000	1930000	10000	2000	1320000	4400000		*
OU	94A	1	350000	270000	166000	1000	1700	103400	50600	*	*
OU	94A	2	258000	170000	142000	5000	1500	129800	29700		*
OU	94B	1	11100000	4300000	1140000	10000	90	2420000	1320000	*	*
OU	94B	2	3500000	3200000	1420000	10000	20	1540000	770000		*
CW	95A	1	149000	237000	105000	2000	3100	67100	19800	*	*
CW	95A	2	138000	224000	109000	2000	2100	70400	23100		*
CW	95B	1	380000	850000	104000	1000	190	80300	24200		
CW	95B	2	530000	890000	137000	1000	330	102300	19800		
OW	96A	1	570000	770000	370000	10000	60	187000	165000		
OW	96A	2	700000	610000	450000	10000	120	253000	121000		
OW	96B	1	5800000	10200000	400000	10000	6600	2860000	1540000	*	
OW	96B	2	4600000	8600000	510000	30000	6400	1980000	2090000		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CU	97A	1	1970000	1320000	93000	2000	2100	1166000	275000	*	
CU	97A	2	2370000	1470000	70000	2000	1800	1067000	242000		
CU	97B	1	1590000	930000	216000	4000	1700	2530000	2090000	*	
CU	97B	2	1780000	750000	197000	5000	2300	2750000	660000		
OU	98A	1	3600000	4700000	250000	5000	800	2750000	3850000	*	
OU	98A	2	3400000	3300000	462000	6000	500	2970000	1430000		
OU	98B	1	1670000	1680000	450000	10000	250000	638000	319000	*	
OU	98B	2	1360000	1430000	650000	10000	220000	660000	220000		
CW	99A	1	240000	ND	5200	200	190	28600	4400	*	
CW	99A	2	260000	ND	4200	1700	260	25300	20900		
CW	99B	1	99000	131000	47000	6000	300	57200	59400	*	
CW	99B	2	94000	104000	28000	1000	330	62700	49500		
OW	100A	1	44000	11400	6500	700	160	55000	18700	*	
OW	100A	2	27000	10000	9400	3200	370	59400	20900		
OW	100B	1	15300	100	2000	100	2100	25300	16500		
OW	100B	2	10300	400	2300	300	2900	22000	14300		
CU	101A	1	25300000	38800000	3900000	100000	23000	2090000	1650000	*	*
CU	101A	2	22000000	35100000	2300000	100000	22000	2860000	1650000		*
CU	101B	1	8900000	15800000	1830000	10000	80	2530000	1980000	*	*
CU	101B	2	7800000	18900000	1790000	10000	190	1870000	1760000		*
OU	102A	1	5400000	6900000	960000	20000	120	550000	297000	*	*
OU	102A	2	6600000	7300000	1120000	10000	90	638000	88000		*
OU	102B	1	7000000	5500000	870000	30000	280	1760000	1870000	*	*
OU	102B	2	8100000	6900000	830000	10000	400	4400000	550000		*

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]
CW	103A	1	187000	420000	104000	1000	60	19800	19800	*	
CW	103A	2	191000	410000	100000	2000	80	16500	37400		
CW	103B	1	190000	480000	263000	1000	10	968000	132000		
CW	103B	2	390000	700000	191000	1000	10	1188000	77000		*
OW	104A	1	4800000	6200000	207000	1000	4200	506000	33000	*	*
OW	104A	2	4800000	4800000	204000	2000	5000	803000	55000		*
OW	104B	1	940000	2130000	810000	10000	6000	440000	121000	*	
OW	104B	2	780000	1820000	560000	10000	6500	363000	22000		
CU	105A	1	1180000	730000	226000	2000	80	286000	55000	*	*
CU	105A	2	1040000	750000	220000	5000	90	308000	176000		*
CU	105B	1	2350000	2700000	231000	3000	120	627000	176000	*	*
CU	105B	2	2460000	2600000	268000	3000	110	550000	154000		*
OU	106A	1	3000000	2500000	390000	10000	800	605000	209000	*	*
OU	106A	2	3100000	3200000	380000	10000	500	748000	220000		*
OU	106B	1	1890000	1690000	326000	5000	1500	462000	132000	*	*
OU	106B	2	1850000	1710000	236000	2000	900	473000	121000		*
CW	107A	1	124000	152000	15300	100	10	17600	6600	*	
CW	107A	2	148000	160000	14800	100	10	26400	11000		
CW	107B	1	89000	140000	7900	600	20	13200	13200		*
CW	107B	2	136000	159000	8300	100	10	24200	12100		*
OW	108A	1	37000	44000	19500	100	270	24090	9130		
OW	108A	2	47000	47000	22600	200	320	25300	9350		
OW	108B	1	400000	410000	19300	100	70	31900	19800		
OW	108B	2	290000	370000	14500	200	40	41800	24200		

Appendix A- Microbial Populations on Organic and Conventional Spring Mix

Sample Type[a]	Sample Number	Duplicate Plate	Mesophilic Counts	Psychrotrophic Counts	Yeast Counts	Molds Counts	Coliform Counts	Heteroferm. LAB[b]	Homoferm. LAB[b]	*S*[c]	*L*[d]

[a] CU: conventional unwashed spring mix, OU: organic unwashed spring mix, CW: conventional washed spring mix OW: organic washed spring mix

[b] Heterofermentative LAB, Homofermentative LAB: lactic acid bacteria

[c] *S*= *Salmonella*; When both duplicate plates had presumptive positive colonies for *Salmonella* the first plate was used to perform further biochemical tests. No positive *Salmonella* were confirmed.

[d] *L*= *Listeria*; Plates that had presumptive positive colonies of *Listeria* were used to perform biochemical tests. No positive *Listeria monocytogenes* were confirmed.

[e] Average of both heterofermentative and homofermentative LAB colonies, rather than separated as in rest of document.

[f] ND: No data