THESIS

MICROBIAL QUALITY OF MIXED SALAD GREENS AND SELECTED FRESH AND DRIED HERBS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY DANIEL LEE WOO ENTITLED MICROBIAL QUALITY OF MIXED SALAD GREENS AND SELECTED FRESH AND DRIED HERBS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

MICROBIAL QUALITY OF MIXED SALAD GREENS AND SELECTED FRESH AND DRIED HERBS

Direct marketing has been growing in the Western U.S., with 2007 sales of direct-marketed agricultural products totaling nearly $142.6 million in Colorado, New Mexico, Oregon, Idaho, Nevada and Washington—more than twice the 1997 sales level for the region. In addition, the number of farms engaging in direct sales to consumers grew by more than 20% to 18,274 in 2007. With increasing foodborne outbreaks linked to produce consumption, more research is needed to fill in the gaps of knowledge on the microbiological quality of leafy salad greens. Limited research has been conducted on the microbial safety and quality of farmers' market leafy salad greens.

This study surveyed the microbial quality of salad greens from Larimer County farmers' markets in conjunction with salad mixes from a local supermarket. Leafy salad greens were obtained weekly for a period of four weeks from September to October, 2009, from both farmers' markets and supermarkets. Total aerobic and coliform counts were assessed within 48 hours of obtaining the samples via plating onto Petrifilm plates, then following one week of storage at refrigeration temperatures to observe changes in the microbial load. In addition, handling methods and temperatures were also recorded.

Gloves and tongs were not used by vendors when handling salad greens. The vendors surveyed also lacked adequate refrigeration or ice for holding salad greens. Farmers' market salad greens were lower than supermarket salad greens in terms of initial aerobic plate counts. Both farmers' market and supermarket salad greens had no detectable levels of *Escherichia coli* and low levels of coliforms. After 1 week of storage, aerobic counts were higher in all samples ($P<0.05$). Coliform counts tended to decrease in all samples but no significant differences were observed ($P>0.05$). The results reiterate the need for consumers to thoroughly wash their salad greens. Further research should be conducted to assess the microbial quality of other produce at local farmers' markets.

Herbs are often used in cooking to add aroma and flavor to foods. Consumers may choose to dry herbs from their garden or purchased from the market. Herbs, like other agricultural produce, may be exposed to a wide range of potential microbial contamination. There is currently little research on safe drying practices of herbs at home. Microwaving herbs is a potentially popular and time-saving approach for drying herbs at home. This research project investigated the impact of three drying methods (microwave, dehydrator, and conventional air drying) for improving the microbial quality of dried parsley and cilantro. Herb samples were obtained weekly from a local supermarket for a 2 month period from January to March, 2010. Standard guidelines from Oregon State University Extension service were followed for microwave drying of herbs. The manufacturer's drying temperatures/times were used for drying herbs in a dehydrator. Herbs were also air-dried for 1 week. Microbial testing was performed using plating onto 3M Petrifilm and when counts were below the detection limit, via the Most Probable Number (MPN) method. Microwave drying provided the greatest reduction in

aerobic counts of bacteria in herbs. All drying methods reduced coliform counts to undetectable amounts; however, the determination of the effectiveness of each drying method in comparison with others for reducing the coliform count was complicated by the low initial load of coliforms in herbs used in the study.

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CHAPTER I

Review of Literature

Produce Consumption

Fresh produce remains an important part of the human diet, providing essential vitamins, phytonutrients, minerals, and fiber (USDA, 2010a). The United States Department of Agriculture encourages the consumption of 3-5 servings of vegetable items and 2-4 servings of fruit items per day in their Food Guide Pyramid (USDA-MYPYRAMID, 2010). Fresh-cut fruits and vegetables represent a large and rapidly growing segment of the fresh produce industry (Sapers et al., 2006). In the United States, the per capita consumption of fresh fruits and vegetables has increased from 254 pounds in 1980 to over 328 pounds in 2000 (Matthews, 2006). The trend is expected to rise with a 24% increase per capita of vegetable consumption through 2020 (Lin, 2004).

Consumer Trends

Consumer demand for minimally processed produce is increasing due to the numerous health benefits associated with a diet filled with fresh fruits and vegetables (Alzamora et al., 2000). Farmers' markets are also increasing in the United States with the number of farmers' markets more than tripling from 1,755 in 1994 to 5,274 in 2009 (USDA-AMS 2009). Three out of four respondents in a nation-wide survey indicated that they had visited a farmers' market within the previous year (Keeling-Bond et al., 2006). This is in large part due to the current green movement's interest in fresh local food and organic production. Consumers also expect salad greens to have a fresh appearance, be of consistent quality, and be reasonably free of defects (Bruhn, 2006). Consumers also desire that minimally processed produce be microbiologically safe and stable (Leistner, 2000).

Leafy Salad Greens

Foodborne illness in relation to fresh produce consumption has also increased in the last twenty years in the United States (Doyle and Erickson, 2008). Between 1990 and 2009, leafy greens were linked to 363 outbreaks involving 13,569 reported cases of illness (Klein et al., 2009). The actual number of illnesses linked to leafy greens could be much higher since many foodborne illnesses go unreported (Sivapalasingam et al., 2004). Because fresh produce generally is eaten raw or minimally processed, foodborne illness in produce may be due to contamination from the environment and postharvesting practices (Johnston et al., 2005). Sources of contamination for fresh produce include contaminated water, raw manure being used for fertilizer, animal contact, poor worker hygiene, and time/temperature abuse during pre- or postharvest handling. The lack of a proper "kill step" in fresh produce also increases the likeliness of foodborne illness (Gorny, 2006).

Vegetable crops, during pre- and postharvest stages, are in close contact with potentially thousands of different types of bacteria, viruses, and other microorganisms. Fresh produce in particular have natural micro-flora, high moisture content, unique surfaces, high rates of metabolic activity and nutrient rich tissues—all of which can encourage pathogen growth (Beuchat, 2006). Leafy salad greens also have a pH range of 5-7, which is favorable for the growth of numerous microbial species (Martinez et al., 2001). These characteristics contribute to the susceptibility of leafy salad greens to microbial contamination.

The safety of vegetable crops grown on any given piece of land can be influenced by current and former agricultural practices. Human pathogens may persist in soils for long periods of times (Gorny, 2006). There is a possible increase of soil contamination if farming land was previously used as a feedlot or for animal grazing since fecal contamination of the soil may be extensive. Because of the variety of soil types, climates, and persistence of pathogens in question, it is difficult to determine the exact risk associated with land use (Gorny, 2006).

The surface of fresh produce often contains high numbers of coliforms that are unavoidable due to the presence of domestic and wild animals near farming and production environments (Beuchat, 1996). Birds, deer, dogs, rodents, amphibians, insects, and reptiles are known to be potential reservoirs for human pathogens and their feces can help spread the pathogens to the farm environment (Beuchat, 1996). Open field agriculture has very few remedies available to deal with periodic infestations by these animals. While a zero tolerance for animal presence would be ideal, such operating procedures are practically impossible to implement (Gorny, 2006). Human pathogens can persist in animal manure for weeks or even months (Fukushima et al., 1999; Gagliardi and Karns, 2000, Islam et al., 2005). If animal manure containing pathogenic organisms comes into contact with crops, the organisms may cause human illness (Pell, 1997). When using manure as a fertilizer, proper storage, treatment and application processes are crucial (Bicudo and Goyal, 2003). Proper composting via thermal treatment will reduce the risk of potential foodborne illness (Gorny, 2006).

Irrigation water is another potential vector by which foodborne pathogens may come in contact with vegetable crops (Gorny, 2006). If contaminated water is used to irrigate crops, human pathogens may persist on soils and surfaces of fresh produce (Kirby, 2004). Contaminated irrigation water was implicated in a O157:H7 mesclun lettuce greens multi-state outbreak of *E. coli* (Hilborn et al., 1999). Wash water used during postharvest handling can also carry human pathogens. *Salmonella* outbreaks have been linked to contaminated water during processing of fresh melons (Gagliardi et al., 2003). These results confirm the suggestion by the Food and Drug Administration (FDA) that "the quality of water in direct contact with the edible portion of produce may need to be of better quality compared to uses where there is minimal contact" (FDA-CFSAN, 1998).

Proper employee hygiene and sanitary equipment is crucial in reducing contamination of leafy salad greens. During harvesting operations, field personnel may contaminate leafy salad greens by simply touching them with an unclean hand or knife blade (Gorny, 2006). Safe food handling training for employees should be emphasized and in a language that is easily understandable (Gorny, 2006). Providing clean, sanitary, well stocked hygiene facilities and enforcing proper use will promote good hygiene and show the public that the farm is committed to food safety (Bihn and Gravani, 2006).

One hypothesis for the greater prevalence of foodborne illness in fresh produce is the growth in number and improvement of surveillance systems for foodborne pathogens by public health agencies. This increase in surveillance is related directly to the heightened awareness that fresh produce may be a potential vehicle for human pathogens (Gorny, 2006). New advanced methods for detecting foodborne pathogens are another reason for the amplified prevalence. These new detection methods allow a more accurate survey of foodborne illness than older methods (Angulo et al., 1998). Another reason for the increased incidence is the rise in global sourcing of produce items to ensure year-round supply of the broad diversity of produce available in grocery stores.

Postharvest Handling of Leafy Greens

Postharvest contamination from the consumer contributes to a large majority of foodborne illnesses related to fresh produce. According to CDC data, approximately 12.3% of all foodborne outbreaks were directly related to fresh produce (Alliance for Food and Farming, 2010). Of that, 81% was due to improper handling after leaving the farm by restaurants and consumers while only 19% was associated with the growing practices on the farm. Basic knowledge of proper handling, storage, and preparation from consumer education programs should minimize microbial hazards of foods prepared at home (Matthews, 2006).

Organic Production

Organic foods are often available at farmers' markets. The popularity of organic foods is quickly growing in the food industry, with sales increasing about 20% a year since 1990 (Winter and Davis, 2006). Researchers have shown that consumers regard organic produce as better tasting and more nutritious when compared to conventional produce (Yiridoe et al., 2005). There has been concern about an increased risk to food safety associated with organic produce production. This assumption may be due to the lack of chemical treatments to reduce microbes on the produce and fertilizers such as animal manure being used. However, there is little scientific evidence to support this reasoning (McMahon and Wilson, 2001).

Safe Handling of Leafy Salad Greens

During a ten year period, from 1998 to 2008, 24 multi-state outbreaks were linked to leafy greens. Recently, this led the FDA to add cut leafy greens to the definition of potentially hazardous foods regarding time-temperature control for safety (TCS) (FDA, 2009a). Loose salad greens have favorable pH, water activity, and nutrient content, which support the growth of foodborne pathogens and therefore can be classified as a potentially hazardous food. Refrigeration at $5^{o}C$ or less can inhibit the growth of pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp. (FDA, 2009b). While a variety of cut lettuces and leafy greens are included in this definition, herbs such as parsley and basil are not (FDA, 2009a). While there are no mandatory food quality and safety requirements for leafy salad greens from the FDA, processing facilities are still subject to good manufacturing practices as outlined in the FDA Code of Federal

Regulations, Title 21, Part 10 (FDA, 2009c). To prevent foodborne illness, recommended handling instruction for growing, harvesting, purchasing, receiving, storing, and preparing cut leafy greens were drafted in July, 2009, by the FDA (FDA, 2009d).

Herbs

From 1980 to 2000, the annual per capita consumption of herbs increased by 60% in the United States (Furth, 2001). Herbs are annual/biennial/perennial plants, that are commonly used to add flavor, color, and aroma to foods and beverages (Shylaja et al., 2004). Herbs are also used for garnishing dishes due to the attractive foliage and color they can provide (Shylaja et al., 2004). In ancient times, herbs were used for perfumes, aphrodisiacs, cosmetics and for medicine (Parry, 1953). Herbs were even used as currency and were only available for the rich (Parry, 1953). These versatile plants are a central part of many cuisines in the world today largely due to an increased consumer demand for low sodium, highly flavorful foods (Przybyla, 1986).

Herbs have been traditionally traded as dry products for reasons of preservation (Peters, 2001). With the advent of modern transportation and technologies, herbs can now be traded fresh and flash frozen. Most consumers however are accustomed to using dried herbs, still to this day. Because of this, many quality specifications are based on dried herbs (Muggeridge and Clay, 2001).

Curly Leaf Parsley

Petroselinum cripsum, or curly leaf parsley, is an upright, much branched plant with green leaves and yellowish green flowers growing in clusters from the main stem (USDA, 2010a). Parsley is mostly cultivated as an annual culinary herb, widely grown in Europe and Western Asia (Charles, 2004). In the United States, the two types of parsley that are most frequently grown are Italian flat leaf parsley and curly leaf parsley (Charles, 2004). The leaves of this plant are most commonly used in garnishing and flavoring Mediterranean dishes (Charles, 2004). Parsley is rich in minerals such as calcium, potassium, iron, and also rich in vitamins A, C, riboflavin, thiamin, and niacin (USDA, 2010c).

Cilantro

Coriandrum sativum is also known as the coriander plant and the leaves of this plant are called cilantro. The plant itself is erect with many branches shooting from the stem (USDA, 2010b). The fresh leaves are a common ingredient in many southeast Asian and Mexican dishes (Sharma and Sharma, 2004). Although cilantro and coriander come from the same plant, their flavor is very different from each other. Coriander leaves have a stronger citrus undertone when compared to the seeds (Sharma and Sharma, 2004). Cilantro is a good source of zinc, thiamin, calcium and vitamins A, C, E, K, and niacin (USDA, 2010d).

Food Safety

As with many other types of fresh produce, fresh spices and herbs may be exposed to a wide range of microbial contamination during pre- and postharvest (Mckee, 1995). Contamination of herbs from the fields can come from the natural environment, fertilizers containing manure, contaminated irrigation water, poor worker hygiene and animal contact (Beuchat and Ryu, 1997). Postharvest contamination may come from tainted harvest equipment, rinse water, improper storage and processing (Gorny, 2006). In 1998 *Shigella boydii* was implicated in a foodborne outbreak associated with a bean salad containing fresh parsley and cilantro (Chan and Blaschek, 2005). In California, 76 cases of *Salmonella* foodborne illness were linked to the consumption of fresh cilantro (Campbell et al., 2001). A microbiological study of a variety of fresh herbs sold in the United Kingdom found that 3.9% of the 3,760 samples collected had unsatisfactory levels of *Salmonella* and *E. coli* (Elviss et al., 2009).

Dried Herbs

Dried herbs can still contain high levels of foodborne pathogens, depending on whether they have received a form of pathogen reduction treatment or not (Mckee 1995). In a study that investigated the increase in the amount of recalls associated with dried herbal spices in the United States, researchers found *Salmonella* spp. to be the most common microbiological contaminant (Vij et al., 2006). A comprehensive study of dried herbs from production and retail premises in the United Kingdom found that 3% of herbs surveyed had contamination from *Bacillus cereus*, *Escherichia coli*, and/or *Clostridium perfringens* (Sagoo et al., 2009). About 1.5% of the herbs also tested positive for

Salmonella contamination. Prevention of foodborne illness from dried herbs lies directly in the application of good hygiene practices and effective decontamination during the growing, harvesting, and processing periods (Sagoo et al., 2009).

Dehydration

Dehydrating herbal spices is one of the oldest methods for preservation (Derosier and Derosier, 1977). Drying herbs at home often uses a form of heat and/or air to carry moisture away from the drying product (Derosier and Derosier, 1977). With a lack of moisture, herbs have a lower chance of being susceptible to spoilage organisms (Van Garde and Woodburn, 1994.) Minimal processing from dehydration may only injure pathogenic bacteria without eliminating them but may also reduce the likelihood of foodborne illness (Uljas and Ingham, 1999). Methanol and aqueous extracts from the leaves of dried parsley and cilantro have been shown to inhibit *Salmonella* and *Escherchia coli* growth (Delaquis et al., 2002; Manderfield et al., 1997).

Microwave drying of herbs is a relatively new method of dehydration which can be convenient for in-home use for small quantities of herbs (Oregon State University Extension, 2007). Microwave treatment may have little effect on the total viable count of spices and herbs. Heat production from microwaves is caused by friction between water molecules, which is reduced as dehydration continues in the microwave (Legnani et al., 2001). Microwave treatment was shown to reduce fecal indicator organisms but not overall viable count in sage, rosemary, and black pepper (Legnani et al., 2001).

A review of the literature confirmed a need for further investigation of the safe handling and processing of leafy green vegetables and fresh herbs.

Objectives of this study were to:

- Survey the microbial quality of mixed salad greens from farmers' markets
- Determine the effects of refrigerated storage on microbial quality of mixed salad greens
- Determine drying methods that provide optimum reduction of microbial counts in parsley and cilantro
- Study differences in drying methods (microwave oven, dehydrator, and conventional air drying) for herbs
- Provide information to consumers regarding the effectiveness of dehydration treatments for herbs

CHAPTER II

Microbial Quality of Mixed Salad Greens

Introduction

Fresh produce, such as leafy salad greens, is an important part of the diet because it provides many nutritional benefits including fiber, minerals, vitamins and phytonutrients (USDA, 2010a). Because of the increased awareness of these benefits, consumer demand for fresh produce has grown in the United States with per capita consumption of fresh produce increasing from 254 pounds in 1980 to over 328 pounds in 2000 (Matthews, 2006). Farmers' markets are also increasing in the United States, with the number of farmers' markets more than tripling from 1,755 in 1994 to 5,274 in 2009 (USDA-AMS 2009). Consumers who purchase produce such as leafy salad greens expect the product to have a fresh appearance, be of consistent quality, be reasonably free of defects, and microbiologically safe to eat (Bruhn, 2006; Leistner, 2000).

During a ten year period, from 1998 to 2008, 24 multi-state outbreaks were linked to leafy greens. Recently, this led the FDA to add cut leafy greens to the definition of potentially hazardous foods regarding time-temperature control for safety (TCS) (FDA, 2009a). Loose salad greens have favorable pH, water activity, and nutrient content, which support the growth of foodborne pathogens and therefore can be classified as a potentially hazardous food. Refrigeration at 5°C or less can inhibit the growth of pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp. (FDA, 2009b).

Foodborne illness associated with the consumption of fresh produce has also increased in the United States in the last twenty years (Doyle and Erickson, 2008). Because leafy greens are eaten raw or minimally processed, there is an increased risk in contracting foodborne illness due to pre- and postharvest handling practices (Johnston et al., 2005). Currently there are no studies regarding the microbial quality of mixed salad greens from local farmers' market. The objectives of this study were to set a baseline for the microbial quality for leafy salad greens collected from Northern Colorado farmers' markets and compare the result with the microbial quality of leafy salad greens available from a local supermarket. Microbial quality of leafy salad greens from both sources was also evaluated after 1 week at refrigerated temperature to assess the impact of storage.

Materials and Methods

Salad Greens Collection

Leafy salad greens were collected at the weekly Larimer County Farmers' Market in Fort Collins, Colorado every Saturday during a four week period, for a total of four sampling days. A total of 7 samples was collected during the months of September and October 2009; 3 samples were from Farm A and 4 samples from Farm B. Three plastic bags weighing approximately 150 grams each were purchased for each salad greens sample to conduct testing in triplicate. Both farms operate in Northern Colorado, Larimer County. The salad greens consisted of a varied mixture of red and green romaine along with other leaf lettuces. In addition, leafy salad green samples were also collected at a local supermarket weekly for a period of five weeks. Sample sizes and numbers were the same as described above for farmers' market vendors. Following purchase, samples were immediately placed in coolers lined with ice packs for transport back to the microbiological analysis laboratory. Samples were stored in a refrigerator at 4°C for no longer than 48 hours before analysis was performed. Samples from the farmers' markets and supermarkets were also evaluated one week after microbiological testing was performed, in order to determine changes in microbial counts during storage at 4°C.

Safe Handling Survey at Farmers' Markets

Observations for safe handling procedures were made at the farmers' markets each time samples were collected. Notations were recorded regarding use of gloves or tongs for handling the salad greens and the use of ice for temperature control. Each vendor was asked when leafy salad greens were harvested and if they had been washed. Immediately after purchase, surface temperature of the leafy salad greens was measured using an infrared thermometer (Fisher Scientific, Pittsburgh, PA) before placing into a cooler lined with ice packs for transport to the laboratory.

Microbiological Analysis

Twenty-five grams of salad greens were aseptically removed using sterile forceps from each sample salad greens bag and placed in triplicate into stomacher bags (VWR, West Chester, PA) followed by the addition of 225ml of Buffered Peptone Water (BPW)

(Beckton Dickinson, Franklin Lakes, NJ). The salad greens were homogenized for 2 minutes in a stomacher (IUL, Barcelona, Spain) at a speed of 480 strokes/second. Following homogenization, serial dilutions were made using 9 mL dilution blanks in lamda phage buffer (100 mmol/L NaCl, 50 mmol/L Tris, 8 mmol/L $MgSO_4$, 0.1 g/L gelatin, pH 7.5). From each dilution, 1mL of solution was plated onto Aerobic Count Plate and coliform/*E.coli* Petrifilm™ (3M, St. Paul, MN) according to manufacturer's instructions. The U.S. EPA standard operating procedures for use of Petrifilm plates in research applications were used for petrifilm plating (EPA, 2006). Coliform/*E. coli* Petrifilm plates were incubated for 24 hours at 37°C while Aerobic Count Plate Petrifilm plates were incubated for 48 hours at 30°C. For Aerobic Count Plate Petrifilm, colonies for plates that contained 25-250 colonies were counted and results were expressed as CFU/g. For coliform/*E. coli* Petrifilm results, coliform colonies that were counted as positives appear red surrounded by a bubble, due to an indicator dye and the trapping of gas produced by the coliforms by the upper film of the Petrifilm plate. *Escherichia coli* colonies that were counted as positives were characterized by a blue precipitate surrounded by a gas bubble; blue colonies with gas were counted as *E. coli*, while blue colonies without gas were not (AOAC Official Methods 986.33 and 990.12).

Statistical Analysis

Analysis was conducted using the Analysis Tool Pack- VBA add-in on Microsoft Excel (Redmond, WA). Twenty samples were collected from farmers' markets and supermarkets. Results were transformed into log scale and expressed as CFU/g. A one-way ANOVA and paired T-test with unequal variances statistical analysis were conducted. A probability value of less than 5% was defined as significant.

Microbiological Criteria

Currently there are no microbial limits set as standards for salad greens in the United States. However, in the United Kingdom, microbiological guidelines by the Health Protection Agency have been written regarding prepared salad greens. Acceptable Aerobic Plate counts are below 7 $\log_{10}$ CFU/g while unsatisfactory counts are anything above 7 $\log_{10}$ CFU/g (Gilbert et al., 2000). Acceptable total coliform counts and *E. coli* counts should not exceed 4 $\log_{10}$ CFU/g in total.

Results and Discussion

Safe Handling Survey

Results of the safe handling survey are shown in Table 1.1. Local vendors from Farm A and Farm B were not using gloves or tongs in any of our visits while handling and bagging leafy salad greens. Vendors also were not practicing time/temperature precautions when selling the greens. Surface temperatures of the salad greens recorded by infrared thermometer are displayed in Table 1.1. The data showed that on average Farm A's salad greens were 13.26°C and Farm B salad greens were 14.17°C at time of purchase. Both vendors surveyed said that leafy greens were washed with cold running water the morning of sale. Methods for washing the leafy greens were not described.

Aerobic Plate Counts

The mean aerobic bacterial counts for leafy greens from both farms and a local supermarket are presented in Figure 2.1. Aerobic bacterial counts for leafy greensafter 1 week of storage for all vendors are also presented in Figure 2.1. Aerobic counts of farmers' market samples from Farm A ranged from 5.74 to 6.20 $\log_{10}$ CFU/ml. Farm B aerobic bacterial counts ranged from 6.11 to 6.76 $\log_{10}$ CFU/gram. Aerobic counts on salad greens from Farm A and Farm B were found to be similar (P>0.05). Local supermarket aerobic plate counts ranged from 6.82 to 7.58 $\log_{10}$ CFU/ gram. Supermarket counts were higher than both Farm A and Farm B (P<0.05).

Aerobic plate counts were significantly higher in all leafy greens samples after one week of storage (P<0.05). Counts after 1 week of storage for Farm A ranged from 7.48 to 7.59 $\log_{10}$ CFU/ml and counts for Farm B after 1 week ranged from 6.22 to 7.22 $\log_{10}$ CFU/ gram. Counts after 1 week of storage for the local supermarket ranged from 7.70 to 8.20 $\log_{10}$ CFU/gram. Interestingly the aerobic count for Farm A after 1 week of storage had a significant similarity with the fresh supermarket sample (P>0.05).

Coliform and *Escherichia coli* Counts

In this particular study, no detectable presence of *Escherichia coli* was found in both the initial fresh samples of leafy greens and after one week storage therefore all samples were statistically similar (P>0.05). Mean coliform plate counts for leafy greens from both farms and a local supermarket are presented in Figure 2.2. Mean coliform counts after 1 week of storage for all vendors are also presented in Figure 2.2. Coliform plate counts from Farm A ranged from 1.43 to 1.85 $\log_{10}$ CFU/gram and counts for Farm B were 0.22 to 1.63 $\log_{10}$ CFU/gram. The range of coliform counts for the local supermarket was 1.23 to 2.34 $\log_{10}$ CFU/gram.

Coliform levels after 1 week of storage were negligible and even decreased although statistically no correlation can be made ($P>0.05$). Coliform counts after 1 week of storage for Farm A ranged from 1.12 to 1.94 $\log_{10}$ CFU/gram and coliform counts for Farm B ranged from 0.94 to 1.48 $\log_{10}$ CFU/gram. After 1 week of storage, range of coliform counts from the local supermarket was 1.00 to 1.92 $\log_{10}$ CFU/gram.

Farmers' markets vendors surveyed for safe food handling practices displayed some concerning results. Vendors were not using gloves when handling the leafy salad greens. The Colorado Department of Public Health and Environment code for retail foods, section 3-301 requires that gloves be used by restaurants and vendors when handling ready-to-eat salad greens (CDHPE, 2007). However, fresh leafy greens are not classified as a ready-to-eat food and therefore do not fall under the Colorado Department of Public Health and Environment guidelines (CDHPE, 2007b). Bare hand contact with leafy greens by vendors is still not recommended and consumers are strongly urged to wash leafy greens after purchase (CDHPE, 2007b). Due to farmers markets often being held during the summer, a lack of refrigeration or ice methods for holding greens is also quite alarming. The lack of ice present at farmers' markets is a direct violation of the FDA Food code, which lists leafy greens as a potentially hazardous food requiring time-temperature control for safety (FDA, 2009a). The FDA recommends that leafy salad greens be stored below 5^{o} C to inhibit microbial growth (FDA, 2009b). Implementation of safe handling practices could reduce foodborne illness associated with produce from farmers' markets. Additional surveys and more information on washing techniques are needed to fully investigate safe handling procedures.

Farmers' markets leafy salad green samples had lower aerobic counts in comparison to the supermarket. All samples were relatively high in aerobic counts and the supermarket samples had counts above the satisfactory level recommended by the HPA (Gilbert et al., 2000). Coliform counts in all samples were well below the 4 log_{10} CFU/gram recommended by the HPA and the presence of *E. coli* was not detected. Although safe handling practices during sale were not observed, thorough washing of salad greens prior to sale may be the cause for the low counts. A similar study regarding the microbial quality of organic and conventional leafy greens in Minnesota found higher coliform counts in organic leafy greens along with *E. coli* presence in both organic and conventional samples (Mukherjee et al., 2003). Because of the conflicting results, further studies are needed with more produce types and different vendors before conclusions regarding microbial quality at Larimer County farmers' markets can be accessed. Leafy greens washing practices before arrival at a supermarket should also be studied since there is little information on whether or not leafy greens were thoroughly washed.

After 1 week of storage at 4°C, aerobic counts in all leafy green samples increased to above the satisfactory level recommended by the HPA. Coliform counts after storage relatively decreased but were not statistically different. The lack of coliform growth could be attributed to the low storage temperature. Other studies have shown an increase in microbial counts in packaged leafy greens after 1 week of storage at 5°C and significantly higher increases were found to be directly correlated to higher storage temperature (Jacxsens et al., 2001). Research with inoculated leafy greens that have been stored for 1 week could provide better insight on the effects of storage.

Conclusions

Coliform counts for leafy greens were well below unsatisfactory levels. However, aerobic plate counts for both farmers' market samples and grocery store leafy green samples were relatively high. Storage at 4°C for 1 week may pose a greater risk in aerobic bacteria though effects on coliform count cannot be concluded. Based on the results of this study, consumers should thoroughly wash their salad greens before consumption, regardless of whether it was bought at a farmers' market or a supermarket.

Table 2.1 *Farmers' Market Collection Information and Handling Practices.*

Vendor	Date of Collection (2009)	Use of Gloves or Tongs	Use of Ice or Temperature Control	Mean Temperature on Surface (^{o}C)
Farm A	Sept. 26	No	No	14.6
Farm B	Sept. 26	No	No	12.0
Farm A	Oct. 3	No	No	12.4
Farm B	Oct. 3	No	No	15.2
Farm B	Oct. 10	No	No	15.0
Farm A	Oct. 17	No	No	12.8
Farm B	Oct. 17	No	No	12.8

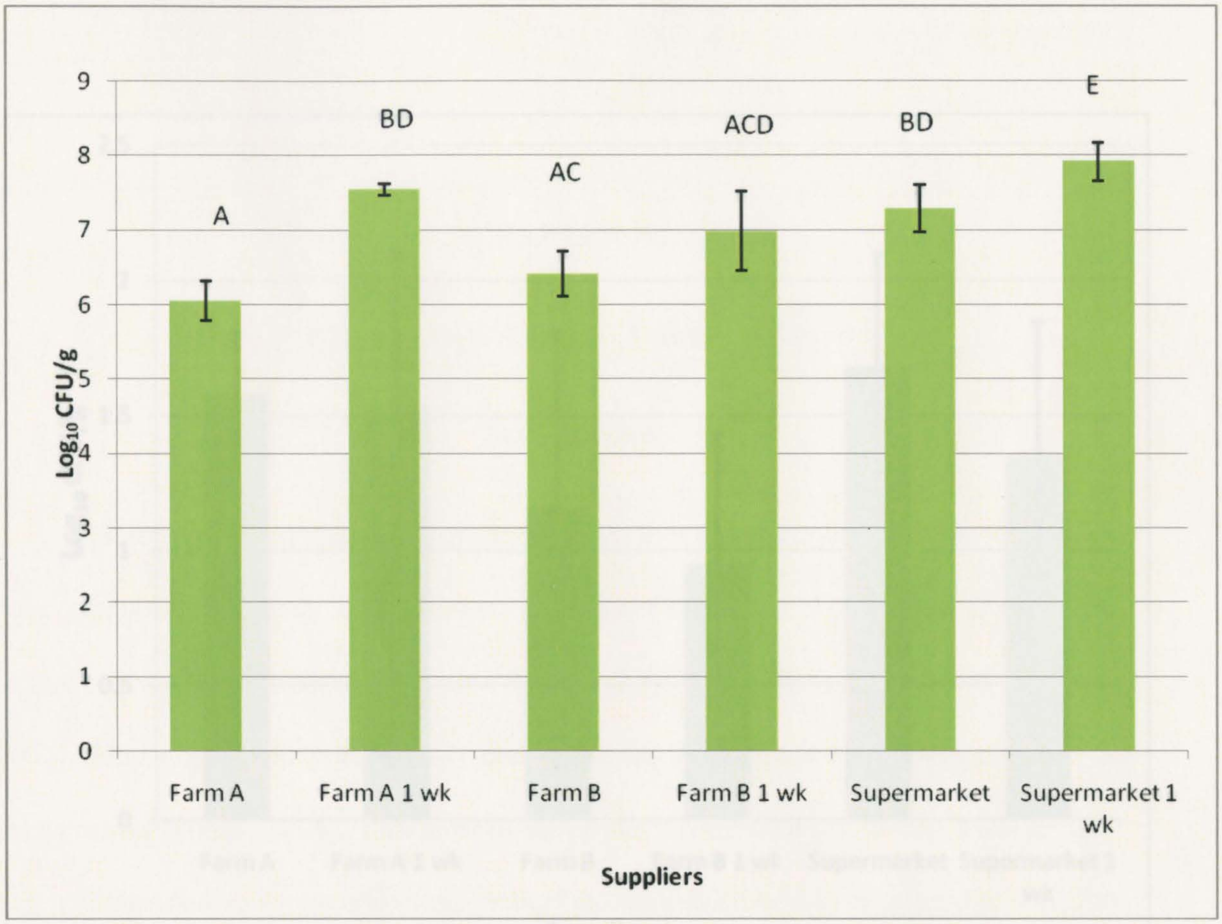

Figure 2.1 *Total aerobic counts in leafy salad greens from different suppliers before and after refrigerated storage.*

Each bar represents the mean bacterial counts of leafy greens from each vendor. Error bars indicate the standard error. Data labels marked by different letter groupings are significantly different ($P<0.05$).

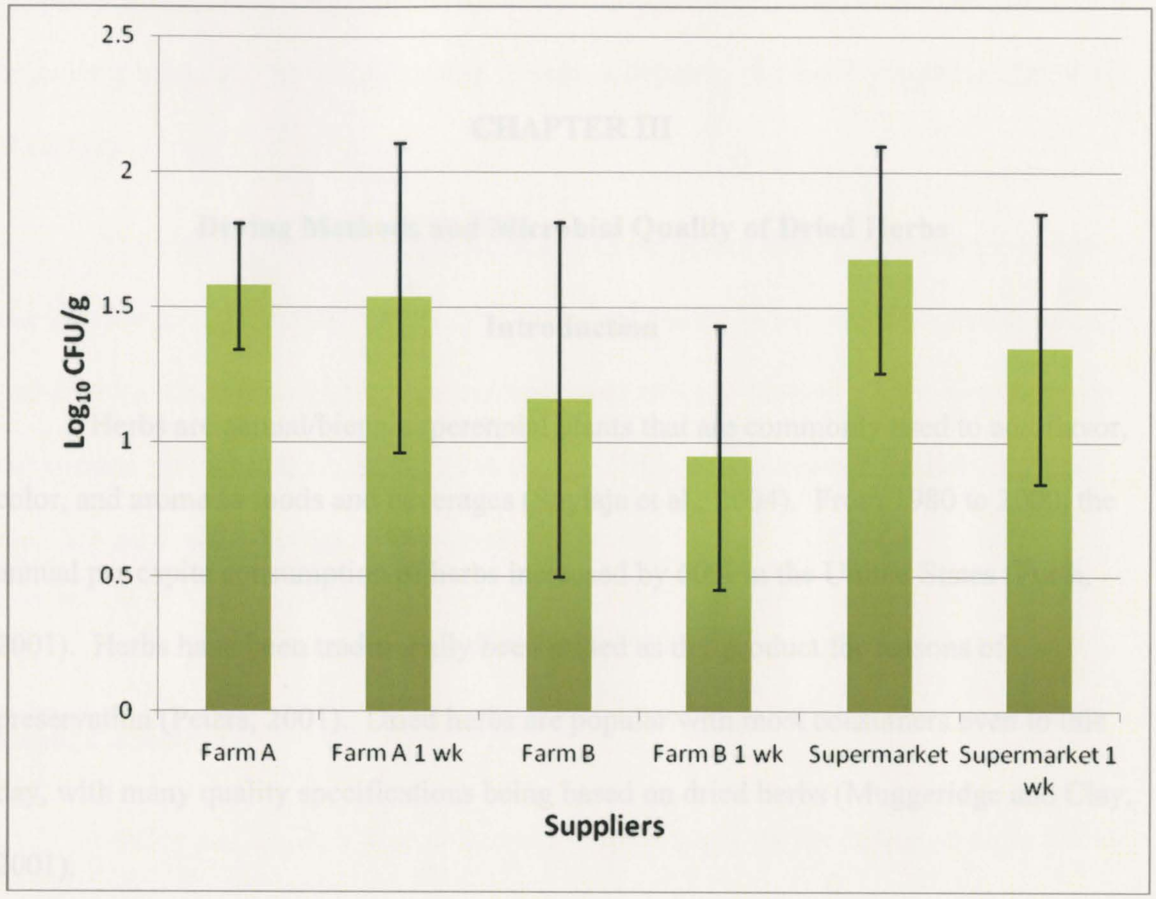

Figure 2.2 *Total coliform counts in leafy salad greens from different suppliers before and after refrigerated storage.*

Each bar represents the mean bacterial counts of leafy greens from each vendor. Error bars indicate the standard error. One way ANOVA table showed no significant differences between samples.

CHAPTER III

Drying Methods and Microbial Quality of Dried Herbs

Introduction

Herbs are annual/biennial/perennial plants that are commonly used to add flavor, color, and aroma to foods and beverages (Shylaja et al., 2004). From 1980 to 2000, the annual per capita consumption of herbs increased by 60% in the United States (Furth, 2001). Herbs have been traditionally been traded as dry product for reasons of preservation (Peters, 2001). Dried herbs are popular with most consumers even to this day, with many quality specifications being based on dried herbs (Muggeridge and Clay, 2001).

Herbs, like other agricultural produce, may be exposed to a wide range of potential microbial contamination (McKee, 1995). Dehydration can lower the chance of herbs being susceptible to spoilage organisms (Van Garde and Woodburn, 1994). However, dried herbs can still contain high levels of foodborne pathogens, depending on whether they have received a form of pathogen reduction treatment or not (Mckee 1995).

Microwave drying of herbs is a fairly new method of dehydration which can be convenient for in-home use for small quantities of herbs (Oregon State University Extension Services, 2007). Heat production from microwaves is caused by friction between water molecules, which is reduced as dehydration continues in the microwave (Legnani et al., 2001). Microwave treatment was shown to reduce fecal indicator organisms but not overall viable count in sage, rosemary, and black pepper (Legnani et al., 2001).

The objective of this study was to determine which drying methods for in home use provide most favorable reduction of microbial counts in two common herbs: parsley and cilantro. Common dehydration methods used at home such as microwave oven, air dehydrator, and normal air drying were tested. Differences between the dehydration methods were also observed in this study.

Materials and Methods

Herbs Collection

Parsley and cilantro were collected at a local supermarket during an eight week period from mid-January to mid-March of 2010. A total of 6 samples was collected, 3 samples each of fresh parsley and cilantro. Three plastic bags weighing approximately 150 grams each were purchased for each herb sample to conduct testing in triplicate. The supermarket operates year round and is located in Northern Colorado, Larimer County. Following purchase, samples were immediately placed in coolers lined with ice packs for transport to the microbiological analysis laboratory. Samples were stored in a refrigerator at $4^{o}C$ until analysis was performed.

Drying Methods

Three methods of drying herbs were followed in this study.

Conventional air drying. Two to three sprigs of herbs were tied at base of stems with a piece of twine and hung to air dry for a period of one week. Herbs were dried away from direct sunlight and at a room temperature of 22°C.

Microwave oven drying. Guidelines from Oregon State University Extension were followed for microwave drying of herbs (OSU Extension, 2007). Adjustments were made in regards to the time intervals used to check herbs during the microwave drying process. Instead of 30 second intervals, 15 second intervals were used in order to reduce risk of fire and charring of herbs.

Food Dehydrator. A Nesco 500-watt food dehydrator was used for the third drying method with the heat source and fan situated above the drying trays (Nesco Model FD40, Two Rivers, Wisconsin). Following manufacturer instructions, herbs were arranged in single layers on multiple stacked perforated trays at a temperature of 35°C for a period of 12 hours (Nesco, 2010).

Microbial Analysis

For the control, twenty-five grams each of fresh parsley and cilantro were aseptically removed using sterile forceps from each sample bag and placed in triplicate into stomacher bags (VWR, West Chester, PA) followed by the addition of 225ml of Buffered Peptone Water (Beckton Dickinson, Franklin Lakes, NJ). For drying treatment samples, 2.5 grams of dried herbs were aseptically removed using sterile forceps for each sample bag and placed in triplicate into stomacher bags. The herbs were homogenized for 2 minutes in a stomacher (IUL, Barcelona, Spain) at a speed of 480 strokes/second. Following homogenization, serial dilutions were made using 9 ml dilution blanks in lamda phage buffer (100 mmol/L NaCl, 50 mmol/L Tris, 8 mmol/L $MgSO_4$, 0.1 g/L gelatin, pH 7.5). From each dilution, 1 ml volumes of solution were plated onto Aerobic Count Plate and coliform/*E.coli* Petrifilm™ (3M, St. Paul, MN) according to manufacturer's instructions. The U.S. EPA standard operating procedures for use of Petrifilm in research applications were used for petrifilm plating. Coliform/*E. coli* Petrifilm plates were incubated for 24 hours at $37^{o}C$ while Aerobic Count Plate Petrifilm plates were incubated for 48 hours at $30^{o}C$. For Aerobic Count Plate Petrifilm, colonies from plates that contained 25-250 colonies were counted and results were expressed as CFU/g. For coliform/*E. coli* Petrifilm results, coliform colonies that were counted as positives appear red surrounded by a bubble, due to an indicator dye and the trapping of gas produced by the coliforms by the upper film of the Petrifilm plate. *Escherichia coli* colonies that were counted as positives were characterized by a blue precipitate surrounded by a gas bubble; blue colonies with gas were counted as *E. coli*, while blue colonies without gas were not (AOAC Official Methods 986.33 and 990.12). A 3 tube most probable number (MPN) technique was also used to detect coliform presence in herbs from drying treatments. One mL samples from lamda phage buffer dilution blanks were placed into test tubes filled with 9mL of McConkey broth (Beckton Dickinson, Franklin Lakes, NJ) and a single inverted Durham tube. After incubation at $35^{o}C$ for 48 hours, all tubes showing formation of acid and gas were considered positive.

Statistical Analysis

Analysis was conducted using the Analysis Tool Pack- VBA add-in on Microsoft Excel (Redmond, WA). A total of six samples was collected from farmers' markets and supermarkets. Results were transformed into log scale and expressed as CFU/g. A one-way ANOVA and paired T-test statistical analysis were conducted. A probability value of less than 5% was defined as significant.

Microbiological Criteria

Currently there are no microbial limits set as standards for fresh and dried herbs in the United States. Fresh spices are similar to fresh produce and similar microbiological criteria may be used (FDA, 2009c). Acceptable Aerobic Plate counts are below 7 $\log_{10}$ CFU/g while unsatisfactory counts are anything above 7 $\log_{10}$ CFU/g (Gilbert et al. 2000). Acceptable total coliform counts and *E. coli* counts should not exceed 4 $\log_{10}$ CFU/g in total (Gilbert et al., 2000). The European Spice Association has set microbial quality standards for dried spices imported into Europe. Total aerobic plate count for dried spices should not exceed 5 $\log_{10}$ CFU/gram (Muggeridge and Clay, 2001). *E. coli* and coliform counts should each not exceed 2 $\log_{10}$ CFU/gram in dried herbs (Muggeridge and Clay, 2001).

Results and Discussion

Aerobic Plate Counts

Baseline aerobic plate counts for parsley and cilantro before dehydration treatments are presented in Figures 3.1 and 3.2. Aerobic plate count values for fresh parsley ranged from 6.09 to 6.80 $\log_{10}$ CFU/gram. Cilantro aerobic plate count values ranged from 6.96 to 7.14 $\log_{10}$ CFU/gram. Parsley and cilantro aerobic counts were found to be different ($P<0.05$).

Microwave dehydration effects on aerobic plate counts for parsley are presented in Figure 3.1. Aerobic plate counts values for microwave dried parsley ranged from 3.64 to 5.00 $\log_{10}$ CFU/gram. Aerobic plate counts for microwave treated parsley were found to be lower than the control and other parsley dehydration treatments ($P<0.05$). On average, microwave treated parsley showed a 2.1 log reduction compared to the control.

Microwave dehydration effects on mean aerobic plate counts for cilantro are presented in Figure 3.2. Aerobic plate counts for cilantro ranged from 4.51 to 4.78 $\log_{10}$ CFU/gram. The aerobic plate counts of microwave treated cilantro samples was found to be statistically lower than the cilantro control and other dehydration treatments as well ($P<0.05$). Microwave treated cilantro showed on average a 2.46 log reduction from the control. Microwave treatments for cilantro and parsley showed statistically similar reduction in aerobic counts compared to the control ($P>0.05$).

Drying effects on mean aerobic plate counts for parsley from using a dehydrator are presented in Figure 3.1. Aerobic plate count values for dehydrator dried parsley ranged from 4.94 to 5.93 $\log_{10}$ CFU/gram. Dehydrator drying treatment counts for parsley were found to be statistically lower than the control but statistically higher than the microwave treatment ($P<0.05$). On average, dehydrator treated parsley showed a 0.93 log reduction of aerobic bacteria from the control.

Dehydrator dried cilantro mean aerobic counts are presented in Figure 3.2. Aerobic plate count values for dehydrator dried cilantro ranged from 5.73 to 6.10 $\log_{10}$ CFU/gram. Dehydrator drying treatment counts for cilantro were found to be statistically lower than the control but statistically higher than the microwave treatment. Dehydrator dried cilantro showed on average a reduction of 1.14 log in aerobic bacteria when compared to the cilantro control. Dehydrator treatments for parsley and cilantro showed statistically similar reduction in aerobic counts from the control (P>0.05).

Air drying effects on mean aerobic plate counts for parsley are presented in Figure 3.1. Aerobic plate counts for air dried parsley ranged from 5.26 to 5.97 log_{10} CFU/gram. Air dry treatment counts for parsley were found to be lower than the control (P<0.05) but statistically higher than microwave treatment (P<0.05). On average, air dried parsley showed a 1.19 log reduction of aerobic bacteria compared to the control.

Mean aerobic plate counts for air dried cilantro are presented in Figure 3.2. Air drying effects on mean aerobic plate counts for cilantro ranged from 5.69 to 6.21 log_{10} CFU/gram. Air dried cilantro showed on average a reduction of 1.07 log_{10} CFU/gram in aerobic bacteria when compared to the cilantro control. Air dry treatments for parsley and cilantro showed statistically similar reduction in aerobic counts from the control (P>0.05).

Coliform/*Escherichia coli* Counts

Baseline coliform counts for parsley and cilantro are presented in Figures 3.3 and 3.4. Initial baseline coliform counts for parsley ranged from 1.37 to 1.60 log_{10} CFU/gram while initial coliform counts for cilantro ranged from 1.30 to 1.80 log_{10} CFU/gram. Coliform counts in both parsley and cilantro were found to be statistically similar (P>0.05). Subsequently, no *Escherchia coli* were detectable after dehydration treatments. All dehydration methods used in this study were able to reduce coliform counts to non-detectable levels in the 3-tube MPN method. Presence of *Escherchia coli* were not detected in control samples for parsley and cilantro. A previous study by our lab regarding mixed salad greens collected and tested following identical protocols shows similar counts of aerobic bacteria and coliforms compared to control samples of parsley and cilantro. Both mixed salad greens and fresh herbs had non-detectable *E. coli* counts.

Although U.S standards have not been established, parsley aerobic plate counts are close to the microbial limit of 7 $\log_{10}$ CFU/gram set by the Health Protection Agency in the United Kingdom and cilantro aerobic plate count actually exceeded these microbial limits in some samples (Gilbert et al., 2000). Coliform counts in both parsley and cilantro were well below the microbial limit of 4 $\log_{10}$ CFU/gram set by the H.P.A. (Gilbert et al., 2000). *Escherichia coli* did not exceed any limits since no presence was detected. One particular study shows that the extracts of parsley and cilantro have a significant effect on reducing growth of *E. coli* (Wong and Kits, 2005). This may be a possible explanation for the lack of *E. coli* presence in fresh parsley and cilantro. Based on these initial counts, fresh parsley and cilantro should always be thoroughly washed before being consumed.

Microwave treatment was shown to be most effective at reducing aerobic plate counts in both parsley and cilantro when compared to other dehydration methods. Reductions in aerobic counts were in generally around 1 log more than air drying and dehydrator methods. A related study regarding microwave treatment of black pepper, rosemary, and thyme found that microwave treatment reduced overall coliform count but had no overall effect on aerobic count (Legalani, 2001). The reduction in aerobic counts from microwave treatments could be due to the fact that fresh parsley and cilantro have higher moisture content than the dried herbs used in the related study. Higher moisture content results in a higher rise of internal temperature and evaporation of water in foods during the use of the microwave (Li and Ramaswamy, 2008). Further research with different wattage microwaves and herbs should be conducted in order to determine optimal reduction in microbial counts.

Air drying and dehydrator methods both proved to be statistically similar in reducing aerobic plate counts to lower levels. The similarity could be due to the fact that both the food dehydrator and air drying had comparable temperatures when drying. Since there is a lack of a heat treatment during dehydration for both methods, it is not surprising to observe higher reduction in microbial counts with microwave treatment. Further research on different brands of food dehydrators should be conducted to compare effects versus air drying on microbial counts of dehydrated herbs.

All three drying methods reduced coliform counts for parsley and cilantro to non-detectable levels. Since there was initially a low coliform count in both control samples, conclusions on the best dehydration method against coliforms cannot be made. Researchers have indicated that dehydration alone may not improve the safety of home dried foods if high levels of pathogens are present (DiPersio et al., 2003; DiPersio et al., 2004). Further studies should be done using fresh herbs inoculated with *E. coli* and coliforms to observe which drying treatment produces the optimum reduction in microbial counts.

Conclusions

All three of the dehydration methods used in this study reduced microbial counts in both aerobic bacteria and coliforms to significantly lower levels. Microwave drying provided the greatest reduction in aerobic bacteria, with counts being reduced around one log more than the other drying methods. Both dehydrator and air drying treatments produced statistically similar reductions in aerobic counts. Definite conclusions regarding drying method effectiveness against coliforms and *Escherchia coli* could not be made due to the initial low counts of these microorganisms in the fresh herb controls. Results from this study were made available to consumers in outreach fact sheet (Appendix B) that will be distributed by the Colorado State University Extension Services. Further studies with different herbs and microbial inoculation should be conducted to investigate the true effectiveness of each home-use dehydration treatment.

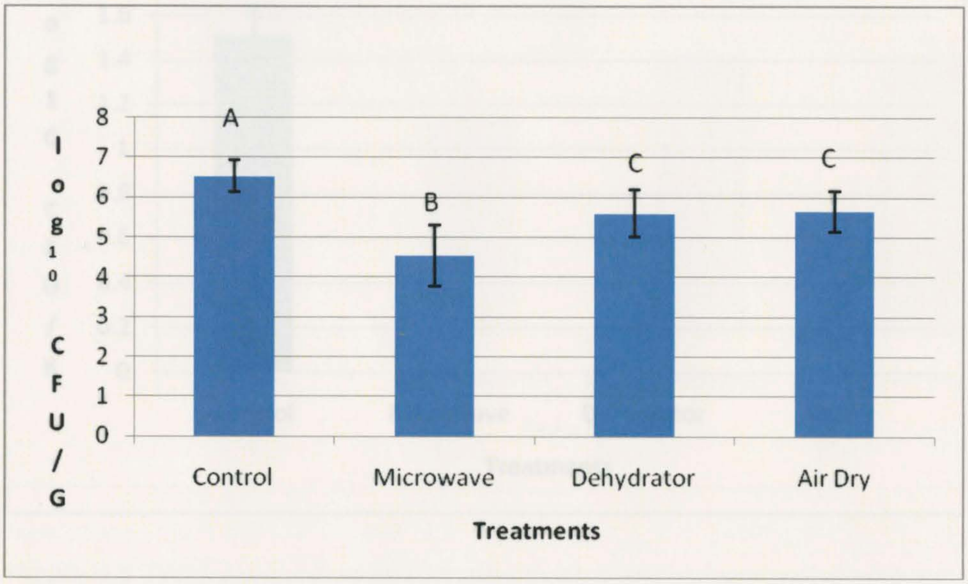

Figure 3.1 *Total aerobic counts in fresh parsley and subsequent drying treatments*

Each bar represents the mean bacterial counts of different parsley treatments. Error bars indicate the standard error. Data labels marked by different letters are significantly different (P<0.05).

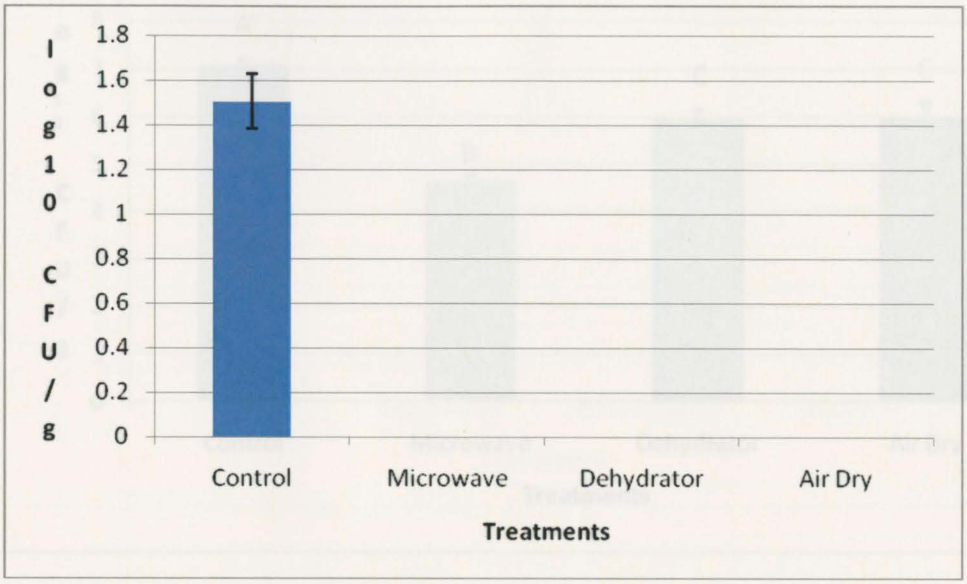

Figure 3.2 *Total coliform counts in fresh parsley and subsequent drying treatments.*

Each bar represents the mean bacterial counts of different cilantro treatments. Error bars indicate the standard error. Statistical evaluation showed no differences between treatments (P>0.05).

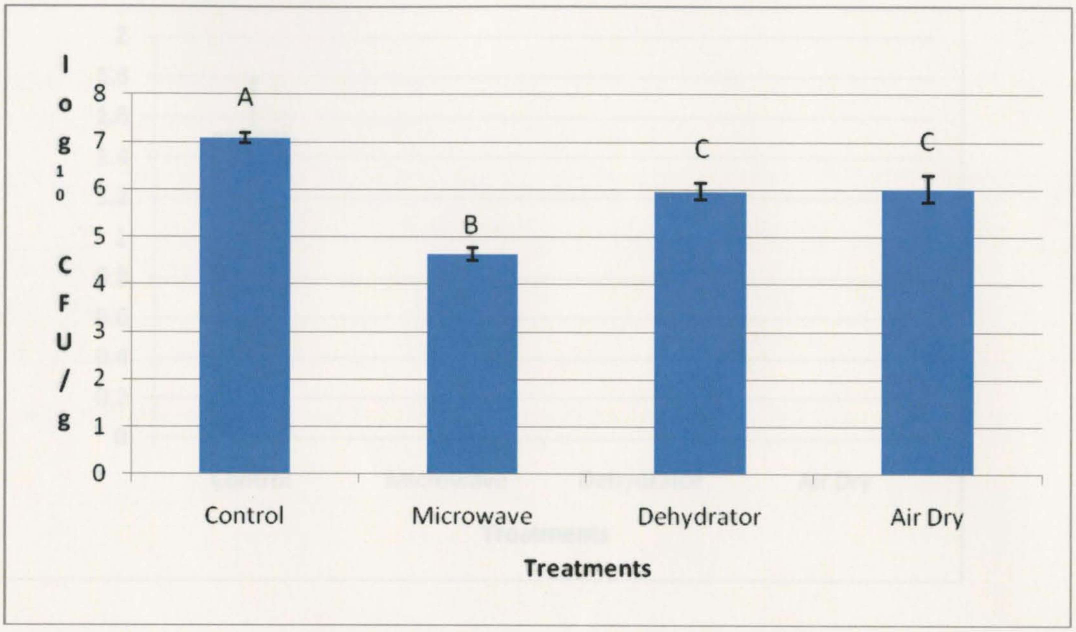

Figure 3.3 *Total aerobic counts in fresh cilantro and subsequent drying treatments*

Each bar represents the mean bacterial counts of different parsley treatments. Error bars indicate the standard error. Data labels marked by different letters are significantly different (P<0.05).

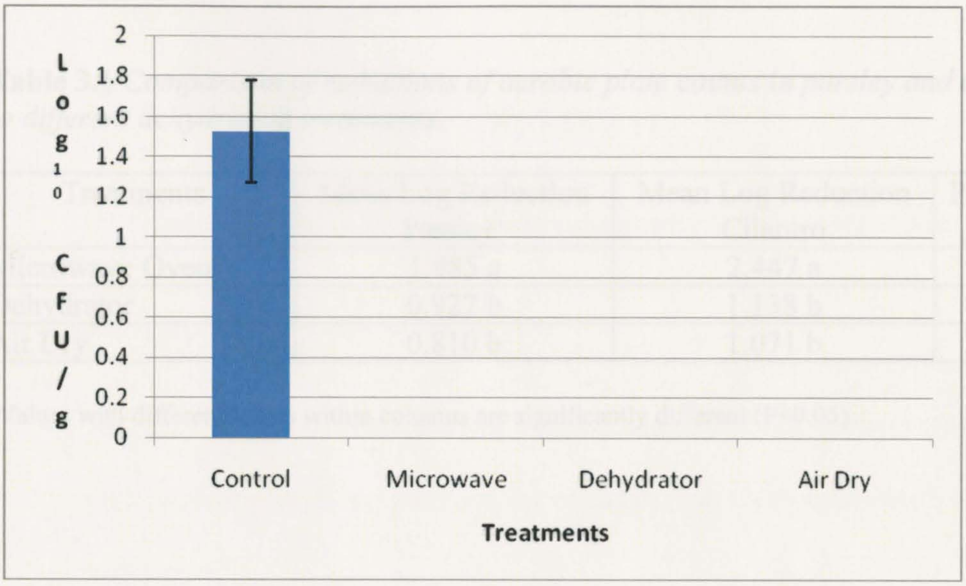

Figure 3.4 *Total coliform counts in fresh cilantro and subsequent drying treatments.*

Each bar represents the mean bacterial counts of different cilantro treatments. Error bars indicate the standard error. Statistical evaluation showed no differences between treatments (P>0.05).

Table 3.1 *Comparison of reductions of aerobic plate counts in parsley and cilantro due to different dehydration treatments.*

Treatments	Mean Log Reduction Parsley[1]	Mean Log Reduction Cilantro	P-value between herbs
Microwave Oven	1.985 a	2.447 a	0.168
Dehydrator	0.927 b	1.138 b	0.085
Air Dry	0.810 b	1.071 b	0.099

[1]Values with different letters within columns are significantly different ($P<0.05$).

CHAPTER IV

General Conclusions and Recommendations for Further Studies

Microbial Quality of Mixed Salad Greens

- All leafy greens samples showed high counts of aerobic bacteria and presence of coliforms, highlighting the need for consumers to thoroughly wash their leafy salad greens.
- Handling practices commonly observed at the farmers' markets such as bare hand contact of leafy greens and lack of temperature control/ice for leafy greens illustrate that more food safety education regarding safe handling of fresh produce is necessary.
- Aerobic counts for leafy greens and supermarket samples were relatively high according to standards in other countries, i.e from the Health Protection Agency in the United Kingdom.
- Both vendors from the farmers' markets had similar aerobic counts in leafy greens. Supermarket greens were higher in aerobic counts in comparison to both farms from farmers' markets.

- Coliform counts for both farmers' markets and supermarket samples were low according to standards from HPA. No significant differences were found in coliform counts for both farmers' market and supermarket samples.
- One week of storage caused a negative impact in aerobic counts in both farmers' market samples and supermarket samples, with all counts in samples increasing.
- Coliform counts in both farmers' market samples and supermarket samples decreased after 1 week of storage, although no statistical conclusions can be made.

Further Studies

- Microbial quality should be surveyed on more farms and different type of produce to get true assessment for northern Colorado farmers' markets.
- Additional surveys on washing techniques for leafy greens at farmers' markets and supermarkets should be conducted.
- Research with inoculated leafy greens that have been stored for 1 week could provide better insight on the effects of storage.

Drying Methods and Microbial Quality of Dried Herbs

- All three in home dehydration treatments reduced microbial counts in both parsley and cilantro with microwave drying providing the greatest reduction in aerobic bacteria counts.

- Food dehydrator and air drying were statistically similar in reducing aerobic bacteria counts for both herbs illustrating the fact that neither can provide a form of heat treatment as effective as a microwave oven.

- All three dehydration treatments reduced coliform counts to non-detectable levels and show the need for further studies due to low initial coliform levels.

Further Studies

- Studies involving dehydration treatments with different types of herbs could show if similar results can be produced.

- Research with different wattage microwaves and dehydrators should be conducted to see which are most effective in reducing microbial counts in herbs.

- Dehydration treatments with inoculated fresh herbs should be conducted to determine the extent of reduction in coliforms and other indicator organisms.

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APPENDIX A

Statistical Analysis

ANOVA: Single Factor APC Salad Greens

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Farm A	3	18.14844	6.04948	0.069856
Farm B	4	25.61891	6.404728	0.08896
Supermarket	5	36.44624	7.289247	0.101275
Farm A 1 wk.	2	15.07429	7.537144	0.006639
Farm B 1 wk.	3	20.46978	6.82326	0.279049
Supermarket 1 wk.	3	23.73528	7.91176	0.065944

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.698003	5	1.539601	14.29038	4.47E-05	2.958249
Within Groups	1.508316	14	0.107737			
Total	9.20632	19				

ANOVA: Single Factor Coliform Salad Greens

SUMMARY

Groups	Count	Sum	Average	Variance
Farm A	3	4.723368	1.574456	0.055107
Farm B	4	4.617967	1.154492	0.428897
Supermarket	5	8.381553	1.676311	0.176136
Farm A 1 wk.	2	3.06279	1.531395	0.330415
Farm B 1 wk.	3	2.823865	0.941288	0.237999
Supermarket 1 wk.	3	4.034762	1.344921	0.25199

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.382693	5	0.276539	1.134736	0.387322	2.958249
Within Groups	3.411843	14	0.243703			
Total	4.794536	19				

ANOVA: Single Factor Parsley APC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	3	19.60423	6.534744	0.152512
Microwave	3	13.59865	4.532884	0.59392
Air Dry	3	17.16879	5.722929	0.157876
Dehydrator	3	16.82256	5.60752	0.336342

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.08295	3	2.02765	6.53738	0.015192	4.066181
Within Groups	2.4813	8	0.310162			
Total	8.56425	11				

ANOVA: Single Factor Parsley Coliform

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	3	4.514043	1.504681	0.014869
Microwave	3	0	0	0
Air Dry	3	0	0	0
Dehydrator	3	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.094146	3	1.698049	456.7879	2.79E-09	4.066181
Within Groups	0.029739	8	0.003717			
Total	5.123885	11				

ANOVA: Single Factor Cilantro APC

SUMMARY

Groups	Count	Sum	Average	Variance
Control	3	21.25926	7.086421	0.012854
Microwave	3	13.91734	4.639112	0.017294
Air Dry	3	18.0451	6.015032	0.07837
Dehydrator	3	17.84443	5.948143	0.03864

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	9.033046	3	3.011015	81.84449	2.41E-06	4.066181
Within Groups	0.294316	8	0.036789			
Total	9.327362	11				

ANOVA: Single Factor Cilantro Coliform

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	3	4.579555	1.526518	0.064424
Microwave	3	0	0	0
Air Dry	3	0	0	0
Dehydrator	3	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.243081	3	1.747694	108.5126	8.07E-07	4.066181
Within Groups	0.128847	8	0.016106			
Total	5.371928	11				

APPENDIX B

Preserving and Using Herbs — no. 9.335

by P. Kendall, D. Woo, and M. Bunning[1]

Quick Facts...

- Herbs are used to add flavor, aroma, and color to foods and beverages.
- The flavor of herbs comes from oils in the cell walls of the leaves and seeds.
- Herbs can be dried using a food dehydrator, oven, microwave oven, or by air drying.
- Dried herbs can be stored effectively up to one year.

Herbs grow in temperate areas of the world and generally the leaves or seeds of the plant are used. In contrast, spices grow in tropical zones and come from bark, berries, flower buds, roots, or seeds. These versatile plants play an important role in the cuisine of many cultures and consumer demand in the U.S. for spicy and flavorful foods has increased. Herbs are often used as complements to food dishes and to flavor beverages, vinegars, and oils. Herbs and spices can sometimes replace or reduce salt and sugar in foods. Herb leaves can be used fresh, or they can be dried or frozen for later use. Their flavor comes from aromatic oils in the cell walls of the plants. Chopping or grinding breaks the cell walls and releases the flavor. Heat increases the rate at which some herbs release their flavors.

Using Herbs

For best results, use herbs to complement, not disguise, the flavor of food. Generally, 1/4 teaspoon of dried herbs per four servings is adequate. The flavor of dried herbs is about three to four times stronger than fresh herbs. To substitute dried herbs for fresh in a recipe, use 1/4 to 1/3 as much. These amounts can be used as guidelines: 1/4 teaspoon powdered herb = 1 teaspoon dried herb = 1 tablespoon chopped, fresh herb. Chopping the leaves very fine will create more cut surface and allow additional flavor components to be released. An herbal infusion involves steeping plants with desired flavors or characteristics in water or oil. Herbal infusions are used to flavor drinks, such as teas and cocktails, and culinary products, such as salad dressings, vinegar, and oil.

Whether fresh herbs are grown in the home garden or purchased, they should be thoroughly washed before using. Remove damaged leaves and rinse well under cool running water or dip and swish in a bowl of cool water. Shake gently to remove excess water and dry with paper towels.

The flavor characteristics of different herbs vary widely and this influences their culinary use (Table 1). Best flavor results from adding herbs during the last part of cooking. Herbs in uncooked foods, such as salad dressings, dips and fruits, need time to blend flavors, so add them as far in advance of serving as possible. Wrap whole herbs in a cheesecloth bag before adding them to cooked dishes. This makes it easy to remove them before serving. Basil, cilantro, dill, mint, oregano, parsley, rosemary, sage, savory, tarragon, and thyme add interest to salads and rosemary, thyme or basil may be added as a secondary flavor to sorbet.

Table 1. Herb classification and characteristics (Modified from Snider, 2007).

Flavor	Herbs	Characteristics
Delicate	Burnet, chervil, chives, parsley	May be used in fairly large quantities and combine well with most other herbs
Medium	Basil, celery leaves, tarragon, marjoram, mint, oregano, savory, thyme	Use in moderate amount (1-2 teaspoons for 6 servings)
Strong	Bay leaf, rosemary, sage	Will impart a dominant flavor
Sweet	Mint	Used in sweet dishes and desserts
Savory	Oregano, tarragon, chives, dill	Often used to flavor meat and cheese dishes.

Herbal Teas and Beverages. Traditionally, a true tea is made from the leaves of the tea plant, *Camellia sinensis*, but beverages made from the leaves, flowers, bark, or roots of other plants have become widely known as herbal teas. Herbal teas do not go through the same curing process as black or green tea so it is important to brew herbal teas using water that has been heated to a temperature high enough to destroy harmful microorganisms. It is recommended to brew herbal teas at 180-200°F for 5-15 minutes. Herbal teas should not be used in making sun tea, however brewed herbal tea can be added to sun tea that has been made with black tea labeled for sun tea use.

Herb Flavored Vinegars and Oils. Herbs may be added to vinegar or oil and used in the seasoning of salads and sauces. Generally, cider, white or wine vinegar is used and tarragon is one of the herbs often used to add flavor. Flavored vinegars should retain good quality when stored in a cool dark pantry for two to three months or the refrigerator for six to eight months. Some recipes call for herbs to be blanched for 10 seconds before adding to oil. It is important to store homemade herb infused oils in the refrigerator and use within 10 days because they have the potential to support the growth of *C. botulinum* bacteria. Commercially made herb/oil mixtures are acidified to prevent bacterial growth and these may be stored at room temperature. For more information on using herbs to flavor culinary preparations, see CSU Extension Fact Sheet # 9.340, Flavored Vinegars and Oil.

Herbs for Health. Besides culinary uses, herbs have a history of being used for health and medicinal purposes. However, herbal products are classified by the U.S. Food and Drug Administration (FDA) as dietary supplements, not drugs, and therefore are not tested for safety or efficacy. Medicinal herbs should be used with caution because they may contain substances that trigger side effects or interact with medications. Components of black and green tea, such as catechins and theobromine, have been associated with health benefits but most herbal teas contain very low amounts of these compounds. Herbal teas also tend to have lower antioxidant values but lemon myrtle, guava and oregano teas are exceptions. For information on using herbs for health, see CSU Fact Sheet # 9.370, Herbals for Health?

Drying Herbs

Drying is the traditional method for preserving herbs. To minimize wilting, gather herbs in the morning of a dry day, just after dew has evaporated. Rinse thoroughly before using and dry with paper towels. Label all herbs before they are dried because many look alike when dry. Herbs are dry when they are crispy dry, crumble easily and stems break. When collecting the seeds of herbs, try drying inside a paper bag with holes cut in the side of the bag. Sun drying is not recommended because herbs can lose flavor and color.

Air drying. Tie two to three sprigs of fresh herbs at the base of stems with twine and hang away from direct sunlight at room temperature or lay on cheesecloth stretched on frames or netting screens. This method may be used for sturdier herbs. More tender leaf herbs, such as basil, tarragon, lemon balm, and mint, are higher in moisture and should be dried quickly to prevent mold growth. These can be hung inside a paper bag with vent holes and placed in a warm location with good air circulation.

Food dehydrator. Arrange herbs on drying trays in single layers; good air circulation between trays is important. To preserve aromatic oils, dry at 95°F to 115°F. For more information on dehydration methods, see CSU Fact Sheet # 9.308, Drying Vegetables and #9.309, Drying Fruits.

Microwave oven. Drying in a microwave oven can be a good option for small amounts of herbs and appears to be the best drying method for reducing microbial contamination of herb leaves. Check the microwave oven owner's manual for specific herb drying directions. Generally a single layer of herbs is placed between two paper towels on a microwave-safe plate. Some types of paper towels made from recycled materials may not be recommended for microwave oven use. Herbs need to be thoroughly dry before placing in the microwave oven otherwise residual water will cause the herbs to cook instead of dry. A general guide is to dry 1 cup of herbs on high for one to two minutes in 700-1,200 watt ovens; 2-4 minutes in 650-700 watt ovens; and 3-6 minutes in 500-600 watt ovens. It is important to stop every 15 seconds to check the herbs and periodically turn them over. Although some microwave drying instructions suggest a longer time, in Colorado's dry climate, it is necessary to check every 15 seconds to reduce the risk of fire and charring of the herbs.

Oven drying. The oven light of an electric range or the pilot light of a gas range may furnish enough heat for overnight drying of herbs. Place single layer of herbs between two layers of paper towels.

Storing Herbs

Dried herbs should be stored in a cool, dry place and most keep well for up to a year. Their strength can be judged by their aroma. Dried herbs can be stored whole or crushed, but whole herbs retain their flavor longer. To ensure optimum quality, store in rigid, opaque containers with airtight seals. Choose ceramic jars or darkened glass containers to help protect the herbs against light deterioration. Make sure herb leaves are completely dry to prevent mold growth during storage. Label all storage containers with the herb's name and date.

Quick-frozen herbs will keep up to two years in the freezer if well wrapped. To tray freeze herbs, wash, drain, trim and chop. Seal in airtight plastic bags, label with the herb's name, and date. Basil can be frozen in ice cube trays and filled with water. When frozen, the cubes should be stored in plastic bags. Frozen herbs are best used in cooked dishes as they will become limp when thawed.

Many popular herbs are members of Lamiaceae and Apiaceae, the mint and carrot families. Table 2 summarizes the flavor profile, harvesting, and preservation methods for several herbs that can be grown or purchased fresh in Colorado. Fresh basil, cilantro, parsley, chives, rosemary, and many herb seedlings are often available at Colorado farmers' markets. For more information on growing herbs in Colorado, see CSU Extension Fact Sheet #X.XXX, Growing Herbs.

Table 2. Herb Characteristics and Preservation Information.

Characteristics	Preserving
Anise (seeds), *Pimpinella anisum*; Carrot Family	

Annual. Grown for small oval, greenish-brown seeds with licorice flavor. Used to flavor cookies, candies, pickles, beverages, breads, and fig dishes.	Cut stems of seed heads after seeds have developed but while they are still green. To dry, place on frames of stretched cheesecloth in a cool dry room or heat in a 100 °F oven for 15 minutes. When dry, remove seeds and store in airtight container.
Basil, sweet (leaves), *Ocimum species;* Mint Family	
Annual. Good border plant. Tender green leaves have sweet flavor with wild pungency. Used in Italian and Thai dishes, excellent with tomatoes, cheese, stews, meats, soups and green salads.	When the plant starts to flower, cut stems 6 to 8 inches above ground, about ¼ inch above a stem node. Hang upside down in warm, dry, dark room, or dry individual leaves on cheesecloth or netting screens. Basil can be dried in a microwave oven at a low setting. Lay basil between paper towels. It may take up to 3 minutes, check every 15 seconds, turning periodically. When dry, leaves crumble easily. Store whole or crushed in airtight containers. To freeze, wash leaves, pack in ice cube trays and fill with water. When frozen, remove cubes and store in plastic bags. Defrost in a strainer and use as fresh.
Bay (leaves), *Laurus nobilis*; Laurel Family	
Perennial evergreen shrub. Move indoors for winter in Colorado. Leaves are aromatic with a sweetish odor and pungent flavor. A classic ingredient in French bouquet. Used in sauces, pickling, stews, and with meats and potatoes. Bay leaves are tough and should be removed before serving.	Pick individual leaves to use fresh or preserve. Dry leaves on screens in cool, dry, airy, shaded place. When dry, pack in airtight containers. To freeze, see basil.
Burnet, *Sanguisorba minor*; Rose Family	
Perennial herb with a light cucumber flavor, used in flavoring butter, cottage cheese, cream cheese, salads and salad dressings.	Select young leaves, older leaves may be bitter. May be used in place of mint leaves.
Caraway (seeds), *Carum carvi*; Carrot Family	
Biennial with feathery foliage and creamy white flowers. Grown for its hard, brown, savory seeds. Mostly used whole	Cut plants to ground level when flowers and stalks turn grayish-brown, about a month after flowering. Hang in small bunches by stems in airy place over cloth- or paper-lined containers. Seeds shake out

in rye breads, sauerkraut, cheeses, potato salads, meats and stews.	easily when fully ripe. Store in airtight containers.
Chervil (leaves), ***Anthriscus cerefolium*****; Carrot Family**	
Annual that resembles a fine-leaved parsley and fennel combined. Has a light, licorice flavor with a wild taste of pepper. Gives delightful flavor to salads and salad dressings, meats, fish, soups, omelets and stews.	Pick only young, tender leaves just before the buds break, fresh chervil has a short storage life. To dry or freeze, see basil.
Chives (leaves), ***Allium schoenoprasum*****; Onion Family**	
Perennial. Attractive border plant. Similar to green onion, but milder and finer leaves. Used for light, oniony flavor in salads, dips, sauces, vegetables, soups, fish, etc.	Use leaves fresh by snipping off the tops with scissors. Chives lose their color and flavor when dried. To freeze, wash and chop finely, then continue as for basil.
Cilantro, ***Coriandrum sativum*****; Carrot Family**	
Annual. Delicate, lacy foliage with pinkish-white flowers. May reach 20 to 30 inches tall. Used in Asian and Latin American dishes. Goes well with corn, cucumbers, avocado, rice, fish, and chili peppers.	Flavor is agreeable to some and disagreeable to others. High heat reduces flavor. Freezes well in ice cubes.
Coriander (seeds), ***Coriandrum sativum*****; Carrot Family**	
Annual – same plant is source of cilantro. Small, orange-flavored seeds used in cold cuts, curry powder, cakes, cookies, poultry dressings, French dressing and Scandinavian cooking.	Cut off seed heads as they ripen and dry in an airy place on screens over cloth-lined container. When seeds are dry, shake out of heads and store in airtight containers. Flavor improves if stored a month before using.
Dill (seeds, leaves), ***Anethum graveolens;*** **Carrot Family**	

Annual. Feathery foliage, flower umbels. Grows 2-3 feet tall. Seeds have slightly bitter taste. Used in soups, pickles, cheese dishes, breads, sauces, meats and fish. Dill weed has delicate bouquet. Used to flavor fish sauces, salads, dips, potatoes and meats.	Pick young leaves just as flowers open. Cut leaves and spread in a thin layer to dry until brittle. Crumble leaves and store in an airtight container. To collect seeds, cut flower umbel stalks or pull entire plant from ground. Hang upside down in a sunny place to dry. Shake seeds out when dry. Store in airtight containers.
Fennel (stems, leaves, seeds), *Foeniculum vulgare*; Carrot Family	
Annual. Often reseeds. Bright green, feathery foliage with yellow flowers. Yellowish-brown seeds with sharp, sweet, licorice-like flavor. Use leaves as garnish or flavoring in sauces and salads. Use seeds to flavor sausages, breads, salads, salad dressings, pickles, cheese spreads, soups and sauces.	Young stems can be used like celery. Pick young leaves to dry, as for basil. Cut off flower stems before seeds fall. Hang over a cloth in a warm, dry place until seeds can be shaken out. Store in airtight containers.
Marjoram, sweet (leaves), *Origanum majorana;* Mint Family	
Perennial; treated as an annual since it rarely overwinters. Small bush with white flowers. Gray-green leaves with slightly bitter undertone. May be used fresh or dried to season vegetables, lamb, sausage, eggs, poultry, cheese dishes, potato salad, stuffings and soups.	Cut stems just before buds begin to flower, leaving a few leaves to send up another crop. Tie in bundles and dry in an airy, shady place. When dry, crumble and store in airtight containers. To freeze, pack small bunches in plastic bags and place in freezer. Blanch before freezing if storing more than 2-3 months.
Mint, spearmint, peppermint, apple mint, orange mint (leaves); Mint Family	
Perennial. Purple flowers. Refreshing odor and flavor. Often used as garnish. Flavor combines well with lamb, peas, fish sauces, candies, chocolate and vegetables. Crush leaves just before adding to a dish.	Pick young, fresh leaves to dry. Hang in bunches in a warm, dry place away from strong sunlight. When dry, crumble and store in airtight containers. To freeze, see basil.
Oregano (leaves), *Origanum* species; Mint Family	
Perennial treated as an annual	See sweet marjoram for preserving.

because it doesn't overwinter. Flavor similar to sweet marjoram, but stronger and more sage-like. Liberally used in Spanish and Italian dishes, pizza; component of chili powder.	
Parsley (leaves), *Petroselinum crispum*; Carrot Family	
Annual. Good border plant. Finely curled, aromatic leaves are rich in vitamins A and C. Used as flavoring or garnish for soups, salads, eggs, meat and poultry dishes, creamed vegetables and hot breads.	Parsley can be dried as for basil, but the flavor is better if frozen. To freeze, see basil.
Rosemary (leaves), *Rosmarinus officinalis;* Mint Family	
Perennial. Small, pine-like bush in mint family. Pale blue flowers. Leaves have a spicy odor and warm, piny taste. Used as a garnish and to flavor vegetable and meat dishes, cream soups, sauces and jellies. Makes a good tea.	If brought inside to winter, provides fresh leaves throughout the year. To dry, cut stems and hang upside down in a cool, airy place. When dry, crumble and store in airtight containers.
Saffron, *Crocus sativus*; Iris Family	
Annual. An autumn crocus with delicate, lilac flowers. Yellow stigmas are pleasantly bitter, give a yellow hue to foods. Used sparingly in sauces, cookies, cakes, chicken, gravies and Spanish rice.	Remove stigma in late fall and dry on a cloth in a warm room. Store stigma in airtight containers.
Sage (leaves), *Salvia officinalis;* Mint Family	
Perennial. Imported shrub with gray leaves. Don't use Western U.S. varieties, as these taste like turpentine. Strong bitter flavor. Used sparingly in stuffings, soups, stews, sausage and herb breads.	Pick leaves in spring before flower buds form, or flavor becomes musty. Hang in bunches in a warm, dry place away from strong sun. When dry, crumble and store in airtight containers. To freeze, pack in small plastic bags. To store more than three months, blanch before freezing.

Summer savory (leaves), *Satureja hortensis;* Mint Family	
Annual. Bushy plant with long, narrow leaves and weak, woody stems. Used in poultry, soups, gravies, stuffings, salads, bean dishes, sauces for fish or veal.	Cut leafy tops and use only young, tender leaves. Remove woody stems. To dry, see mint.
Tarragon (leaves), *Artemisia dracunculus*; Sunflower Family	
Perennial. Slender, dark green leaves with sweet anise scent. Considered essential in many French dishes. Goes well with eggs, poultry, fish, shellfish and many vegetables. Used as flavoring in pickles and vinegar.	Use fresh young leaves and stem tips. To dry, see mint. To freeze, see sage.
Thyme (leaves), *Thymus vulgaris;* Mint Family	
Perennial. Small shrub with tiny, brownish-green leaves. The leaves have unexcelled aroma and flavor. Good with roast meats, fish chowders, sauces, soups, gumbos, stews, stuffings and salads. Makes a flavorful tea.	Cut sprigs before the plant flowers. Hang in a dry, shady place for a few weeks, then rub leaves from stems and store in airtight containers. To freeze, see sage.

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Appendix C

Colorado Department of Public Health and Environment Memo

STATE OF COLORADO

Bill Owens, Governor
Jane E. Norton, Executive Director

Dedicated to protecting and improving the health and environment of the people of Colorado

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Colorado Department of Public Health and Environment

FOOD AND DRUG LETTER #01-01

Date: May 11, 2001

Subject: What are the requirements for salad mixes and mixed greens offered for sale at farmers' markets?

INTERPRETATION

- Packaged, ready-to-eat salad mixes (i.e., fresh cut produce) shall be processed in an approved, GMP-compliant facility or licensed retail food establishment.
- Unpackaged mixed greens sold as a ready-to-eat food shall be processed in a GMP-compliant facility or licensed retail food establishment.
- Raw, unpackaged mixed greens with minimal or no post-harvest processing are not a ready-to-eat food and are not subject to the *Colorado Wholesale Food Regulations* or the *Colorado Retail Food Establishment Rules and Regulations*. Consumers should wash the product if it is to be eaten raw.

BACKGROUND & GUIDANCE

- Vendors operating at farmers' markets and selling only uncut fresh fruit and vegetables are exempt from the requirements of the *Colorado Retail Food Establishment Rules and Regulations*.
- Harvesters or growers who also process the fruits and vegetables are considered processors and must comply with the requirements of the *Colorado Wholesale Food Regulations (GMP's)*. That is, processing must be conducted in an approved GMP-compliant facility.
- "Minimal processing" at the farm, such as rinsing gross debris from raw produce, is not considered processing and, therefore, not subject to the GMP's.
- All food vendors, whether processors or retailers, must comply with the provisions of the Colorado Food and Drug Act, §25-5-4, Colorado Revised Statutes. (aka the Pure Food and Drug Law).

"Fresh fruits and vegetables" refers to produce that is likely to be sold to consumers in an unprocessed or minimally processed (i.e., raw) form. Fresh produce may be intact, such as strawberries, whole carrots, or radishes, or may be cut during harvesting, such as stalks of celery, and broccoli. The consumer recognizes that the product has not been processed and should be washed prior to consumption.

"Fresh cut" produce includes pre-cut, packaged, ready-to-eat salad mixes. These salad mixes are considered processed and must be produced in an approved GMP-facility.

"Mixed greens" refers to a fresh produce specialty item that may or may not be subject to post-harvest processing steps at the grower. The produce is offered to the consumer in its raw form, unpackaged. Mixed greens are often displayed in a manner that suggests to the consumer that the product is a ready-to-eat food. The additional processing/handling steps and the marketing method may present an increased food-safety risk because the consumer may not recognize that the product should be washed prior to consumption.

There are five major areas of concern related to microbial contamination of fresh produce.

- Water quality
- Manure/municipal biosolids
- Worker hygiene
- Field, facility and transport sanitation; and
- Traceback

Fresh produce is often washed at the farm to remove gross debris. This is not considered processing. Water used during the post-harvest handling of produce often involves a high degree of water-to-produce contact and may serve as a source of contamination. In addition, reusing the wash water may result in the build-up of microbial loads, including undesirable pathogens from the crop.

Thoroughly washing fresh produce is an important step in reducing microbial food safety hazards since most contamination is on the surface of the fruit or vegetable. Past outbreaks of foodborne illness associated with fresh and minimally processed produce have usually been the result of produce becoming contaminated with fecal material from water or food handlers. All fresh produce should be washed thoroughly before consumption.

Produce sold at farmers' markets is sold directly from the grower to the customer. To reduce the risk of foodborne illness associated with raw and minimally processed agricultural products, it is important to advise customers that the raw produce should be washed if it is to be eaten raw.

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~WRL3870.tmp
May 11, 2001

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STATE OF COLORADO

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RETAIL FOOD ESTABLISHMENT INTERPRETATION #02-2

DATE: April 26, 2002

SUBJECT: Farmers' Markets

Note: This retail food establishment interpretation supersedes Retail Food Establishment Interpretation #01-03 (dated September 27, 2001).

Farmers' markets have evolved over the years from venues for small vegetable and fruit producers to marketplaces that encompass much more than uncut fruit and vegetables. This letter is designed to provide guidance to local health departments so that vendors at farmers' markets are treated equitably throughout the State. The various food-related activities at farmers' markets can be divided into five categories, which are distinguishable by the products that are sold and/or the type of retail food establishment (RFE) license that is issued:

Category 1. Vendors who sell uncut fruit and vegetables. If a vendor is selling only uncut fruit and vegetables, the vendor is exempt from retail food establishment licensure pursuant to 25-4-1602(14)(j), C.R.S. (1998), even if offering samples.

Category 2. Vendors who sell wrapped, non-potentially hazardous foods (phf) for off-premises consumption. If a vendor is selling only wrapped, non-potentially hazardous foods for off-premises consumption, the vendor is exempt from obtaining a RFE license, even if offering samples. For example, wrapped loaf bread falls in this category.

Category 3. Vendors who sell unwrapped, non-potentially hazardous foods for off-premises consumption. If a vendor is selling unwrapped non-potentially hazardous foods for off-premises consumption, the vendor must obtain a RFE license. For example, unwrapped loaf bread falls in this category.

Category 4. Vendors who sell wrapped, potentially hazardous foods for off-premises consumption. If a vendor is selling wrapped, potentially hazardous foods for off-premises consumption, the vendor must obtain a RFE license. For example, cheese, shell eggs, frozen meats, and roasted chiles fall in this category.

Category 5. Vendors who sell potentially hazardous foods in individual portions for immediate consumption. If a vendor is selling potentially hazardous foods in individual portions for immediate consumption, the vendor must obtain a RFE license.

IN ADDITION to the licensing issues noted in Categories 1-5 above, the following items should be checked when conducting an inspection at a farmers' market:

For Categories 1-5, the following items apply Food must be protected from contamination at all times.

Vendors who prepare samples at the market must follow the minimum sanitation guidelines that were developed and distributed to vendors in 2001 ("Preparing and Offering Food Samples to Consumers"). These guidelines state that the person preparing the samples at the market site must have an available handwashing facility and must prepare the samples in a sanitary manner. Vendors who offer samples prepared at their commissary do not need a handwashing facility.

In addition to the items above, for Categories 3, 4, 5, the following items
Apply Vendors must provide evidence of commissary use Vendors must provide a Colorado sales tax account number in order to obtain a RFE license Vendors in category 4 may use an approved ice chest (e.g., Igloo or Coleman cooler) to transport and store shell eggs and prepackaged potentially hazardous foods, as long as the foods for sale are maintained at 41 degrees or less.

In addition to the items above, for Category 5, the following items apply Vendors are subject to plan review fees; this includes menu submission. Vendors must provide a potable water system under pressure. The system must be of sufficient capacity to furnish adequate hot and cold water for food preparation, utensil cleaning, and sanitizing. All liquid waste must be stored in a retention tank that is at least 15 percent larger than the water supply tank. Liquid waste must be discharged from the retention tank to an approved sewage disposal system.

Vendors who handle, package, or prepare food for sale must have a conveniently located handwashing facility available for employee handwashing. This facility must be capable of providing an unassisted, continuous flow of warm water. The handwashing facility must be of adequate pressure and size to facilitate proper handwashing. Soap and individual paper towels must also be provided.

Vendors may be prohibited from selling some or all potentially hazardous foods for immediate consumption, unless they have a mobile unit or pushcart that is commercially designed and approved to handle food preparation and service. The equipment must be certified or classified for sanitation by an American National Standards Institute (ANSI) accredited certification program.

Vendors must use clean and sanitized knives, utensils, and cutting boards. These utensils and food-contact surfaces of equipment must be cleaned and sanitized every four hours, or whenever contamination occurs. Lastly, additional requirements may be imposed as necessary to protect against health hazards associated with the vendor's operation. The attached flowchart is intended to assist you in determining the licensing requirements for vendors at farmers' markets.

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