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Misting Effects of the Microbial Quality of Retail Leaf Lettuce

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Cover Page Footnote

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1. Introduction

The consumption of fresh produce has increased due to national nutrition initiatives such as the 5-a-Day campaign, Fruits and Vegetables More Matters™, Healthy People 2010, and the USDA Dietary Guidelines for Americans (USDHHS, 2005; USDHHS, 2009; USDHHS, 2000; USDA, 2005). Although there are health benefits of consuming fresh fruits and vegetables, the safety of these foods are also of concern. Fresh fruits and vegetables may be highly contaminated as freshly harvested produce contains a variety of microorganisms (Maddeen, 1992; Liao and Fett, 2001; Burnett and Beuchat, 2001; Buechat, 1996). The normal microflora of fresh produce consists mainly of soil microorganisms. The types and number of microorganisms differ based on the type of produce, geographic area, weather conditions prior to harvest as well as agricultural production practices such as use of contaminated irrigation or process water, use of biosolids or manure for fertilizer (Burnett and Beuchat, 2001; USDA, 2001). Intrinsic factors, such as pH, structure of the outer surfaces and protective cuticle, and the presence of any antimicrobials dictate the type and number of microorganisms on the surfaces of fresh produce. The Food and Drug Administration and the United States Department of Agriculture published voluntary guidelines in 1998 to identify and implement practices that would decrease the risk for pathogenic microbial contamination of produce based on good agricultural and manufacturing practices (USFDA, 2008). Commodity specific food safety guidelines for lettuce and leafy greens (Gorney et al., 2006) have been recently developed by industry associations in response to numerous foodborne illness outbreaks from these produce items. However, these guidelines fail to address safety issues and recommendations for the produce departments in grocery stores that store, display, and sell fresh produce.

Numerous research studies have focused on agricultural practices that affect the microbial state of raw produce (Steele and Odumeur, 2006; USFDA, 2001; Wachtel et al., 2002). Also research is reported on the microbial safety of minimally processed fruits and vegetables, including modified atmosphere packaged produce (Beuchat and Ryu, 1997; Francis et al., 1999; Francis and O'Beirne, 2001; Nguyen-the and Carlin, 1994; Richert et al., 2000), salad bars (Albrecht et al., 1995; Gourama et al., 1991) and consumer handling of produce (Li-Cohen and Bruhn, 2002).

Very limited published research on grocery store practices in the produce department exists. Thunber et al., 2002 found a variety of *Listeria* spp., an enterotoxigenic isolate of *Staphylococcus* spp. and a toxigenic species of *Bacillus* spp. on fresh produce at retail markets.

In 1989, an outbreak of Legionnaire's disease in Bogalusa, Louisiana was attributed to a produce mist machine (Anonymous, 1990). The water reservoir was contaminated with *Legionella pneumophilia* and became airborne via the automatic mister in the produce department display case. As a result of this outbreak, this type of "fogger" misting machine is no longer used in produce departments (Anonymous, 1990). Currently a sprinkler type system is used which generates droplets that are much larger and less likely to be inhaled and there is no evidence that these systems present a risk for spreading Legionnaire's Disease (Anonymous, 1990; Sharifzadeh, 1990). The Food and Drug Administration has issued guidelines on cleaning and maintaining misting machines in grocery store produce departments (Sharifzadeh, 1990).

Misting is done to keep vegetables fresh and prolong their shelf life. Barth et al., 1990 examined the effect of misting on the ascorbic acid content of broccoli at retail but did not examine the microbial effect of misting. Limited studies have explored the effect of misting on the microbial population of produce. Mohd-som, et al., 1995 conducted a controlled study of misting versus non-misting in a laboratory setting. They concluded that misting with chlorinated water had a washing effect and reduced aerobic plate counts, coliforms, yeasts and molds due to the chlorination. Broccoli was held at 4°C which may not be representative of retail display temperatures. In a recent study conducted in the United Kingdom, Brown et al., 2004 conducted a controlled misting (humidification) experiment and reported that misting had no adverse effects on the microbial quality of produce. No studies or surveys have been reported that have been conducted in retail settings comparing the microbial quality of misted and non-misted produce.

Although misting can maintain freshness of vegetables, and thus extend shelf life, it provides water at the surface of vegetables that could increase microbial growth. Increase in microbial growth can result in proliferation of both pathogenic and spoilage organisms especially under temperature abuse conditions. Therefore, the objective of our study was to determine the microbial quality of leaf lettuce displayed at the retail level in misted or non-misted display cases as available to consumers.

2. Methods

Leaf lettuce (not pre-packaged) was purchased from three (3) local grocery stores that mist their leaf lettuce and three (3) local grocery stores that do not mist their leaf lettuce, over a three month period in the fall of the year. Leaf lettuce (misted only) was obtained from the top, middle and bottom shelves from the display cases where leaf lettuce was displayed in levels (three heads per shelf level). Non-misted leaf lettuce was displayed on only one level in the grocery stores where leaf lettuce was obtained. Three replications of leaf lettuce from each grocery store were conducted. Leaf lettuce was analyzed within three hours of purchase. After purchase, leaf lettuce was stored in a laboratory refrigerator and analyzed within 2 hours. To prepare leaf lettuce for analysis, two inches was removed from the stem end of the leaf lettuce head before chopping and mixing each head of leaf lettuce. From each chopped head of leaf lettuce, a 25 g sample was blended (Osterizer™) with 225 ml buffered peptone water (Difco, Becton Dickinson) for 2 min and used for the microbial analyses. Appropriate serial dilutions were made in buffered peptone water for each analysis (9, 28). Samples were analyzed for aerobic plate count (APC) using plate count agar (Difco), yeast, mold, using the Yeast and Mold Count Plate Petrifilm and *Enterobacteriaceae* (EB) Petrifilm (3M Microbiology, St. Paul, MN) following the manufacturers' instructions.

Data were analyzed using SAS version 9.1.3 (SAS Institute, Inc., Cary, NC, USA). GLM and Pearson correlation coefficients were determined between variable values. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

Results of the microbiological quality of leaf lettuce are listed in Table 1. For leaf lettuce obtained from grocery stores that mist produce, APC ranged from 1.7×10^6 to 8.59×10^6 CFU/g compared to 1.6×10^5 CFU/g for non-misted leaf lettuce. APC counts were statistically significant ($p = 0.0169$) between the misted and non-misted leaf lettuce but no significance was found for the APC counts for the three shelf levels for the misted leaf lettuce. For leaf lettuce obtained from grocery stores that mist produce, yeast counts ranged from 2.2×10^4 to 8.1×10^4 CFU/g compared to 1.1×10^4 CFU/g for non-misted leaf lettuce. APC counts found on leaf lettuce from grocery stores in this study are in agreement with values reported by Liao and Fett, 2001. Yeast counts were statistically significant ($p = 0.043$) between the misted and non-misted leaf lettuce but not significant for the three shelf levels for the misted leaf lettuce. For leaf lettuce obtained from grocery stores that mist produce, mold counts ranged from 7.6×10^2 to 2.9×10^4 CFU/g compared to 1.2×10^4 CFU/g for non-misted leaf lettuce which were not statistically significant between misted and non-misted leaf lettuce. Mold counts were statistically significant ($p = 0.024$) for the three shelf levels of misted leaf lettuce. For leaf lettuce obtained from grocery stores that mist produce, EB ranged from 1.7×10^6 to 3.4×10^6 CFU/g compared to 1.2×10^4 CFU/g for non-misted leaf lettuce. EB counts were statistically significant ($p = 0.049$) between the misted and non-misted leaf lettuce but not significant for the three shelf levels for the misted leaf lettuce.

The APC, Yeast and EB load may be higher on the misted produce due to the length of time the leaf lettuce remains on the shelf, the misting process may increase the APC, Yeast and EB levels, the handling practices of consumers may increase the microbial loads, and the growing region or source of the produce may contribute to the initial microbial load. Although length of time the leaf lettuce remains on the shelf, the handling practices of consumers and the growing region or source of the leaf lettuce would also contribute to the microbial load of the non-misted leaf lettuce as well. Since there are no standardized cleaning methods for display units in retail grocery stores, different cleaning conditions used in the display unit vary greatly from store to store and within store (Quinlin, 2004). Seasonality may also play a role in the microbial quality of produce. Volkman et al., 2006 reported that seasonality had a significant effect in broccoli under misted and non-misted situations with higher levels of mold on broccoli obtained from retail in the spring ($p = 0.019$).

Table 1. Total Plate Count, Yeast, Mold and *Enterobacteriaceae* isolated from lettuce obtained from grocery stores that mist produce and grocery stores that do not mist produce.

	APC (CFU/gm)	**Yeast** (CFU/gm)	**Mold** (CFU/gm)	**Enterobacteriaceae** (CFU/gm)
Misted Lettuce[a]				
Top Level (n=9)	$3.5 \times 10^6 \pm 8.5 \times 10^6$ [b]	$5.2 \times 10^4 \pm 1.5 \times 10^5$ [c]	$1.3 \times 10^3 \pm 1.5 \times 10^3$ [e]	$3.4 \times 10^6 \pm 8.5 \times 10^6$ [d]
Middle Level (n=9)	$2.3 \times 10^6 \pm 3.9 \times 10^6$ [b]	$2.2 \times 10^4 \pm 7.6 \times 10^4$ [c]	$7.6 \times 10^2 \pm 9.5 \times 10^2$ [e]	$2.3 \times 10^6 \pm 3.9 \times 10^6$ [d]
Bottom Level (n=9)	$1.7 \times 10^6 \pm 3.0 \times 10^6$ [b]	$8.1 \times 10^4 \pm 1.8 \times 10^5$ [c]	$2.9 \times 10^4 \pm 6.5 \times 10^4$ [e]	$1.7 \times 10^6 \pm 3.0 \times 10^6$ [d]
Non-Misted Lettuce				
One Level (n=9)	$1.6 \times 10^5 \pm 3.2 \times 10^5$ [b]	$1.1 \times 10^4 \pm 2.2 \times 10^4$ [c]	$1.2 \times 10^4 \pm 2.2 \times 10^4$	$1.2 \times 10^4 \pm 1.2 \times 10^4$ [d]

a. In misted produce, leaf lettuce was obtained from 3 levels within the display unit.

b. Average APC counts for non-misted lettuce were significantly lower than misted lettuce ($p = 0.0169$).

c. Average Yeast counts for non-misted lettuce were significantly lower than misted lettuce ($p = 0.043$).

d. Average Enterobacteriaceae counts for non-misted lettuce were significantly lower than misted lettuce ($p = 0.049$).

e. Average Mold counts for different shelf levels for misted lettuce were statistically significantly ($p = 0.024$).

In a preliminary study, Quinlin, 2004 reported higher average APC and yeast counts on misted leaf lettuce (not significant) than non-misted leaf lettuce and average mold counts approached significance (p = 0.073) on misted leaf lettuce. Quinlin, 2004 also reported that misted leaf lettuce that were at higher levels in the produce display case had a significantly higher levels of mold (p = 0.016) than leaf lettuce at lower levels. Mold counts for the different shelf levels in our study were also significant (p = 0.024) but with the lowest shelf with the highest mold count. Although shelf height did not affect the APC, Yeast and EB, this finding raises some interesting questions. Does the misting processes wash mold from leaf lettuce at the higher levels and deposit the mold on leaf lettuce at the bottom level? Or does water accumulate at the lower shelves and facilitate mold growth? Does misting have an effect on distribution of mold spores in the display case. In this study, we did not analyze the shelves which could have biofilms containing mold spores. From a preliminary study, Beattie, 2008 found that there was a significant increase in APC counts and EB on surfaces under misted produce at lower shelf levels.

4. Conclusions

From our study, we have demonstrated that there is an increase in the number of microorganisms on leaf lettuce that was misted, but we did not analyze for specific pathogenic microorganisms on the misted and non-misted leaf lettuce. Results of this study indicate a need for further research to follow leaf lettuce and/or other produce from field to grocery store to delineate the effect of misting on microorganisms in general and specific pathogens that may be on the produce.

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