The Effects of Growing Method and the Food Processing System on Bacterial Concentration in Spinach

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Received June 6, 2009

The purpose of this study was to examine the bacterial effects of the industrial food processing system on bacterial contamination and the differences between conventionally and organically grown produce therein. This is significant because every day millions of people consume these products. The hypothesis was that organically grown and locally grown spinach will have the lowest levels of bacterial concentration, respectively, when compared to conventional and bagged spinach. Three samples each of local organic, loose, domestic organic, bagged organic and bagged conventional spinach were taken by rinsing a portion of each leaf in water. 500 μL of this rinse was spread on nutrient agar plate and was incubated for 24 hours at 36ºC. Bacterial colonies in 10 randomly selected 1 cm^2 areas were counted, and the colonies' morphologies were noted, as was any fungal growth. The data were averaged and analyzed using a one-way ANOVA test (α=0.05). There was no statistically significant difference between the bagged organic and conventional test groups. Local organic spinach had the least number of bacterial colonies with a total of 247 colonies, and bagged conventional had the most with 1813. These results support the hypothesis. Future research could test other types of produce as well. Other possibilities include focusing exclusively on testing organic produce against conventionally grown produce.

Introduction

Every day, millions of people all around the world consume produce that has been processed, packaged and shipped. A study by the Freedonia Group found that packaged produce will be purchased by more consumers, as its market share in the United States will increase by 4.2% annually until 2012 (2). Packaged foods are already a staple of diets everywhere, and they are here to stay. Such growth in the industry comes even in the midst of far-reaching outbreaks related to such foods. In September and October 2006, 199 people from 26 states were infected by *Escherichia coli* 0157:H7 (*E. coli*) in an outbreak traced to fresh spinach. 3 died, including 1 child (3). In September 2008, Gadi Frankel, a professor at London's Imperial College, said that "people are eating more salad products, choosing to buy organic brands and preferring the ease of 'pre-washed' bagged salads from supermarkets. All of these factors, together with the globalisation of the food market, mean that cases of salmonella and E coli poisoning caused by salads are likely to rise in the future" (5). A study published in the Journal Applied and Environmental Microbiology links the age of lettuce leaves to the size of populations of *E. coli*. The study found that leaves on the inner part of the head consistently had 10 times more growth than older leaves on the middle part (6). As agriculture is increasingly commercialized and industrialized, leaves are picked earlier and earlier, and the likelihood of *E. coli* contamination increases drastically.

Produce is defined as any fruit or vegetable that is purchased fresh in either loose or packaged. Food processing is defined here as the system of industrially harvesting, packaging and shipping produce. This study will also examine organically and conventionally grown produce. Foods grown without chemical fertilizers and pesticides or certified organic by the US Department of Agriculture are organic. Foods grown with these chemicals are conventionally grown. Locally grown produce is defined as produce grown within South Carolina. Domestically grown produce is defined as produce grown within the United States but outside of South Carolina.

Contamination is common in all aspects of food processing. A study by Lillard, "The Impact of Commercial Processing Procedures of the Bacterial Contamination and Cross-contamination of Broiler Carcasses," measured bacterial levels at six points in a broiler processing plant and found that bacterial concentration increased as carcasses moved through the plant (7). If processing has such dramatic effects on meat, what effects does processing have on produce? A study by Brackett, "Microbiological Consequences of Minimally Processed Fruits and Vegetables," partially answered this question when it found that minor changes in fruits' and vegetables' environments, such as temperature and humidity, can affect how prevalent microbes are (1). Though Brackett's research answered this question partially, no study has completely answered it. This study, however, will attempt to using the following methods: Loose organic, packaged organic and packaged conventional spinach were purchased from grocery stores in the Columbia, SC area. Leaves with excessive brownness and/or stagnant water were avoided. Local organic spinach was picked at a local community garden that uses organic growing methods. Three leaves from each of the 4 groups, along with 3 control tests, were tested. Bacterial levels and colony morphologies were compared between groups. Data were analyzed with a one-way ANOVA (α=0.05) test.

This research was conducted because food is a crucially important aspect of all people's lives. Millions of people around the world consume organic produce, packaged produce and domestically grown produce. As food is produced farther and farther away from people and people are more and more disconnected from their food source, it's important that they know what they are consuming, bacteria and all. The purpose of this research was to study bacterial levels in organically and conventionally grown produce and local, domestic and packaged produce.

It was hypothesized that organically grown and locally grown produce will have the lowest levels of bacterial concentration, respectively. The null hypothesis is that bacterial concentration will not vary between growing methods and growing locations. The independent variable is the growing method and the growing location. The dependent variable is the number of bacterial colonies that are present after incubation.

Methods

Spinach leaves and bags were selected using guidelines suggested by experts in food safety and in the industry: leaves with excessive brownness were avoided, as were bags with excessive water in them. Once purchased, leaves were refrigerated until needed. The amount of time between purchase and testing was minimized. Before testing began, Petri dishes were poured with nutrient agar using instructions outlined in "Instructions for Bottled Media," a booklet written by Carolina Biological Supply. Poured plates were stored in a refrigerator until needed. Throughout preparation and testing, proper sterile technique was followed. 5 milliliters of sterile water was added to each sterile 50mL centrifuge tube. A 2x2cm square was then cut from each of the 12 spinach leaves [3 leaves per group (local organic, loose organic, bagged organic and bagged conventional)] and added to its corresponding tube. After the leaf was added, each tube was shaken for 10 seconds. The leaf was removed with sterilized forceps. 500 microliters of each sample was taken with the micropipette, added to a Petri dish containing nutrient agar and spread evenly on the plate. The plate was then taped and placed into an incubator set at 36ºC and incubated for 24 hours. Each plate held only the sample from one leaf. After incubation, plates were stored in a refrigerator until they were ready to be counted. PetriStickers were placed on the bottom of each plate. A set of 10 random numbers was generated. These numbers represented 10 1 cm^2 squares on the sticker. These squares were counted by hand with the assistance of a stereo microscope. Colonies' morphology and any fungal growth were noted in addition to the bacterial counts. The same set of random numbers was used when counting the remaining plates. This process was repeated for each of the remaining 14 trials. This process was repeated thrice more without leaves to act as a negative control. The bacterial counts for each of the three plates within each group were averaged, and the standard deviation was calculated. The results were then analyzed using a one-way ANOVA (α=0.05) test.

Results

Table 1 displays the total number of bacteria counted in a 10 cm^2 per plate, the total number of colonies counted by experimental group and the mean for each group, as well as counts and the mean for the control group. Asterisks (*) denote plates that had observed fungal growth. As demonstrated by the data, bagged conventional leaves had the most bacterial growth (1813 colonies), followed, in order, by bagged organic (953), loose organic (514) and, finally, local organic (247). All local organic plates had fungal growth, as did one bagged conventional plate. Standard deviation was highest in bagged conventional tests (±212.74), followed closely by bagged organic (±211.33). Loose organic plates had the third highest standard deviation (±123.62), and local organic had the lowest deviation of the experimental groups (±10.02). The control plates had the lowest deviation of all groups (±3.61).

Table 1: Bacterial Colony Counts for Loose Organic, Bagged Organic, Bagged Conventional and Control Plates in a 10 cm^2 Area

Plate #	Loose Organic	Bagged Organic	Local Organic	Bagged Conventional	Control
1	306	296	*92	783	0
2	63	118	*72	*369	2
3	145	539	*83	661	7
TOTAL	514	953	247	1813	9
MEAN	171.3	317.7	82.3	604.3	3.0
STDEV	123.62	211.33	10.02	212.74	3.61

Table 2 displays the average number per plate of bacteria for each colony morphology observed, ordered by group. There were four colony morphologies observed in this study. The smooth white morphology was the most prevalent of the four observed. Smooth yellow was the second most common in three groups, while it was not present in local organic tests. Clear and fried egg morphologies followed in third and fourth, respectively. In each of the experimental groups, smooth white bacteria were the most common. In the control group, clear was the dominant morphology. The smooth yellow and fried egg morphologies were found most often in loose organic tests. Bagged conventional followed loose organic closely in its smooth yellow counts. Clear bacteria were most commonly found in bagged organic tests, as compared to the other groups.

A single factor ANOVA test (α=.05) was used to analyze data and compare groups. 2 of the 4 experimental groups, loose organic and bagged organic, were found to have a statistically insignificant difference from the control group ($p>0.05$). Bagged conventional and local organic data, however, did yield statistically significant results when compared to the control group, with p-values of .008 and .0001, respectively. When bagged conventional and bagged organic data were compared, there was no statistically significant difference at α=0.05, suggesting that the difference in bacterial levels of organically and conventionally grown

Table 2: Average Bacterial Colony Counts by Colony Type per Plate

Bacteria Type	Loose Organic	Bagged Organic	Local Organic	Bagged Conventional	Control
Smooth White	110	349	81	547	0
Smooth Yellow	57	39	0	55	0
Fried Egg	4	3	0	1	0
Clear	0	19	1	1	3

Table 3: Results from Single Factor ANOVA (α=0.05) Tests Comparing Groups

P-values	Loc Org	Loose Org	Bagged Org	Bagged Con	Control
Loc Org		0.282	0.126	0.013	0
Loose Org			0.359	0.038	0.078
Bagged Org				0.173	0.061
Bagged Con					0.008
Control					

spinach is insignificant. No tests comparing any two organic groups (local, loose and bagged) yielded statistically significant results. A test between the loose organic and bagged conventional groups yielded a p-value of .038, suggesting a statistically significant difference. Local organic spinach showed similar results, yielding a p-value of .013 when compared to bagged conventional spinach.

Conclusions

The purpose of this research was to examine the bacterial repercussions of the industrialization that dominates the food system today, as well as to examine the difference of organically and conventionally grown foods. The difference in bacterial levels of organically and conventionally grown spinach was insignificant. No tests comparing any two organic groups (local, loose and bagged) yielded statistically significant results. The data would suggest that spinach purchased locally or loose and domestically that were grown organically have a statistically significant lower amount of bacteria than bagged conventional. That no two similar (organic or bagged) groups had statistically significant differences suggests that bacterial concentration increased in small increments. Nonetheless, when extremes (loose organic and local organic v. bagged conventional) were compared, differences were statistically significant. These data would tend to support the hypothesis that organically grown and locally grown produce will have the lowest levels of bacterial concentration, respectively.

Attempts were made to decrease sources of error, but possible sources of error existed nonetheless. Before testing, leaves were not rinsed, so local organic leaves, which were picked at Heathwood's community garden, had soil visibly present, which is likely the source of the observes fungal growth. Another possible source of error was the type of spinach tested. Both the loose and bagged organic test groups used baby spinach, as opposed to the bagged conventional group, which was marketed simply as spinach. Another possible source is that there simply were not enough tests for definite conclusions to be made.

Future research possibilities abound. One possibility is to test other types of produce to determine if similar results are found. Another is to test for pathogenic bacteria, such as *E. Coli* or *Salmonella*. Such testing would take into account 'good bacteria.' Future research could also examine the effects that chemical pesticides and fertilizers have on bacterial concentration. Moving forward, food processing practices should be reviewed to explain why and where bacterial contamination is occurring. No matter the source of contamination, these data display some of the adverse reciprocal effects of being separated from food sources. If this study causes one consumer to reconsider what he or she purchases and eats, whether that means they start to buy locally or organically, start their own garden, or otherwise alter their habits, or simply to wash his or her produce, it will be considered a success. Food is an essential component of all people's lives, and the more knowledgeable they are about what they eat, the better off they are.

Acknowledgements

I would like to thank Mr. Jim Morris for use of his lab and Heathwood Hall Episcopal School for funding and use of its community garden. I would also like to thank Mrs. Michelle Myer for her incredibly useful advice. Finally, I simply cannot thank my Biology teacher, Mrs. Lisa Norman, enough for her patience, advice and wisdom, all of which were invaluable during the course of planning, experimentation and analysis.

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