The Effectiveness of Alcohol to Reduce Bacterial Counts in Craft Beer

A Thesis Presented to the
Faculty of the
Conrad N. Hilton College of Hotel and Restaurant Management
University of Houston

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

Martina Bahr
May 2016

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Dedication

I’d like to dedicate this paper to those who have made my academic journey possible. To my parents who have given me continual support, to my professors who have taught me past words in the textbook, and to my mentors who have given me invaluable hands on experience. This is for those who have lit an undying fire and desire to continually learn about food and beer through knowledge, patience, and pure enthusiasm.

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Table of Contents

Title Page..i

Signature Page...ii

Dedication..iii

Acknowledgements..iv

Table of Contents..v

List of Tables..viii

List of Figures...ix

Abstract..x

CHAPTER I..1

Introduction..1

Objective of Study...2

Justifications..3

CHAPTER II...4

Literature Review..4

Sanitation and Cross Contamination...4

HACCP...5

Common microorganisms and outbreaks.....................................7

Basil……………..…………………………………………...........8

Brewery Sanitation and Cross Contamination……………..………9

Craft Beer…………………………………………………...........13

Craft beer trends...……………………………………………..14

CHAPTER III ……………………………………………………………16

Methodology…………………………………………………………..16

Research Design……………………………………………………16

Sample Selection………………………………………………...17

Data Collection……….…………………………………………18

Beer preparation………………………………………........18

Basil beer preparation…….………………………….……. 19

Aerobic plate counts..………...………………………………19

Inoculum preparation..……………..…………………………20

Sample preparation and inoculation……..……………………20

Plating……………….……………………..……….…………20

Data Analysis………………………………………………………21

CHAPTER IV……………………………………………………………22

Results……………………………………………………………………22

CHAPTER V……………………………………………………………………..….27

Discussion……………………………..………………………………..….27

Limitations…………………………..………………………………31

Conclusion……………………………………………………...…31

References…………………………………………………………………….....33

List of Tables

Table 1: Hazard Analysis Critical Control Point Summary……………………………….6

Table 2: Beer Styles with a Potential Target ABV of 3.2%..…………………………....16

Table 3: ANOVA Test between Basil, Beer, Basil Beer, and Two Day Aged Basil Beer…………………………………………………………………………….…...22

Table 4: Multiple Comparisons of ANOVA Testing- Tamhane Posthoc Test………..….23

Table 5: ANOVA Test between Salmonella, Inoculated Sterile Beer, and Inoculated Unsterile Beer…………………………………………………………………….…...26

Table 6: Multiple Comparisons of ANOVA Testing- Tukey Posthoc Test……………..….26

Table 7: Descriptive Statistics- Aerobic Plate Counts…………………………………..…30

Table 8: Descriptive Statistics- Spread Plating……………………………………….…....31

List of Figures

Figure 1: Number of Breweries in the U.S. from 1873 to 2015……………………….……...14

Figure 2: Cumulative Graph Showing the Number of Regional Breweries,
Microbreweries, and Brewpubs from 1994 to 2015……………………………….……….14

Figure 3: Swarming Bacteria on Basil…………...…………………………………....…24

Figure 4: Swarming Bacteria on Basil Beer……………………..…………………....25

Figure 5: Swarming Bacteria on Two-Day Aged Basil Beer……..……………………..25

Figure 6: Mean Log of Colony Forming Units on Aerobic Plate Counts…………..…..29

Figure 7: Mean Log of Colony Forming Units on Spread Plating……….……………...30

Abstract

The most noted craft beer trends of 2015 and 2016 were sessionable and food product infused beers. This experiment aimed to examine these craft beer trends' ability to lead to potential foodborne illness in beer by evaluating alcohol's ability to reduce bacterial counts. In a two-part experiment, the ability for alcohol in a 3.2% ABV beer to kill spoilage bacteria on basil, then *Salmonella* was investigated. In the first part, aerobic plate counts of general bacterial growth on basil, beer, fresh basil beer, and two day aged basil beer were examined. Beer was able to reduce the bacterial growth found on basil. Two day aged basil beer had reduced growth when compared to fresh basil beer. In the second part, basil, sterile beer, unsterile beer, fresh basil beer, and two day aged basil beer were inoculated with 9.0 Log_{10} CFU of *Salmonella* Typhimurium 53647. Sterile and unsterile beer both significantly reduced *Salmonella* contamination. The results imply that aging beer reduces bacterial loads of food product infused beer and that beer is able to significantly reduce the growth of microbial contamination in 3.2% ABV beer, while foodborne illness in sessionable beer poses potential risk.

CHAPTER I

Introduction

Although many argue that brewing developed before the first evidence of written language, the history of mass production brewing can be traced back with the development of society and modern day agriculture (Sinclair & Sinclair, 2010). The first evidence of beer agriculture and manufacturing in the Fertile Crescent, Mesopotamia, and Egypt points directly back to the development of grain-based fermented beverages by the ancient Chinese in 7000 BC (Meussdoerffer, 2009). This suggests that the development of agriculture and manufactured beer contributed to the development of the modern day beverage industry. More specifically, many different innovations such as irrigation systems, food preservatives, food packaging, pasteurization, transportation, and pesticides shaped today's modern day food safety practices (IRS, 2015).

In the United States (U.S.), government agencies such as the Occupational Safety and Health Administration (OSHA) and the Food and Drug Administration (FDA) ensure safety and sanitation through many regulations necessary to protect the general population's health. Many of these regulations were developed in reaction to outbreaks of foodborne illness. As the modern U.S. food and beverage industry began to develop through the awareness of sanitary practices and the various governmental regulations, the tastes and desires of the nation shaped as well (Law, 2004 & Brewers Association, 2015). It is important to mention that from 1919 to 1933, Prohibition hindered and restricted growth of beer manufacturing, so many of the beer production regulations were created preventatively. The brewing industry in the U.S. shrunk to 44 brewing companies by the end of the 1970s due to effective marketing campaigns promoting light-adjunct lagers

(Brewers Association, 2015). Though marketing campaigns had been effective through the 1970s, by the 1980s U.S. beer consumers began to yearn for flavors that more closely resembled beers in other countries (Brewers Association, 2015 & Hieronymus, 2016). Homebrewers started making these flavorful styles on their own and a few opened their own small breweries (Papazian, 2016). These small breweries became known as microbreweries, and eventually known as craft breweries, which have rapidly grown ever since (Brewers Association, 2015 & Papazian, 2016). As the craft beer industry continues to develop in the U.S., the need for continual research on craft beer trends in these establishments remain necessary.

Objective of Study

It is widely recognized, in both governmental and academic research, that there are many opportunities for foodborne illness developing in food production through failure to adequately sanitize facilities, wash product, and prevent cross contamination (De Jong et al., 2008, FDA, 2012, & Marriott & Gravani, 2006). Viruses and pathogens can survive and grow in beverage production facilities if there is an absence of effective food safety procedures. It is also recognized that sanitation in the process of alcohol production is vital to preventing faulty or harmful products (Goldammer, 2008). There is a notable gap when evaluating microorganism growth in beer with specifically less than 5% alcohol by volume (ABV) (Goldammer, 2008 & Menz et al., 2010), This research, based off current craft beer trends, aims to investigate if the lower alcohol content in sessionable beers is able to able to reduce or kill bacterial growth in a craft beer setting.

Justifications

As the U.S. craft beer industry continues to grow, new trends to create unique, yet still traditional styles, take hold each year. The top two craft beer trends in the U.S. of 2015 and predicted for 2016 are more sessionable (low ABV) and food product infused beers (Bernstein, 2015, Kopp, 2016, & Watson, 2016). By lowering intrinsic hurdles that are natural in beer, bacterial contamination and foodborne illness becomes a potentially greater risk. With 9.4 million annual foodborne illness outbreaks in the U. S., there is continual need to prevent as many outbreaks as possible (CDC, 2015, Minnesota Department of Health, 2013, Scallan et al., 2011 & U.S. Department of Health and Human Services, 2014). The basis of this preventative research is evaluate if sessionable beers are able reduce or kill bacterial contamination caused by food product infusion.

CHAPTER II

Literature Review

Sanitation and Cross Contamination

It is recognized that proper sanitation, alongside washing food products, washing equipment, and mitigating cross contamination, can help prevent foodborne illness from viruses, pathogens and parasites that can be naturally found in food supply (Marriott & Gravani, 2006). Of the 36.4 million illnesses that are domestically acquired, 9.4 million have been proven to be foodborne, while cross contamination is one of the leading causes (CDC, 2015, Minnesota Department of Health, 2013, Scallan et al., 2011 & U.S. Department of Health and Human Services, 2014). The three most vital points to control food safety are from a biological, chemical, and physical agents (University of Rhode Island, 2016 & Valigra, 2013). Additionally, the best ways to prevent foodborne illness include proper personal hygiene, proper cleaning and sanitizing of all food contact surfaces and equipment, good basic housekeeping and maintenance, and food storage for the proper time at safe (Valigra, 2013). When sanitizing equipment, establishments should look to use "a chemical or heat to reduce the number of microorganisms or other contaminants to a level that is not harmful. The first step is cleaning; the second step is sanitizing (National Food Service Management Institute, 2009)."

As previously mentioned, cross contamination is one of the leading causes of foodborne illness (CDC, 2015, Minnesota Department of Health, 2013 & U.S. Department of Health and Human Services, 2014). Cross contamination is the physical transfer of harmful bacteria, usually from raw food product's contact with other food products, tools, or surfaces (Minnesota Department of Health, 2013). By ensuring

sanitation, creating control points, establishing a steady schedule, keeping up with storage, and employee education cross contamination can be avoided (Carrera, 1996 & Roetker, 2005).

HACCP. Bacterial transfer can be unseen to the naked eye, making it vital for food service establishments to implement proper sanitation procedures that work to avoid cross contamination. To ensure that food products meet quality and food safety standards legally, food production establishments can institute Good Manufacturing Practices (GMPs). These systems cover building, facility, and equipment maintenance while clearly define terms and expectations of production and process controls (FDA, 2004). Hazard Analysis Critical Control Point (HACCP) should be utilized to further assure food safety. HACCP plans are a management procedure which examines and controls all biological, chemical, and physical hazards (FDA, 2015). Because HACCP plans are often poorly implemented, utilized, or updated by the majority of food companies, many cross contamination issues arise (Mortimore & Wallace, 2013). A specific HACCP plan should be designed and applied to each stage of food production from receiving to distribution and consumption (Pierson, 2012). Although HACCP plans are not required for beverage production, they would be highly beneficial in order to reduce the risk of cross contamination. The creation and implementation of a HACCP plan are on the following page in Table 1 (Minnesota Department of Agriculture, 1998).

Table 1

Hazard Analysis Critical Control Point Summary

Step	Principle	Use
1	Conduct a hazard analysis	• Identify hazards present in process • Evaluate if the hazard is "reasonably likely to occur" • If it is likely to occur, list any preventative measures
2	Identify Critical Control Points (CCPs)	• Identify a point, step, or procedures that is controllable and can eliminated, prevented, or reduced to acceptable levels • Evaluate unique conditions in certain facility • CCPs include, but are not limited to freezing, cooking, smoking, acidification
3	Establish Critical Limits for each CCP	• Identify the maximum or minimum value to which a hazard must be controlled at to prevent, eliminate, or reduce to an acceptable level of occurrence • These are boundaries for safety of each CCP and are often specific numerical values
4	Establish CCP monitoring procedures	• Consists of observations of measurements to check that CCPs are under control • These tell you where a problem has occurred, track the system's operations, identify dangerous trends, and provides written documentation of compliance • Must include who will monitor, what will be monitored, when it is done, and how it is done
5	Establish corrective actions	• These are procedures to follow once a failure to meet a critical limit occurs • Must determine the disposition of a non-complying product • Must correct the cause of non-compliance to prevent recurrence • Must show that the CCP is under control once again
6	Establish recordkeeping procedures	• Establish day-to-day "working" logs as a continual recording of information • Records include the HACCP plan itself and all supporting documentation • Includes records, including product codes, that document all daily monitoring, deviation and corrective action logs, as well as verification logs
7	Establish verification procedures	• Validates the plan's adequacy in controlling the food safety hazards during analysis and ensures effective implementation • Should include initial validation, ongoing verification activities, reassessment of the plan, and reassessment of the hazard analysis

Note. Adapted from "An Introduction to the 7 HACCP Steps," by the Minnesota Department of Agriculture, Dairy and Food Inspection Division, *Understanding How to Develop a HACCP Plan,* 1998, p. 6-8.

Common Microorganisms and Outbreaks. The most common microorganisms that lead to foodborne illnesses are *Norovirus*, *Salmonella spp., Clostridium perfringens,* and *Campylobacter spp.* (Scallan et al., 2011).

The most common pathogen, *Norovirus,* is an extremely contagious virus that stems from food and water by contact with contaminated surfaces (CDC, 2013). Prevention of an outbreak begins with simply proper handwashing, washing food products, and by following general sanitation procedures (CDC, 2013). Though alcohol-based hand sanitizers have been found to aid in the removal of *Norovirus,* they are not as effective at removing *Norovirus* as proper handwashing with soap and water (Blaney et al., 2011). This information suggests that *Norovirus* is able to survive in low ABV beers because alcohol alone cannot. *Norovirus* has been identified as the most common cause of gastroenteritis in the world. Furthermore, "between 250 and 350 million Americans are estimated to suffer acute gastroenteritis annually, with 25% to 30% thought to be caused by foodborne illnesses" (McCabe-Sellers & Beattie, 2004). Usually transmitted through leafy greens, fresh fruits and shellfish, *Norovirus* in restaurants is spread mostly through ready-to-eat food handled with bare hands (CDC, 2013). This was an issue for Chipolte in 2015 after an outbreak of *Norovirus* effected 88 customers and employees, spread through their ready-to-eat menu items (Food Safety News, 2015). When brewing low ABV beers and infusing them with fresh, raw food products, the risk of cross contamination with *Norovirus* is potential.

Nontyphoidal *Salmonella spp.,* is a common bacteria spread from animals to humans, person-to-person, through animal feed, and through the consumption of contaminated food (WHO, 2015). This is especially threatening to those with

compromised immune systems as it can survive in both dry and wet environments, while prevention of spread must be controlled in all parts of the food supply chain, including processing, manufacturing, and preparation (WHO, 2015). *Salmonella* is found in animal product, fresh produce, and even spices (FDA, 2012). Annually, there are about 1.2 million illnesses and about 450 deaths associated with *Salmonella* in the U.S. (CDC, 2015). In 2006, over 190 cases in two months were reported in this outbreak when multiple investigations began in over 21 states that found the source to be raw, large, round tomatoes from Ohio tomato fields. As this area had previously not been linked to any *Salmonella* outbreak, this study reveals the importance of spread prevention at all levels of food service production including restaurants (Behravesh et al., 2011). Because *Salmonella* is so common in natural food, especially leafy greens, it's reasonable to believe that it could contaminate beer if infused with fresh produce, such as basil.

Basil. Fresh produce, such as basil, has been associated with many foodborne illness outbreaks. In February 2004, two clusters of *Cyclospora* outbreaks in Illinois and Texas revealed basil and lettuce as sources (Outbreak Database, 2016). In February 2005, the FDA linked a multistate *Cyclospora* outbreak with 592 illnesses directly to basil imported from Peru (Outbreak Database, 2016). In June of 2005, the FDA was called into a New Haven, Connecticut restaurant to investigate an outbreak of *Cyclospora* presumed to link back to basil (Outbreak Database, 2016). In April 2014, the Lisy Corporation of Miami, Florida voluntarily recalled their sweet basil due to an FDA sampling that identified *Salmonella* (Food Safety News, 2014). Lastly, also in 2014, amongst an 18 state outbreak of *Salmonella,* the CDC confirmed 11 due to contaminated raw, fresh basil

(CDC, 2014). It is important to note that 50 beers have been brewed with basil in the U.S. as of 2016 (“Basil beer,” n.d.).

Brewery Sanitation and Cross Contamination

Because breweries produce beer for public consumption, it’s clear to see that sanitation and the prevention of cross contamination are also an integral part of the brewing process. Brewery sanitation has been researched in the U.S. post-prohibition.

Although ethanol, low pH, lox oxygen levels, hops, carbon dioxide, sulfur dioxide, lack of nutrients, and steps of the brewing process, such as mashing, boiling, pasteurizing, filtering, and bottle conditioning restrict most pathogenic activity in beer, there are a few notable microorganisms that are known cause spoilage (Vriesekoop et al., 2012). While most microorganisms found in beer create quality issues which stem from the contamination of wort, they are also able to elevate the beer’s pH level, create ropiness, alter the fermentation cycle, and create acetification and acidification (Hui et al., 2003 & Vriesekoop et al., 2012). Proper sanitation in a brewery has the ability to halter each of these problems and there are two main ways to sanitize product or equipment: pasteurization and chemical sanitation (Hui et al., 2003).

With pasteurization, beer is heated to destruct harmful microorganisms (Goldammer, 2008 & Hui et al., 2003). There are two ways that breweries are able to pasteurize their beer- before packaging called flash pasteurization or after packaging called tunnel pasteurization (Goldammer, 2008). Typically, flash pasteurization is used for reduction of harmful microorganisms in bulk beer, like kegs, and tunnel

pasteurization is used for smaller packaged beers (Lea & Piggott, 2003). Flash pasteurization is heating beer up to the range 71° C to 79° C for 15 to 60 seconds (Goldammer, 2008). Tunnel pasteurization is when packaged bottles are sprayed with hot water on a conveyor belt until the inner-bottle temperature meets the outer-water temperature to sterilize the beer and gradually cooled back down (Goldammer, 2008). Because pasteurization has extremely specific times and temperatures, a common quality problem is overheating which can cause unwanted effects on flavor and haziness in beer (Hui et al., 2003). When a brewery does not want to risk overheating product, they may choose to substitute pasteurization with filtration (Hui, Y. et al. 2003). Sterile filtration involves pushing beer through primary diatomaceous earth filters and cartridge membrane filters to trap microorganisms and clarify the beer (Goldammer, 2008).

When sanitizing equipment, alkaline-based detergents, such as sodium hydroxide and sodium hydroxide/hypochlorite solutions, and acid-based detergents, such as phosphoric acid and nitric acid are utilized (Goldammer, 2008). Alkaline-based detergents, used to clean organic soils, are widely popular (Goldammer, 2008). These solutions cut fatty oils, fats, starches, proteins, starches, and carbohydrates while they dissolve proteinaceous materials, tannin deposits, and other organic matters (Goldammer, 2008). Acid-based detergents are normally used in conjunction with alkaline detergents to clean beerstone, water scale, and aluminum oxide; they are the most effective way to clean an area heavy with bacteria (Goldammer, 2008).

Cross contamination in breweries is difficult to detect. Some bacteria in brewing yeast act so similar to cultured yeast that contamination often goes unnoticed by brewers (Lewis & Bamforth, 2006). These microorganisms can lead to both foodborne illness and

quality issues. On the other hand, some bacteria in wild yeasts act so different than others, that they actually become out-grown and eliminated (Lewis & Bamforth, 2006). From a quality control standpoint it is clear that specific carbonation, filtration, and packaging methods are advantageous for clean yeast versus wild yeast beers and need to be treated as critical control points for breweries to mitigate cross contamination (Mader, 2015). Aside from keep breweries as clean and sanitary as possible, adenosine triphosphate (ATP) testing can be used in the beverage industry to test for living microorganism counts. More specifically, ATP is an energy molecule that is found in all living organisms and when it reacts with a liquid-stable reagent used in ATP testing, the living molecules emit light in proportion to the sample taken ("ATP bioluminescence," 1996). This is read through the use of a lumniometer which reads the light emitted. If utilized at breweries, ATP meter testing can reveal living organisms on equipment that can potentially lead to cross contamination (Roady, 2015). ATP meters are one of the most reliable ways to evaluate growth of beer spoilage microbes on surfaces (Storgårds, 2000).

The most common gram-positive bacteria found in a brewery are *Lactobacillus* and *Pediococcus.* With gram-positive bacteria, hop compounds create antibacterial activity and usually prevent the possibility of contamination (Sakamoto & Konings, 2003). However, despite the antimicrobial hurdles found in beer, *Lactobacillus* is the most common microorganism accredited with beer spoilage. The higher the ethanol content, the lesser the chance of *Lactobacillus* spoilage (Vriesekoop et al., 2012). *Lactobacillus* is usually considered to be unwanted in a brewery, unless used with extreme care to create sour beers (Nummer, 2012). *Lactobacillus frigidus, Lactobacillus*

brevissimilis, and *Lactobacillus brevis* have all been identified as biogenic amine-forming contaminants, which can be toxic to human health if found in beer (Goldammer, 2008 & Storgårds, 2000). Usually these bacteria spread in the raw malts, hops, and yeasts through the method of brewing and storage (Storgårds, 2000). Though not common, biogenic amine in beer can affect neural transmitters in the central nervous system and interact negatively with the vascular system (Kalac & Krizek, 2003). The other most common bacteria that produces unwanted and off flavors in beer is *Pediococcus.* Though this is in the same family as *Lactobacillus*, *Pediococcus damnosus* only spoils beer through producing off flavors (Vriesekoop et al., 2012). These unwanted, buttery flavors come from the production of diacetyl (Gindreau, Walling, & Lonvaud-Funel, 2001). Between cross contamination with *Pediococcus* and *Lactobacillus,* 70% of beer spoilage incidents occur (Sakamoto & Konings, 2003).

The other common bacteria found in beer include: *Aerobacter (Klebsiella), Obesumbacterium* and *Zymomonas* (Bokulich & Bamforth, 2013 & Kleyn & Hough, 1971). *Acetobacter* creates ropiness, turbidity, vinegary off-flavor in beer (Goldammer, 2008). Ropiness is when the beer becomes too viscous and pours an unexpected stream described as “oily” (Hayes, 2013). This happens when wort is exposed to oxygen and grows on the surface of beer during, “…wort at pitching and early fermentation, wort inadvertently aerated at racking, and cask-conditioned or open-fermented beers” (Goldammer, 2008). This bacteria reveals the common stages at which bacteria can enter and grow in beer. *Obesumbacterium* is nearly intolerant of ethanol and low pH but remains one of the main spoilers in beer due to improper sanitation, pitching yeast, and the wort cooling process (Maugueret & Walker, 2002 & Lewis & Bamforth, 2006). This

is one of the few spoilage bacteria that has been observed to survive fermentation as it separates yeast and continues into the next steps of fermentation (Lewis & Bamforth, 2006). Though not common to survive the fermentation process, *Obesumbacterium proteus* will decrease the rate of fermentation and lead to a faulty product (Maugueret & Walker, 2002). This bacteria is also able to contribute to the formation of *N*-nitroso compounds during fermentation which can convert to nitrosamines and pose a potential health hazard (Maugueret & Walker, 2002). *N*-nitroso has been found to attribute to gastric cancer (Bruning-Fann & Kaneene, 1993). Finally, *Zymomonas* spp. is able to grow in up to 6% of ethanol and 3.5 to 7 pH. It has a set of primary metabolites and secondary metabolites in which the secondary metabolites pose a threat to health. The secondary metabolites in *Zymomonas* spp. are hydrogen sulfide and acetaldehyde which mainly thrive in cask-conditioned beers or develop in primed beers during the fermentation and packaging stage (Lewis & Bamforth, 2006 & Preedy & Watson, 2004). Because this bacteria is able to survive in beers with under 6% alcohol, this study aims to evaluate if it is possible for other microorganisms, specifically foodborne pathogens, to survive as well.

Craft Beer

Despite beer drinking in the U.S. declining, craft beer production is growing. (Forbes, 2015). The number of breweries, as a whole, in the U.S have rapidly grown (see Figure 1) since 1994 (Brewers Association, 2016). The most notable growth of craft breweries (see Figure 2) in the U.S. was from 2008, with 450 breweries, to 2015, with 2,397 craft breweries (Brewers Association, 2016).

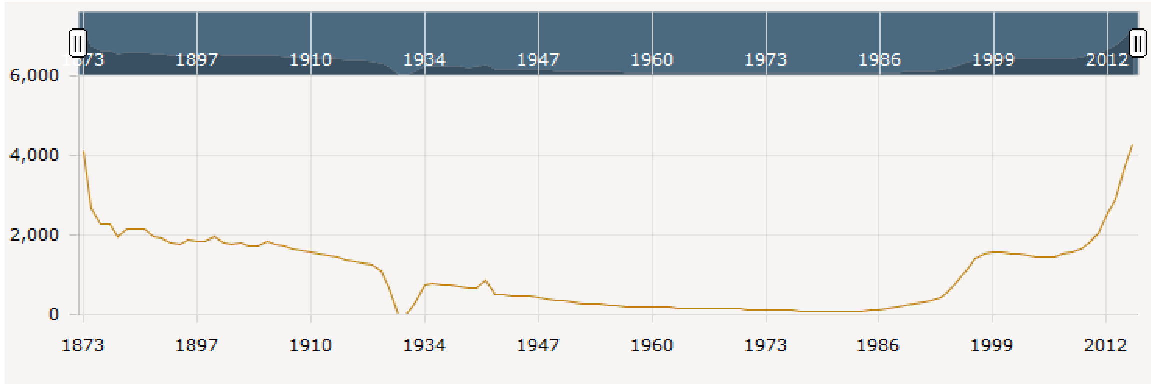

Figure 1. Number of breweries in the U.S. from 1873 to 2015. From "Historical U.S Brewery Count," by Brewers Association, n.d.. Copyright 2016 by Brewers Association. Retrieved from https://www.brewersassociation.org/statistics/number-of-breweries/

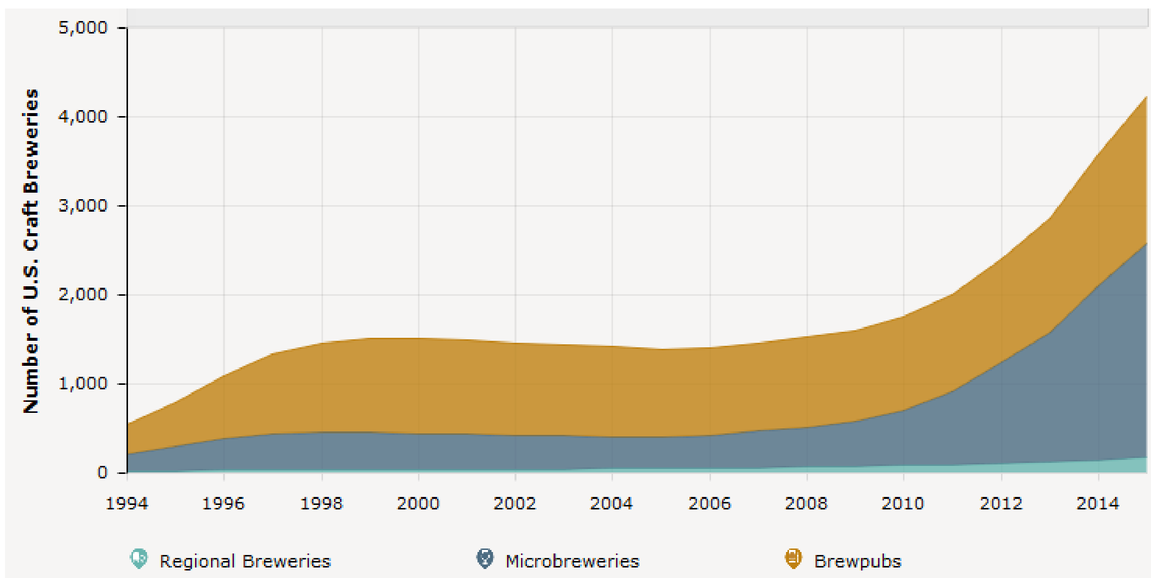

Figure 2. Cumulative graph showing the number of regional breweries, microbreweries, and brewpubs from 1994 to 2015. From "U.S. Craft Brewery Count by Category," by Brewers Association, n.d.. Copyright 2016 by Brewers Association. Retrieved from https://www.brewersassociation.org/statistics/number-of-breweries/

Craft beer trends. The two most noted trends of 2015 and predicted trends 2016 are creating more sessionable beers and naturally flavored beers (Bernstein, 2015, Kopp, 2016, & Watson, 2016). Craft beer communities such as Craftbeer.com, predict more session beers, lagers, and easy drinkers (Herz, 2016). The Brewers Association defines

sessionable beers as having 5.0% ABV or lower (2015). Many other styles of beer can also have an ABV as low as 0.5% (See Table 2).

Original Gravity Magazine not only noted these lighter and session beers with less ABV, but also barrel aging with fruits, herbs, vegetables and roots (Kopp, 2016). Fruit infused beers are expected to boom in 2016 (Watson, 2016). Flavors like apple which mainly trended in 2015 were noted by the Business Insider (Taylor, 2015). Wine Magazine even exclaimed that, "brewers are raiding the kitchen and creating a new breed of beer. Are you ready for a pint of pad thai?" (Bernstein, 2015). Uncorkd predicted plantains, black pepper, ginger, squash, and more (Thacker, 2015). By lowering the alcohol content and infusing beer with fresh kitchen flavors, the opportunity arises for cross contamination to cause foodborne illness in a beer.

Table 2

Beer Styles with a Potential Target ABV of 3.2%

Beer Style	Minimum ABV	Maximum ABV
Ordinary Bitter	3.00%	4.20%
Scottish-Style Light Ale	2.80%	3.50%
Sweet Stout or Cream Stout	3.20%	6.30%
Berliner-Style Weisse	2.80%	3.40%
German-Style Leichtes Weizen	2.50%	3.50%
Belgian-Style Table Beer	0.50%	3.50%
Grodziskie	2.70%	3.70%
Chili Pepper Beer	2.50%	13.30%
American-Style Fruit Beer	2.50%	12.00%
Belgian-Style Fruit Beer	2.50%	12.00%
Field Beer	2.50%	13.30%
Pumpkin Beer	2.50%	12.00%
Chocolate or Cocoa Beer	2.50%	12.00%
Coffee Beer	2.50%	12.00%
Herb and Spice Beer	2.50%	12.00%
Specialty Beer	2.50%	25.00%+
Specialty Honey Beer	2.50%	12.00%

Note. Adapted from "2015 Beer Style Guidelines," by Brewers Association, 2015. Copyright 2016 by Brewers Association. Retrieved from https://www.brewersassociation.org/educational-publications/beer-styles/

CHAPTER III

Methodology

Research Design

Aerobic plate counts (APC) were performed to examine general aerobic bacterial growth on each the basil, beer, basil beer, and two-day aged basil beer. APC procedures are an approved method to count the level of microorganism presence in a certain product (Maturin & Peeler, 2001). The aerobic plate count procedures in this section were adapted from the Official Methods of Analysis (Latimer, 2012). To examine the reduction of pathogen growth in beer, each variable was inoculated with *Salmonella* Typhimurium 53647. This plating procedure was also adapted from Official Methods of Analysis (Latimer, 2012). Additionally, the variable inoculation method was adapted from Neal, et al. (2008).

Sample Selection

Alternate Universe, an alt beer made by 8th Wonder Brewery in Houston, Texas is 4.7% alcohol by volume (ABV) with 26 international bittering units (IBUs) ("Alternate Universe," 2016). Because of its initially low ABV and IBUs, this beer was chosen for this experiment. The sample beer was degassed and diluted to sessionable ranges of less than 5.0% ABV, with a targeted 3.2% ABV.

There are many types of nontyphoidal *Salmonella* including *Salmonella* typhimurium, the second most common stereotype (Robinson, 2013). *Salmonella*

Typhimurium 53647, a nonpathogenic strain of *Salmonella,* is derived *Salmonella enterica* (ATCC, 2014). *Salmonella enterica* and *Salmonella bongori* are the two main species of *Salmonella* that are able to cause illness in humans through contamination of water, while *Salmonella* typhimurium is common in the U.S. (FDA, 2012). *Salmonella* was chosen because it has been associated with outbreaks in basil in the U.S. It requires high water activity environments and is able to grow with or without high levels of carbon dioxide (Lawley, 2013). *Salmonella* has a high D-value and high antibiotic resistance, which would have the lowest microbiological growth under these conditions compared to other bacteria (Stopforth et al., 2008). With such high temperatures required to kill *Salmonella*, this implies that it should be able to survive in a fermenting or bottle-conditioning environment which occurs at room temperature. The rational is that if fermentation or bottle conditioning can kill *Salmonella,* it will be able to kill all other pathogens.

Data Collection

Beer preparation. To lower the alcohol content of the sample beer, the conversion equation of V1*C1 = V2* C2 was used where V1 was unknown, C1 was the known 4.7% ABV, V2 was the target of 1000 mL, and C2 was the target of 3.2% ABV. To change the ABV from 4.7% to 3.2%, 680.851 mL of beer needed to be diluted with 319.149 mL distilled water. For the most optimal results, the beer was first degassed by placing it on a mixer (VWR VMS-C7) in a 1500 mL sterilized beaker for 30 minutes, then diluted with the distilled water. For each trial, the 1000 mL of prepared beer was split into two sterilized 500 mL media bottles. For the plating portion of the experiment,

beer was sterilized using an autoclave (Sanyo MLS-3781) to mimic a pasteurization process.

Basil beer preparation. To create the basil beer used in the experiment, 500 mL beer, 5 g basil, and 4.405 g dextrose corn sugar were mixed to mimic practices commonly used in craft breweries when fermenting or bottle conditioning beer. To ensure a thorough mix, this basil beer was placed in a stomacher bag and mixer (AEX Labratore) for 120 seconds. This basil beer was used in each trial for the fresh basil beer aerobic plate counts, then aged for two days to examine any change during the secondary fermenting or bottle conditioning process.

Aerobic plate counts. Trials were prepared in triplicate**.** In each of the trials, three samples of beer, basil, basil beer, and two-day aged basil beer were serially diluted seven times and plated, including the initial dilution. To perform aerobic plate counts (APCs) for each variable, three sterilized 150 mL beakers were filled with 90 mL peptone (0.1%). 10 mL of each variable were then added, with the exception of basil where 10 g was added instead, and this was then placed in the mixer (AEX Labratore) for 120 seconds. 1 mL of these initial dilutions were then plated on 3M Petrifilm for APCs labelled as dilution zero. Six sequential dilutions of each variable were then performed by placing 1 mL of the previous dilution into a sterilized test tube filled with 9 mL peptone (0.1%) and placing 1 mL of each dilution onto 3M Petrifilm for APCs representing a 1/10 reduction. Each of these dilutions were then placed into an incubator (Fisher Scientific) held at 37°C. After 36 to 48 hours of incubation, the basil, beer, basil beer, and two day aged basil beer APC colony forming units (CFU) were enumerated under a colony counter (Leica Quebec Darkfield).

Inoculum preparation. The *Salmonella* cocktail was prepared over three days. 1 mL *Salmonella* Typhimurium 53647 was subcultured and transferred for two consecutive days in 9 mL Sigma-Aldrich Tryptic Soy Broth (TSB) in an incubator (Fisher Scientific) held at 37°C for 24 hours.

Sample preparation and inoculation. The sample beer was examined both sterile and unsterile to mimic the pasteurization process. To sterilize sample beer, the beer was prepared as previously mentioned and sterilized in an autoclave (Sanyo MLS-3781). The sterile beer, unsterile beer, basil beer, and two day aged basil beer were individually measured into 10 mL portions in stomacher bags. 1 mL of the *Salmonella* cocktail and 90 mL peptone (0.1%) were added to each bag then placed into a mixer (AEX Labratore) for 120 seconds to ensure through inoculation. For basil preparation, bruised, cut, or decaying leaves were removed. The basil was then randomly distributed into 10 g portions in individual stomacher bags, and 1 mL of the *Salmonella* cocktail was added to each bag. Each bag was then mixed using an approved method of shaking by hand for 30 seconds from side to side to prevent spillage and ensure even coating. 90 mL peptone (0.1%) was then added to each bag and placed into a mixer (AEX Labratore) for 120 seconds to ensure through inoculation.

Plating. Trials were prepared in triplicate**.** In each of the trials, three samples of inoculated sterile beer, unsterile beer, basil, basil beer, and two-day aged basil beer were serially diluted seven times and spread plated, including the initial dilution. 1 mL of the initial dilutions were spread plated on prepared Sigma-Aldrich Tryptic Soy Agar (TSA) labelled as dilution zero. Six sequential dilutions of each variable were then performed by placing 1 mL of the previous dilution into a sterilized test tube filled with 9 mL peptone

(0.1%) and spread plating 1 mL of each dilution onto TSA representing a 1/10 reduction. The control, *Salmonella*¸ was spread plated using 0.1 mL for each dilution. Each of these dilutions were then placed into an incubator (Fisher Scientific) held at 37°C. After 36 to 48 hours, the inoculated basil, sterile beer, unsterile beer, basil beer, and two day aged basil beer CFU were enumerated using a colony counter (Leica Quebec Darkfield).

Data Analysis

Upon completion of APC and plating enumeration, an average of colony forming units (CFU) in each dilution of each variable were converted to Log 10 values and the geometric means were determined. Because the *Salmonella* was plated at 0.1 mL, it was then multiplied by 10, using an approved method, making it comparable to each other variable plated at 1 mL (Zhu, 2008) ANOVA testing was run on each variable compared to the controls of beer and *Salmonella*. Once the ANOVA testing was performed, correlation of any variables exceeding the significance level were reported.

CHAPTER IV

Results

Two experiments were conducted to determine if the alcohol contained in beer could reduce or kill aerobic bacteria or *Salmonella.* APC were utilized to examine general bacterial contamination in basil, beer, basil beer, and two-day aged basil beer. Next, basil, sterile beer, unsterile beer, basil beer, and two-day aged basil beer were then inoculated with *Salmonella* Typhimurium 53647 and plated to examine microbial reduction. For the APC data analysis, ANOVA testing was run to look for significant differences in general bacterial contamination of each variable. The ANOVA test revealed that contamination between basil, beer, basil beer, and two-day aged basil beer was statistically significant using the *F*-value at the 0.001 level (see Table 3).

Table 3

ANOVA Test between Basil, Beer, Basil Beer, and Two-Day Aged Basil Beer

Source	Sum of Squares	*df*	Mean Square	*F*	Significance
Between Groups	25.358	3	8.453	18.313	0.000*

* Significant at the 0.001 level.

After running the Welch and the Brown-Forsythe robust tests, the *F* value confirmed that the results of the ANOVA test were robust at the 0.001 level. Furthermore, the Levene test was below 0.01 which indicated that the homogeneity variance was violated and the Tamhane posthoc test was chosen to examine the mean differences (see Table 4). The Tamhane posthoc test showed that basil had a significant difference when compared with the beer, basil beer, and two-day aged basil beer. This

was expected because of the large bacterial contamination on the basil. Furthermore, the beer and basil beer had a significant difference. Between the beer and the two-day basil beer, the lower level of bacterial growth suggested that overtime the beer will reduce bacterial contamination.

Table 4

Multiple Comparisons of ANOVA Testing- Tamhane Posthoc Test

Source	Mean Difference	Std. Error
Basil x Beer	2.25493*	0.3095
Basil x Basil Beer	1.18706*	0.32041
Basil x Two-Day Aged Basil Beer	1.75051*	0.42723
Beer x Basil Beer	-1.06786*	0.15038
Beer x Two-Day Aged Basil Beer	-5.0442	0.32012
Basil Beer x Two-Day Aged Basil Beer	0.56344	0.33068

* Significant at the 0.05 level.

Plate samples of the inoculated basil, basil beer, and two-day aged basil beer, had bacterial swarming (see Figure 3, 4, & 5). Gram staining was conducted on the bacteria, which was identified as Gram-positive. Because of the reduction of variables, ANOVA testing was run to look for a significant difference in only *Salmonella*, inoculated sterile beer, and inoculated unsterile beer. The ANOVA test showed that contamination between these variables was significant at the 0.001 level using the F value (see Table 5). After running the Welch and Brown-Forsythe robust tests, the F value confirmed that the results were robust at the 0.001 level. The Levene test was 0.817, above 0.05, which indicated homogeneity of variance. The Tukey posthoc test was chosen to examine the

homogeneity and showed that there was significant difference in both the sterile and unsterile beer when compared *Salmonella* (see Table 6). This suggests that the alcohol content will help reduce *Salmonella* to a safe level. There was an insignificant difference between the sterile and unsterile beer.

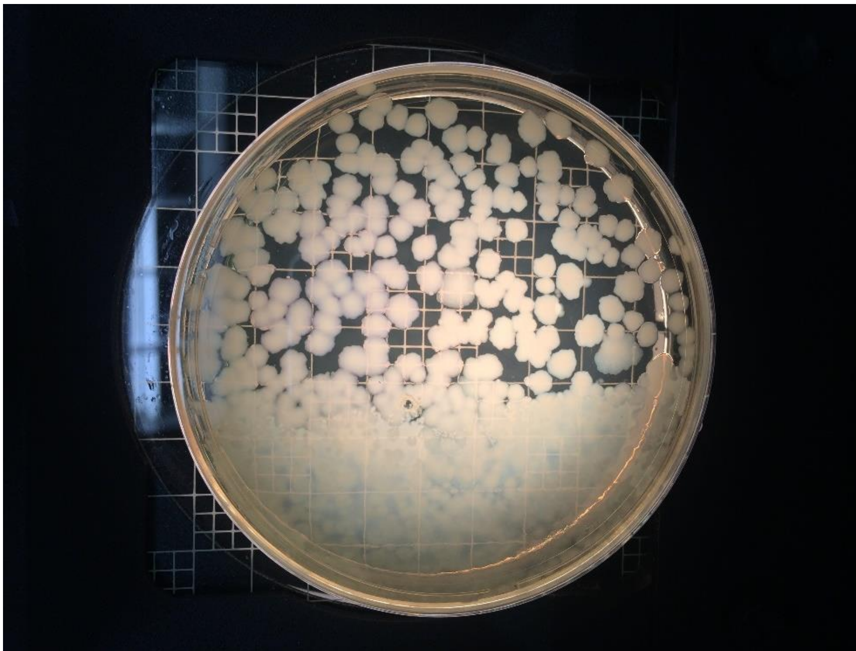

Figure 3. Swarming bacteria on basil.

Figure 4. Swarming bacteria on basil beer.

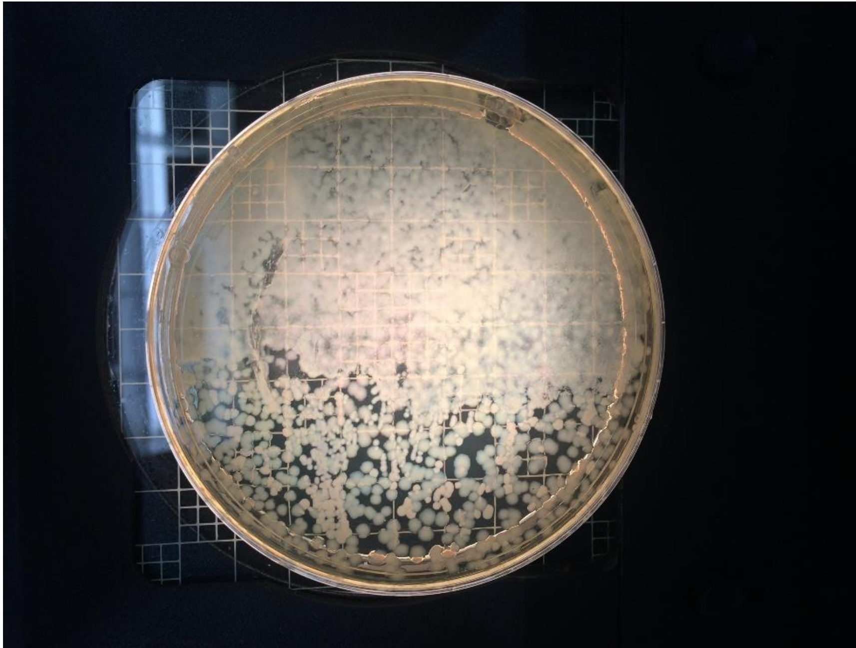

Figure 5. Swarming bacteria on two-day aged basil beer.

Table 5

ANOVA Test between Salmonella, Inoculated Sterile Beer, and Inoculated Unsterile Basil Beer

Source	Sum of Squares	*df*	Mean Square	*F*	Significance
Between Groups	10.231	2	5.116	26.190	0.000*

* Significant at the 0.01 level.

Table 6

Multiple Comparisons of ANOVA Testing- Tukey Postoc Test

Source	Mean Difference	Std. Error
Salmonella x Unsterile Beer	1.97133*	0.29464
Salmonella x Sterile Beer	2.01628*	0.29464
Unsterile Beer x Sterile Beer	0.04495	0.20834

* The mean difference is significant at the 0.05 level.

CHAPTER V

Discussion

In this experiment, the ability for alcohol in a 3.2% ABV beer to kill spoilage bacteria as well as *Salmonella* was investigated. The experiment began with APC testing using 3M Petrifilm to evaluate general bacterial growth on basil, beer, basil beer, and two-day aged basil beer. Next, spread plating was performed by inoculating basil, sterile beer, unsterile beer, basil beer, and two-day aged basil beer with *Salmonella* Typhimurium 53647 to examine the ability to grow in each variable.

Despite the large levels of initial bacterial growth on basil, beer was able to reduce the growth to a non-detectable limit on 3M Petrifilm. When comparing the beer to the basil beer, there were significant differences on APC. Initial aerobic bacterial growth on basil and beer was 2.37 mean Log_{10} CFU/g and 0.12 mean Log_{10} CFU/mL, respectively. When comparing the basil beer to the two-day aged basil beer, microbial loads were reduced. This signaled that the beer was able to lower general bacterial contamination overtime (Figure 6 & Table 7). Aerobic bacterial contamination was reduced in basil beer by 1.07 mean Log_{10} CFU/mL and 1.63 mean Log_{10} CFU/mL in two-day aged basil beer.

One half of the variables in the spread plating experiment were innumerable because of an unknown Gram-positive swarming bacteria that compromised the basil, basil beer, and two-day aged basil beer. Each variable with swarming bacteria had basil in them; the sterile and unsterile beer had no evident swarming bacteria. It was concluded that basil was the source of unknown bacterial contamination due to the amount of

general aerobic bacterial growth observed in the APC. Despite the reduction of variables, valuable results suggest that both sterile and unsterile beer can reduce *Salmonella* significantly. When sterile and unsterile basil beers were compared directly, there were no significant differences (Figure 7 & Table 8). *Salmonella* contamination was reduced in unsterile beer and sterile beer by 1.97 mean Log_{10} CFU/mL and 2.02 mean Log_{10} CFU/mL, respectively.

General aerobic bacterial contamination and *Salmonella* were reduced in 3.2% ABV beer. If craft breweries were to create sessionable, food product infused beers, the possibility for foodborne contamination exists. With a significant reduction in general aerobic bacteria from beer to fresh and two-day aged basil beer, the APC results suggest that craft breweries should age or bottle condition these beers to reduce aerobic microbial loads. This is reflected in the difference of bacterial contamination of two-day aged basil beer and fresh basil beer; the mean difference of bacterial contamination from basil was reduced in two-day aged beer compared to fresh basil beer by 0.56 mean Log_{10} CFU/mL. The plating study suggests that with sterilization or pasteurization of beer, the ability for *Salmonella* to grow is slightly reduced when examining the mean difference of 0.04 mean Log_{10} CFU/mL between sterile and unsterile beer. More importantly, this study suggests that 2.5% ABV may not be enough and shows that when craft breweries infuse their beer with food product, the stronger the ABV, the better. As mentioned in earlier chapters, American fruit beers, herb and spice beers, and any other specialty beers should have a minimum of 2.5% ABV (Brewers Association, 2016). Microbial loads were reduced only 99% during plating and *Salmonella* survived in both sterile and unsterile beer.

A common assumption is that intrinsic hurdles, such as alcohol content, will reduce or kill bacterial growth, though this has been proven to be untrue. In 2005, it was assumed that the pH of oranges would inhibit the growth of *Salmonella* in freshly squeezed orange juice. This safety assumption was found to be untrue when 14 people became ill from the consumption of Orchid Island Juice (Outbreak Database, 2016). In 2008, another similar assumption caused foodborne illness outbreaks in salsa. 17 people fell ill at a California restaurant because pH alone was not enough to prevent *Salmonella* growth (Outbreak Database, 2016). This safety assumption is confirmed once again to be untrue with the growth of *Salmonella* in the 3.2% ABV beer.

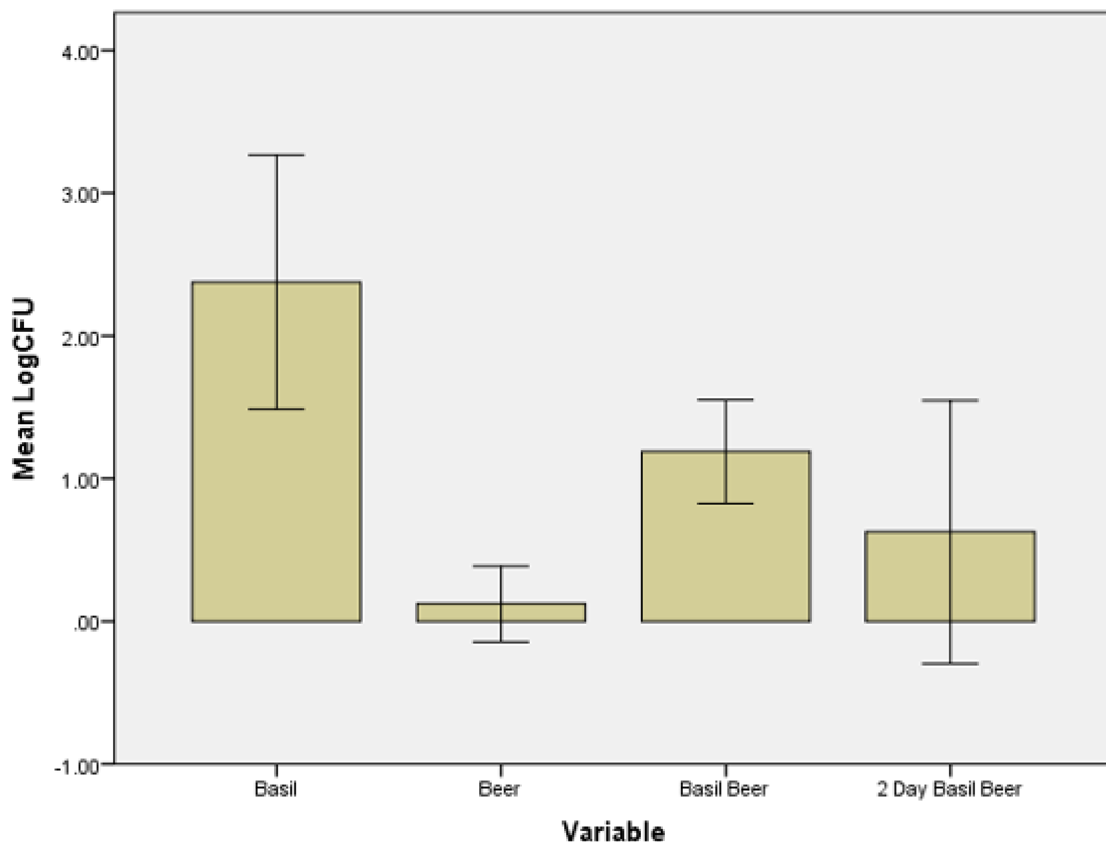

Figure 6. Mean Log of CFU on aerobic plate counts. Error bars are +/- 1.0 unit standard deviation, per variable.

Table 7

Descriptive Statistics- Aerobic Plate Counts

Source	Mean	Std. Deviation
Basil	2.3748	0.88955
Beer	0.1199	0.26616
Basil Beer	1.1878	0.36427
Two Day Basil Beer	0.6243	0.92273

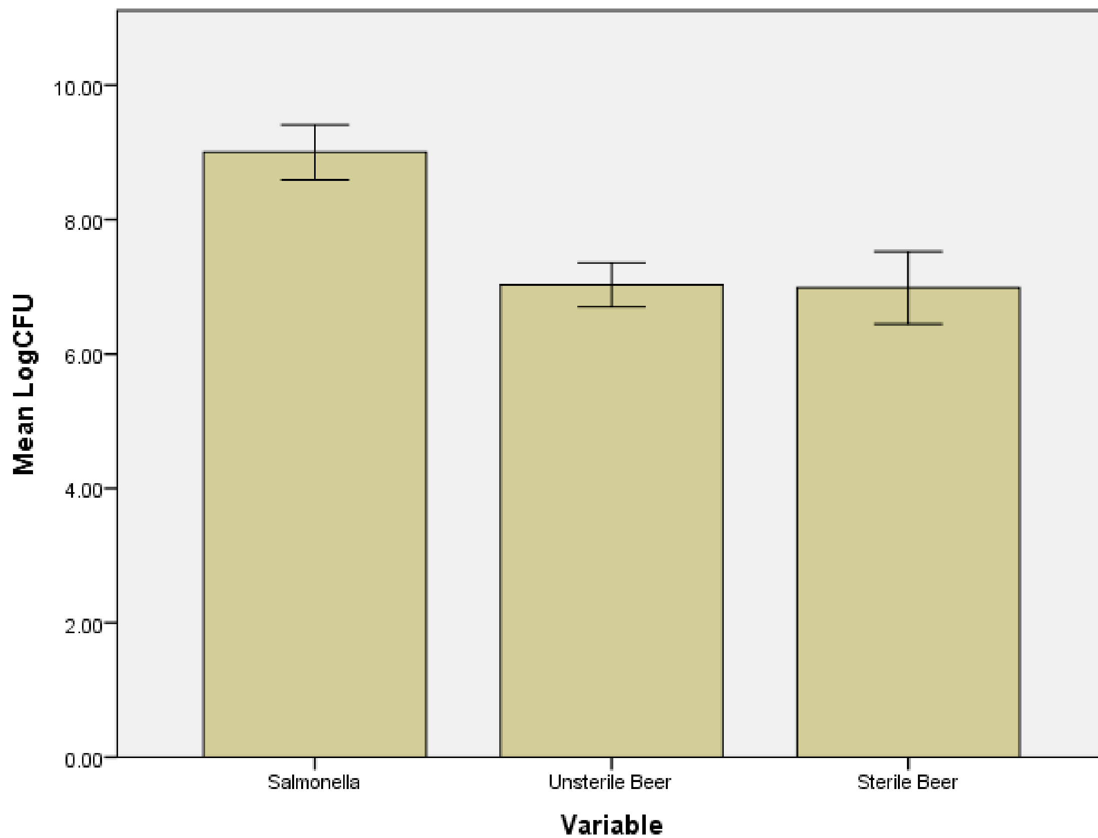

Figure 7. Mean Log of CFU on spread plating. Error bars are +/- 1.0 unit standard deviation, per variable.

Table 8

Descriptive Statistics- Spread Plating

Source	Mean	Std. Deviation
Salmonella	9.0018	0.40851
Unsterile Beer	7.0305	0.32622
Sterile Beer	6.9856	0.53976

Limitations. This experiment had limited results because of the Gram-positive swarming bacteria found on the basil on the inoculated basil, basil beer, and two-day aged basil beer. This reduced the amount of results specifically pertaining to the bottle-conditioning procedures where basil beer was made and aged for two days. Additionally, this experiment utilized only alt bier; many other styles with different ABVs and IBUs would be useful to examine in an effort to create a bacterially safe standard incorporating specific intrinsic hurdles such as IBUs. Finally, only one purveyor of beer was utilized-beer from 8th Wonder Brewery. Further studies could be conducted to see what results would be yielded from different breweries.

Conclusion. A 3.2% ABV beer reduced general aerobic bacterial contamination and *Salmonella*. If sessionable beers are directly contaminated with *Salmonella*, or other bacteria from food product infusion, the possibility for foodborne illness exists. This research implies that craft breweries should increase the ABV of their food product infused sessionable beers, implement HACCP plans, and age or bottle condition their food product infused beers in hopes to reduce bacterial contamination.

With craft breweries aiming to create unique beers that grasp the attention of the growing industry, risks should be evaluated. Craft breweries should continually work to prevent the possibility of foodborne illness. Though sessionable beers have not posed a threat of foodborne illness outbreaks alone, when they're also food product infused, the risk becomes possible. The best practice for craft breweries is to implement HACCP plans. When HACCP plans are put into place, they must be properly utilized in order to be effective. The *Salmonella* outbreak in 2005 associated with orange juice is just one of the many cases of foodborne illness outbreaks that highlight this importance (Outbreak Database, 2016). Craft breweries should also age or bottle condition their food product infused beers in hopes to reduce bacterial contamination over time. Keeping up with current research will also help craft breweries, as it will allow them to continually evaluate risks. Further academic research should look to examine modern craft brewery trends, especially with sessionable beers, because lower ABV beers also lower the intrinsic hurdles for microbial growth (Vriesekoop et al., 2012).

References

Alternate Universe- Altbier. (2016). *8th Wonder Brewery.* Retrieved from http://8thwonderbrew.com/brews/alternate-universe-altbier

ATP bioluminescence: Truths and myths in evaluating plant cleanliness. (1996). *Food in Canada, 56*(6), 13.

Basil beer. (2016). *Untappd.* Retreived from https://untappd.com/search?q=basil+beer

Behravesh, C.B., Blaney, D., Medus, C., Bidol, S.A., Phan, Q., Soliva, S., Daly, E.R., Smith, K., Miller, B., Taylor, T. Jr., Nguyen, T., Perry, C., Hill, T.A., Kleiza, A., Moorhead, D., Al-Khaldi, S., Braden, C., Lynch, M.F. (2012). Multistate outbreak of salmonella serotype typhimurium infections associated with consumption of restaurant tomatoes, USA, 2006: hypothesis generation through case exposures in multiple restaurant clusters. Epidemiology and Infection, 140, pp 2053-2061. Doi: 10.1017/S0950268811002895

Bernstein, J. M. (2015, March 24). Top food-infused craft beers worth trying. *Wine Enthusiast.* Retrieved from http://www.winemag.com/2015/03/24/top-food-infused-craft-beers-worth-trying/

Blaney, D. D., Daly, E. R., Kirkland, K. B., Tongren, J. E., Kelso, P. T., & Talbot, E. A. (2011, May). *Am J Infect Control, 39*(4), 296-301. doi: 10.1016/j.ajic.2010.10.10.010

Bokulich, N. A. & Bamforth, C. W. (2013). The microbiology of malting and brewing. *Microbiological and Molecular Biology Reviews, 77*(2), 157-172. doi: 10.1128/MMBR.00060-12

Bruning-Fann, C.S. & Kaneene, J.B. (1993). The effects of nitrate, nitrite and N-nitroso compounds on human health: a review. *Veterinary and Human Toxicology, 35*(6), 521-538. Retrieved from http://europepmc.org/abstract/med/8303822

California restaurant salsa 2008. (2016). *Foodborne Illness Outbreak Database.* Retrieved from http://www.outbreakdatabase.com/details/california-restaurant-salsa-2008/?organism=Salmonella&vehicle=salsa

Carrera, N. (1996). How to avoid cross-contamination. *Restaurant Hospitality*, *80*(6), 98.

Centers for Disease Control and Prevention. (2010). Surveillance for foodborne disease outbreaks --- United States, 2007. *Morbidity and Mortality Weekly Report, 59*(31), 973-979.

Centers for Disease Control and Prevention. (2014). *Options for evaluating environmental cleaning.* Retrieved from http://www.cdc.gov/HAI/toolkits/Appendices-Evaluating-Environ-Cleaning.html

Centers for Disease Control and Prevention. (2015). *Foodborne germs and illness.* Retrieved from http://www.cdc.gov/foodsafety/foodborne-germs.html

Centers for Disease Control and Prevention. (2015). *Salmonella.* Retrieved from http://www.cdc.gov/salmonella/general/

Chase, L. (2014). *Quality assurance in the brewpub.* Retrieved from https://www.brewersassociation.org/articles/quality-assurance-in-the-brewpub/

De Jong, A. E. I., Verhoeff-Bakkeness, L., Nauta, M. J., & De Jonge, R. (2008, August). Cross-contamination in the kitchen: Effect of hygiene measures. *Journal of Applied Microbiology, 105*(2), 615-624.

Does the declining U.S. beer trend spell doom for brewers?. (2015, June 29). *Forbes.* Retrieved from http://www.forbes.com/sites/greatspeculations/2015/06/29/does-the-declining-u-s-beer-trend-spell-doom-for-brewers/2/#4c8840f54dbb

Food Safety News. (2014). Sweet basil recalled for possible salmonella contamination. *Food Safety News.* Retrieved from http://www.foodsafetynews.com/2014/04/jars-of-sweet-basil-recalled-for-possible-salmonella-contamination/#.VrP-WbIrLIU

Food Safety News. (2015). Report reveals source of foodborne illness outbreak at Iowa high school. *Food Safety News.* Retrieved from http://www.foodsafetynews.com/2015/11/investigation-reveals-source-of-foodborne-illness-outbreak-at-iowa-high-school/#.VqZieVKxVQo

Food Safety News. (2015). Seattle Chipotle closed for ‘repeated food safety violations. *Food Safety News.* Retrieved from http://www.foodsafetynews.com/2015/12/seattle-chipotle-location-closed-for-repeated-food-safety-violations/#.VqZsUVKxVQo

Fresh basil 2005. (2016). *Foodborne Illness Outbreak Database.* Retrieved from http://www.outbreakdatabase.com/details/fresh-basil-2005/?organism=Cyclospora

Gindreau, E., Walling, E., Lonvaud-Funel, A. (2001, April). Direct polymerase chain reaction detection of ropy Pediococcus damnosus strains in wine. *Journal of Applied Microbiology, 90*(4), 535-542. doi: 10.1046/j.1365-2672.2001.01277.x

Goldammer, T. (2008). *The brewer's handbook*: *The complete book to brewing beer.* Virginia: Apex Publishers.

Hayes, R. (2013). *Food microbiology and hygiene*. New York, New York: Springer Science & Business Media.

Herz, J. (2016). Predicting 2016 craft beer trends. *Craft Beer.* Retrieved from http://www.craftbeer.com/craft-beer-muses/predicting-2016-craft-beer-trends

Hieronymus, S. (2016). The American beer story. *Craft Beer.* Retrieved from http://www.craftbeer.com/the-beverage/history-of-beer/the-american-story

History of craft brewing. (2015). *Brewers Association.* Retrieved from https://www.brewersassociation.org/brewers-association/history/history-of-craft-brewing/

Hui, Y., Bruinsma, B., Gorham, J., Nip, W., Tong P., & Ventresca, P. (2002). *Food plant sanitation: Food science and technology.* Boca Raton, Florida: CRC Press.

Internal Revenue Service. (2015). *Food industry overview- history of food industry.* Retrieved from https://www.irs.gov/Businesses/Food-Industry-Overview---History-of-Food-Industry

Kakko, L. (2011). *Good manufacturing practice (GMP).* Retrieved from Tampere University of Applied Science website: www.mf.uni-mb.si/mf/instituti/IPweb/html/KakkoL%20GMP%20in%20food.pdf

Kalac, P., & Krizek, M. (2003, January). A review of biogenic amines and polyamines in beer. *Journal of the Institute of Brewing.* doi:10.1002/j.2050-0416.2003.tb00141.x

Kleyn, J., & Hough, J. (1971). The microbiology of brewing. *Annual Reviews in Microbiology*, *25*(1), 583-608.

Kopp, S. (2016, February 4). New trends in American craft beer by the Brewers Association. *Original Gravity Magazine.* Retrieved from http://www.originalgravitymag.com/2016/02/04/new-trends-in-american-craft-beer-by-brewers-association/

Latimer, G. D. (2012). *Official methods of analysis 19th edition.* Rockville, Maryland: AOAC International.

Lawley, R. (2013, February 2). Salmonella. *Food Safety Watch.* Retrieved from http://www.foodsafetywatch.org/factsheets/salmonella/

Law, M. (2004, October 11). History of food and drug regulations in the United States. *EH.net Encyclopedia.* Retrieved from http://eh.net/encyclopedia/history-of-food-and-drug-regulation-in-the-united-states/

Lea, A. G. H., & Piggott, J. (2003). *Fermented beverage production*. New York, New York: Springer Science & Business Media.

Lewis, M., & Bamforth, C. (2007). *Essays in brewing science*. New York, New York: Springer Science & Business Media.

Mader, N. (2015). Preventative measures for cross-contamination in breweries. *Master Brewers Association of the Americas.* Retrieved from http://www.mbaa.com/meetings/annual/proceedings/Pages/20.aspx

Marriott, N. G., & Gravani, R. B. (2006). *Principles of food sanitation*. [Springer-Verlag New York version]. doi:10.1007/b106753

Maturin, L. & Peeler, J.T. (2001, January). Bacterial Analytical Manual: Aerobic plate count. *U.S. Food and Drug Administration.* Retrieved from http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm

Mauguert, T. M.-J., & Walker, S.L. (2002). Rapid detection of Obesumbacterium proteus from yeast and wort using polymerase chain reaction. *Letters Applied in Microbiology, 35*(4), 281-284. doi: 10.1046/j.1472-765X.2002.01179.x

McCabe-Sellers, B. J. & Beattie, S. E. (November 2004). Food Safety: Emerging trends in foodborne illness surveillance and prevention. *Journal of the American Diabetic Association*. *104*(11), 1708-1717. doi:10.1016/j.jada.2004.08.028

Menz, G., Vriesekoop, F., Zarei, M., Zhu, B., & Aldred, P. (2010, February 13). The growth and survival food-borne pathogens in sweet and fermenting brewers' wort. *International Journal of Food Microbiology. 140*, 12-25.

Meussdoerffer, F. G. (2009). A Comprehensive History of Beer Brewing. In *Handbook of Brewing: Processes, Technology,* Markets [Wiley-VCH Verlag GmbH & Co. KGaA]. doi:10.1002/9783527623488.ch1

Minnesota Department of Agriculture, Dairy and Food Inspection Division. (1998). *A guide to understanding how to develop a HACCP plan.* Retrieved from http://docplayer.net/164674-Meeting-the-requirements-of-the-1998-minnesota-food-code.html

Minnesota Department of Health. (2013). *Food contamination and foodborne illness prevention.* Retrieved from http://www.health.state.mn.us/foodsafety/prevention.html

Mortimore, S., & Wallace, C. (2013). *HACCP: A practical approach.* New York, New York: Springer Science & Business Media.

National Food Service Management Institute. (2009). *Serving it safe.* Retrieved from http://www.nfsmi.org/documentlibraryfiles/PDF/20091028020533.pdf

Neal, J. A., Cabrera-Diaz, E., Marquez-Gonzales, M., Maxim, J. E., & Castillo, A. (2008, June 27). Reduction of Escherichia coli O157:H7 and Salmonella on baby spinach, using electron beam radiation. *Journal of Food Protection, 71*(2), 2415-2420.

New Haven Restaurant 2005. (2016). *Foodborne Illness Outbreak Database.* Retrieved from http://www.outbreakdatabase.com/details/new-haven-restaurant-2005/?organism=Cyclospora

Number of Breweries. (2015). *Brewers Association*. Retrieved from https://www.brewersassociation.org/statistics/number-of-breweries/

Nummer, B. A. (2012, July 25). *Brewing with lactic acid bacteria.* Retrieved from https://www.morebeer.com/articles/brewing_with_lactic_acid_bacteria

Orchid Island Juice Company orange juice 2005. (2016). *Foodborne Illness Outbreak Database.* Retrieved from http://www.outbreakdatabase.com/details/orchid-island-juice-company-orange-juice-2005/?vehicle=orange

Papazian, C. (2015, February 18). 2015 Beer Style Guidelines. *Brewers Association.* Retrieved from https://www.brewersassociation.org/educational-publications/beer-styles/

Papazian, C. (2016). The revival. *Craft Beer.* Retrieved from http://www.craftbeer.com/the-beverage/history-of-beer/the-revival

Pierson, M. D. (2012). *HACCP: Principles and applications.* New York, New York: Springer Science & Business Media.

Preedy, V., & Watson, R. (2004). *Reviews in Food and Nutrition Toxicity.* Retrieved from https://books.google.com/books?id=iv8vnwJYrS8C&dq=Zymomonas+spp&source=gbs_navlinks_s

University of Rhode Island. (2016). *Causes and prevention of foodborne illness*. Retrieved from http://web.uri.edu/foodsafety/cause-and-prevention-of-foodborne-illness/

U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health. (2009). *Biosafety in microbiological and biomedical laboratories.* (HHS Publication No. CDC 21-1112). Retrieved from http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

U.S. Department of Health and Human Services. (2014). *Foodborne illnesses.* Retrieved from http://www.niddk.nih.gov/health-information/health-topics/digestive-diseases/foodborne-illnesses/Pages/facts.aspx#2

U. S. Food and Drug Administration. (2001). *BAM: Aerobic plate count.* Retrieved from http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm

U. S. Food and Drug Administration. (2004). *Good manufacturing practices (GMPs) for the 21st century- food processing*. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/CGMP/ucm110877.htm

U.S. Food and Drug Administration. (2012). *Bad bug book: Handbook of foodborne pathogenic microorganisms and natural toxins. (2),* 3-12. Retrieved from http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/default.htm

U. S. Food and Drug Administration. (2015). *Hazard analysis critical control point (HACCP).* Retrieved from http://www.fda.gov/Food/GuidanceRegulation/HACCP/

Restaurant basil and mesclun/spring mix salad products 2004. (2016). *Foodborne Illness Outbreak Database.* Retrieved from http://www.outbreakdatabase.com/details/restaurant-basil-and-mesclunspring-mix-salad-products-2004/?organism=Cyclospora

Roady, L. (2015, May 19). The story of Avery Brewings quality assurance ATP testing. *Craft Brewing Business.* Retrieved from http://www.craftbrewingbusiness.com/equipment-systems/story-avery-brewings-quality-assurance-atp-testing/

Robinson, S. (2013, August 19). The big five: Most common Salmonella strains in foodborne illness outbreaks. *Food Safety News.* Retrieved from http://www.foodsafetynews.com/2013/08/the-five-most-common-salmonella-strains/#.Vw5KnnqxVnI

Roetker, B. (2005). Avoiding cross-contamination. *Restaurant Hospitality, 89*(10), 118-120. Retrieved from http://search.proquest.com.ezproxy.lib.uh.edu/docview/236850099?accountid=7107

Sakamoto, K., & Konings, W. N. (2003). Beer spoilage bacteria and hop resistance. *International Journal of Food Microbiology. 89,* 105-124 Retrieved from http://diyhpl.us/~bryan/papers2/paperbot/Beer%20spoilage%20bacteria%20and%20hop%20resistance.pdf

Salmonella enterica subsp. enterica (ex Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC 53647). (2014). *ATCC.* Retrieved from http://www.atcc.org/products/all/53647.aspx

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M., Roy, S. L., Jones, J. L., Griffin, P. M. (2011, January). Foodborne illness acquired in the United States- major pathogens. *Emerging Infectious Diseases, 17*(1).

Sinclair, T., & Sinclair, C. J. (2010). *Bread, beer and the seeds of change: Agriculture's imprint on world history.* Boston, Massachusetts: CABI.

Stopforth, J. D., Suhalim, R., Kottapalli, B., Hill W. E., Samadpour, M. (2008, March). Thermal inactivation d- and z- values of multidrug-resistant and non-multidrug-

resistant Salmonella serotypes and survival in ground beef exposed to consumer-style cooking. *Journal of Food Protection, 71*(3), 509-515.

Storgårds, E. (2000). *Process hygiene control in beer production and dispensing.* Finland: Valtion Teknillinen Tutkimuskeskis Publications.

Taylor, K. (2015, December 30). The beer industry is going through 4 seismic changes that are impacting how America drinks. *Business Insider.* Retrieved from http://www.businessinsider.com/4-beer-industry-trends-to-expect-in-2016-2015-12

Thacker, K. (2015, October 1). Beverage trends for 2016: Beer. *Uncorkd.* Retrieved from https://www.uncorkd.biz/blog/beverage-trends-2016-beer/

Valigra, L. (2013). *5 Essential tips for effective sanitation.* Retrieved from http://www.foodqualityandsafety.com/article/five-essential-tips-for-effective-sanitation/

Vriesekoop, F., Krahl, M., Hucker, B., & Menz, G. (2012). 125th anniversary review: Bacteria in brewing: The good, the bad and the ugly. *Journal of the Institute of Brewing*, *118*(4). doi: 10.1002/jib.49

Watson, B. (2016, February 2). Diversity amongst craft beer trends. *Brewers Association.* Retrieved from https://www.brewersassociation.org/insights/craft-beer-trends/

World Health Organization. (2015). *Salmonella (non-typhoidal).* Retrieved from http://www.who.int/mediacentre/factsheets/fs139/en/

Zhu, F. (2008). *Dilution theory and problems.* Retrieved from Florida State University, Department of Biological Sciences website: www.bio.fsu.edu/courses/mcb4403L/dilution.pdf