Spring 5-10-2019

Effects of Salt Concentration on the Physicochemical Properties and Microbial Safety of Spontaneously Fermented Cabbage

Surbhi Khanna
University of Maine, surbhi.khanna@maine.edu

EFFECTS OF SALT CONCENTRATION ON THE PHYSICOCHEMICAL PROPERTIES AND MICROBIAL SAFETY OF SPONTANEOUSLY FERMENTED CABBAGE

By

Surbhi Khanna

B. Tech. SRM University, 2016

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Food Science and Human Nutrition)

The Graduate School

The University of Maine

December 2018

Advisory Committee:

L. Brian Perkins, Research Assistant Professor of Food Science, Co-advisor

Beth L. Calder, Associate Professor of Food Science and Extension Food Science Specialist, Co-advisor

Jennifer J. Perry, Assistant Professor of Food Microbiology

Robson A.M. Machado, Assistant Extension Professor and Food Science Specialist

EFFECTS OF SALT CONCENTRATION ON THE PHYSICOCHEMICAL PROPERTIES AND MICROBIAL SAFETY OF SPONTANEOUSLY FERMENTED CABBAGE

By Surbhi Khanna

Thesis Advisors: Dr. Brian Perkins and Dr. Beth Calder

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
(in Food Science and Human Nutrition)
December 2018

Fermented foods, including sauerkraut, have gained consumer popularity due to an increased awareness of reported health benefits associated with probiotics. Sauerkraut is typically produced at 2−3% salt concentrations or higher, which helps to reduce the growth of pathogenic and spoilage microorganisms. The FDA has recently issued guidance to the food industry to voluntarily reduce sodium in products due to health concerns associated with high sodium consumption. Therefore, interest in lower salt fermented foods may increase. However, information on quality and safety of lower sodium sauerkraut is limited. Two separate studies were conducted to evaluate the physicochemical properties and quality of spontaneously fermented cabbage at four salt concentrations: 1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w) and to investigate the microbial safety of these sauerkrauts when inoculated with foodborne pathogens.

Both studies utilized randomized complete block designs. Four different sauerkraut NaCl concentrations were used (1.0, 1.5 and 2.0%), with a 2.5% NaCl control and blocked by week. For both studies, cabbage was purchased from 3 locations, washed, shredded, salted and packed into glass jars. Sauerkraut was fermented under anaerobic conditions at approximately 22°C until pH levels reached a fermentation endpoint $\leq$3.70. In study 1, the microbiological (lactic acid bacteria, aerobic plate counts, fungi, coliforms) and physicochemical analyses (pH, titratable acidity, organic acids, sugars) were conducted periodically on brine and solid subsamples during the fermentation process. Initially, a significant ($p<0.05$) decrease in pH was noted among all samples on day 3. All treatments reached a pH level of $\geq$3.70 by fermentation day 14 and titratable acidity increased rapidly over fermentation time for all samples. Lactic acid bacteria populations increased throughout fermentation for all salt concentrations reaching 6-8 log (CFU/mL) by day 14. No fungi or coliforms were detected after fermentation was complete. No significant ($p>0.05$) differences across salt treatments were noted among the organic acids and sugars analyzed, and a lactic:acetic acid ratio of 4:1 was achieved for all treatments, which indicates quality sauerkraut samples were produced.

In study 2, sauerkraut samples were reproduced using the same salt concentrations, but inoculated with a pathogen cocktail containing two strains each of STEC, *S. aureus* and *L. monocytogenes* at 10^5 CFU/mL to determine the safety of these sauerkraut samples. Significant ($p<0.05$) increases in all pathogen populations were observed on day 1 followed by a sharp decrease until the fermentation endpoint ($\leq$3.70) was reached, which coincided with an increase in acidification across all treatments. The salt levels did not significantly ($p\geq0.05$) affect pathogen survival. By day 6, *L. monocytogenes* was below the detection limit (1 CFU/mL) for all treatments even after enrichment. Although STEC and *S. aureus* were not detected by day 21 in

sauerkraut samples, these pathogens were detected in all samples after reaching the endpoint pH and after enrichment. However, no pathogens were detected in sauerkraut samples after they were refrigerated (4°C) for 1 week after fermentation was complete. These microbial results reinforce the importance of good manufacturing and hygiene practices and that refrigerating after fermentation helps to ensure the safety of fermented foods.

DEDICATION

I dedicate this manuscript to my parents (Rajesh Khanna, Rita Khanna), my siblings (Priyanka Arora and Neeraj Khanna) and my boyfriend (Siddharth Chatwal).

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisors, Dr. Beth Calder and Dr. Brian Perkins for their immense support and guidance throughout the course of my master's degree which has helped me grow as a researcher. A special thanks to Dr. Calder for always being there and contributing to both my personal and professional growth. I could not have asked for better advisors.

I would also like to thank my committee members Dr. Jennifer Perry and Dr. Robson Machado for their inputs. A special thank you to Dr. Perry for her guidance and insights. It was extremely valuable to attend each and every lab meeting and to be a part of her lab team. I have learnt a lot from her and will always remember to apply those principles in my career.

Katherine Davis-Dentici, thank you for bringing positivity and enthusiasm over the past two years. I couldn't have imagined completing my research without her support and everyday learnings on how to perform experiments efficiently. I am also very grateful to Dr. Bill Halteman for guiding me through the statistical analysis of my research.

Last two years wouldn't have been fun without my friends: Dhriti Nayyar, Praveen Sappati and Bohuee Kang. They all are family to me and I will never forget the discussions and hilarious moments we used to have in the graduate room. Those moments will always bring a smile to my face. A special mention to Sarah Brochu, Dhafer Alshaibani, Alison Brodt and Kilee Nile for helping me during my research work.

Lastly, there are no words to express my profound gratitude to my parents, siblings and my boyfriend for their encouragement, love and blessings, the four value pillars, which have enabled me to look forward and continue to work hard throughout the research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1. History of Fermentation

The word fermentation is derived from the Latin word, fermentare, which means “to leaven” (Katz 2012) and can be defined as the breakdown of sugars in food or beverages by microorganisms, including bacteria and fungi (Giraffa 2004). The primary purpose of fermentation is preservation; however, it is also used to enhance the organoleptic properties of food by delivering unique flavors, which can result in new food products variation. Typical attributes of fermented foods can vary widely and are dependent upon the fermentation process. The sensory attributes can differ between cultures, availability of ingredients, taste preferences, environmental factors, raw materials and technological advancements (Prajapati and Nair 2008). Today, a plethora of fermented foods and beverages are available to the consumers due to the various possibilities of food and microbe combinations. For example, fermented vegetables and yogurt, use lactic acid bacteria (LAB) to convert mono- and disaccharides sugars into lactic acid. On the other hand, natto and tempeh (made of fermented soy beans) uses fungi and *Bacillus* microorganisms to carry out fermentation (Marco and others 2017).

Humans transitioned from hunting to cultivating food in the Middle East approximately 15,000 years ago (Borgstrom 1968). The first fermented foods consumed by humans were likely fermented fruits. Scarcity of food would have led the hunter-gatherers to consume rotten and fermented fruits (Battcock and Ali 1998). Evidence suggests that barley was fermented into beer and grapes were fermented into wine approximately 5,000 years ago (Borgstrom 1968). Availability of fermented beverages, such as beer and wine, was of utmost importance at that point in time due to a lack of safe drinking water. Fermentation of milk into yogurt, and other

sweet and savory milk-based products has been a common practice in the Middle East and the Indian subcontinent (Oberman 1985). Another type of food fermentation, bread making, originated in Europe approximately 3,500 years ago(Sugihara 1985). Fermented rice is a staple cereal in China and many other parts of Southeast Asia (Wang and Hesseltine 1970).

Approximately one-third of the human diet includes fermented foods, which is a significant proportion of diets across all regions of the world, including the United States. The historical demographic heterogeneity of fermented foods can be observed overtime due to the influence of different traditions and cultures (Borresen and others 2012). Fermentation is one of the oldest food preservation methods, which has helped our ancestors survive extreme conditions, including cold winters and droughts. Fermentation can be used to preserve food and beverages, and also an efficient process to make ready to eat foods and beverages; thereby making it one of the most important processes to produce food and beverages with extended shelf-life (Ferrerira and others 2007). The fermentation process creates unique flavor profiles and traditions ranging from Korean kimchi and Indian chutneys, to globally consumed sauerkraut, yogurt and cheese (Foroutan 2012).

1.2. Current Fermented Foods Market

Although the practice of fermenting foods originated from various cultures around the world, it has expanded from individual households to the food industry. Fermented foods are now produced on an industrial scale with the intent of providing a mass supply to the market. Some examples of fermented foods include pickled vegetables, sourdough breads, alcoholic beverages, sausages, yogurts, vinegars, cheeses, and vegetable protein amino acid/peptide sauces and pastes (Steinkraus 1997). In terms of market size, the U.S. pickled vegetable industry,

including both fermented and acidified foods, is a $2 billion market with the predominant fermented vegetables being cucumber pickles, olives and sauerkraut (Breidt and others 2013).

The acceleration and industrialization of food production over the last few years has resulted in the decline of fermented food diversity especially in the Western world. However, fermented foods and in particularly fermented vegetables, pickles, cheese and yogurts have regained popularity amongst health-conscious consumers, due to the increase in consumer awareness associated with the health benefits of consuming probiotics in natural foods (Chilton and others 2015).

1.3. Fermented Vegetables

The primary reason vegetables have been fermented for centuries is to extend their shelf life. When compared to other preservation techniques, such as canning and freezing, vegetable fermentation is easier to perform and more cost effective. Hence, fermentation of vegetables is an affordable way of preserving produce worldwide (Pederson and Albury 1969, Stamer and others 1971). Fermented vegetables are wholesome and nutritious with diverse flavors, aromas and textures. Fermenting foods remove anti-nutritional compounds, including naturally occurring toxins via the action of microorganisms during fermentation (Nout 1994, Tamang 2015). As an example, various steps are employed during the processing of gari and fufu, which are fermented cassava products of Africa, such as peeling, washing, grating, fermentation, dewatering and roasting which help to minimize the residual cyanide levels of cassava (Babalola 2014). Bitter varieties of cassava tubers contain cyanogenic glycoside, an anti-nutritional compound, which are detoxified as a result of the action of *Lactobacillus* and *Streptococcus* during the fermentation process to produce gari and fufu (Lambri and others 2013, Babalola 2014). Fermentation has also been reported to enrich raw fruits and vegetables by increasing the

bioavailability of vitamins, essential amino acids and bioactive compounds, as well as other food components. Hence, fermented products play a significant role in providing a nutritious food source to the world (Battcock and Ali 2001, Thapa and Tamang 2015).

During the lactic acid fermentation process, typically low-acid vegetables are exposed to acid-producing microorganisms with the objective of obtaining a pH of 4.6 or lower during a natural acidification process, regardless of whether acid is added or not. Lactic acid and other organic acids are produced by the microbial action a during the fermentation process by lowering pH levels, which helps to outcompete pathogenic bacteria and deter spoilage microorganisms. (Pe´rez-Dı´az and others 2013). Naturally fermented vegetables are typically fermented by the natural flora present on the vegetables, and lactic acid fermentation occurs due to the activity of lactic acid bacteria (LAB) on the surface of the vegetables (Karovicova and others1999). Vegetables typically serve as a source of water-soluble vitamins such as C and B-complex, provitamin A, phytosterols, dietary fibers, minerals, and phytochemicals for the human diet (Gebbers 2007). Vegetables are an adequate medium for natural lactic acid fermentation, regardless of the low sugar content, primarily due to their neutral pH and presence of minerals and vitamins (Buckenhuskes 1997). Not only does the fermentation process help to retain nutrients and colored pigments, it also enhances the organoleptic and nutritional quality of fermented fruits and vegetables (Dahal and others 2005). Human nutrition can be enhanced as a result of consuming fermented fruits and vegetables since these foods supply vitamins, minerals and carbohydrates, assist in balanced nutrition and have been reported to reduce the risk of diarrhea and cirrhosis of the liver due to its probiotic properties (Yamano and others 2006). Moreover, fermented fruits and vegetables also contain colored pigments such as flavonoids, lycopene, anthocyanins, β-carotene, and glucosinolates, which act as antioxidants to help

scavenge harmful free radicals, that can contribute to degenerative diseases such as cancer, arthritis, and aging may slow the normal aging process (Kaur and Kapoor 2001). Some examples of commonly fermented vegetables include cabbage, cucumbers, beets, turnips, cauliflower, celery, radishes, and carrots. However, cucumbers, pickles, sauerkraut and olives dominate the commercial market, as previously mentioned (Montet and others 2014, Roberts and Kidd 2005).

1.4. Sauerkraut

The literal translation of sauerkraut in German is "sour cabbage". Sauerkraut is produced by lactic acid fermentation of chopped and salted white cabbage (*B. oleracea*, var. capitata). Although sauerkraut is typically made with only salt and cabbage, other sauerkraut varieties may include other vegetables such as carrots, onions, garlic and beets (Gardner and others 2001).

Sauerkraut is a traditional dish consumed in many parts of the world including Central and Eastern Europe, U.S. and Asia (Wacher and others 2010). Sauerkraut has been produced worldwide for more than 150 years to prevent the spoilage of cabbage, while increasing its shelf-life (Steinkraus 2002).

The main constituents of white cabbage are carbohydrates (4.18–5.51 g/100 g), dietary fiber (1.9–2.9 g/100 g), followed by protein (1.27–1.37 g/100 g), minerals (0.3–0.7 g/100 g), fat (0.06–0.20 g/100 g), and vitamins, especially vitamin C (30−40 mg/100 g) (Souci and others 2000). Cabbage is known for its high nutritional value and high levels of bioactive compounds. It also contains other phytochemicals, mainly phenolic compounds and glucosinolates (GLS). GLS, a group of nitrogen and sulfur containing plant metabolites, is responsible for the distinct flavor and odor of Brassica vegetables (Verkerk and others 2009). GLS themselves are not biologically active but are hydrolyzed during the fermentation process to produce a wide range of biologically active compounds (Frías and others 2016).

The lactic acid fermentation process facilitates chemical changes in cabbage, thereby increasing the bioavailability of vitamins and nutrients in the final sauerkraut product. Lactic acid bacteria assist in releasing the nutrients present in cabbage by breaking down sugar via fermentation, allowing for more ease of nutrient absorption in the human body. Hence, sauerkraut has enhanced digestibility when compared to raw cabbage, contributing to a higher nutritive value. Sauerkraut contains organic acids, mostly lactic and acetic acids (1–2% (w/w)), along with propionic, malic, and succinic acids, ethanol, ethyl acetate, acetaldehyde, and carbon dioxide (Trail and others 1996), which are the byproducts of fermentable sugars present in cabbage. Sauerkraut contains phenolic compounds such as gallic acid, ranging from 0.44–1.06 mg gallic acid equivalents/100 g (Ciska and others 2005).

1.5. Health Benefits of Sauerkraut

Sauerkraut contains high levels of biologically active compounds, which results in a wide range of proposed health benefits for consumers, including probiotics, antioxidants and anticarcinogenic properties (Hayes and others 2008, Tse and Eslick 2014).

1.5.1. Probiotics

By definition, probiotics are live bacteria that provide positive health benefits to humans (Prado and others 2008). These proposed benefits include reduced serum cholesterol levels, improved gastrointestinal function, enhanced immune system function, and lower risk of colon cancer (Kechagia and others 2013, Prado and others 2008). Many studies have shown that maintenance of health gut microflora protects against gastrointestinal disorders and has a positive effect on overall health (Quigley 2013, Sekirov and others 2010, Guarner and others 2003). The bacterial cultures in probiotics help to reduce the adverse effects of harmful bacteria by nurturing the growth of beneficial intestinal microbiota. Hence, they enhance the body's natural defense

mechanisms. LAB is considered to be the most important group of probiotic organisms due to their numerous reported advantages, including the treatment of diarrhea, constipation, irritable bowel syndrome, and infections, including urogenital, urinary and a treatment for candida infections (Kechagia and others 2013). LAB also enhances the immune system which aids in the prevention of numerous illnesses and also stimulates the digestion of lactose. LAB is able to reach its site of action by surviving the passage through the upper gastrointestinal tract (GIT) and is able to perform its functions in the gut (Di Cerbo and others 2016). In addition, LAB provides the functional requirement of probiotics which includes tolerance to human gastric juice and bile, adherence to epithelial surfaces, persistence in the human GIT, immune stimulation, antagonistic activity toward intestinal pathogens (such as *Helicobacter pylori*, *Salmonella* spp., *L. monocytogenes*, and *Clostridium difficile*), and the capacity to stabilize and modulate the intestinal microbiota (Dimitriadi and others 2013).

Most LAB research is focused on dairy products, such as yogurt; however, sauerkraut also promotes LAB growth (Dougla and others 2008, Orgeron and others 2016).The FDA recommends a therapeutic dose of LAB should is in the range of 10^6–10^{10} CFUs per day (Sarowska and others 2013). Research done by Minelli and Benini (2008) also suggests that in order to confer benefits, an effective dose of probiotics falls within the 10^6–10^8 CFU/ serving size range. A study was conducted to determine if various serving sizes of homemade sauerkraut (2 Tbsp., 1/2 cup and 1 cup) met this recommended range (Orgeron and others 2016).The results showed that homemade sauerkraut can supply an efficient amount of LAB to promote health benefits in small (2 tbsp.) and large (1 cup) serving sizes. This suggests that the amount of LAB supplied by sauerkraut is sufficient to meet the recommended standards (10^6–10^8 CFU).

Based on these findings, sauerkraut is on its way to receiving “probiotic superfood” status (Orgeron and others 2016).

1.5.2. Antioxidant Properties

As the name suggests, an antioxidant is a substance that inhibits oxidation. Antioxidant’s important protective properties include slowing the aging process and reducing the risk of pathogenesis of many chronic health problems, such as cardiovascular diseases, neurodegenerative diseases, and cancer (Persson and others 2014, Pisoschi and Pop 2015). Sauerkraut has been reported to prevent oxidation due to the presence of high levels of vitamins C, E and phenolic compounds, which act as potent free radical scavengers (Podsędek 2007). Moreover, the presence of vitamin C helps in reducing C-reactive protein (CRP) levels involved in inflammation. Together with phenolic compounds, vitamin C acts as an electron donor for eight human enzymes, neutralizing superoxide and hydroxyl radicals (Ellulu and others 2015). On the other hand, vitamin E can donate a hydrogen atom resulting in antioxidant capabilities and protection against cardiovascular diseases due to the inhibition of LDL oxidation (Podsędek 2007). One study compared the antioxidant activity of sauerkraut along with raw cabbage and reported that sauerkraut’s antioxidant activity was significantly higher than that of raw cabbage. These results suggest that the fermentation process may release various bioactive compounds that may be bound to cell walls, which result in enhanced antioxidant activity (Hunaefi and others 2013).

1.5.3. Anticarcinogenic Properties

Anticarcinogens are compounds that inhibit the growth of cancer cells. Various studies and experiments have found that there is a decrease in DNA damage and cell mutation rates in cancer patients due to the presence of high levels of anticarcinogens, such as glucosinolates,

ascorbigen, and ascorbic acid. Ascorbigen is a derivative of L-ascorbic acid found in cruciferous vegetables and increases the activity of the cytochrome isoenzymes CYP1A1 and CYP1A2, which may be responsible for preventing the biotransformation of carcinogens (Wagner and Rimbach 2009). Sauerkraut contains high levels of these anticarcinogenic compounds; however, their concentration depends on the conditions of the cabbage fermentation.

According to Martinez-Villaluenga and others (2009), producing sauerkraut at low-salt concentration levels improved ascorbigen content, with the highest concentration being observed in lower sodium sauerkraut (0.5% NaCl) produced from cabbage cultivated in winter the using natural fermentation. Several studies have also reported the chemo-preventive effects of sauerkraut against different types of cancer. The antiestrogenic activity of sauerkraut extracts at low concentrations (5–25 ng/mL) in estrogen-dependent human breast cancer (MCF-7) cells has been reported (Ju and others 2000). A controlled case study performed on Polish migrant women demonstrated that sauerkraut consumption of greater than 3 servings per week during adolescence and adulthood resulted in a 72% reduced risk of breast cancer, when compared to women whose consumption was less than or equal to 1.55 servings per week (Nelson 2006). Epidemiological studies demonstrate that the dietary intake of isothiocyanates reduced the occurrence of breast, lung, and colon cancer (Zhao and others 2001, Seow and others 2002) and sauerkraut contains isothiocyanates in the range of 22 μmol/100 g (Peñas and others 2010). Hence, a weekly consumption of 200–250 g of sauerkraut may provide effective isothiocyanate levels to utilize the potential anticarcinogenic capabilities of sauerkraut. The studies listed above support that sauerkraut may have promising anti-carcinogenic properties.

1.6. Sauerkraut Production

The production of sauerkraut is an important industry in different parts of the world, including the U.S., Germany, Korea and Vietnam. Approximately 79% of the total sauerkraut consumption in the United States takes place at home (Sandra and others 2006). Moreover, 75% of the total consumption of sauerkraut in the United States takes place in the Midwest and East, which implies that consumers eat sauerkraut less frequently in Southern and Western regions of the U.S. (Lucier and Lin 2002). Additionally, upper income households appear to prefer sauerkraut, which indicates it is more of a specialty food item (Sandra and others 2005). According to a recent report from the Agriculture Marketing Resource Center (2017) the United States is the world's sixth largest cabbage producer with 78% of the production located in New York, Texas, California, Florida and Georgia. The per capita consumption of fresh cabbage increased from 8.2 pounds to 8.8 pounds between 1991 and 2001, which is increase of 7.3 percent. In 2001, approximately 88% of the total cabbage produced (3 billion pounds) was consumed fresh, while the other 12% was consumed as processed, mainly in the form of sauerkraut (Lucier and Lin 2002). A recent poll conducted by the Great Lakes Kraut Company reported that two out of three Americans in the United States consume sauerkraut. The consumption of sauerkraut has increased to 1.5 pounds per person per year and attributes to 387 million pounds of sauerkraut each year (Sandra and others 2006, USDA 2003). The trend for cabbage consumption also reflects a strong consumer interest in more convenient, fermented products such as sauerkraut (Sandra and others 2006).

Sauerkraut currently sold in the market is processed the same as sauerkraut made in years past. The small-scale sauerkraut production starts trimming the outer leaves of fresh cabbage and removing the central cores. Cabbage is further shredded to 0.7–2.0 mm thick strips and salted

with 2−3% sodium chloride (NaCl) (Perez-Dıaz and others 2013, Holzapfel 2003). The addition of salt is necessary for 3 keys steps in sauerkraut production to: a) increase the osmotic pressure which allows water and sugars to be pulled from the cabbage cell walls, which are used as nutrients by LAB, b) prevent spoilage and pathogenic microorganisms from growing, and c) prevent texture softening as a result of the decrease in endogenous pectolytic enzyme activity, naturally found in cabbage. Cabbage is pressed and mixed with salt, and then packed tightly into fermentation vessels to exclude air (Man 2008). Some processors add additional brine or weights to cover the top layers to ensure the cabbage is submerged under a salt brine. Fermentation vessels are then covered with a lid, sometimes fitted with an airlock, to allow the development of anaerobic conditions and cabbage is left to ferment between one week and several months at ambient temperatures, typically between 16−24°C. After fermentation, sauerkraut is typically consumed as fresh or stored in a refrigerator to further extend its shelf life. Some sauerkraut products are thermally processed (canned) to produce shelf stable products (Wolkers-Rooijackers and others 2013).

1.7. Sauerkraut Fermentation

Sauerkraut is usually produced by spontaneous fermentation relying on naturally existing LAB on raw cabbage (Nguyen and Carlin 1994). For spontaneous fermentation to take place, favorable conditions of anaerobiosis, which includes temperature and salt concentration needs to be established. The fermentation process results in the conversion of sugars (fructose, glucose and sucrose) into lactic acid, ethanol and acetic acid (Nguyen and Carlin 1994).

Spoilage bacteria, such as *Pseudomonas, Enterobacter*, yeasts and molds, are present on raw cabbage before fermentation. The populations of these microorganisms range from 10^4–10^6 CFU/g, whereas the LAB population on raw cabbage is typically much lower, 10^2–10^3 CFU/g

(Peñas and others 2010, Breidt and others 2013). Pressing the shredded cabbage reduces the oxygen levels among the cabbage layers during the fermentation process and also inhibit the growth of dominant aerobic bacterial communities. However, these conditions support the rapid increase in the growth of different types of heterofermentative and homofermentative LAB species during cabbage fermentation. Fermentation is first initiated by the heterofermentative LAB and dominated mainly by *Leuconostoc mesenteroides* due to their reduced acid tolerance, microaerophilic properties and shorter generation time than other LAB species (Johanningsmeier and others 2007). They convert the fermentable sugars of cabbage, primarily glucose and fructose, into lactic acid, mannitol, acetic acid, ethanol and carbon dioxide (Pederson and Albury 1969). A reduction in pH occurs due to the formation of lactic and acetic acids which results in the development of anaerobic conditions essential for fermentation. The reduction of oxygen is necessary to ensure that spoilage microorganisms do not proliferate, and LAB continues to grow in microbial succession. When the acid content increases to 0.3–0.7 % (w/w), and the pH level decreases below 4.0, *Leuconostoc* species are replaced by more acid-tolerant homofermentative LAB, such as *Lactobacillus plantarum* and *Lactobacillus brevis* (Franco and others 2012). These organisms continue to ferment the residual sugar, while continuously producing lactic acid until a fully fermented and stable product is produced (Fleming 1991). It is important to have the correct succession of these bacterial communities to obtain sauerkraut with the desired level of acidity and desired organoleptic properties. At the end of fermentation, sauerkraut should typically contain approximately 1% acetic acid (w/w) and 2% (w/w) lactic acid, as this acidity ratio provides the expected sauerkraut flavor that consumers enjoy (Breidt and others 2013).

As previously mentioned, sauerkraut production occurs through a spontaneous fermentation process relying on indigenous microbes, which is unlike other fermented foods,

such as alcoholic beverages and dairy-based products. These products are produced by controlled fermentation, as starter cultures are typically added prior to the fermentation process. This fermentation is accomplished by adding certain bacteria or fungi at known concentrations to the raw materials at the beginning of the production process. The purpose of adding starter cultures is to ensure batch consistency, maintain quality characteristics and to increase the speed and efficiency of the fermentation process (Giorgio and Sivasankari 2014).

Breidt and others (1995) have investigated utilizing starter cultures during sauerkraut fermentation to ensure uniformity of the product and to minimize the variation in quality. A nisin-resistant *L. mesenteroides* strain alone, or in combination with a nisin-producing *L. lactis* strain, have been applied to control cabbage fermentation. This paired starter culture system directed the progression of the microflora in the controlled fermentations, by inhibiting the growth of naturally present LAB, thus improving the uniformity of sauerkraut quality (Breidt and others 1995). However, studies have demonstrated the negative effects of using starter cultures during the sauerkraut fermentation process (Kristeck and others 2004, Axelsson 2004, Battcock and Ali 1998). It has been reported that using *L. mesenteroides* as a starter culture during sauerkraut production resulted in a suitable flavor but did not allow the sauerkraut to ferment to completion due to an altered sequence of substantial bacterial growth. Moreover, some studies have demonstrated that the addition of gas-producing rod-shaped bacteria, such as *Lactobacillus pentoaceticus,* to the sauerkraut disturbs the balance between acetic and lactic acids resulting in more acetic acid and less lactic acid than normal (Battcock and Ali 1998). A consequence of adding *L. pentoaceticus* can result in an incomplete fermentation, which affects the flavor of the sauerkraut. When non-gas producing rods (*Lactobacillus cucumeris*) were used as a starter culture, it resulted in an incomplete fermented product, as well. In addition, the resulting product

was bitter and had increased susceptibility to yeast spoilage (Kristeck and others 2004, Battcock and Ali 1998). These studies illustrate the challenges associated with developing an appropriate starter culture for sauerkraut, including the use of the right bacterial strain combinations to, produce an adequate fermentation and to obtain optimum sauerkraut sensory qualities. Moreover, the use of starter cultures for sauerkraut is not scalable at a household level. Even small-scale businesses prefer to make sauerkraut using traditional practices, including spontaneous fermentation, since the proper sequence of fermentation and adequate sauerkraut quality are possible with natural LAB cultures (Niksic and others 2005, Fleming 1991). Hence, starter cultures appear not to be a necessity to be a necessary and may be difficult to produce quality sauerkraut fermentations.

1.8. Factors Affecting Sauerkraut Fermentation

The complex nature of sauerkraut fermentation involves several physical, chemical and microbiological factors that can influence the final sauerkraut quality. The two essential factors that influence the growth of the two main LAB species during sauerkraut fermentation are salt concentration and temperature (Fleming 1991).

1.8.1. Temperature

The growth of heterofermentative species in sauerkraut, such as *L. mesenteroides,* is initiated at low temperatures (~ 18°C), whereas the growth of the homofermenter *L. plantarum,* is favored at higher temperatures (~ 32°C). As previously mentioned, these two bacteria can alter sauerkraut quality (Pederson and Albury 1969). Fermentation at higher temperatures (above 32°C) often leads to a low concentration of acetic acid and will not attain as high a total acidity level even though the pH is lower due to limited growth of heterofermentative LAB species. The off-flavor developed from sauerkraut due to altered acidity profiles may be linked to the

acidification process of the cabbage. Also, sauerkraut fermented at higher temperatures will be more susceptible to yeast spoilage and will darken readily. However, sauerkraut fermented at temperatures between 18°C to 22°C results in a quality product compared to sauerkraut fermented at 26°C and above, as the heterofermentative lactic acid bacteria grow better at these lower temperatures (Fleming and others 1985). In general, sauerkraut fermented at relatively lower fermentation temperatures has better or more consistent color, flavor and quality compared to sauerkraut fermented at higher temperatures.

1.8.2. Salt Concentration

The production of high-quality sauerkraut is also dependent on the addition and even distribution of salt. Salt is an important addition to sauerkraut production to encourage LAB growth which outcompete spoilage and pathogenic microorganisms and helps to further govern the desired sensory qualities of the final product (Holzapfel and others 2008). Salt withdraws water and nutrients from the cabbage tissues, and the absorbed nutrients serve as substrates for the growth of lactic acid bacteria. Salt, in combination with acid produced, is responsible for restricting the growth of undesirable microorganisms and delaying the enzymatic softening of the cabbage. Therefore, it is common to observe undesirable flavors and softening of sauerkraut due to insufficient salt. The typical salt concentrations added to cabbage for sauerkraut fermentation can range between 2 to 10% (w/w) (Xiong and others 2016). The concentration of salt added to cabbage prior to sauerkraut fermentation also helps to determine the growth of LAB species. Since *L. mesenteroides* is more salt sensitive than other LAB, lower salt concentration (1–2.5%) are more advantageous for its growth, whereas higher salt concentrations (3.5% or more) favor the growth of homofermentative LAB that produce little carbon dioxide essential for flushing out entrapped air among the shredded cabbage (Pederson and Albury 1969). In addition, yeast

growth (most commonly pink yeast) becomes more prevalent at higher salt concentrations due to the inhibition of heterofermentative bacteria, thus negatively affecting the sauerkraut quality. Moreover, higher salt concentrations favor the growth of homofermentative LAB which results in higher lactic acid levels in relation to other end products such as acetic acid, carbon dioxide and alcohol. Pederson and Albury (1954) demonstrated that lower ratios of heterofermentative bacteria to homofermentative bacteria during fermentation resulted in a lower total acidity produced in the final sauerkraut product which contributes toward the flavor and aroma of the final product (Breidt and others 2013).

The unique flavors of sauerkraut are often derived from the salt concentration and the ratio of organic acids after a complete and adequate fermentation process. Other factors such as adequate salt concentration and fermentation temperatures, are also important to promote the proper ratios of heterofermentative to homofermentative LAB and organic acids, which contribute to the desirable flavors, textures and qualities of sauerkraut.

1.9. Health Concerns Associated with Sodium Consumption

Increasing interest in lowering sodium consumption has recently occurred in the United States and Europe because excess sodium has been linked to high blood pressure levels and other health concerns (Dickinson and Havas 2007, He and MacGregor 2009). Furthermore, excess sodium may also increase the acidity in body fluids, which can cause loss of body calcium and potentially lead to osteoporosis and renal stones, loss of muscle mass and age-related renal insufficiency (Dickinson and Havas 2007). The World Health Organization (WHO) also concluded that excess sodium can cause hypertension, which may lead to cardiovascular diseases. In order to curb the problems associated with high sodium intake, the WHO has

advocated reformulating processed and prepared foods with lower sodium levels (WHO 2007). Consequently, the food industry is responding by developing more reduced sodium foods.

According to the Dietary Guidelines for Americans (USDA 2010), most Americans consume more sodium than required. The average intake of sodium for Americans (2 years and older) was approximately 3,400 mg/day, which is substantially higher than the recommended daily sodium intake of less than 2,300 mg/day. Moreover, people 51 years and older, and those who suffer from hypertension, diabetes or chronic kidney diseases, should not consume more than 1,500 mg/day of sodium as per the recommended dietary guidelines (Bautista and others 2013).

The majority of sodium in western diets comes from sodium chloride (table salt) (Frassetto and others 2008). Bibbins and others (2010) found that reducing salt intake to 3g per day could drop the annual number of cardiovascular disease in the U.S. by 60,000 to 120,000 cases and reduce annual deaths from these causes by between 44,000 and 92,000. Consequently, the Center for Science and the Public Interest (CSPI) has petitioned the U.S. Food and Drug Administration (FDA) to revoke the generally recognized as safe status (GRAS) of sodium chloride and reclassify the molecule as a food additive (FDA 2007). Moreover, the FDA has issued directives for the food industry to reduce the sodium content intake to less than or equal to 23,00 mg/day (from approximately 3,400 mg/day, currently) in both processed and prepared foods. The FDA's goal to reduce sodium intake is consistent with the 2015 Dietary Guidelines Advisory Committee (DGAC) report, Healthy People 2020, and the two reports by the Institute of Medicine on sodium (FDA 2011).

Salt is typically added to food products during processing (Mattes and Donnelly 1991). The addition of salt during the processing of fermented vegetables is important to ensure a safe

and quality fermentation process (Breidt and others 2007). Sauerkraut, a common fermented vegetable product, consumed by a significant number of Americans contains a typical salt concentration between 2−10% NaCl. On average, sauerkraut contains 661 mg of sodium in 100 g (USDA 2012). Therefore, the importance of reducing the use of salt in all food products, including fermented foods, has gained the attention of consumers and food producers. However, it is essential to consider the effects of low-salt content on the quality, safety and organoleptic characteristics of fermented foods, such as sauerkraut.

1.10. Sodium Content in Fermented Foods

A plausible solution to lowering the sodium levels of fermented foods is to use substitutes or replacements of sodium chloride with other salt mixtures to reduce the sodium chloride levels in brines. According to Viander and others (2003) and Wiander and Ryhänen (2005), sauerkraut juice made at low salt concentrations of 5 g/kg of a mineral salt mixture (consisting of 57% Sodium Chloride (NaCl), 28% potassium chloride (KCl), 12% magnesium sulphate ($MgSO_4$), 2% lysine hydrogen chloride (HCl), and 1% silicon dioxide (SiO_2)) obtained adequate sensory evaluations and lactic acid bacteria growth. However, lower salt sauerkraut that contained only 5 g/kg of sodium chloride lacked the desired crispy texture and had an odd taste that needed to be masked by using additives, such as spices. These studies indicated that the use of mineral salt, such as KCl with a low sodium chloride content, resulted in sauerkraut juices with a milder taste compared with sauerkraut juices produced with ordinary salt. The softer texture of sauerkraut in these studies were dependent on the lower salt concentrations. The lower salt concentration (0.5% w/w or 5g/kg) also reduces the fermentation rate because NaCl has the capability to extract liquid from sliced cabbage, thereby enhancing the growth of lactic acid bacteria (Viander and others 2003 and Wiander and Ryhänen 2005). Based on these studies, it

appears that sodium chloride is difficult to replace in fermented vegetable products. Hence, it is essential to conduct thorough research to understand the effects of salt reduction on consumer acceptance and shelf life of fermented vegetables. Another important consideration when producing modified fermented foods is evaluating the safety of the product before recommending any substitutions of sodium chloride with salts such as potassium chloride or calcium chloride (Bautista and others 2013).

Researchers have also studied the use of starter cultures in lower salt sauerkraut fermentations. When *L. mesenteroides* was added as a starter culture to reduce the amount of salt added to sauerkraut, researchers found out that the firmness was negatively affected with a decrease in salt concentration. The lower salt concentrations (1% NaCl) used may have contributed to the production of softening enzymes leading to a less crunchy sauerkraut (Johanningsmeier and others 2007). Even though the structural integrity or softness of sauerkraut was maintained at lower salt concentration (1% NaCl) by the addition of a starter culture, the concentration of sodium chloride appeared to play a crucial role in retaining more crunchy textural qualities (Johanningsmeier and others 2007). Therefore, using a starter culture at lower salt concentrations did appear to affect the texture of the final sauerkraut product and may also cause strong off-flavor due to changes in organic acid profiles to develop, which are not desired by consumers (Plengvidhya and others 2004). However, research is needed to investigate whether spontaneously fermented cabbage can be safely fermented by home-fermenters and the food industry when using a lower salt brine concentration (<2.5% salt concentration) to produce high-quality, safe, lower sodium sauerkraut products that provide similar organoleptic characteristics as sauerkraut produced at salt concentrations of 2.5% or higher.

1.11. Fermented Vegetables and Food Safety

As previously mentioned, there are numerous reported benefits of consuming fermented foods. However, it is important to measure the benefits of producing modified fermented foods against the associated risks of foodborne illness. It is crucial to mitigate the risks associated with microbial pathogens, food spoilage (Nout 1994), and mycotoxin production from fungi (Kinosita and others 1968, Onilude and others 2005) in fermented foods. Moreover, contamination may occur during the primary production of raw materials or during and after processing as a result of inadequate hygiene or packaging (Nout 1994). Using adequate salt concentrations in the sauerkraut formulation and storing sauerkraut at refrigerated temperatures after fermentation is complete might not always be sufficient to mitigate the risks associated with unwanted pathogen growth in food products. The highly acidic conditions and pasteurization after fermentation are typically sufficient to prevent pathogenic infection of fermented food products (Nout 1994). However, up to half of the pickled vegetable market consists of products which can be preserved without thermal processing and include fermented products (Breidt and Caldwell 2011). Moreover, pasteurization of the final fermented product is not often performed when using traditional methods to produce homemade fermented foods in Europe, the Balkans, and the United States (Nisik and others 2005).

A variety of pathogens, including *Salmonella* and *Shigella* species, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli*, including acid-resistant enterohemorrhagic O157:H7, O145, and other pathogenic serotype strain, have been detected on the surface of vegetables that could be destined for use in the production of fermented foods (Beuchat 2000 and Brackett 1999). However, the most common pathogenic infections associated with fermented food consumption

includes *Clostridium botulinum*, pathogenic *E. coli*, *L. monocytogenes*, *S. aureus* and *Salmonella typhimurium* (Nout 1994). These pathogens are considered to be a significant cause of foodborne diseases in the U.S. and other developed nations (Nisik and others 2005). The growth of unwanted pathogens is hindered by LAB due to nutrient competition and the production of lactic and acetic acids, which act as bacterial inhibitors (Adams 1990). However, LAB cannot hinder the growth of *L. monocytogenes* and *S. typhimurium* because these organisms have the ability to tolerate low-pH environments and can survive in acidic fermentation environments (Paramithiotis and others 2012, Niksic and others 2005). One of the most common foodborne illness pathogens is Shiga toxin-producing *Escherichia coli* (STEC), which causes 6% of confirmed outbreaks associated with leafy vegetables (Herman and others 2015). It has been reported that *Escherichia coli* O157:H7 is the most acid resistant pathogen detected in fermented vegetables (Breidt and Caldwell 2011). Even lower pH products (pH of 3.70 or below) such as contaminated apple ciders and apple juices have been associated in outbreaks (Besser and others 1993, CDC 1997). The 2012 outbreak related to kimchi was caused by enterotoxigenic *E. coli* O169 (Cho and others 2014, Park and Lee 2014). One possible hypothesis reported was that kimchi had not been fermented and ripened completely, which may have allowed the pathogen to survive (Cho and others 2012). Another microorganism that has been associated with low pH foods and can tolerate higher salt concentrations is *S. aureus* and has been reported to be found in fermented olive oil products (Bevilacqua and others 2010, Pereira and others 2008). *C. botulinum* may also thrive and produce a harmful neurotoxin in conditions of insufficient acid production and anaerobic fermentation (Henney and others 2010). For *C. botulinum,* a pH value of ≤ 4.6 is the upper limit that prevents spore outgrowth and neurotoxin production in fermented foods (Breidt and Caldwell 2011). Although some fermented foods have been reported to be

contaminated with pathogens, it has been acknowledged that it is uncommon for fermented foods to be associated with foodborne illnesses outbreaks due to the antagonistic effect of LAB. However, the aforementioned outbreaks and research suggest that the presence of foodborne pathogens on raw plants, along with the increased tolerance against low pH and the presence of salt in foods implies that foodborne pathogens must still continue to be a concern in fermented vegetables.

Inatsu and others (2004) studied *Escherichia coli* O157:H7, *Salmonella enteritidis, S. aureus*, and *L. monocytogenes* survival in both commercially produced and laboratory-prepared kimchi. These products were separately inoculated with each pathogen at 5−6 log (CFU/g) and incubated at 10°C. Populations of *E. coli* O157:H7 were found to remain at high levels (4–5 log (CFU/g) throughout the incubation period in both products. *S. enteritidis* demonstrated similar results, but only for the product prepared under a laboratory setting. The populations of *S. aureus* decreased to its detection limit (200 CFU/g) after 16 days, and the population of *L. monocytogenes* reached the similar level after 20 days in both the commercially and laboratory-prepared products. Therefore, this study clearly suggests that *E. coli* O157:H7, *S. enteritis, S. aureus* and *L. monocytogenes* could contaminate kimchi and persist through fermentation posing a potential risk of illness to consumers. A study by Niksic and others (2005) investigated the survival of *L. monocytogenes* and *E. coli* O157:H7 during and after lactic acid fermentation of sauerkraut produced from both whole-head and shredded cabbage. Acid-tolerant strains of *E. coli* and *L. monocytogenes* were isolated from both shredded and whole-head sauerkraut at different salt concentrations (1.8%, 2.25% and 3.0% NaCl) and fermentation temperatures (18°C and 22°C). These pathogens were detected after 15 days of fermentation in both shredded and whole-head fermented cabbage (sauerkraut). In 2011, Breidt and Caldwell assessed brine obtained from

a commercial kitchen at various stages of fermentation to address the knowledge gap in survival of *E. coli* O157:H7 in cucumber fermentation. The time required to obtain the 5−log reduction standard was positively correlated with the pH value ranging from 3.2 to 4.6 and the NaCl composition of brines (5.5−8.7% NaCl). According to Breidt and Caldwell (2011), as acid accumulated and pH was reduced in the brine, the 5-log reduction time was correspondingly reduced. The author found out that brine pH values below 3.3 required less than 4 days to achieve a 5-log reduction regardless of temperature (10°C or higher). Interestingly, one commercial brine with highest salt concentration of 7.82% NaCl had a 5–log reduction time that was lower (at least 6 days shorter) than expected based on the pH level which might be due to other unidentified factors such as preservatives. There was a decrease in the cell count of pathogens below the detection limit (2 log (CFU/mL) within 2−3 days, depending on pH, since *E. coli* O157:H7 was not able to compete with either *L. plantatrum* or *L. mesenteroides* at 30°C. However, the amount of published research on the survival rate of foodborne pathogens at reduced salt concentrations is limited.

Approximately 93 foodborne illnesses have been reported to be caused by the consumption of fermented and pickled vegetables in the U.S. and South Korea (Cho and others 2014). These illnesses could have been caused by high infection levels of these pathogenic bacteria on the raw vegetable ingredients prior to fermentation or by improper fermentation process including inadequate fermentation temperature and salt concentration (Burnett and others 2000, Takeuchi and Frank 2000). Therefore, it is critical to validate the safety of fermented cabbage (sauerkraut) produced salt with lower salt concentration brines of less than 2.5% NaCl.

1.12. Safety Concerns of Using Lower Salt Brines for Sauerkraut Production

As previously mentioned, salt concentration has a significant influence on controlling pathogen growth in foods and also plays a critical role to ensure the food safety of fermented foods (Henney and others 2010). Studies demonstrate that high salt concentrations result in higher osmotic pressures which stops the metabolism of pathogens and restricts their growth in fermented foods (Bautista and others 2013). Therefore, reducing sodium content has the potential to increase food spoilage rates and the presence of pathogens. For low-sodium foods, it is important to ensure that product reformulation, changes in processing, and changes in handling are considered so that the product has an adequate shelf life and to prevent pathogen growth.

There has been evidence signifying that lower salt concentrations may increase the likelihood of *C. botulinum* toxin formation (the organism responsible for botulism) in certain fermented foods (Henney and others 2010). In addition, the growth of other foodborne pathogens, such as *L. monocytogenes, Bacillus cereus, S. aureus, Y. enterocolitica, A. hydrophila, Clostridium perfringens, and Arcobacter*, may occur at faster rates in fermented foods with lower salt concentrations (Dsa and Harrison 2005, Reddy and Marth 1991). Therefore, it is crucial to investigate the risk of pathogenic infection in reduced salt sauerkrauts to validate the microbial safety of these products. Various compounds, such as potassium chloride (Barbut and others 1986) and mixtures of potassium lactate and sodium diacetate (Devlieghere and others 2009), have been marginally effective at reducing toxin production and inhibiting the growth of pathogens. One of the approaches recommended by researchers include partially replacing NaCl with other salts, such as potassium chloride and calcium chloride, in fermented products (Bautista-Gallego and others 2008, Reddy and Marth 1991, Yumani and others 1999). However, the salt alternatives might be less effective than NaCl, which may result

in these alternatives having to be used at higher concentrations, thus negatively affecting product quality and safety (Bautista-Gallego and others 2008).

Some research has highlighted the effects of foodborne pathogens on the microbial safety of spontaneously fermented vegetables. Karagozlu and Erg€onül (2011) investigated the survival of *S. typhimurium* strains in sauerkraut during fermentation. *S. typhimurium* strains were inoculated in sauerkraut, and counts were determined on days 0, 1, 2, 4, 6 and 7 days of fermentation. It was observed that the initial count of *S. typhimurium* was 6.61 log CFU/g on inoculation day and declined to <1.0 log CFU/g on the 7 day of fermentation (Karagozlu and Erg€onül 2011**)**. Adams and Nicolaides (1997) recorded a one-log cycle increase in *S. aureus* before observing a decline after 4 days, while documenting the sensitivity of *S. aureus* to fermentation. The author discussed that some pathogens are still able to grow for a limited period until the acid produced by the LAB has reached inhibitory levels. Another study conducted by Cho and others (2011) researched the survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during kimchi fermentation supplemented with pork meat. The population of *E. coli* O157:H7 gradually decreased during the fermentation at 4°C and was no longer detected in cabbage kimchi samples after 14 days, whereas population of *L. monocytogenes* gradually decreased and did not survive in after 15 days. Argyri and others (2013) studied the fate of *E. coli* O157:H7, *Salmonella Enteritidis* and *L. monocytogenes* during the storage of fermented green table olives. The results demonstrated that the growth of these pathogenic strains was not supported during storage at 20°C. According to Argyri and others (2013), these pathogens may survive for a long period, approximately 2–3 days to achieve 1 log reduction in the stressful environment of a fermented product with a low pH value (4.2) and higher salt

concentration (6%). However, the literature lacks definitive evidence concerning the safety of fermented vegetables, especially sauerkraut, at reduced salt concentrations.

1.13. Research Justification

In summary, prior studies indicate the desired quality of sauerkraut, while reducing salt concentration, can be accomplished by replacing the salt with other mineral salts and/or adding a starter culture at the beginning of the fermentation process. Some studies have also investigated the safety of fermented vegetables but at high salt concentrations (3% or higher). However, the survival of bacterial pathogens (STEC, *Salmonella, S. aureus* and *L. monocytogenes*) in naturally fermented vegetables, especially at lower salt concentrations, still require additional research. Moreover, there will be an increased interest to ferment food at lower salt concentrations due to recent FDA guidance on the reduction of salt content in foods, making this research even more critical.

Fermented foods have become more popular due to enhanced consumer awareness of the health benefits of probiotics. There has been an evident increase in the production of fermented foods by small businesses in the U.S., including value-added products such as sauerkraut and other fermented vegetables (Calder, 2018). One of the most popular fermented vegetables consumed in significant amounts by consumers is sauerkraut. Raw cabbage is fermented naturally at home or produced by small-scale businesses (Niksic and others 2005) and typically require a brine concentrations of 2% or higher. Therefore, it is critical to investigate the safety and quality of fermented sauerkraut at salt concentrations below 2%. This research may generate additional opportunities to market value-added, naturally fermented foods at lower salt concentrations to an expanding market of health-conscious consumers.

It is imperative to ensure that lactic acid bacterial growth is supported at lower salt concentrations and enough acid is produced to hinder any spoilage and pathogenic microorganism growth, without any deterioration of sauerkraut organoleptic qualities.

1.14. Objectives

The overall aim of this research is to assess the safety and quality of value-added, naturally fermented vegetables at lower salt concentrations. The results from this study will provide guidance for not only small agricultural-based businesses, but also home-fermenters to produce safe and quality fermented foods with a lower sodium content. Therefore, this research may help to improve the agricultural-based economy of states with small food businesses, such as Maine. Results generated from this study will provide crucial information on the physicochemical, chemical and microbial changes during fermentation and microbial safety of sauerkraut produced at salt levels below 2.5%. The specific research objectives are as follows:

Objective 1: To determine the effects of four different salt concentrations (1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w)) on the pH and titratable acidity level as well as lactic acid bacteria growth and presence of spoilage microorganisms during the spontaneous fermentation of sauerkraut.

Objective 2: To analyze the effects of different salt concentrations (1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w)) on the fermentable sugars (glucose, fructose and sucrose) and organic acids (lactic acid and acetic acid) during spontaneous fermentation of sauerkraut.

Objective 3: To evaluate the effects of salt concentrations (1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w)) on the survival of bacterial pathogens (STEC, *S. aureus* and *L. monocytogenes*) during spontaneous fermentation of cabbage.

CHAPTER 2

EFFECTS OF SALT CONCENTRATION ON THE MICROBIOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF SPONTANEOUSLY FERMENTED CABBAGE

2.1. Justification and Objectives

The increasing interest in functional foods has prompted a market opportunity for fermented vegetables (Cetin 2011). Both from a traditional and commercial perspective, sauerkraut has become one of the most popular natural fermented vegetable products in the world (Perez-Diaz and others 2013). The production of sauerkraut requires a salt concentration typically in the range of 2−3% NaCl or higher (Thakur and Kabir 2015). Due to the recent FDA guidelines (FDA 2011) to voluntarily reduce sodium in processed foods, the use of sodium in foods has gained attention and with increasing consumer awareness. Consumers and food processors are interested in reducing sodium in food products due to the associated health concerns of consuming excess salt (Thakur and Kabir 2015). Hence, research has investigated ways to reduce and partially replace NaCl in foods with other minerals. Viander and others (2003) studied the impact of reducing salt with other alternatives and the effect of the quality on fermented foods. In their study, NaCl was replaced with 28% KCl, and the shelf-life and sensory qualities of the sauerkraut juice were investigated. Research has also been conducted on adding lactic acid bacterial starter cultures at the beginning of the sauerkraut fermentation process to reduce salt content (Wiander and Korhonen 2015). However, literature lacks evidence on the effectiveness of lowering the salt concentration in naturally fermented cabbage (sauerkraut). Moreover, research lacks any results that indicate the physicochemical properties or quality of naturally fermented sauerkraut at lower salt concentrations.

Hence, the focus of this study is to evaluate the spontaneous fermentation of sauerkraut produced with four different NaCl concentrations and to evaluate the quality characteristics of the final sauerkraut products.

The specific objectives of this study were to assess the changes in physicochemical characteristics and the microbial profiles in sauerkraut samples over time until a pH of 3.70 or lower is obtained: 1) to determine the effects of four different salt concentrations (1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w)) on the pH and titratable acidity levels, as well as lactic acid bacteria growth and the presence of spoilage microorganisms during the spontaneous fermentation of sauerkraut and 2) to analyze the effects of different salt concentrations (1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w)) on the fermentable sugars (glucose, fructose and sucrose) and organic acids (lactic acid and acetic acid) during spontaneous fermentation of sauerkraut.

2.2. Materials and Methods

2.2.1. Experimental Design

A randomized complete block design (RCBD) was used to evaluate spontaneously fermented sauerkraut prepared at four different NaCl concentrations (1.0%, 1.5%, 2.0% and 2.5% (w/w)) as treatments and blocked by the number of weeks (n=3) (Table 2.1.). The 2.5% NaCl treatment represents the control since most sauerkraut is typically produced with a salt brine between 2–3% or higher, based on the total formulation weight. In this experimental design approach, the number of blocks (n=3) equals to the number of replications, with a total of 12 samples over a period of three weeks. All the samples were tested for microbiological and physicochemical analyses on fermentation days 0, 1, 3, 7, 10 and 14.

Table 2.1. The experimental design of spontaneously fermented sauerkraut

Treatments (NaCl (w/w))					
Blocked by Week		1.0%	1.5%	2.0%	2.5%
	Week 1	W	X	Y	Z
	Week 2	WA	XA	YA	ZA
	Week 3	WB	XB	YB	ZB

W= 1.0% NaCl Sample, Week 1 X = 1.5% NaCl Sample, Week 1

Y= 2.0% NaCl Sample, Week 1 Z = 2.5% NaCl Sample, Week 1

WA= 1.0% NaCl Sample, Week 2 XA = 1.5% NaCl Sample, Week 2

YA= 2.0% NaCl Sample, Week 2 ZA = 2.5% NaCl Sample, Week 2

WB= 1.0% NaCl Sample, Week 3 XB = 1.5% NaCl Sample, Week 3

YB= 2.0% NaCl Sample, Week 3 ZB = 2.5% NaCl Sample, Week 3

2.2.2. Sample Preparation

For each week, fresh cabbage (*Brassica oleracea*) was purchased from three different local retailers, including Walmart, Shaws and Hannaford (Bangor, ME) prior to processing. A total of 12 cabbages, four from each store were purchased each week. Only whole, undamaged cabbage heads were selected for this project. Prior to shredding, three-four outer leaves and the white stem base of the cabbages were removed. Edible parts of the cabbage were washed thoroughly in cold, potable water and cut into six pieces each. Cabbage was shredded using Robot-coupe (CL 50 series E, Robot-coupe USA. Inc., Ridgeland, MS) and mixed thoroughly with gloved hands in a large, stainless-steel mixing bowl to provide a homogenized sample. For each treatment, 3,000 g of cabbage were weighed into four different bowls and covered with aluminum foil and stored in a walk-in cooler (3–4°C) overnight.

2.2.3. Processing

The shredded cabbage was salted with non-iodized Kosher salt (Morton Salt Inc., Chicago, IL) at four different concentrations of 1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w), which was approximately 30 g, 45 g, 60 g, 75 g of salt for 3,000 g of cabbage, respectively. Cabbage and salt were mixed manually for 5−6 minutes via gloved hands and allowed to stand for 15 minutes at room temperature (22°C) to draw out the natural juices. The shredded cabbage was packed tightly into labelled 1-gallon glass jars. For each treatment, 100 g of brine consisting of 100 g of water and 1.0 g, 1.5 g, 2.0 g, or 2.5 g of salt were added to the 1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w) treatments, respectively. After adding the brine, clean and sanitized glass weights were placed on top of the cabbage to keep the sauerkraut packed under the brine. Glass jars were closed using lids with twin bubble airlocks (B01AKB4G9E, Home Brew Ohio, Sandusky, OH). These glass jars were incubated at room temperature (22°C) for up to 14 days until a pH level of 3.70 or lower was obtained. A flow chart illustrating the sauerkraut preparation process is shown in Figure 2.1.

Figure 2.1. Preparation of sauerkraut

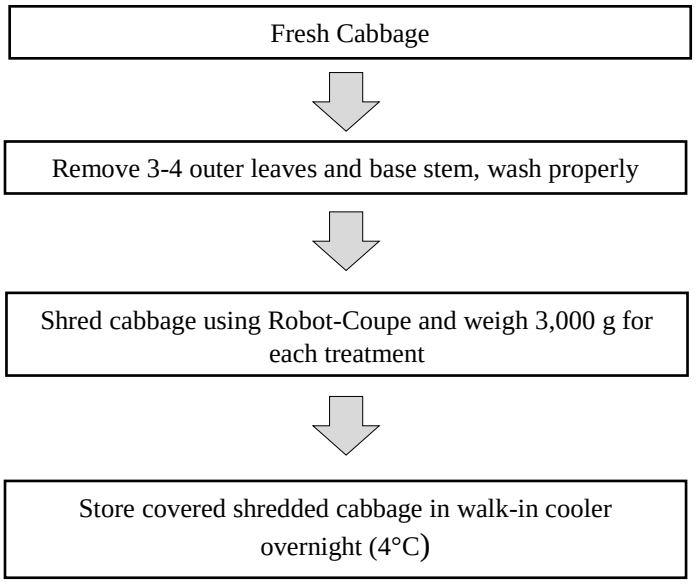
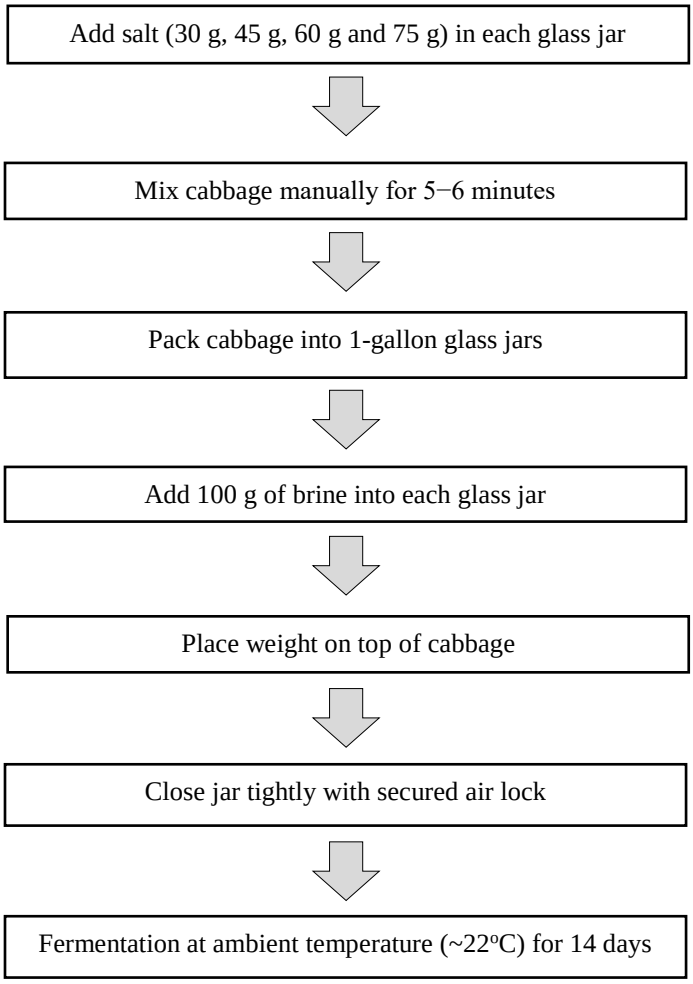


Figure 2.1. Continued



2.2.4. Sampling

Brine and solids were aseptically sampled from glass treatment jars during fermentation days 0, 1, 3, 7, 10 and 14. Brine samples were used for microbiological analyses, sugar and organic acid analyses, and solids were analyzed for pH and titratable acidity levels, color and salt analyses. Brine (2–7 mL) was collected from the center of the glass jars using a sterile pipette, and solids (10 g) were sampled using an aseptic technique with sterilized tongs from the top,

bottom and center of the glass jars and mixed to reduce variability. Room temperature was recorded for each testing day and ranged from 21–22°C.

2.2.5. Physiochemical Analyses

2.2.5.1. pH

Approximately 10−15 g of sauerkraut were removed in triplicate from each glass jar. Samples were ground using a Magic Bullet (Nutri Bullet, Los Angeles, CA), and pH levels were measured using a pH meter (Orion Star A111, Thermo Scientific., Waltham, MA) in triplicate. The flat surface pH probe (Thermo Scientific™ Orion™ AquaPro™ Flat Surface 9135, Waltham, MA) was carefully cleaned between samples with distilled water and was calibrated with pH 4 and 7 buffer solutions prior to use. The flat surface probe was placed directly into the pureed sauerkraut samples until a consistent reading was obtained. The readings were taken in duplicate for each sample and pH values were averaged. These samples were retained for titratable acidity.

2.2.5.2. Titratable Acidity (TA)

The TA was determined using a potentiometric titration method (Neilsen 1998). Distilled water was added to approximately 10−15 g of pureed sauerkraut in a 1:10 (w/w) ratio. The samples were titrated with a standard alkali solution (0.1 N NaOH) in triplicate. The volume of 0.1 N NaOH required to achieve a pH level of 8.15−8.20 was recorded. The measured volume was then plugged into the following equation to obtain the titratable acidity value and was reported as % lactic acid:

TA (% lactic acid) = [mL NaOH used] x [0.1 N NaOH] x [milliequivalent factor] x [100]/ sample weight (g)

2.2.6. Microbiological Analyses

2.2.6.1. Raw Cabbage

Microbial analysis was conducted on raw cabbage prior to fermentation and brine samples of fermented sauerkraut treatments. Raw shredded cabbage samples (10 g) were aseptically collected with sterile tongs and placed into sterile stomacher bags prior to salting on day 0. For microbial analyses, 10g of cabbage were diluted with 90 mL of sterile 0.1% peptone (BD, 26 Sparks, Maryland) water in the stomacher bag and homogenized (Interscience BagMixer®, Woburn, MA) for 2 minutes. The serial dilutions were plated onto DeMann Rogosa Sharpe agar (MRS) (Alpha Biosciences, Baltimore, MD), tryptic soy agar (TSA) (Alpha Biosciences, Baltimore, MD) and acidified potato dextrose agar (APDA) in duplicate to enumerate lactic acid bacteria (LAB), total aerobic microflora (APC) and yeast and molds, respectively. Acidified potato dextrose agar (APDA) plates were made using potato dextrose agar (Alpha Biosciences, Baltimore, MD) with 10% tartaric acid added (pH 3.5). The MRS and TSA plates were incubated for 48 hours at 30°C and 35−37°C, respectively. APDA plates were incubated for five days at room temperature. After incubation, plates with 25−250 colonies were counted and duplicate values were averaged. Coliforms were enumerated using the most probable number (MPN) method in lactose broth (Acumedia, Lansing, MI) with three Durham tubes (USFDA, 2010, Hardy Diagnostics, 2018). After 24 hours, the test tubes were examined for turbidity and bubbles. The scoring of the MPN was determined by comparing the number of positive test tubes to a table from the Bacteriological Analytical Manual (FDA 2010).

Brine samples (2–3 mL) were removed from the center of the glass jars to determine APC, LAB, fungi and coliform counts. Yeasts, molds and coliforms were checked once a week (days 0, 7 and 14) due to their slow growth. Lactic acid bacteria and APC counts

were analyzed on each testing day of the study. All treatments were analyzed in duplicates and the values were averaged.

2.2.7. Colorimetric Analyses

Colorimetric analyses were performed on raw cabbage on day 0, and for sauerkraut samples on days 0, 7 and 14 using a colorimeter (LabScan XE, Hunter Labs., Reston, VA). Sauerkraut samples (20 g) were obtained from each treatment and three replications were performed per sample. The CIELAB color scale (L*, a* and b* values) was set for D65/10° and values were recorded by the Hunter colorimeter software. Before each use, the colorimeter was warmed for 30 minutes and was standardized prior to use with white and black tiles for a port size of 1.75 inches or 44 mm. Colorimeter sample glass cups (353002, Corning, Durham, NC) were evenly filled with sauerkraut samples. After the initial color reading, each sample cup was rotated 120° for a total of three times. The rotations were repeated to achieve three readings, which were then averaged.

2.2.8. High Performance Liquid Chromatography (HPLC) Analyses

2.2.8.1. Chemicals

Sulfuric acid (H_2SO_4) and certified ACS organic standards including 85% lactic and glacial acetic acids and glucose were purchased from Fisher Scientific (Fair Lawn, NJ). The sugar standards (sucrose and fructose) were purchased from Sigma (St. Louis, MO). The 0.01 M H_2SO_4 was used as the mobile phase and was prepared by dissolving 0.53 mL sulfuric acid in 1 L deionized water

2.2.8.2. Equipment

An Agilent Technologies (Santa Clara, CA) model 1100/1200 Hewlett-Packard HPLC system was used for this study. The degasser (1100 series), thermostatted column compartment

(1200 series), and refractive index detector (RID) (1200 series) were from Agilent Technologies. The pump (1100 series ISO pump, upgraded to a quat pump) and the autosampler (1100 series) were from Hewlett-Packard (Palo Alto, CA). The computer used with this system was a Dell Optiflexx 755 formatted with Windows XP Professional, version 5.1.2600. Measurements were taken using ChemStation Software for LC by 3D Systems, version 8.0401(481) (Agilent Technologies). The standard and sample preparation required equipment including 100 mL volumetric flask(s), 20−100 μL automatic pipets, 200−1000 μL automatic pipets, 1 mL HPLC vials with caps and an analytical balance to weigh the sugar standards.

2.2.8.3. Standard Preparation

For HPLC calibration and performance evaluation, sugar standard solutions (fructose, glucose and sucrose) and organic acids (acetic and lactic acid) were prepared by diluting the required amount of each standard into a 0.01 M H_2SO_4 mobile phase. The stock standard solutions for lactic and acetic acid were made by dissolving 1mL of each acid into 99 mL of 0.01 M H_2SO_4. These acid standard curves were made by dissolving 50 mL, 10 mL, 5.0 mL and 2.5 mL of each stock solution into 99 mL of 0.01 M H_2SO_4. For acetic acid (AA), one more concentration was created by dissolving 1.25 mL of 1.0% AA stock solution into 99 mL 0.01 M H_2SO_4. Hence, standard concentrations ranged from 3.33−111.01 mM for lactic acid (LA) and 1.66−166.52 mM for AA was produced to make the standard curves.

A 5-point standard curve was produced for all sugar analyses, (glucose, fructose and sucrose) with standard concentrations ranging from 138.73−2774.65 mM for glucose and fructose, and 7.30−730.37 mM for sucrose. For glucose and fructose, the 1.0% stock solution was made by dissolving 1 g of glucose and 1 g of fructose into separate 99 mL of 0.01 M H_2SO_4. From these stock solutions, 50 mL was removed and dissolved into 50 mL 0.01 M H_2SO_4 to

create 0.5% of each glucose and fructose standard solution. Again, 50 mL was pipetted from these 0.5% standard solutions for each sugar and dissolved into 50 mL of 0.01 M H_2SO_4 to make a 0.25% standard solution. The 0.125%, 0.0625% and 0.025% standards were produced in a similar manner for both fructose and glucose. Similarly, the sucrose stock solution was made by dissolving 0.5 g of sucrose into 0.01 M H_2SO_4. From this 0.5% stock solution, 50 mL was removed and added to 50 mL of 0.01 M H_2SO_4 to produce a 0.25% standard sucrose solution. The remaining sucrose standard concentrations (0.125%, 0.0625%, 0.025% and 0.0025%) were prepared using the same process by adding 50 mL of the prior concentration into 50 mL of 0.01 M H_2SO_4.

2.2.8.4. Sample Preparation

Brine samples were collected on each sampling day from all salt treatments. A 5−7 mL brine sample was removed from each glass jar and placed into a -80°C freezer to preserve the samples before analyzing. All samples were thawed at 4°C overnight prior to analyses. Brine samples were diluted with mobile phase (0.01 M H_2SO_4) at a 1:4 dilution. The 100 µL sample was diluted with 300 µL of mobile phase in triplicate. Before injecting, all prepared samples were filtered through a 0.45 µm syringe filter (Advanced Microdevices, Ambala, India). Using a pasteur pipette, 1 mL of filtered sample was removed and placed into a small sample vial for HPLC analysis.

2.2.8.5. Chromatography Conditions

A RID detector (Agilent Technologies 1200 Series Refractive Index Detector) was used in this study. A flow rate of 0.6 mL/min with a run time of 15−20 minutes was used, and 20 µL of both sample and standards were injected into the system. The mobile phase was 0.01 M H_2SO_4 dissolved in HPLC grade water. The column used was a Hi-Plex, 300x65 mm with a 5x3

Hi-Plex H guard column (Agilent Technologies) operated at 50°C. The analytical triplicates were averaged, and organic acids and sugars were reported in mM.

2.2.9. Statistical Analyses

Data were analyzed using SPSS (IBM SPSS statistics 22) and R-program. Shapiro-Wilk's normality test and the Levene equality of variances were used to assess the normality of data prior to further analyses. One-way analyses of variance (ANOVA) was conducted to determine significant differences among the four salt treatments and within each day of fermentation. Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses to determine significant differences among means. In cases where data did not satisfy normality, homogeneity or independence, the statistical analyses were performed on residuals. A significance level of $p \leq 0.05$ was chosen for all statistical analyses.

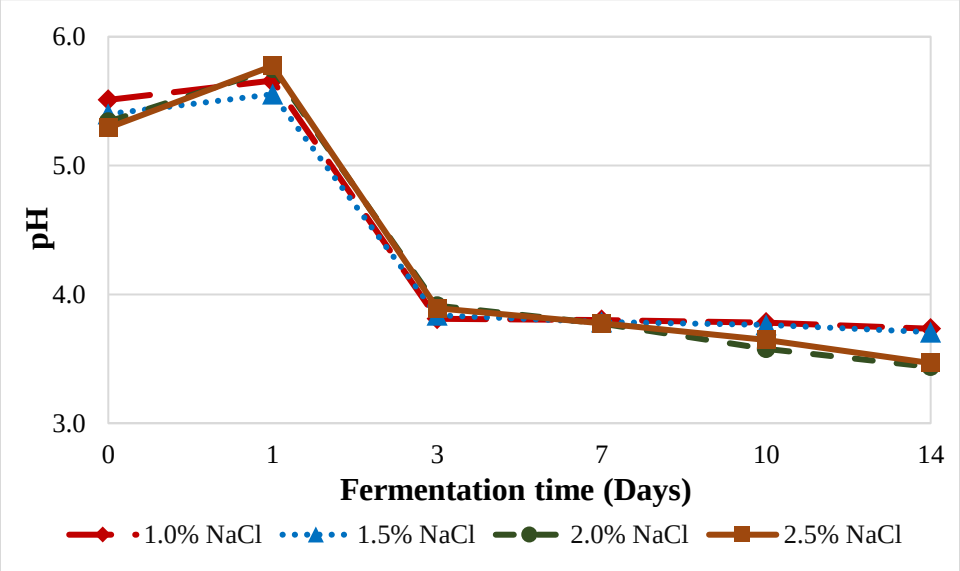
2.3. Results and Discussion

2.3.1. pH Levels

Salt concentration appeared to have no significant ($p \geq 0.05$) effect on sauerkraut pH levels, and all treatments reached the target pH level of 3.70 or less within 14 days of fermentation (Figure 2.2). A significant ($p < 0.05$) decrease in pH values occurred on day 3, as initial sauerkraut sample pH values were 5.29–5.51 on day 0, and were reduced to a pH level range of 3.80–3.90 on day 3. This rapid decrease in pH levels most likely helped to minimize the impact of spoilage bacteria and influence the quality of the finished sauerkraut product, as discussed by Viander and others (2003). During primary fermentation, the lower pH is a result from the lactic acid production due to the metabolic activity of *L. mesenteroides* and other heterofermentative LAB, which generally lower the pH levels to approximately 3.9–4.5. This lower pH level favors the growth and predominance of acid-resistant, homofermentative LAB, such as *L. plantarum*, which further

lowers the pH to approximately 3.4–3.7 before inhibiting their own growth. When LAB growth is inhibited, this stage is commonly known as secondary fermentation. In general, the adequate progression of the primary and secondary fermentations is sufficient to stabilize and preserve the fermented vegetables and prevent spoilage (Steinkraus 1992).

Figure 2.2. Mean sauerkraut pH levels at different salt concentrations during spontaneous fermentation over time

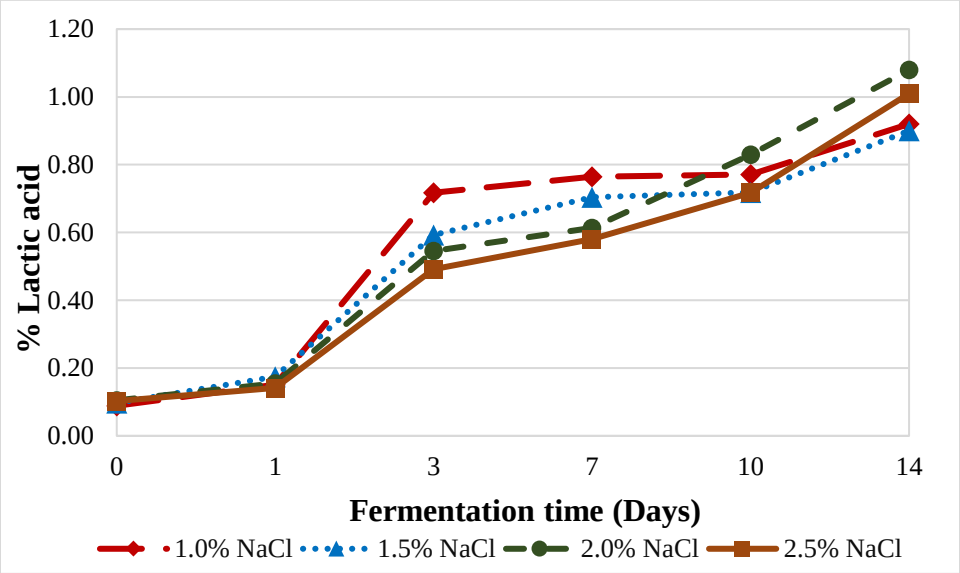


Each value represents the mean pH level (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant pH ($p \leq 0.05$) differences were detected among days over time.

During the mid-fermentation phase (day 7 to day 10), the pH decreased at a slower rate for all salt treatments. This slower reduction in pH levels, could be attributed to the reduced amount of available fermentable sugars in sauerkraut for LAB growth as the lower growth rate occurs as these nutrients are depleted, which was mentioned by Hayek and Ibrahim (2013). The pH values continued to decrease significantly ($p<0.05$) from an average of 3.80 on day 7 to a lower pH average of 3.44 by the end of fermentation for all salt treatments. Thakur and Kabir (2015) also reported similar pH values (3.57–3.72) for naturally fermented sauerkraut after14 days of fermentation, but their salt concentrations ranged from 2–4% NaCl (w/w).

2.3.2. Titratable Acidity (TA)

Figure 2.3. Mean sauerkraut titratable acidity changes at different salt concentrations during spontaneous fermentation over time



Each value represents the mean TA level (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant TA ($p \leq 0.05$) differences were detected among days over time.

As fermentation progressed and pH levels decreased, a rapid and significant ($p<0.05$) increase in titratable acidity was observed in sauerkraut samples for all salt concentrations after day 1 (Figure 2.3), which was expected. The percent TA (% TA) was reported as percent lactic acid. Titratable acidity (TA) is an important criterion to monitor the progress of lactic acid fermentation. The increase in %TA during sauerkraut fermentation indicates growth of LAB in the sauerkraut and utilization of sugars present in the cabbage to produce lactic acid and other by-products, as discussed by Vatansever and others (2016).

On day 0, the titratable acidity ranged between 0.09−0.10% TA for all salt treatments. A significant increase ($p<0.05$) in %TA among all sauerkraut samples were observed on day 3 (0.49–0.72% lactic acid) compared to day 0. On day 3, the 1.0% salt treatment had a slightly higher TA level than the other treatments, but the difference was not significant. According to

Zhang and others (2016), the slight differences in TA level on days 3 and 7 may be attributed to the higher growth of LAB at the lower salt concentrations, which may result in slightly higher amounts of lactic acid produced. Zhang and others (2016) evaluated the changes in TA and pH during the fermentation of chinese paocai prepared at three different salt ranges: 1–3%, 4 –5%, and 10% NaCl concentrations. They noticed that the paocai produced with lower salt levels appeared to have higher LAB growth rates and produced more lactic acid by day 6 of fermentation compared to paocai fermented at higher salt concentrations.

However, by the end of fermentation for this study, the %TA ranged between 0.90−1.08 % lactic acid for all salt treatments, with the 2.0% NaCl treatment having the highest %TA (1.08% lactic acid). However, no significant ($p \geq 0.05$) TA level differences were noted among the different salt treatments by the end of fermentation. Therefore, all salt treatments appeared to produce adequate lactic acid and could be employed to produce successful sauerkraut fermentation.

For the 1.0 and 1.5% salt treatments, the final %TA observed in this study were slightly lower than the ideal TA range of 1.0−1.5% lactic acid as suggested by Hong and Park (2000). The fermentation rate and microbial competition depends on both temperature and salt concentrations. Pederson and Albury (1969) also achieved ideal TA levels when they fermented sauerkraut at a 2.0% salt level at 18ºC. However, this study was conducted at a slightly higher fermentation temperature at approximately 22ºC, compared to their temperature of 18ºC.

2.3.3. Microbiological Analyses

2.3.3.1 Lactic Acid Bacteria (LAB)

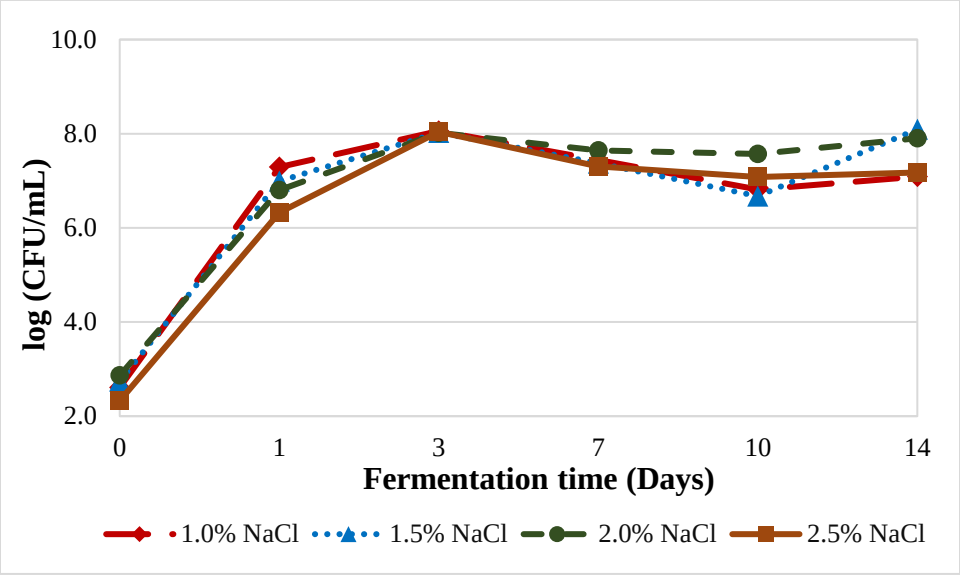
Table 2.2. Microbiological analyses of raw cabbage

Raw Cabbage	LAB log (CFU/g)	APC log (CFU/g)	Yeast and mold log (CFU/g)	Coliform MPN/g
Batch 1	2.1	4.6	2.3	460
Batch 2	2.0	4.5	2.5	240
Batch 3	2.7	3.9	2.3	150

Each value represents the mean microbial count log (CFU/g) (n=3).

Several researchers have recorded the LAB population of raw cabbage typically ranges between 2–3 log (CFU/g) (Peñas and others 2010, Breidt and others 2013). The LAB count of raw cabbage in this study was similar and ranged from 2.0–2.7 log (CFU/g) (Table 2.2). On fermentation day 1, a significant increase (p<0.05) in LAB counts was noted, which ranged from 6.33−7.29 log (CFU/mL) for all salt treatments (Figure 2.4). The 2.5 % NaCl treatment (control) had the lowest LAB count, while the 1.0% NaCl treatment had the highest count. It is important to note that the initiating strain of fermentation is usually heterofermentative LAB, which contributes towards the total acid (lactic and acetic acid) production, as noted by Wouters and others (2013). By day 3, the LAB count reached a peak level of 8 log (CFU/mL) for all salt treatments (Figure 2.4). The LAB count increased significantly (p<0.05) between days 1 and 3. LAB helps to inhibit or reduce pathogens and may eventually affect the safety and quality of the final product (FDA, 2011). One important attribute of LAB is their ability to produce antimicrobial compounds including lactic acid, thereby contributing to product safety by creating unfavorable conditions for pathogens and spoilage organisms (Soomro and others 2002, Krockel 2013).

Figure 2.4. Mean Lactic Acid Bacteria (LAB) counts present in brine at different salt concentrations during spontaneous fermentation over time



Each value represents the mean LAB count log(CFU/mL) (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant LAB ($p \leq 0.05$) differences were detected among days over time. Detection limit <100 CFU/mL.

During the mid to late stages of fermentation, the population of LAB slightly decreased for most treatments and reached a final level of 7−8 log (CFU/mL) after 14 days of fermentation for all salt treatments (Figure 2.4). The decrease in LAB after fermentation day 3 could most likely be attributed to the decline in the dominant heterofermentative LAB species due to the increase in acidity, as well as a simultaneous drop in pH levels in the brine, as mentioned by Perez and others (2013). When the acidity of 0.3 to 0.7% lactic acid and pH level below 4.0 is reached, heterofermentative bacteria may slow down and begin to die off. The activity initiated by the heterofermentative LAB is continued by the homofermentative LAB until an acidity level of 1.0% lactic acid is attained (Franco and others 2012). However, the different salt treatments did not appear to have any significant ($p \geq 0.05$) effect on LAB growth over time. Jagannath and others (2012) also experienced similar LAB counts with naturally fermented sauerkraut samples that contained 2.5% NaCl and held at 25ºC for 15 days. The researchers

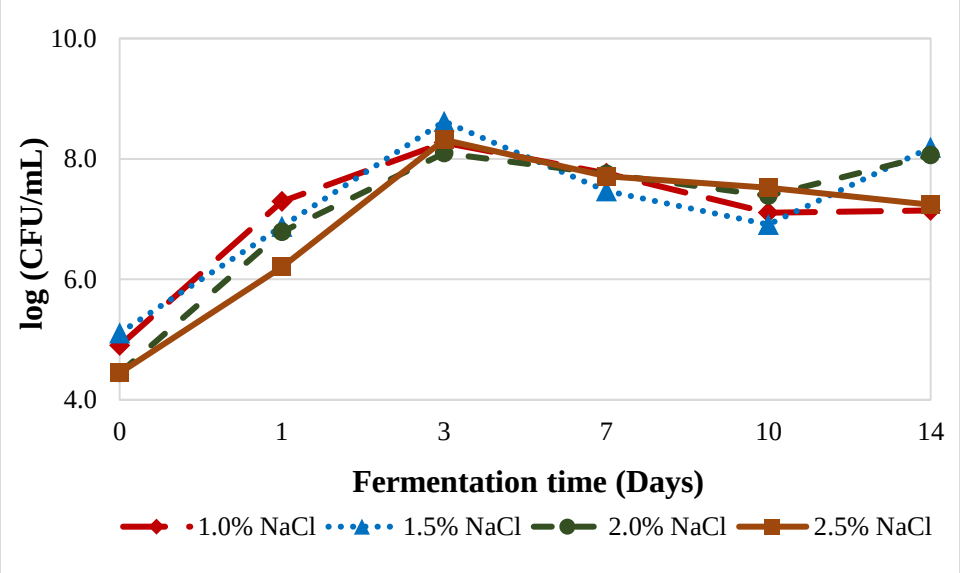
reported that in the case of spontaneous fermentation, the LAB count increased within the first 2–3 days, reached a peak level of 8.3 log (CFU/g) and later decreased to a level of 6.5–7.5 log (CFU/g) after 15 days of sauerkraut fermentation. Their observed microbiological changes are consistent with the findings of this study even though the researchers used much higher fermentation temperatures. Xiong and others (2016) also studied the effects of low salt concentrations on Chinese sauerkraut fermentation for 7 days. Their results were quite similar to this study, as they observed LAB counts as high as 8.0 log (CFU/mL) for the 2.0% and 5.0% NaCl treatments on fermentation day 1. However, these LAB levels were achieved in this study by day 3 for all salt treatments. The differences in LAB growth might be attributed to the different preparation methods used to make fermented Chinese cabbage, which involves other ingredients, such as the addition of crystal sugar, hot red pepper, garlic, ginger and Chinese prickly ash. Therefore, the LAB results from this study indicates that sauerkraut can be fermented at reduced salt concentrations and will reach the desired LAB count of 7−8 log CFU/mL after 2 weeks of fermentation.

2.3.3.2 . Aerobic Plate Count (APC)

The natural microbial population of cabbage, or any other vegetables, is dominated by total aerobes, LAB, yeasts and molds. The typical microbial community of vegetables is usually *Enterobacteriaceae*, LAB, and yeasts, which dominate during initial fermentation stages (Plengvidhya and others 2007). The population of total aerobes on raw cabbage typically range from 4–6 log (CFU/g) and yeast and mold range from 2–3 log (CFU/g) (Peñas and others 2010, Breidt and others 2013) However, the microbial counts for fresh produce vary with season, maturity stage, environmental humidity, temperature, and the use of pesticides (Samish and others 1963, Medina-Pradas 2016). In this study, the total aerobic plate counts on raw cabbage

prior to fermentation ranged between 3.9−4.6 log (CFU/g) (Table 2.2). The growth of aerobic bacteria over fermentation time followed a similar trend as the LAB. On fermentation day 1, aerobic plate counts (APCs) rapidly increased and ranged between 6.2−7.3 log (CFU/mL) for all salt treatments. On day 3, APC reached a peak level of 8.10−8.62 log (CFU/mL) for all samples, similar to the LAB counts. After day 7, the total aerobes started to decrease and by day 14, reached a total APC count of 7.2−8.1 log (CFU/mL) (Figure 2.5).

Figure 2.5. Mean sauerkraut aerobic plate counts (bacteria) at different salt concentrations during spontaneous fermentation over time



Each value represents the mean APC counts log(CFU/mL) (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant APC ($p \leq 0.05$) differences were detected among days over time. Detection limit <100 CFU/mL.

Pérez-Díaz and others (2018) investigated the total aerobes in commercially fermented cucumber brine at 6% NaCl. They also observed that total aerobes increased from 4.46 log (CFU/mL) in brined cucumbers after 1 day to 7.34 log (CFU/mL) on fermentation day 7, which was similar to findings in this study. Fleming and others (1987) noticed total aerobes followed a similar growth pattern as LAB in sauerkraut samples produced with 2.0% NaCl and held at 18ºC for 2 weeks of fermentation. These researchers reported that the total aerobic plate counts

present in sauerkraut samples ranged between 7−8 log (CFU/mL) by day 16. Jagannath and others (2012) also observed APC counts of 8.2 log (CFU/mL) in sauerkraut produced with 2.5% NaCl that was naturally fermented for 15 days. These results indicate that the salt concentrations in this study did not appear to have a significant ($p \geq 0.05$) effect on the aerobic plate counts during sauerkraut fermentation.

2.3.3.3. Fungi and Coliforms

Table 2.3. Mean yeasts, molds and coliforms present in sauerkraut brine at different salt concentrations during spontaneous fermentation over time

Salt concentrations (%NaCl)	**Yeasts and molds log (CFU/mL)**			**Coliforms (MPN/mL)**		
	Day 0	Day 7	Day14	Day 0	Day 7	Day 14
1.0	3.35	ND	ND	490	ND	ND
1.5	3.17	ND	ND	393	ND	ND
2.0	3.13	ND	ND	131	ND	ND
2.5	2.75	ND	ND	43	ND	ND

Each value represents the mean log (CFU/mL) (n=3). Detection limit <100 CFU/mL for yeasts and molds. Non-detectable = ND

The initial population of yeast and mold counts on day 0 ranged from 2.75 to 3.35 log (CFU/mL) and were reduced to undetectable levels by day 7 for all sauerkraut samples (Table 2.3). The coliform counts on day 0 ranged between 43 –490 (MPN/mL) for all salt treatments, with the 2.5% salt treatment having the least number of coliforms, and the 1.0% salt treatment having the highest coliform count. These results indicate that coliforms do not tolerate salt well. After 7 days of fermentation, no fungi were detected in any samples. According to Barth and others (2010), the pectinase produced by fungi (yeasts and molds) softens vegetables and leads to the deterioration of both flavor and quality. Coliforms, which are often used as an indicator organism for *E. coli*, were also not detected on fermentation days 7 and 14 for all samples. These results indicate that the fermentation process, regardless of salt concentration, appeared to be effective in reducing yeasts, molds, and coliforms in sauerkraut samples. These results suggest

that the quality and possible safety of sauerkraut may not be affected by the lower salt concentrations, as no spoilage organisms were observed or detected over fermentation time even at the reduced salt concentrations. However, the inoculation study (study 2), will address the potential for pathogen survival in lower salt, fermented sauerkraut samples. Additionally, the sauerkraut samples were tightly packed in the glass jar prior to fermentation to ensure the cabbage was submerged under brine and therefore, did not allow for air exposure. Exposure to air may enhance mold or other spoilage microorganisms to grow during fermentation.

Pérez-Díaz and others (2018) assessed the non-LAB microbiota in commercially fermented cucumbers and found the presence of spoilage microorganisms during fermentation were inhibited by reduced levels of dissolved oxygen and a decrease in pH levels below 4.5. In this study, the pH level of 3.5–3.8 was achieved by day 3, and hence no potential spoilage microorganisms were observed on fermentation days 7 and 14. Thus, the results indicate that as pH decreased by day 3, fungi and coliforms were susceptible to an increase of total acids produced over fermentation time produced by LAB growth, which were able to suppress unwanted microbial growth for all salt treatments.

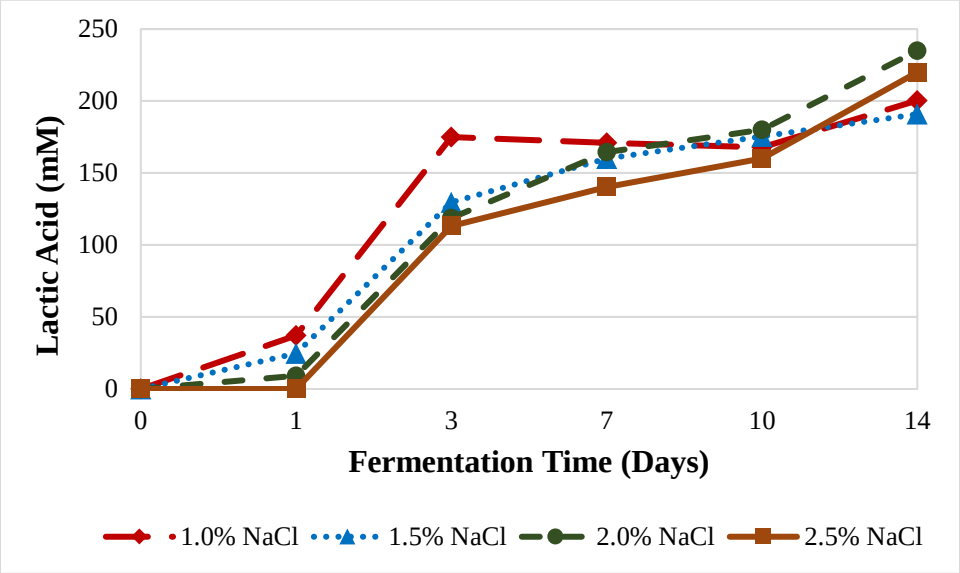
2.3.4. HPLC Analyses

2.3.4.1. Lactic Acid

Based on the organic acid results, lactic acid was the primary organic acid produced in sauerkraut samples during fermentation for all treatments. During the fermentation process, an increase in lactic acid was observed and followed a consistent trend with the titratable acidity results. The concentration of lactic acid increased slowly within the first days of fermentation for all salt treatments. However, lactic acid increased significantly ($p<0.05$) on day 3 compared to days 0 and 1 for all salt treatments (Figure 2.6). Heterofermentative LAB including, *L.*

mesenteroides, ferments glucose using the phosphoketolase pathway to produce lactic acid, CO_2 and acetic acid and may explain the increase of lactic acid over fermentation time (White 2007). The 1.0% NaCl–treated sample had a slightly higher lactic acid level compared to the other salt treatments on day 3, which also corresponds with the %TA results. However, this difference in lactic acid levels was not significant. This difference might be due to the heterofermentative LAB growth at early stages of fermentation, which may be able to produce slightly more lactic acid at lower salt concentrations (Franco and others 2012).

Figure 2.6. Mean lactic acid levels in sauerkraut at different salt concentrations during spontaneous fermentation over time



Each value represents the mean lactic acid level (mM) (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant lactic acid ($p \leq 0.05$) differences were detected among days over time.

During sauerkraut fermentation, there is a rapid turnover of LAB species. The dominant species present in fermentation shifts within 2 to 3 days from heterolactic LAB species that produce less acid, to more homolactic fermenting LAB species that produce more acid (Plengvidhya and others 2004). The lactic acid content increased for all salt treatments after day 3 (Figure 2.6), which was most likely due to the presence and action of these homofermentative

lactic acid bacteria, which have better acid and salt tolerance (Jiajia and others 2012). These homofermentative organisms primarily produce lactic acid from glucose and fructose via the Embden-Meyerhoff-Parnas pathway (Breidt and others 2007).

The lactic acid levels significantly ($p<0.05$) increased on day 14 compared to day 7 for all the salt treatments. By the end of sauerkraut fermentation (day 14), the lactic acid concentration for all samples was ranged between 190−230mM, with the 2.0% NaCl treatment having the highest lactic acid level of 230mM (Figure 2.6). However, no significant differences ($p\geq0.05$) were detected in regard to lactic acid concentration by the end of fermentation, which indicates that salt concentration did not affect lactic acid production in the sauerkraut samples.

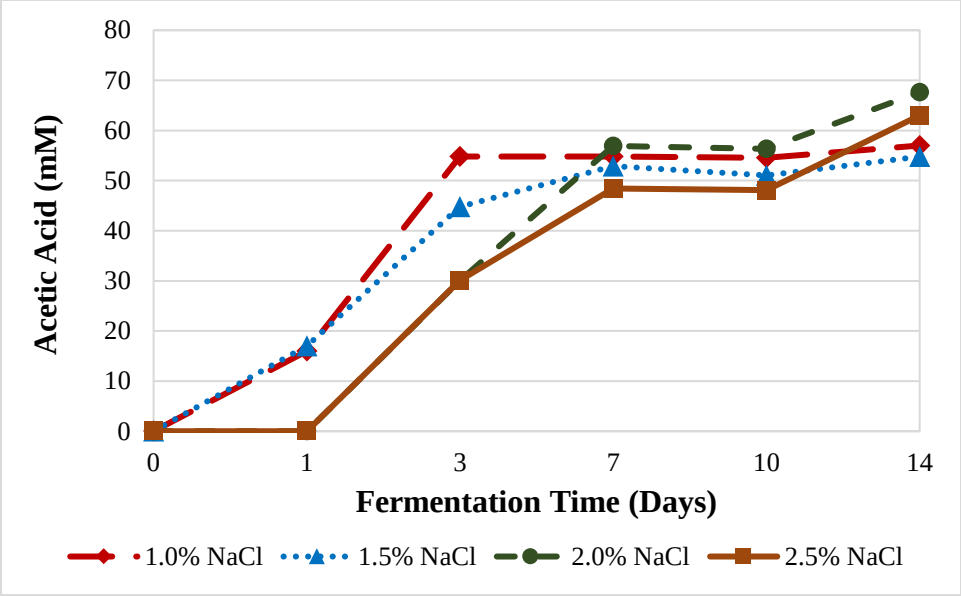
Xiong and others (2016) reported a similar trend in lactic acid production when Chinese sauerkraut was naturally fermented at three different salt concentrations of 2%, 5% and 8% NaCl (w/w). Likewise, lactic acid rapidly increased within the first 3 days of fermentation, followed by a slow increase until reaching the end of fermentation. However, the researchers reported that salt had a significant impact on lactic acid production over fermentation time in sauerkraut, the higher the salt concentration was, the lower the lactic acid production. This difference in results could be due to the differences in salt concentrations between the two studies, as Xiong and others (2016) studied higher and more variable salt concentrations.

2.3.4.2. Acetic Acid

Another organic acid that is produced from fermentation is acetic acid. The acetic acid concentration plays a major role in imparting sour flavor to sauerkraut. Acetic acid levels increased from day 0 to day 1 in the lower salt sauerkraut samples (1.0 and 1.5% NaCl) compared to the other salt treatments (Figure 2.7), which indicates the higher salt treatments were slightly slower to initiate acetic acid production. The slightly higher acetic acid levels

observed at the lower salt treatments on day 3 might be attributed to a more favorable environment for heterofermentative LAB growth at early stages of fermentation due to the lower salt concentrations. Although the acetic acid levels initially varied for some treatments, by day 7 all salt treatments had similar acetic acid levels. By days 7 and 10, acetic acid levels stabilized and then slightly increased on day 14. By the end of fermentation, the 2.0% NaCl sauerkraut sample had the highest acetic acid level of 67.72 mM, followed by 2.5%, 1.0% and 1.5% NaCl treatments with 62.99, 57.00 and 54.78 mM of acetic acid, respectively (Figure 2.7). The acetic acid level was lower than the lactic acid levels day 14 for all salt treatments with a final acid ratio of 1:4 (acetic acid: lactic acid). An important parameter to evaluate final sauerkraut quality is the lactic:acetic acid ratio. According to Pederson and Albury (1969) and Medina-Pradas and others (2016), the ideal ratio is 4:1. Fleming and others (1987) reported similar findings for organic acid formation between the two acid levels (acetic and lactic acid) of 1:4 when sauerkraut was fermented with a salt concentration of 2.0−2.5% NaCl (w/w). In this study, although there were differences in organic acid concentration (lactic and acetic acid) during fermentation, no significant differences ($p \geq 0.05$) were noted on day 14. At the end of the fermentation process, sauerkraut should contain approximately 1% lactic acid to obtain a quality final product with acceptable sensory characteristics (Breidt and others 2013).

Figure 2.7. Mean acetic acid levels in sauerkraut at different salt concentrations during spontaneous fermentation over time



Each value represents the mean acetic acid level (mM) (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant acetic acid ($p \leq 0.05$) differences were detected among days over time.

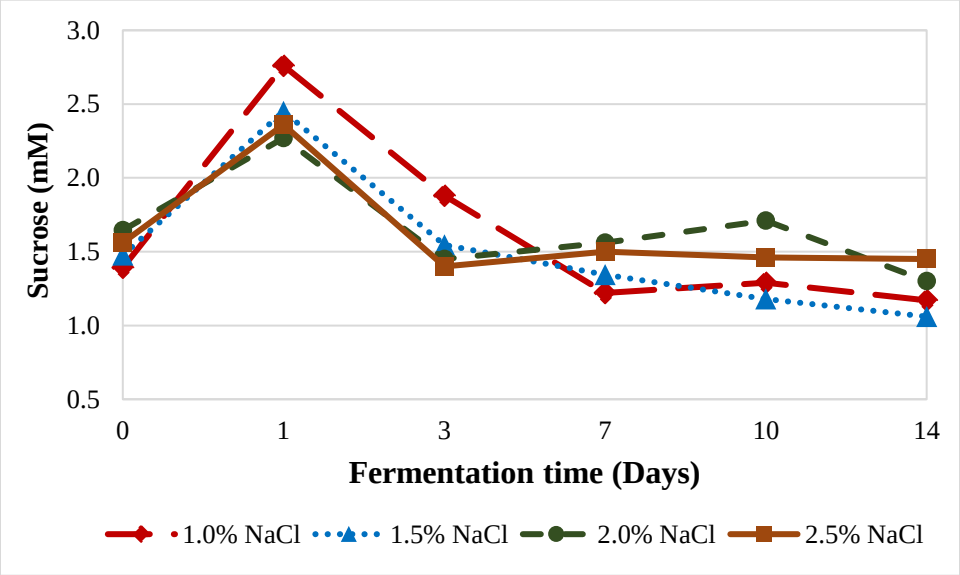
These results indicate that salt concentration had no significant ($p \geq 0.05$) impact on the formation of acetic and lactic acid by the end of sauerkraut fermentation. The lactic acid to acetic acid ratio was found out to be 4:1 for all salt treatments in this study, which further justifies that the lower salt brines were able to produce quality sauerkraut, although a sensory evaluation should be conducted in the future to determine the consumer acceptance of the lower salt brine sauerkrauts compared to the control treatment (2.5% brine).

2.3.4.3. Sucrose

Residual sugars in cabbage, which are broken down and released during the fermentation of sauerkraut, play a vital role as they support primary and secondary fermentation by yeast and LAB cultures (Jagannath and others 2012). The three main fermentable sugars present in cabbage are glucose, fructose and sucrose. Prior studies have reported that raw cabbage contains higher glucose and fructose concentrations compared to sucrose (Fleming and others 1987, Rosa

and others 2001). Similar sugar results were found in this study, with glucose and fructose being the primary fermentable sugars present in the largest concentrations, compared to sucrose among all samples.

Figure 2.8. Mean sucrose levels in sauerkraut at different salt concentrations during spontaneous fermentation over time



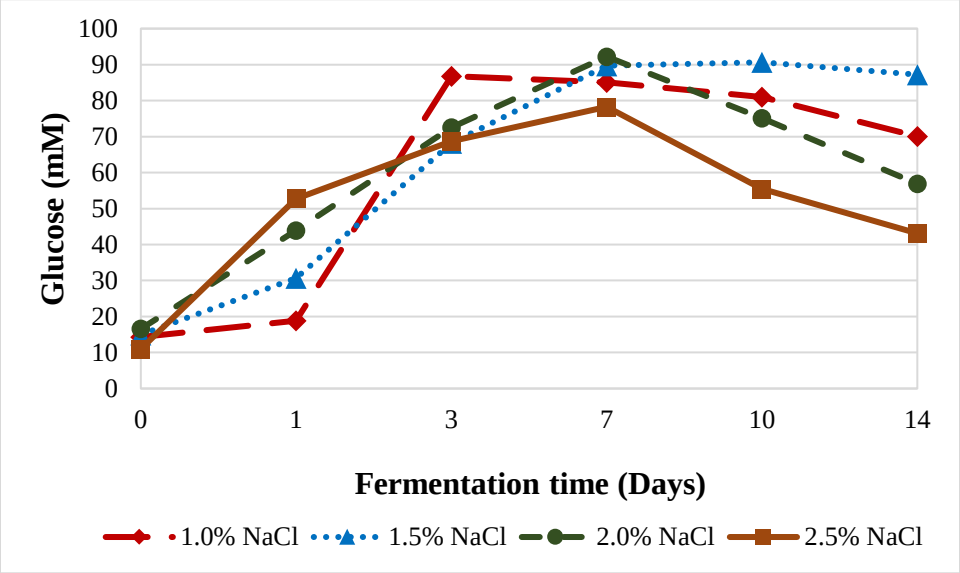
Each value represents the mean sucrose levels (mM) (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant sucrose ($p \leq 0.05$) differences were detected among days over time.

Sucrose concentrations ranged from 1.39 to 1.56 mM on day 0, and then significantly increased on day 1 for all treatments, which corresponds to diffusion of sucrose from the cabbage to brine as soon as fermentation started, as brine samples were tested in this study (Xiong and others 2014) (Figure 2.8). However, all sauerkraut samples had sharp decline in sucrose levels on day 3 that slowly stabilized by day 14 for all the salt treatments, reaching a final sucrose concentration of 1.06−1.40 mM. According to Xiong and others (2016), this rapid decrease in sucrose content is mainly due to the presence of heterofermentative LAB, which produce a high-activity dextransucrase enzyme that catalyzes the decomposition of sucrose into lactate, ethanol, acetate, mannitol, dextran and carbon dioxide. The rate of sucrose consumption slowed down

during the mid to late stages of fermentation because of homofermentative LAB, which has comparatively lower utilizing capacity of sucrose than heterofermentative LAB. Sucrose content was small (1.06–1.45 mM) in all sauerkraut samples. The low sucrose levels could suggest that sucrose was hydrolyzed throughout the fermentation, resulting in some increase in glucose and fructose levels (Xiong and others 2014). By the end of the fermentation process, the 1.5% NaCl treatment had the lowest sucrose content and the 2.5% NaCl brine samples had the highest concentration. However, the differences in sucrose levels among the different salt treatments were not significant ($p \geq 0.05$) by day 14.

2.3.4.4. Glucose

Figure 2.9. Mean glucose levels in sauerkraut at different salt concentrations during spontaneous fermentation over time



Each value represents the mean glucose levels (mM) (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant glucose ($p \leq 0.05$) differences were detected among days over time.

A significant difference ($p<0.05$) was observed in glucose levels between days 0 and 7 for all salt treatments (Figure 2.9). On day 0, glucose concentrations ranged between 11.00−16.58 mM among the different sauerkraut samples. The glucose concentration rapidly increased and peaked

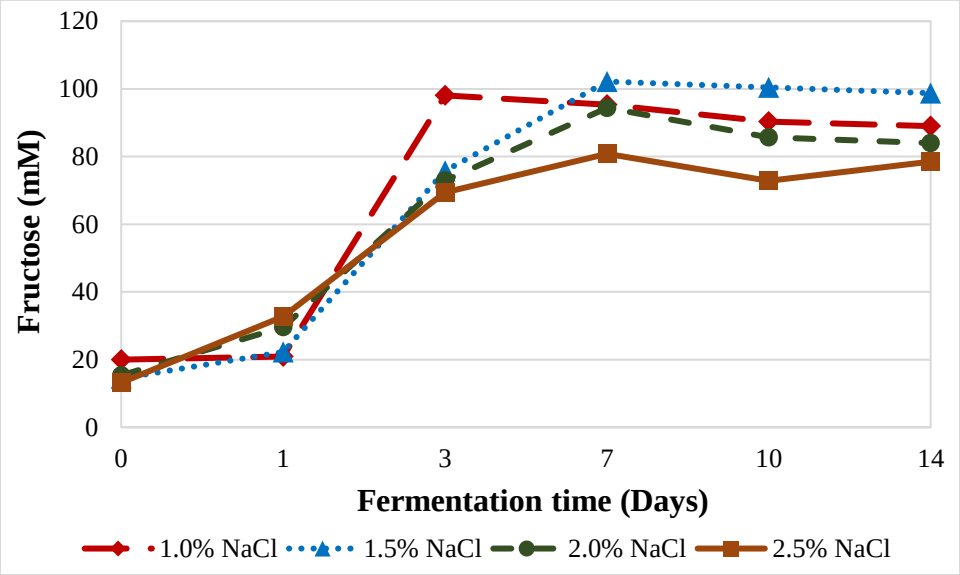
at day 7 to glucose concentrations of 78.25−92.21 mM for samples. Xiong and others (2016) recorded a similar glucose concentration of 65–76 mM in brine samples after 7 days of sauerkraut fermentation at 2% NaCl levels.

After day 7, glucose concentrations started to slightly decrease until the end of the fermentation on day 14, and final concentrations ranged from 43.11 to 87.17 mM. The heterofermentative LAB are active during the initial stages of fermentation, including *L. mesenteroides*. These LAB can tolerate fairly high sugar concentrations (up to 50% sugar) and utilize glucose as an electron acceptor to produce CO_2 and lactic acid, which rapidly lowers the pH level in fermented foods (Erten 2000). However, after day 7, glucose concentrations decreased, which might be the result of *L. plantarum* and other homofermentative LAB that utilizes Embden-Meyerhof-Parnas pathway to converts glucose into lactic acid (Xiong and others 2016). Any differences in glucose concentrations were not significant ($p \geq 0.05$) among the different salt treatments.

2.3.4.5. Fructose

The initial fructose content ranged between 13.21−19.11 mM for all the salt treatments (Figure 2.10). Compared to glucose, the fructose levels did not rapidly increase until after day 1 and peaked after day 3. By day 7, the fructose concentrations ranged between 80.81−102.21 mM for all samples. After day 7, fructose levels gradually decreased and reached a final concentration of 78.54−98.76 mM on day 14 for all treatments. The brine concentration appeared to not have any significant ($p \geq 0.05$) effects on fructose levels throughout fermentation. Fructose concentrations were relatively higher than the glucose levels during sauerkraut fermentation.

Figure 2.10. Mean fructose levels in sauerkraut at different salt concentrations during spontaneous fermentation over time



Each value represents the mean fructose levels (mM) (n=3). No significant differences were detected among salt treatments at each analysis day ($p \geq 0.05$). However, significant fructose ($p \leq 0.05$) differences were detected among days over time.

However, the sugars were not completely depleted after 14 days of sauerkraut fermentation. These results were in accordance with Wolkers and others (2013). The researchers studied the effects of reduced salt concentrations (15 g/kg NaCl, 9g/kg NaCl and 40% partial sodium replacement with mineral salts) and reported that the total sugars decreased during fermentation, but were not completely depleted by the end of fermentation in their reduced salt studies. Wolkers and others (2013) noted that presence of some sugar at the end of the fermentation are result of natural sugar extraction by salt from cabbage and their absorption by LAB and therefore are no direct indicator of microbial activity.

2.3.5. Colorimetric Analyses

Table 2.4. Mean L*, a* and b* values of sauerkraut at different salt concentrations during spontaneous fermentation over time

Salt concentrations (% NaCl)	L*			a*			b*		
	Day 0	Day 7	Day14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
1.0	61.70^{b}± 1.68	62.51^{b} ± 0.21	65.79^{a} ± 1.21	3.63^{a} ± 0.38	1.43^{b} ± 0.08	1.38^{c} ± 0.17	22.23^{b} ± 2.03	22.23^{b} ± 1.04	24.14^{a} ± 1.39
1.5	59.47^{b} ± 1.17	62.27^{b} ± 1.21	66.08^{a} ± 1.21	3.95^{a} ± 0.4	1.42^{b} ± 0.11	1.34^{c} ± 0.20	22.08^{b} ± 2.35	21.24^{b} ± 1.27	23.47^{a} ± 2.12
2.0	62.71^{b} ± 0.71	60.50^{b} ± 1.69	63.99^{a} ± 1.70	4.09^{a} ± 0.39	0.60^{b} ± 0.24	0.20^{c} ± 0.24	23.60^{b} ± 2.12	21.87^{b} ± 2.10	24.27^{a} ± 1.39
2.0	62.12^{b} ± 1.13	63.18^{b} ± 1.03	64.37^{a} ± 0.76	3.83^{a} ± 0.26	1.69^{b} ± 0.51	1.10^{c} ± 0.15	23.15^{b} ± 0.65	20.62^{b} ± 2.20	24.01^{a}± 1.05

Each value represents the mean L*, a*, b* values $\pm$ s.d. (n=3). No significant differences were detected among salt treatments on day 14 ($p>0.05$). Means with different letters are significantly ($p\geq0.05$) different across columns for each color value.

Color is the first characteristic that consumers rely on when perceiving product quality. In the case of sauerkraut, non-enzymatic browning discoloration can occur during fermentation, which is considered a defect, thereby affecting the quality of the final product (Lonergan and Lindsay 1979, Johanningsmeier and others 2005). Therefore, it is imperative to analyze any color changes during sauerkraut fermentation when modifying salt concentrations.

Colorimetric L* values indicates light to dark color differences in foods, and the scale ranges from 0 to 100, where 0 is black and 100 is white. The salt concentration did not appear to significantly ($p\geq0.05$) affect L* values of sauerkraut samples. However, fermentation time (days) did have significant ($p<0.05$) effects on sauerkraut color (Table 2.4). The L* value significantly ($p<0.05$) increased for all samples by day 14 for all salt treatments compared to day 0, which indicates the sauerkraut samples were slightly, but significantly lighter in color over time. A lighter sauerkraut color at the end of fermentation is a desired quality attribute for sauerkraut by consumers (Johanningsmeier and others 2007).

The a* and b* values are a measure of any red (positive a* values) to green (negative a* values) changes and yellow (positive b* values) to blue (negative b* values) color changes over fermentation time, respectively. The a* values were significantly ($p<0.05$) higher on day 0 and decreased overtime compared to both days 7 and 14 for all treatments, which suggests that the sauerkraut samples were less red in color and approached the green scale over fermentation time. Similarly, b* values significantly ($p<0.05$) increased after day 7 for all salt treatments compared to day 0. These results indicate that after fermentation, the sauerkraut samples were developing a more yellow color, irrespective of the salt brine concentration. These color changes are most likely attributed to the breakdown of chlorophyll, which is the predominant green pigment. Chlorophyll is sensitive to changes in pH, and the low pH environment during fermentation, may have caused the changes of green to more yellow color over fermentation time in the sauerkraut samples (Hunaefi and others 2013).

2.4. Study 1 Conclusions

Due to the recent FDA guidance on the voluntary reduction of sodium in foods (FDA 2011), both consumers and food processors are interested in developing or modifying food formulations to lower salt concentrations, including fermented foods. Therefore, it is important to determine the viability, safety and quality of lower sodium fermented products to benefit both home-fermenters and the food industry.

The results of study 1 indicate that spontaneous, natural fermentation of sauerkraut can occur at lower sodium levels. Chemical and microbiological analyses of sauerkraut samples during spontaneous fermentation reflected that salt concentrations (< 2.5% NaCl) had no significant effects ($p\geq0.05$) on the measured parameters. The available sugars (glucose, fructose and sucrose) for fermentation were not completely depleted after 14 days of fermentation among

all treatments, and the desired level of lactic acid (approximately 1%) was produced by the end of fermentation for most sauerkraut samples. The lactic acid to acetic acid ratio was 4:1, which also suggests that the finished sauerkraut samples were similar to acceptable quality standards. During the fermentation process, a sharp decline in pH and an increase in titratable acidity occurred in all samples regardless of salt treatments, as well as adequate growth of lactic acid bacteria. A pH of 3.70 or lower was achieved for all salt treatment within 14 days of fermentation, which marked the completion of fermentation. Moreover, the growth of lactic acid bacteria and production of organic acids was not affected by salt concentration differences. The rapid decrease in pH appeared to play a vital role in minimizing the influence of spoilage microbes in sauerkraut samples, as no fungi and coliforms were detected among all samples after fermentation was complete. Therefore, these results indicate that quality sauerkrauts can be produced at lower salt concentrations. However, microbial safety was not addressed in this study and will be addressed in Chapter 3 to determine any differences in pathogen survival among these sauerkraut samples.

CHAPTER 3

EFFECTS OF LOW SALT CONCENTRATION ON THE MICROBIAL SAFETY OF SPONTANEOUSLY FERMENTED CABBAGE

3.1. Justification and Objectives

Food safety is an important aspect of the food preparation process, and there is a slight, but potential foodborne illness risk when both home-prepared and commercially-produced fermented foods are not prepared correctly (Nout 1994, Bodmer and others 1999). Although acidic conditions and high NaCl levels have been thought to provide adequate safety of fermented vegetables, there have been reports that indicate that some acid resistant pathogens can occur in such foods (Medina-Pradas and Arroyo-López 2015). These pathogens can be found on vegetables when contaminated with cattle manure close to harvest or by using contaminated water for irrigation. For example, there was an outbreak of *L. monocytogenes* reported in Canada in 1983 due to coleslaw produced from cabbage contaminated with sheep manure (Niksic and others 2005). *E. coli* O157:H7 is exceptionally acid tolerant and can also be present on contaminated cabbage (Niksic and others 2005). However, evidence is lacking on the safety of sauerkraut produced at lower salt concentrations (<2.5% NaCl). An increased interest in fermented vegetables, coupled with the FDA guidance to reduce salt content, further solidifies the need to validate the safety of these value-added, fermented vegetables.

The overall objective of this research was to evaluate the effects of salt concentrations (1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w)) on the survival of bacterial pathogens (STEC, *S. aureus* and *L. monocytogenes*) during spontaneous fermentation of cabbage. These food-borne pathogens were selected due to their ability to tolerate low pH conditions and the potential to be present on raw cabbage.

3.2. Material and Methods

3.2.1. Experimental design

A randomized complete block design (RCBD) was used to prepare fermented cabbage (sauerkraut) at four different NaCl concentrations (1.0%, 1.5%, 2.0% and 2.5% (w/w)). Treatments (NaCl concentration) were blocked by replicate (n=3). In this experimental design approach, number of blocks (n=3) equals to the number of replications, with a total of 12 samples over a period of three weeks. The samples were tested throughout fermentation until a pH of 3.70 or lower was achieved. There were four control jars, one for each salt concentration, that were not inoculated.

3.2.2. Bacterial Strains

Sauerkraut was inoculated using following strains: *L. monocytogenes* strains included American Type Culture Collection (ATCC) 1911 and ATCC 19115, Shiga-toxin producing *E. coli* (STEC) strains BAA-1653 and BAA-184 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA), and *S. aureus* strains: *S. aureus* COW and *S. aureus* GFP were obtained from The University of Maine, Animal Diagnostic Lab from Dr. Melody Neely, and included a mastitis isolate and green fluorescent laboratory strain, respectively. All stock cultures were maintained at -80°C and were sub-cultured prior to use.

3.2.3 Preparation of Inocula

A cocktail was prepared which included two strains of each of the three foodborne pathogens: STEC, *S. aureus* and *L. monocytogenes*. STEC, *S. aureus* and *L. monocytogenes* strains were grown overnight at 37°C, 37°C and 30°C, respectively in 10 mL of tryptic soy broth (TSB) (Alpha Biosciences, Inc., Baltimore, MD). After incubation, inocula concentration (10^5 - 10^8 CFU/mL) was confirmed by plating appropriate dilutions onto Levine Eosin Methylene Blue

agar (EMB), Baird Parker Agar (BP) (Difco, Becton, Dickinson, Sparks, MD) or Oxord Listeria Agar Base (MOX), including Modified Oxford Antibiotic Supplement (Difco, Becton, Dickinson, Sparks, MD) plates. EMB and BP plates were incubated at 37^{o}C for 2 days to enumerate STEC and *S. aureus* strains, respectively. MOX plates were incubated at 30^{o}C for 1 day to enumerate *L. monocytogenes*.

3.2.4. Preparation of Fermented Cabbage (Sauerkraut)

Fresh cabbage was purchased from three different local retailers for each of the three weeks: Walmart, Shaws and Hannaford (Bangor, ME). A total of 4 cabbage heads (3.6−4.1 kg), were purchased from each store for each testing week. Three to four outer leaves, along with the white stem were removed prior to shredding since the outer leaves of cabbage may have been damaged and to remove inedible parts. The cabbage heads were washed thoroughly in cold potable water and cut into six pieces. The cabbage was shredded using a Robot-coupe (CL 50 series E, Robot-coupe USA. Inc., Ridgeland, MS) and mixed manually with gloved hands in a large mixing bowl to ensure a homogenized sample. Cabbage (500 g) was weighed for each treatment and respective controls.

3.2.5. Processing

Shredded cabbage was salted with non-iodized Kosher salt at four different concentrations of 1.0%, 1.5%, 2.0% and 2.5% NaCl (w/w). Cabbage and salt were mixed manually for 5−6 minutes with gloved hands and allowed to stand for 15 minutes to express liquid. The cabbage was then packed tightly into sterile 24 oz. glass jars. The glass jars were inoculated with a pathogen cocktail containing 2 strains of each pathogen at approximately 10^5 CFU/g. There were four control jars, one for each salt concentration, that were not inoculated. Additionally, 10 mL of brine for each salt concentration corresponding to 1.0%, 1.5%, 2.0% and

2.5% NaCl were added in all jars. Sterile glass weights were placed in jars to keep the sauerkraut submerged under the brine. Glass jars were closed using lids with airlocks and incubated at room temperature (~ 22°C) until a pH of 3.70 or lower was achieved. All the inoculated glass jars were refrigerated for one week after the completion of fermentation. Figure 3.1 illustrates the sauerkraut preparation process and storage for this study.

Figure 3.1. Preparation of sauerkraut samples

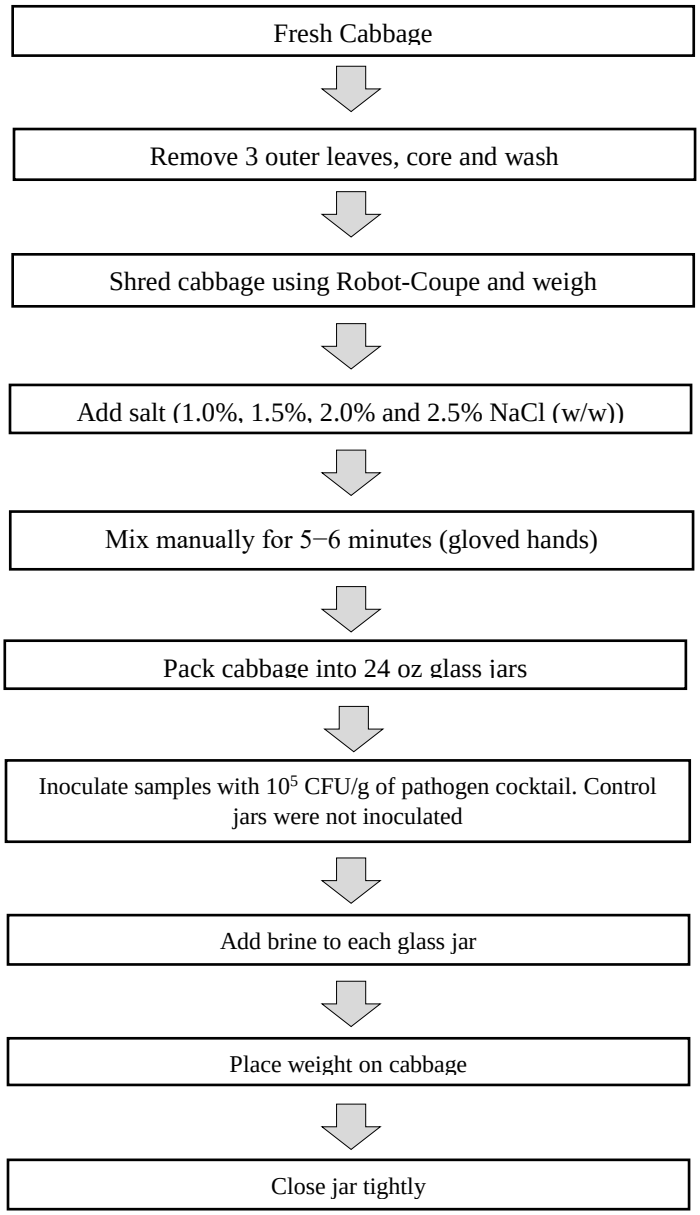
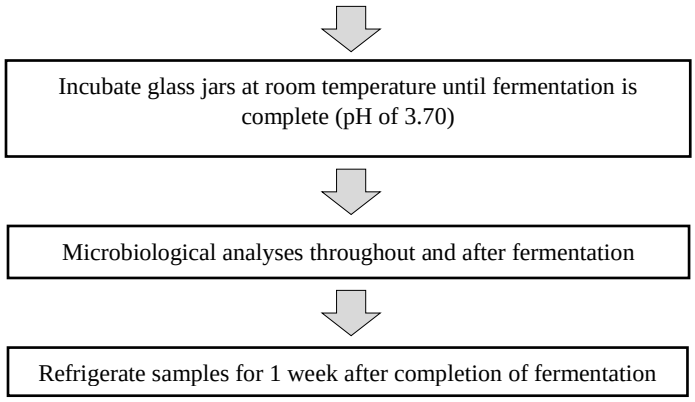


Figure 3.1. Continued



3.2.6. Sample Collection

Brine samples (1 mL) were aseptically collected during the fermentation process on days 0, 1, 2, 3, 4, 6, 9, 12, 15 and 18 from the center of the inoculated glass jars using sterile pipettes. On each sampling day, the pH was monitored to measure acidification levels of all the control samples using a pH probe (Orion Star A111, Thermo Scientific., Waltham, MA). The ambient temperature was also recorded on each sampling day until the end of fermentation. Upon completion of fermentation, all brine samples were refrigerated for 1 week. After 1 week of refrigeration, brine samples were collected to enumerate the number of bacterial pathogens present.

3.2.7. Bacterial Enumeration and Detection

3.2.7.1. Surface Plating and Thin Agar Overlay Method

On each testing day, the presence of both healthy and injured cells was assessed for all bacterial pathogens. The healthy cells were enumerated using surface plating on selective media. Since the injured cells may not grow on the selective media, their presence was assessed utilizing a thin agar layer method. This one-step procedure is based on the diffusion of selective agents from the underlying selective medium to the upper non-selective medium (Wesche and Ryser 2014). The non-selective overlay was prepared by combining half strength tryptic soy agar

(Alpha Biosciences, Inc., Baltimore, MD) into tryptic soy broth (Alpha Biosciences, Inc., Baltimore, MD) and was then autoclaved in 10 mL test tubes. The overlay solidifies at room temperature and was melted prior to use using a microwave and cooled prior to pouring onto the selective agar plates.

Brine samples (1 mL) were subsampled from each inoculated sample jar using a sterile pipette. The samples were serially diluted in peptone, and surface plated onto the EMB agar, MOX agar and BP agar in duplicates for enumeration of STEC, *L. monocytogenes* and *S. aureus*, respectively. The healthy STEC cells and *S. aureus* populations were enumerated 2 days after incubation at 37^{o}C. The healthy *L. monocytogenes* cells were enumerated at 30^{o}C after 1 day of incubation. The overlay cells were recovered by overlaying non-selective plating medium onto the pathogens' selective pre-poured plate medium in duplicate. The selective media used to enumerate STEC, *L. monocytogenes* and *S. aureus* was EMB agar medium, MOX agar and BP agar, respectively. The overlay was checked to ensure it completely covered the selective agar media plate. All the plates were allowed to sit on the benchtop at room temperature for 5 to 10 minutes to solidify. After plating, the STEC and *S. aureus* plates were then incubated at 37^{o}C for 2−3 days depending upon the visibility of colonies. The *L. monocytogenes* plates were incubated for 1 day at 30^{o}C. Both healthy and overlay cells of all three pathogens were counted after incubation. The injured cell count was determined by subtracting overlay cells from healthy cells. The results for both healthy and injured cells were reported as log (CFU/mL) using the following calculation:

$$\text{Injured cells} = \text{Overlay cells}/\text{Overall cells} - \text{Healthy cells}$$

3.2.7.2. Enrichment

After fermentation was complete, the enrichment was performed to confirm the presence or absence of all three bacterial pathogens because the levels were too low for detection. Enrichment provides a high sensitivity bacterial detection method by enhancing the growth of target microorganisms that are present in smaller numbers (Gill 2017). Thereby, enrichments help to ensure that there are no false negatives and to determine if there are bacterial pathogens at extremely low levels.

Sauerkraut (25 g) from the each of inoculated jars was aseptically placed into separate, sterile stomacher bags. The sauerkraut samples were then homogenized in selective enrichment broth for 2 minutes with the use of a stomacher (BagMixer 400, interscience Laboratories Inc., Woburn, MA). The selective enrichment broths utilized to detect STEC and *L. monocytogenes* were *E. coli* enrichment broth (EC medium, Alpha Biosciences, Inc., Baltimore, MD) and buffered listeria enrichment broth (BLEB) (Alpha Biosciences, Inc., Baltimore, MD), respectively. For *S. aureus*, the tryptic soy broth (Alpha Biosciences, Incs. Baltimore, MD) had 10% sodium chloride (NaCl) (Aqua Solutions, Inc., Deer Park, TX) and 1% sodium pyruvate added (Alfa Aesar, Ward Hill, MA) as the selective enrichment broth. This selective enrichment broth is used to enhance the detection of this organism by providing the essential nutrients for growth and selects against the growth of other organisms.

The stomacher bags contained a homogenized mixture of inoculated sauerkraut samples and selective enrichment broth, which were then incubated for enrichment. The STEC and *S. aureus* bags were incubated at 37^{o}C for 1 day. The *L. monocytogenes* bags were incubated at 30^{o}C for 1 day. After incubation, 100 µL of selective enrichment broths were surface plated and streaked onto their selective agar plates in duplicate, and then placed in an incubator. The

presence or absence of the STEC and *S. aureus* colonies were recorded after the incubation period of 2 days at 37^{o}C. The MOX plates for *L. monocytogenes* detection were incubated at 30^{o}C for 1 day. After the incubation period of 1 day, the presence or absence of *L. monocytogenes* colonies were recorded. The sauerkraut samples refrigerated for one week were again assessed for all three bacterial pathogen populations using surface plating on selective media as previously described.

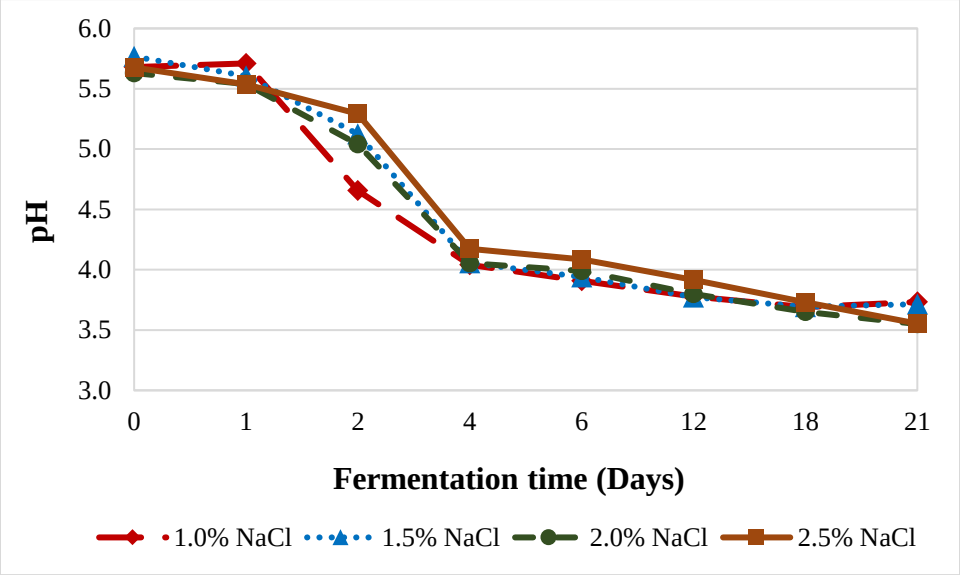
3.2.8. Statistical Analysis

This study was replicated three times. Data were analyzed using SPSS software, version 25 (SAS Software, Cary, NC). Shapiro-Wilk's normality test and Levene equality of variances were implemented to assess data prior to further analyses. One-way ANOVA was used to determine any significant differences among treatments with a significance level of $p \leq 0.05$ and Tukey's HSD test was selected for post-hoc analyses to determine significance difference among means.

3.3. Results and Discussion

3.3.1. pH

Figure 3.2. The effects of salt concentration on pH levels during spontaneous sauerkraut fermentation



Each value represents the mean pH levels (n=3). No significant differences were detected among treatment days throughout fermentation ($p \geq 0.05$).

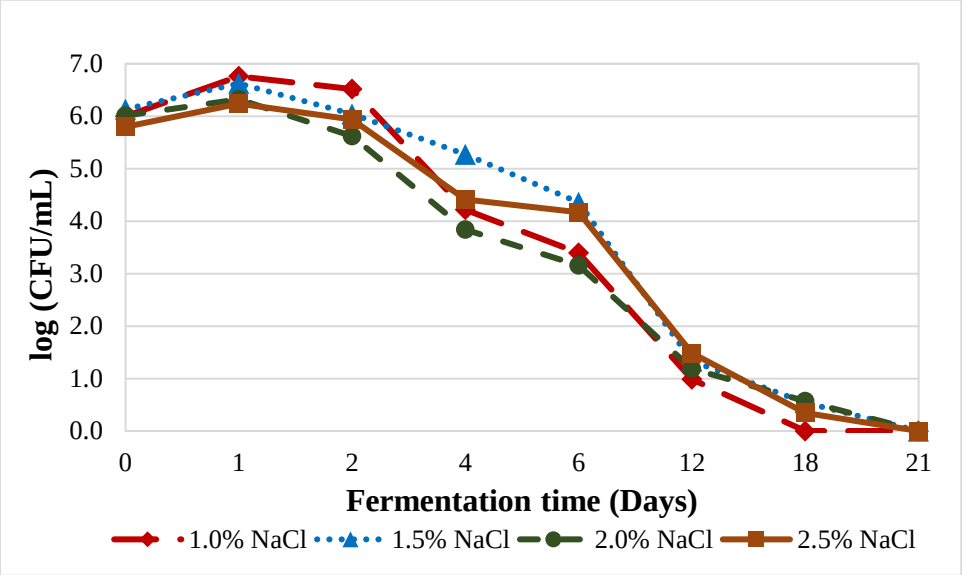
All salt concentration treatments reached the fermentation pH endpoint of 3.70 or less by day 21, which marked the completion of fermentation. When analyzing the rate of pH level changes, the pH values decreased rapidly until day 4 for all salt treatments. The 1.0% NaCl-treated sample had a slightly lower pH level on day 2 compared to the other salt treatments on (Figure 3.2). However, all the treatments had similar pH levels by day 4, and pH levels decreased at a slower rate until the completion of fermentation. By day 21, pH levels ranged from 3.50 to 3.70. Hence, the pH values continued to decrease over fermentation time for all salt concentrations. The rapid decrease in pH at the beginning of the fermentation process is crucial for the production of quality sauerkraut since it helps to outcompete spoilage microorganisms (Viander and others 2003). This reduction in pH levels can be attributed to the growth of lactic acid bacteria, which produces lactic acid and decreases the pH levels (Johanningsmeier and

others 2005). A similar pH trend was observed in study 1, with an observed rapid decrease in pH values within the first 3 days of fermentation, followed by slower decrease until reaching 3.70 or lower by fermentation day 14. However, it took 21 days to complete fermentation in study 2, possibly because this study was conducted at slightly lower temperatures (18–21^{o}C), which may have increased the fermentation time.

3.3.2. *Staphylococcus aureus* (*S. aureus*)

No significant ($p \geq 0.05$) differences were observed regarding *S. aureus* survival rates across all salt treatments (Figure 3.3). The *S. aureus* population on day 0 ranged from 5.80 to 6.13 log (CFU/mL) and increased to a maximum of 6.24−6.76 log (CFU/mL) for all salt concentrations on day 1 (Figure 3.3). This increase in *S. aureus* populations is a concern, as it may present a risk of toxin production since the levels exceeded 6 log (CFU/mL) (Bhunia and Ray 2014). However, *S. aureus* populations decreased after 1–2 days in conjunction with a decrease in pH levels for all salt treatments. It was worth noting that the higher salt treatments had *S. aureus* levels at or below 6 log (CFU/mL) on day 2, while the lower salt treatments were just above 6 log (CFU/mL). However, *S. aureus* levels decreased over fermentation time, reaching non-detectable levels (1 CFU/mL) for all salt treatments by day 21 except for the 1.0% NaCl treatment. The 1.0% NaCl treatment reached its detection limit (1 CFU/mL) on day 18 (Figure 3.3).

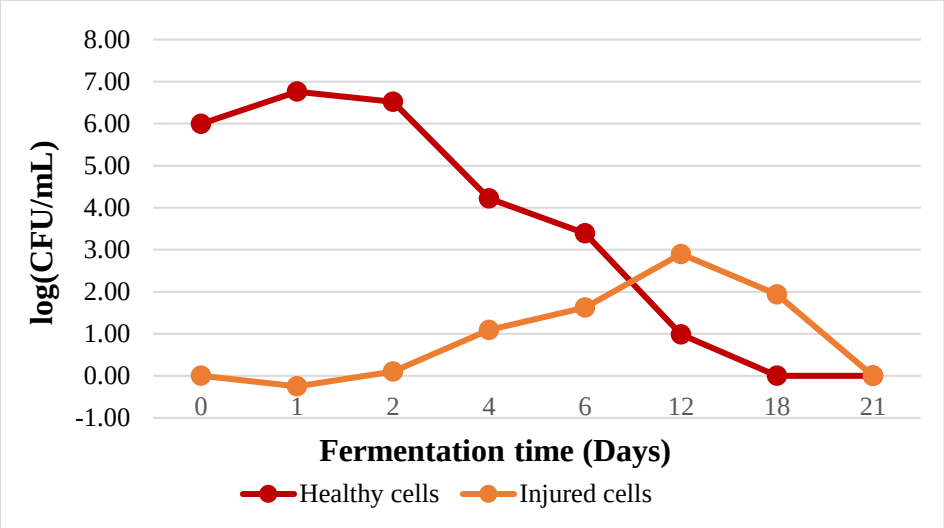
Figure 3.3. The effects of salt concentration on *S. aureus* survival during spontaneous sauerkraut fermentation overtime



Each value represents the mean log (CFU/mL) (n=3). On day 21, no significant ($p \geq 0.05$) differences were detected among salt treatments. Detection limit 1 CFU/mL

The 1% NaCl treatment had a faster acidification period on day 2 compared to the other salt treatments and may have injured the *S. aureus* cells at a faster rate. On day 2, the 1% NaCl sauerkraut samples had a mean pH value of 4.65, whereas the other salt treatments had pH values that ranged from 5.05–5.29. Therefore, the 1% NaCl treatment could have inhibited the growth of *S. aureus* at a relatively faster rate. Medveďová and Valík (2012), documented the resistance of *S. aureus* to acid tolerance and stated that the inhibition of *S. aureus* starts at pH lower than 5.0, which can decrease the overall growth of *S. aureus*. Moreover, the most effective method to inhibit the growth of *S. aureus* is to acidify the environment as soon as possible because acidic stress, and the drop of intracellular pH, alter the cell membrane structure and leads to a decrease in the activity of several enzymes, which are pH sensitive and hence affects the bacterial growth (Medveďová and Valík 2012).

Figure 3.4. Comparison between injured and healthy *S. aureus* cells for the 1.0% NaCl treatment during spontaneous sauerkraut fermentation over time



Each value represents the mean log(CFU/mL) (n=3). Detection limit 1 CFU/mL

Due to the potential recovery and possibility of becoming functionally normal in a favorable environment, injured cells are just as crucial to analyze as their healthy counterparts (Wu 2008). Therefore, it is imperative not to overlook the presence of injured microorganisms to ensure the safety of food products. In this study, the healthy *S. aureus* cell count dropped, whereas the injured cells increased, throughout the fermentation process until day 12. It can be seen in Figure 3.4, that the *S. aureus* injured cell count was 1.93 log (CFU/mL) in the 1% NaCl sample brine on day 18, while healthy cells were below the detection limit. A similar observation was recorded for the other salt treatments on day 18, which indicates that *S. aureus* was viable until day 18 since injured cells were present (data not shown). These results suggest that *S. aureus* cells have the potential to survive acidic stress that occurs during the sauerkraut fermentation process. Additionally, injured cells may show an extended lag phase, compared to healthy cells in order to synthesize proteins and nucleic acids needed to repair damage (Ray 1989, Shintani 2006). Similarly, Smith and Pablo (1978) also reported the presence of higher numbers of *S. aureus* injured cells compared to healthy cells during sausage fermentation.

Wesche and others 2009 discussed that acid stress during fermentation allows H^+ ions to cross bacterial cell membranes and creates more acidic intracellular pH levels. This low intracellular pH environment leads to an altered cell membrane structure with decreased activity of pH-sensitive enzymes (Wesche and others 2009). Likewise, undissociated organic acids can diffuse across the bacterial cell membranes and lower the internal pH on dissociation. The pH equilibrium shift occurs, which is common in fermented foods such as sauerkraut, and results in acid injury (Wesche and others 2009). This potentially explains the detection of S. *aureus* injured cells in this study. As a result of acid injury/stress during sauerkraut fermentation, some healthy *S. aureus* cells were being stressed/injured and an increase in injured cells was observed until day 12. Eventually as acidification increases due to an increase in lactic acid production, the injured cells begin to die off and hence, reach the detection limit of 1 CFU/mL for all salt concentrations by day 21.

A research study conducted by Inatsu and others (2004) also reported a decrease in *S. aureus* populations over time, in both commercial and laboratory prepared kimchi, inoculated at 5−6 log (CFU/g) and incubated at 10^{o}C for 24 days. They found *S. aureus* levels decreased rapidly from the initial inoculum level to the minimum countable level (200 CFU/g) within 12 days for both commercial and laboratory prepared kimchi samples. In contrast, *S. aureus* reached its detection limit (1 CFU/mL) on day 21 in this study. However, they used a higher detection limit (200 CFU/g) compared to this study (1 CFU/mL). Additionally, they used a different fermentation temperature (10^{o}C) to incubate the prepared kimchi samples. In this study, sauerkraut was fermented at approximately 23^{o}C, and *S. aureus* appeared to grow more rapidly at day 2 at this temperature and persisted for a longer period of time compared to the lower fermentation temperature of 10^{o}C. The differences in product formulations (sauerkraut versus

kimchi) could have also explained the *S. aureus* growth and detection differences over fermentation time.

After the sauerkraut samples reached the detection limit of <1 CFU/mL, all treatments were then enriched to ensure the absence or presence of *S. aureus*. There were noticeable variations in the enrichment results among the three trials at different salt concentrations (Table 3.1.), which suggests that *S. aureus* survived in all sauerkraut samples, even after the completion of fermentation at a pH of 3.70 or lower. This indicates the adaptability of *S. aureus* to survive under acidic conditions in the presence of salt. Hence, it is crucial that proper hygiene practices are followed during processing, especially in manually prepared foods, which may involve skin contact. However, after 1 week of refrigerated storage, *S. aureus* was reduced to below the detection limit (1CFU/mL) for all the salt treatments, which indicates that *S. aureus* may not survive exposures at refrigerated temperatures (4^{o}C), irrespective of the salt concentration. Moreover, toxin production that may have been produced during the initial stages of sauerkraut fermentation could still cause the risk of foodborne illness since sauerkraut is a ready to eat food and the *S. aureus* toxin is heat stable. Although this inoculation study used higher *S. aureus* levels than would be expected, further research is required to validate the production of *S. aureus* toxin during the initial stages of spontaneous sauerkraut fermentation and subsequent survival rates during refrigerated temperatures. Therefore, it is important to conduct further inoculation studies that closely mimic more realistic *S. aureus* levels on cabbage.

Table 3.1. Detection of bacterial pathogens after reaching fermentation endpoint (pH <3.70) and enrichment of sauerkraut samples

Replication	Salt Concentration (% NaCl)	L. monocytogenes (P – Present)	S. aureus (P − Present)	STEC (P – Present)
1	1.0	< 1 CFU/25g	< 1 CFU/25g	P
2	1.0	< 1 CFU/25g	P	< 1 CFU/25g
3	1.0	< 1 CFU/25g	< 1 CFU/25g	< 1 CFU/25g
1	1.5	< 1 CFU/25g	< 1 CFU/25g	< 1 CFU/25g
2	1.5	< 1 CFU/25g	< 1 CFU/25g	< 1 CFU/25g
3	1.5	< 1 CFU/25g	P	< 1 CFU/25g
1	2.0	< 1 CFU/25g	P	< 1 CFU/25g
2	2.0	< 1 CFU/25g	P	< 1 CFU/25g
3	2.0	< 1 CFU/25g	< 1 CFU/25g	< 1 CFU/25g
1	2.5	< 1 CFU/25g	P	P
2	2.5	< 1 CFU/25g	< 1 CFU/25g	< 1 CFU/25g
3	2.5	< 1 CFU/25g	< 1 CFU/25g	< 1 CFU/25g

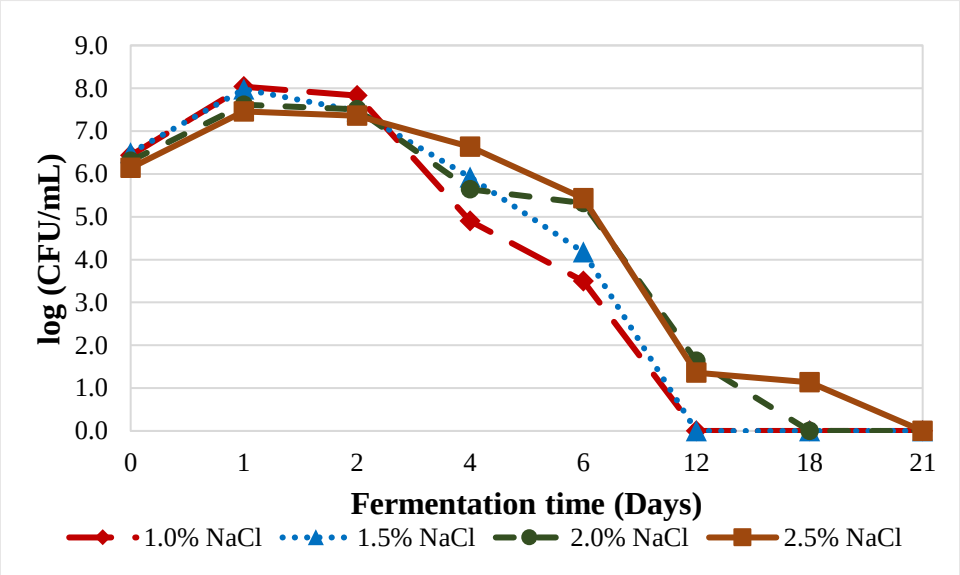
Each value represents the mean bacterial pathogen log (CFU/25g) (n=3). Detection limit 1 CFU/g

3.3.3. Shiga Toxin Producing *E. coli* (STEC)

No significant ($p \geq 0.05$) differences were detected regarding STEC survival rates among the salt treatments. The STEC populations significantly ($p \leq 0.05$) increased between day 0 (6.14−6.43) and day 1 (7.46−8.04) for all sauerkraut samples (Figure 3.5). However, the STEC counts decreased sharply after day 2 and throughout the fermentation period for all salt treatments, which corresponded with a reduction in pH levels. For the 1.0% and 1.5% NaCl – treated samples, STEC reached the detection limit (1 CFU/mL) on day 12, whereas the 2.0% and 2.5% NaCl treatments met the detection limit on day 18 and 21, respectively (Figure 3.5). These results indicate that STEC may survive at these slightly higher salt concentrations for a longer period of time, especially under acidic stress conditions. NaCl may offer a protective effect for STEC under acidic conditions, which might be mediated by the coupling of Na^+ import to H^+ export, thereby permitting STEC to maintain the internal pH and allow for a longer survival period (Champman and others 2006). Another potential reason that STEC may be protected in

acidic environments by the addition of salt is due to the water loss from the cytoplasm resulting in a reduced cytoplasmic cell volume that might effectively concentrate the cytoplasmic constituents, and thereby raise the internal pH of the cell (Champman and others 2006). Casey and Condon (2002) reported that *E. coli* can use NaCl to counteract acidification of the cytoplasm by organic acid production. These theories may explain why STEC was able to persist longer in the 2.0% and 2.5% NaCl sauerkraut treatments, when compared to the lower salt concentrations (1% and 1.5% NaCl).

Figure 3.5. The effects of salt concentration on STEC survival during spontaneous sauerkraut fermentation over time



Each value represents the mean log(CFU/mL) (n=3). On day 21, no significant ($p \geq 0.05$) differences were detected among salt treatments. Detection limit 1 CFU/mL

Similar results were reported by Inastu and others (2004) who studied *E. coli* O157:H7 survival in prepared kimchi. According to the researchers, *E. coli* O157:H7 remained at high levels throughout the incubation period of 7 days compared to the other two pathogens studied (*S. enteritidis* and *L. monocytogenes*). Another study performed by Niksic and others (2005) assessed *E. coli* O157:H7 and *L. monocytogenes* survival during sauerkraut fermentation

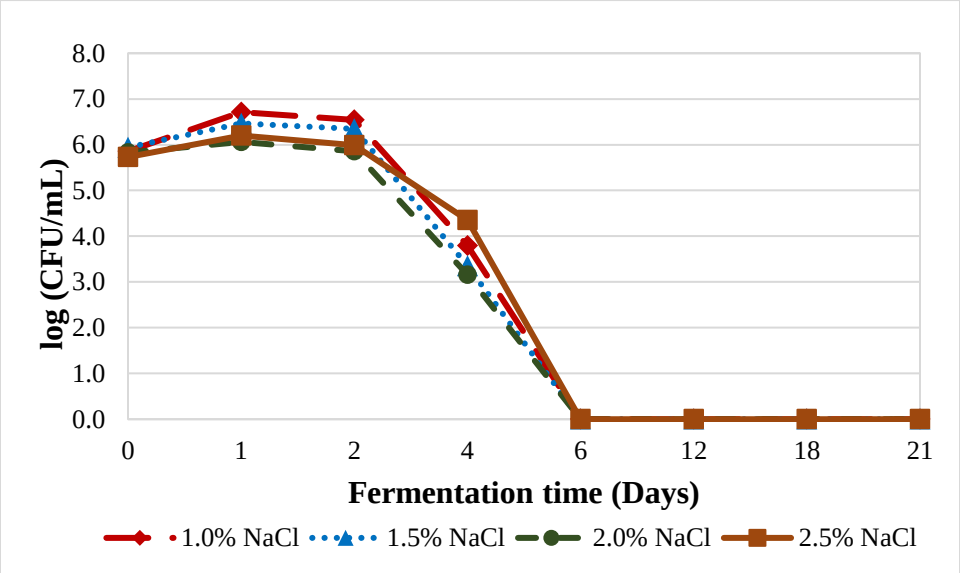
produced from both shredded and whole-head cabbage. Sauerkraut was inoculated with a five-strain cocktail of *E. coli* O157:H7 and *L. monocytogenes,* at three different salt concentrations (1.8%, 2.25% and 3.0%), and two different fermentation temperatures (18ºC and 22ºC). They found that neither salt nor fermentation temperature had a significant effect on pathogen survival among the cabbage types. The authors reported that although *E. coli* O157:H7 persisted in the brines for most of the fermentation, it was not detected at the end of fermentation (15 days for shredded cabbage). These results were in accordance to this study, as STEC remained in the 4 –6 log (CFU/mL) range until fermentation day 6, and then by fermentation day 21, all sauerkraut samples reached the detection limit (1 CFU/mL).

No injured STEC cells were detected in any sauerkraut samples (data not shown), unlike *S. aureus*, which indicates that STEC cells were less apt to be injured and were able to persist under non-selective medium. Hence, STEC cells did not appear to be sensitive to the overlay (non-selective media) method to resuscitate, and thereby able to enumerate on selective media for all salt treatments. In the presence of a selective agent, the injured cells cannot repair the cellular damage (resuscitation) and lose their ability to form a colony (Wu 2008, Palumbo 1989). After fermentation was completed, STEC cells were enriched when their populations were below the detection limit. A plating method was used to confirm the absence/presence of pathogens after the completion of fermentation (pH <3.70) (Table 3.1). For the 1.0% and 2.5% NaCl treatments, one out of the three replications tested positive for STEC (Table 3.1), which suggests STEC has the potential to survive at low pH levels in sauerkraut. The ability of STEC to tolerate high acidic environments has led to *E. coli* O157:H7 outbreaks in the past associated with the consumption of contaminated apple juice and fermented dry salami (Chung and others 2006). Thus, the potential survival of STEC in fermented foods may pose a health risk to consumers.

Moreover, STEC doses required to cause an infection in susceptible human populations are extremely low (<10 cells) (Montet and others 2008), which further amplifies the risk associated with consuming sauerkraut produced without adhering to good manufacturing and sanitation practices. However after 1 week of refrigeration, STEC reached the detection limit (1CFU/mL) for all salt concentrations, which indicates that STEC does not favor refrigerated (4^{o}C) conditions.

3.3.4. *Listeria monocytogenes* (*L. monocytogenes*)

Figure 3.6. The effects of salt concentration on *L. monocytogenes* survival during spontaneous sauerkraut fermentation over time



Each value represents the mean log (CFU/mL) (n=3). On day 21, no significant ($p \geq 0.05$) differences were detected among salt treatments. Detection limit 1 CFU/mL

Salt treatment had no significant ($p \geq 0.05$) effect on the survival of *L. monocytogenes*. Similar to the other two pathogens, *L. monocytogenes* populations increased on day 1, followed by a sharp decrease over the fermentation period. These results show that *L. monocytogenes* was the most affected by the increase in acidity over fermentation time compared to the other two pathogens. *L. monocytogenes* reached the detection limit of 1 CFU/mL for all treatments on day

6 and was non-detectable throughout the rest of the study (Figure 3.6). Moreover, the injured *L. monocytogenes* cells also reached the same detection limit on day 6, which indicates that injured cells were not present. These results indicate that *L. monocytogenes* cells do not have the ability to repair cellular damage in a non-selective medium (Bhunia and others 2014). Enrichment was completed after the fermentation endpoint was reached to ensure the absence of this pathogen. No variation was observed among the 3 trials, and the result obtained was <1 CFU/25g for all salt concentrations (Table 3.1.), which suggests that *L. monocytogenes* did not survive after day 6 of sauerkraut fermentation. *L. monocytogenes* was also not detected in any sauerkraut samples after one week of refrigerated storage.

The presence of *L. monocytogenes* in foods presents a greater challenge than the presence of other foodborne pathogens since it is widely distributed in the environment and can be resistant to diverse environmental conditions. However, *L. monocytogenes* has been shown to survive but cease to multiply at pH values below 4.30 (Bhunia and others 2014). Moreover, lactic acid production by LAB inhibits the growth of *L. monocytogenes* during fermentation. Conner and others (1986) concluded that low pH environments inhibited *L. monocytogenes* in fermented cabbage juice due to the pathogen's inability to adapt to acidic environments. *L. monocytogenes* can proliferate in low–acid foods, but cannot tolerate the environment of acidic and highly acidic foods for long periods of time. Additionally, Conner and others (1986) demonstrated that there was a continuous decrease in the viable populations of *L. monocytogenes* in cabbage juice containing salt concentrations greater than 1.0%, and was not viable after 15 days of fermentation. Similarly, this study demonstrated that *L. monocytogenes* was not able to survive during sauerkraut fermentation and was not detected after fermentation day 6 for all salt treatments, which indicates it did not tolerate pH levels approaching 3.70 or less.

In relation to vegetable fermentation, Niksic and others (2005) isolated acid-tolerant *L. monocytogenes* strains during sauerkraut fermentation using whole-head cabbage, at both 18ºC and 22ºC. They also reported that acid-tolerant strains during sauerkraut fermentation using shredded cabbage were unable to be isolated, most likely due to the increased availability of fermentable carbohydrates and rapid acid development.

It is important to note that raw vegetables used for the production of fermented vegetables could be contaminated at the farm and may serve as vehicles for pathogenic bacteria. Moreover, there is potential for foodborne pathogens, such as *L. monocytogenes* and STEC, to persist and proliferate on vegetables, including cabbage to be used to prepare sauerkraut. Therefore, it is important to consider proper hygiene practices, adequate cleaning of utensils and food contact surfaces, and proper washing of produce when producing fermented products. Good manufacturing and sanitation practices help to prevent or reduce the risk of pathogenic bacteria from contaminating fermented food products and reducing the risk of foodborne illness.

3.4. Study 2 Conclusions

Lactic acid fermentation of cabbage and other vegetables is a common method for preserving fresh vegetables. From a microbiological point of view, it has been widely accepted that the antagonistic effect of LAB, plus low pH levels, protects fermented foods from the potential outgrowth of foodborne pathogens and reduces the risk of foodborne illnesses (Montet and others 2014). However, several studies and recent outbreaks have shown that foodborne illnesses can be associated with both the raw materials, and the ability of pathogens to adapt to fermentation environments, including low pH levels and possibly low salt concentrations.

The salt concentrations (1.0%, 1.5%, 2.0% and 2.5% NaCl) used in this study had no significant effect on the survival of *L. monocytogenes*, *S. aureus* and STEC. Additionally, the

study also analyzed the population decline of these three pathogens over the fermentation period in conjunction with decreasing pH levels. Of the three pathogenic bacteria, *L. monocytogenes* was the least acid resistant and survived until fermentation day 6, which indicates that *L. monocytogenes* cannot tolerate the lower pH environments (pH <3.70) at those salt levels. In contrast, *S. aureus* was the most resistant in regards to enrichment results and reached the detection limit on day 18 for all salt concentrations. In fact, *S. aureus* was detected in all salt treatments even after reaching the fermentation endpoint (pH level $\leq$ 3.70). The STEC count reached the detection limit on fermentation day 12 for the lower salt concentrations, except 2.5% NaCl, which is usually used to prepare sauerkraut. The lower salt brine conditions may be more favorable for LAB growth which may reduce the growth of STEC. However, STEC was detected at both 1.0% and 2.5% NaCl concentrations after selective enrichment was conducted when the fermentation endpoint was reached. These results indicate a potential risk of foodborne illness, irrespective of salt level, primarily because of the low infectious dose of STEC. However, all three bacterial pathogens were not detectable after one week of refrigerated storage.

Overall, these results indicate that although fermented foods are typically considered safe, it is crucial to weigh the health benefits against the risks of foodborne illness as a result of their consumption. As per this study, there is a potential risk of STEC and *S. aureus* survival during fermentation conditions, regardless of pH levels and at low salt concentrations, at levels used in this inoculation study. This implies that the production of naturally fermented, sauerkraut should be produced following proper hygiene and sanitation practices to avoid contamination of sauerkraut with pathogenic bacteria due to their possible presence in raw materials or possible cross-contamination from the environment. The common practice of packaging sauerkraut in sanitized containers and keeping this product refrigerated after fermentation is complete is a

recommended practice, as all pathogens were not able to persist after 1 week of refrigerated storage. This study provides crucial information to guide home-fermenters and the food industry, which enables them to produce value-added sauerkraut without compromising the product safety, especially when there is considerable consumer and FDA pressure to reduce salt concentrations in the quest for high quality, healthier and safe foods.

CHAPTER 4

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The results from these two studies indicate that a safe and high-quality sauerkraut can be prepared at low salt concentrations, when produced under good manufacturing and sanitation practices. Low salt concentrations used in this study (1.0%, 1.5%, 2.0% and 2.5% NaCl) appeared to have no significant effect on spontaneous sauerkraut fermentation. The pH levels, which are one of the most important parameters to evaluate fermentation success and safety, reached a level of 3.70 or lower for all salt concentrations within 14 days of fermentation. Similarly, there was an increase in titratable acidity levels over fermentation time, and reached the desired acidity level of approximately 1.0% for most salt concentrations. The HPLC analyses showed that no significant differences were detected among the salt treatments for both organic acids and fermentable sugars (glucose, fructose and sucrose). The ratio of lactic to acetic acid was 4:1 for all salt concentrations, which implied quality sauerkraut was produced according to Pederson and Albury (1996).

The probiotic characteristics and the inhibition of unwanted microorganisms in sauerkraut is exerted by lactic acid bacteria, which forms the basis of the extended shelf-life and improved microbiological quality of lactic acid fermented products (Wiander and Ryhanene 2005). The results showed that even the reduced salt levels produced adequate lactic acid bacterial growth supporting the feasibility of sauerkraut fermentation even at low salt concentrations of 1.0% NaCl. Moreover, no fungi or coliforms were detected after sauerkraut fermentation was complete for all salt concentrations. This validates that all the sauerkraut samples appeared to have adequate quality characteristics and that reduced salt concentrations do not appear to influence the growth of spoilage microorganisms. One of the major reasons for the popularity of sauerkraut is its unique sensory attributes, which has a direct correlation with the

intensity of its salty taste (Johanningsmeier and others 2007). Therefore, consumer acceptability tests for naturally fermented, lower salt sauerkrauts are crucial to assess the overall acceptance of these products. Moreover, studying the organoleptic properties of lower salt sauerkrauts will help accelerate the potential market opportunities of these products (Albarracin and others 2011). Therefore, a sensory consumer acceptability test is recommended for future research to investigate consumer's acceptance of sauerkraut produced at salt concentrations <2.5% NaCl.

While the results from this study provide a strong foundation for the quality assessments of fermented vegetables produced at lower salt concentrations, studying the effects of reduced salt concentrations on sauerkraut safety is of even greater importance for the food industry and public health. Due to the increased consumer interest to consume lower sodium foods for health purposes (Penas and others 2010), it is imperative to study the safety of sauerkraut produced at lower salt levels. Based on the microbiological results, it can be concluded that the different salt concentrations used in this study had no significant effects on STEC, *S. aureus* and *L. monocytogenes* survival. One of the major findings of this study was the absence of *L. monocytogenes* after fermentation day 6 for all salt concentrations, which indicates that *L. monocytogenes* is not as tolerant to lower pH environments. Moreover, the United States has a zero-tolerance policy for *L. monocytogenes* in processed foods, and these results helps to confirm the safety of naturally fermented, lower salt sauerkrauts after reaching an endpoint pH of $\leq$3.70 in regards to *L. monocytogenes*. However, STEC and *S. aureus* were able to survive the fermentation conditions at all salt concentrations due to the potential adaptability of these pathogens to low-pH environments in the presence of salt. This suggests the potential risk of foodborne illness to consumers at the inoculation levels used in study 2. However, safe, naturally fermented vegetables most likely can be produced at lower salt levels, provided proper

good manufacturing and sanitation practices are followed. We recommend that future validation studies are conducted to assess STEC and *S. aureus*' viability to persist in sauerkraut at lower inoculation levels. However, our results reinforce the importance to store fermented foods under refrigerated conditions after fermentation is complete to further reduce the risk of *S. aureus* and STEC.

Another safety concern, that was not addressed in these studies, is the risk of biogenic amines present in fermented foods. Biogenic amines are a group of biologically active natural compounds, formed mainly by the microbial decarboxylation of amino acids. There are various types of biogenic amines that can be produced during the fermentation process, which can include tyramine, histamine, spermidine and putrescine. Sauerkraut may contain high levels of tyramine and histamine, which are reported to be possible causes of food intolerances since they can cause reactions in the body similar to certain types of allergic reactions (Raak and others 2014). Studying the effects of lower salt brines on biogenic amine formation in spontaneously, fermented cabbage will provide more insight and enable the food industry to determine the chemical safety of these products.

There is an increase in the demand for healthier foods due to consumer awareness and the plethora of food choices. These trends have fostered the demand for lower salt fermented food options. However, a key consideration is the amount of salt used to process fermented foods. Due to several health concerns, such as high blood pressure and other health risks associated with excessive sodium consumption, the FDA has suggested the food industry to voluntarily reduce the sodium levels in foods (FDA 2011). Therefore, there may be an increase in the demand and formulation of fermented vegetables at lower salt levels in the near future. This thesis offers timely information on the quality and safety of value-added, lower sodium

fermented vegetables, directed towards guiding home-fermenters and industry professionals to achieve their goals.

REFERENCES

Adams M. R. 1990.Topical aspects of fermented foods. *Trends Food Sci. Tech.* 1:140-44.

Adams, M. R. and L. Nicolaides. 1997. Review of the sensitivity of different foodborne pathogens to fermentation. *Food Control.* 8:227–239.

Agricultural Marketing Report Center. 2017. Cabbage. Available from: https://www.agmrc.org/commodities-products/vegetables/cabbage

Albarraci, W., I. C. Sa´nchez, R. Grau, and J. S. Barat. 2011. Salt in food processing; usage and reduction: a review. *Int. J. Food Sci. Tech.* 46:1329–1336.

Applications of Biotechnology to Fermented Foods: Report of an Ad Hoc Panel of the Board on Science and Technology for International Development. 1992. In Steinkraus, K.H. (eds), Lactic acid Fermentation, p.5. National Academies Press, Washington DC. Available from: https://www.ncbi.nlm.nih.gov/books/NBK234703/

Argyri, A. A., E. Lyra, E. Z. Panagou, and C. C. Tassou. 2013. Fate of *Escherichia coli* O157: H7, *Salmonella enteritidis* and *Listeria monocytogenes* during storage of fermented green table olives in brine. *Food Microbiol.* 36:1-6.

Axelsson, L. 2004. Lactic Acid Bacteria: Classification and Physiology, p 1-67. In Salminen, S., A.V. Wright and A. Ouwehand (3 eds), Lactic Acid Bacteria: Microbiological and Functional Aspects. Marcel Dekker, New York.

Babalola, O. 2014. Cyanide content of commercial gari from different areas of Ekiti State, Nigeria world. *J. Nutri. Health.* 2:58–60.

Barbut, S., N.Tanaka, and A. J. Maurer. 1986. Effects of varying levels of chloride salts on *Clostridium botulinum* toxin production in turkey frankfurters. *J. Food Sci*. 51:1129–1131.

Barth, M., R. Hankinson, T. Z. Hong, and F. Breidt, 2010. Microbiological Spoilage of Fruits and Vegetables. In Compendium of the Microbiological Spoilage of Foods and Beverages. 10.1007/978-1-4419-0826-1_6.

Battcock, M., and S. Azam-Ali. 1998. Fermented fruits and vegetables: A global perspective. FAO agricultural services bulletin. Food and Agriculture Organization of the United Nations. Rome. Available from http://www.fao.org/docrep/x0560e/x0560e10.htm

Battcock M., and S. Azam-Ali. 2001. Fermented fruits and vegetables: A global perspective. FAO agricultural services bulletin, Rome.

Bautista-Gallego, J., F. N. Arroyo-Lopez, M. C. Duran-Quintana, and A. Garrido-Fernandez. 2008. Individual effects of sodium, potassium, calcium, and magnesium chloride salts on *Lactobacillus pentosus* and *Saccharomyces cerevisiae* growth. *J. Food Prot.* 71:1412–1421.

Beuchat, L. R., and A. J. Scouten. 2002. Combined effects of water activity, temperature and chemical treatments on the survival of *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. *J. Appl. Microbiol.* 92:382–395.

Bautista-Gallego, J., K. Rantsiou, A. Garrido-Fern´andez, L. Cocolin, and F. N. Arroyo-L´opez. 2013. Reduction in Vegetable Fermentation: Reality or Desire? *J. Food Sci.* 78.

Besser, R. E., Susan M. L., J. Todd Weber, M. P. Doyle, T. J. Barrett, J. G. Wells, and P. M. Griffin. 1993. An Outbreak of Diarrhea and Hemolytic Uremic Syndrome from *Escherichia coli* O157:H7 in Fresh-Pressed Apple Cider. *JAMA.* 269(17):2217–2220.

Bevilacqua, A., M. Cannarsi, M. Gallo, M. Sinigaglia, and M. R. Corbo. 2010. Characterization and implications of *Enterobacter cloacae* strains isolated from Italian table olives. *J. Food Sci.* 75:53-60.

Bhunia, A., and B. Ray. 2014. Fundamental Food Microbiology. CRC Press, Boca Raton, FL.

Bibbins, D. K., G. M. Chertow, P. G. Coxson, A. Moran, J. M. Lightwood, M. J. Pletcher, and L. Goldman. 2010. Projected effect of dietary salt reductions on future cardiovascular disease. *J. Med.* 362:590–599.

Bodmer, S., C. Imark, and M. Kneubühl. 1999. Biogenic amines in foods: histamine and food processing. *Inflamm. Res.* 48:296–300.

Borgstrom, G. 1968. Principles of Food Science-Food Microbiology and Biochemistry. American Public Health Association, Macmillan, NY.

Borresen, E.C., A. J. Henderson, A. Kumar, T. L. Weir, and E. P. Ryan. 2012. Fermented foods: Patented approaches and formulations for nutritional supplementation and health promotion. *Food Nutr. Agric*. 4:134–140.

Brackett, R. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. *Posharvest. Biol. Technol.*15: 305-311.

Breidt, F., K.A. Crowley, and H.P. Fleming. 1995. Controlling cabbage fermentations with nisin and nisin-resistant *Leuconostoc mesenteroides*. *Food Microbiol.* 12:109–116.

Breidt, F., J. S. Hayes, and R. F. McFeeters. 2007. Determination of 5-Log reduction times for food pathogens in acidified cucumbers during storage at 10 and 25°C. *J. Food Prot.* 70:2638-2641.

Breidt, Jr. F., and J. M. Caldwell. 2011. Survival of *Escherichia coli* O157:H7 in cucumber fermentation brines. *J. Food Sci.* 76:198-203. *doi: 10.1111/j.1750-3841.2011.02045.*

Breidt, F., R. F. McFeeters, I. Perez-Diaz and C. H. Lee. 2013. Fermented Vegetables, p. 841-855. In Doyle M.P., and R. L. Buchanan (4 ed.), Food Microbiology: Fundamentals and Frontiers. ASM Press, Washington, D.C.

Buckenhuskes, H. J. 1997. Fermented Vegetables. ASM Press, Whasington, D.C.

Burnett, S., L. Chen, J. Beuchat, and L. R. 2000. Attachment of *Escherichia coli* O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. *Appl. Environ. Microbiol.* 66:4679–4687.
Calder, B. L. 2018. Personal communication.

Casey, P. G., and S. Condon. 2002. Sodium chloride decreases the bacteriocidal effect of acid pH on *Escherichia coli* O157:H45. *Int J. Food Microbiol.* 76:199-206.

Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider – Connecticut and New York. *Morbid. Mortal. Wkly. Rep*. 46:4-8.

Cetin, B. 2011. Production of probiotic mixed pickles (Tursu) and microbiological properties. *Afr. J. Biotech.* 10:14926-14931.

Chapman, B., N. Jensen, T. Ross, and M. Cole. 2006. Salt, Alone or in Combination with Sucrose, Can Improve the Survival of *Escherichia coli* O157 (SERL 2) in Model Acidic Sauces. *Appl. Environ. Microbiol.* 72:5165–5172.

Chilton, S. N., J. P. Burton, and G. Reid. 2015. Inclusion of fermented foods in food guides around the world. *Nutrients.* 7:390-404. doi:10.3390/nu7010390.

Cho, G. Y., L. M. Hwa, and C. Changsun. 2011. Survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during kimchi fermentation supplemented with raw pork meat. *Food Control.* 22:1253-1260.

Cho, S., J. Kim, K. Oh, J. Hu, J. Seo, S. Oh, M. Hur, Y. Choi, S. Youn, G. Chung, and Y. Choe. 2014. Outbreak of enterotoxigenic *Escherichia coli* O169 enteritis in school children associated with consumption of kimchi, Republic of Korea. *Epidemiol Infect.* 142:616-623.

Chongde, W**.**, J. Huang, and R. Zhou. 2013. Progress in engineering acid stress resistance of lactic acid bacteria**.** *Appl. Microbiol. Biotech.* 98.

Chung, H. J., W. Bang, and M. A. Drake. 2006. Stress Response of *Escherichia coli. Compr. Rev. Food Sci. Food Saf.* 5:52-61.

Ciska E., M. Karamac, and A. Kosinska. 2005. Antioxidant activity of extracts of white cabbage and sauerkraut. *J. Food Nutri. Sci.* 55:367–373.
Conner, D. E., R. E. Brackett, and L. R. Beuchat. 1986. Effect of temperature, sodium chloride, and pH on growth of Listeria monocytogenes in cabbage juice. *Appl. Environ. Microbiol.* 52:59–63.

Dahal, N. R., T. B. Karki, B. Swamylingappa, Q. Li, and G. Gu. 2005. Traditional foods and beverages of Nepal-a review. *Food Reviews Int.* 21:1–25.

Devlieghere, F., L. Vermeiren, E. Bontenbal, P. P. Lamers, and J. Debevere. 2009. Reducing salt intake from meat products by combined use of lactate and diacetate salts without affecting microbial stability. *Int. J. Food Sci. Tech*. 44:337–341.

Dickinson, B.D.,and S. Havas. 2007. Reducing the population burden of cardiovascular disease by reducing sodium intake: a report of the Council on Science and Public Health. *Arch Int. Med.* 167:1460–8.

Di Cerbo, A., B. Palmieri, M. Aponte, J.C. Morales-Medina, and T. Iannitti. 2016. Mechanisms and therapeutic effectiveness of Lactobacilli. *J. Clin. Pathol.* 69:187–203.

Dimitriadi, D., K. Stavroula, B. Dimitrios, K. M Fakiri, M. Eleni, S. Nikoletta, and K. Gyftopoulou. 2013. Health benefits of Probiotics: A Review. *ISRN Nutr.* 7.

Douglas, L. C., and M. E. Sanders. 2008. Probiotics and prebiotics in dietetic practice. *J. Am. Diet. Assoc.* 108:510–521.

Doyle, M. P., and R. L. Buchanan. 2007. Fermented Vegetables, p. 783–794. In Doyle, M.P., and L. R Beuchat (ed.), Food Microbiology: Fundamentals and Frontiers. ASM Press, Washington, D.C.

Driscoll, O. B., C. G. Gahan, and C. Hill. 1996. Adaptive acid tolerance response in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. *Appl. Environ. Microbiol.* 62:1693-1698.

Dsa, E. M., and M. A. Harrison. 2005. Effect of pH, NaCl content, and temperature on growth and survival of *Arcobacter* spp. *J. Food Prot*. 68:18–25.

Ellulu, M. S., A. Rahmat, I. Patima, A. H. Khaza, and Y. Abed. 2015. Effect of vitamin C on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: a randomized controlled trial. *Drug Des. Dev. and Ther.* 9:3405–3412.

Erten, H. 2000. Fermentation of Glucose and Fructose by *Leuconostoc mesenteroides*. *Turk J. Agric.* 24:527–532.

Etchells, J. L., T. A. Bell, T.A., R. J. Monroe, P. M. Masley, and A. L. Demain. 1958. Populations and softening enzyme activity of filamentous fungi on flowers, ovaries, and fruit of pickling cucumbers. *Appl. Microbiol*. 6:427-440.

Ferreira, I. M. P. L. V. O., R. Eça, O. Pinho, P. Tavares, A. Pereira, and A. C. Roque. 2007. Development and validation of an HPLC/UV method for quantification of bioactive peptides in fermented milks. *J. Liq. Chromatogr. Relat. Tech.* 30:2139–2147

Fleming, H. P., R. F. McFeeters, and M. A. Daeschel. 1985. The *lactobacilli*, *pediococci*, and *leuconostocs*: vegetable products, p. 97-114. In Gilliland, S.E. (ed.), Bacterial starter cultures for foods. CRC Press, Boca Raton, FL.

Fleming, H. P., E. G. Humpharies, and R. F. McFeeters. 1987. A fermentor for study of the sauerkraut fermentation. *Biotech. Bioeng.* 30 (In Press).

Fleming, H.P., R. F. McFeeters, and E. G. Humphries. 1988. A fermentor for study of sauerkraut fermentation. *Biotech. Bioeng.* 31:189-97.

Fleming, H. P. 1991. Mixed cultures in vegetable fermentations, p. 69-103. In Zeikus, J. G., and E. A. Johnson (ed.), Mixed cultures in biotechnology. McGraw-Hill Inc., New York.

Food and Drug Administration. 2007. Salt and Sodium; Petition to Revise the Regulatory Status of Salt and Establish Food Labeling. U. S. Department of Health and Human Services. Maryland. Available from: https://www.federalregister.gov/documents/2007/10/23/07-5216/salt-and-sodium-petition-to-revise-the-regulatory-status-of-salt-and-establish-food-labeling

Food and Drug Administration. 2010. Laboratory Methods - BAM Appendix 2: Most Probable Number from Serial Dilutions. Center for Food Safety and Applied Nutrition. Available from: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm.

Food and Drug Administration. 2011. Federal Register Notice: Approaches to Reducing Sodium Consumption. Public Meeting, October 12, 201. Available at: https://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm253316htm#summary.

Franco, W., S. Johanningsmeier, J. J. Demo, E. Wilson, and L. Moeller. 2012. Cucumber Fermentation. Lactic Acid Fermentation of Fruits and Vegetables. Available at: https://fbns.ncsu.edu/USDAARS/Acrobatpubs/P401-425/p406.pdf.

Frassetto, L. A., R. C. Morris, Jr. Sellmeyer, D. E., and A. Sebastian. 2008. Adverse effects of sodium chloride on bone in the aging human population resulting from habitual consumption of typical american diets. *J. Nutr.* 138:419–422.

Frias, J., C. M. Villaluenga, and E. Peñas. 2016. Fermented Foods in Health and Disease Prevention. Academic Press-Technology & Engineering. London.

Foroutan, R. 2012. The History and Health Benefits of Fermented Food. Food & Nutrition Magazine. Academy of Nutrition and Dietetics. Available at: https://foodandnutrition.org/winter-2012/history-health-benefits-fermented-food./

Gardner, N. J., T. Savard, P. Obermeier, G. Caldwell, C. P. Champagne. 2001. Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *Int. J. Food Microbiol*. 64:261–275.

Gebbers, J. O. 2007. Atherosclerosis, cholesterol, nutrition, and statins—a critical review. *German Med. Sci*. 5:1–11.

Gill, A. 2017. The Importance of Bacterial Culture to Food Microbiology in the Age of Genomics. *Front. Microbiol.* 8:77.

Giorgio, G. 2004. Studying the dynamics of microbial populations during food fermentation. *FEMS Microbiol. Reviews*. 28:251–260.

Giorgio, M., and B. Sivasankari. 2014. Isolation of potential probiotic *Lactobacillus oris* HMI68 from mother's milk with cholesterol-reducing property. *J. Biosci. Bioeng.* 118:153-9.

Gourama, H. and L. B. Bullerman. 1988. Mycotoxin production by molds isolated from “Greek-style” black olives. *Int. J. of Food Microbiol.* 6:81–90.

Guarner, F., and J. R. Malagelada. 2003. Gut flora in health and disease. Lancet. 361: 512-519.

Halász, Á., A. Baráth, and W. H. Holzapfel. 1999. The influence of starter culture selection on sauerkraut fermentation. *European Food Res. Technol*. 208:434–438.

Hayes, J .D., M. O. Kelleher, and I. M. Eggleston. 2008. The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *European J. Nutr.* 47:73–88.

Hayek, S. A. and S. A. Ibrahim. 2013.Current Limitations and Challenges with Lactic Acid Bacteria: A Review. *Food and Nutri. Sci.* 4:73-87.

Henney, J. E., C. L. Taylor, and C. S. Boon. 2010. Strategies to Reduce Sodium Intake in the United States. National Academies Press (US). Washington D. C.

Herman, K. M., A. J. Hall, and L. H. Gould. 2015 Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. *Epidemiol. and Infect.* 143:3011-3021. doi:10.1017/S0950268815000047.

He, F. J., and G. A. MacGregor. 2009. A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. *J. Human Hyperten.* 23:363–84.

Hong, S. I, and W. S. Park. 2000. Application of Time-temperature Indicators for Monitoring Kimchi Fermentation. *Food. Sci. Biotech*. 9:258-262.

Holzapfel. 2003. Sauerkraut, p. 343-360. In: Farnworth E.R. (ed.), Handbook of Fermented Functional Foods. CRC Press, Boca Raton, FL.

Holzapfel, W., U. Schillinger, and H. Buckenhüskes. 2008. Sauerkraut, p. 395-412. In Farnworth E. R. (ed.), Handbook of fermented functional foods, 2nd ed. CRC Press, Boca Raton, FL.

Hunaefi, D., D. N. Akumo, and I. Smetanska. 2013. Effects of fermentation on antioxidant properties of red cabbage. *Food Biotech.* 27:66-85.

Inatsu, Y., B. Latiful, S. Kawasaki, and K. Isshiki. 2004. Survival of Escherichia coli O157:H7, Salmonella Enteritidis, Staphylococcus aureus, and Listeria monocytogenes in Kimchi. *J. Food Prot.* 67:1497-500. doi: 10.4315/0362-028X-67.7.1497.

Jagannath, A., P. S. Raju, and A. S. Bawa. 2012. A two-step controlled lactic fermentation of cabbage for improved chemical and microbiological qualities. *Food Control Qual.* 35:13-20.

Jiajia, W., K. Ma, Y. F. Zhang, and F. S. Chen. 2012. Biodiversity of yeasts, lactic acid bacteria and acetic acid bacteria in the fermentation of "Shanxi aged vinegar", a traditional Chinese vinegar. *Food Microbiol.* 30:289-97.

Johanningsmeier, S. D., H.P. Fleming, and F. Breidt. 2004. Malolactic Activity of Lactic Acid Bacteria during Sauerkraut Fermentation. *J. Food Sci.* 69.

Johanningsmeier, S. D., H.P. Fleming, R. Thompson, and R. F. McFeeters. 2005. Chemical and sensory properties of sauerkraut produced with *Leuconostoc mesenteroides* starter cultures of differing malolactic phenotypes. *J. Food Sci.* 70:343-S349.

Johanningsmeier, S., R. F. McFeeters, H. P. Fleming, and R. L. Thompson. 2007. Effects of *Leuconostoc mesenteroides* starter culture on fermentation of cabbage with reduced salt concentrations. *J. Food Sci*. 72:166–172.

Ju, Y. H., K. E. Carlson, J. Sun, D. Pathak, B. S. Katzenellenbogen, J. A. Katzenellenbogen, and W. G. Helferich. 2000. Estrogenic effects of extracts from cabbage, fermented cabbage, and acidified brussels sprouts on growth and gene expression of estrogen-dependent human breast cancer (MCF-7) cells. *J. Agric. Food Chem*. 48:4628–4634

Karovičová, J., M. Drdák, G. Greif, and E. Hybenová. 1999. The choice of strains of *Lactobacillus* species for the lactic acid fermentation of vegetable juices. *European Food Res. and Tech*. 210:53–56.

Karagozlu, N., and B. Erg€onül. 2011. Survival of *Salmonella typhimurium* NRRL E4463 in Sauerkraut during Fermentation. *Int. J. Food Eng.* 7:1556-3758.

Katz, S.E. 2012. The Art of Fermentation: An In-depth Exploration of Essential Concepts and Processes from Around the World. Chelsea Green Publishing, Vermont.

Kaur, C., and H. C. Kapoor. 2001. Antioxidants in fruits and vegetables—the millennium's health. *Int. J. Food Sci. and Tech.* 36:703–725.

Kechagia, M., D. Basoulis, S. Konstantopoulou, D. Dimitriadi, K. Gyftopoulou, N. Skarmoutsou, N., and E. M. Fakiri, 2013. Health Benefits of Probiotics: A Review. *ISRN Nutr*. 481-651.

Kinosita, R., T. Ishiko, S. Sugiyama, T. Seto, S. Igarasi, and I.E. Goetz. 1968. Mycotoxins in fermented food. *Cancer Res.* 28:2296–2311.

Kröckel, L. 2013. The Role of Lactic Acid Bacteria in Safety and Flavour Development of Meat and Meat Products, p. 130-146. In Lactic Acid Bacteria – R & D for Food, Health and Livestock Purposes. InTech. Germany.

Krisket, S., D. Beslo, H. Pavlovic and A. Krisket. 2004. Effect of starter cultures *L. mesenteroides* and *L. lactis* ssp. lactis on sauerkraut fermentation and quality. *Czech J. Food Sci.* 22:125–132.

Lambri, M., M. D. Fumi, A. Roda, and D. de Faveri. 2013. Improved processing methods to reduce the total cyanide content of cassava roots from Burundi. *Afr. J. Biotech.* 12:2685–2691.

Lonergan, D., and R. C. Lindsay. 1979. Evaluation of Sauerkraut-Like Products From Direct-Acidification of Cabbage. *J. Food Prot.* 42:38-42.

Lucier, G., and B..H. Lin. 2002. Cabbage Heads Higher. Economic Research Service, U.S. Department of Agriculture. Washington, D.C.

Man, C. M. B. 2008. Technological functions of salt in food products, p. 157-173. In Kilcast D., and F. Angus (eds.), Reducing Salt in Foods. Woodhead, Cambridge, UK.

Marco, M. L, D. Heeney, S. Binda, C. J. Cifelli, P. D. Cotter, B. Foligné, M. Gänzle, R. Kort, G. Pasin, A. Pihlanto, E. J. Smid, and R. Hutkins. 2017. Health benefits of fermented foods. *Current Opinion Biotech.* 44:94–102.

Martinez-Villaluenga, C, E. Peñas E, J. Frias, E. Ciska, J. Honke, M. K. Piskula, H. Kozlowska, C. Vidal-Valverde. 2009. Influence of fermentation conditions on glucosinolates, ascorbigen, and ascorbic acid content in white cabbage (Brassica oleracea var. capitata cv. Taler) cultivated in different seasons. *J Food Sci.*74(1):62–67.

Mattes, R. D., and D. Donnelly. 1991. Relative contributions of dietary sodium sources. *J. Am. Coll. Nutr.*10:383–393.

Medina-Pradas, E., and F. N. Arroyo-Lopez. 2015. Presence of toxic microbial metabolites in table olives. *Front. Microbiol.* 873:1-6.

Medina Pradas E., I. M. Perez Diaz, A. Garrido-Fernandez, and F. N. Arroyo-Lopez. 2016. Review of vegetable fermentations with particular emphasis on processing modifications, microbial ecology, and spoilage, p. 211-236. In Bevilacqua A., M. R. Corbo, and M. Sinigaglia (eds), The Microbiological Quality of Food. Cambridge, MA.

Medveďová, A., and L. Valík . 2012. *Staphylococcus aureus*: Characterisation and Quantitative Growth Description in Milk and Artisanal Raw Milk Cheese Production, p. 71-102. In Structure and Function of Food Engineering. InTech. New York.

Minelli, E. B. and A. Benini. 2008. Relationship between number of bacteria and their probiotic effects. *Microb. Ecol. Health Dis.* 20:180-183.

Montet, D., G. Loiseau, and N. Z. Rozis. 2006. Microbial technology of fermented vegetables, p. 309-343. In Ray R. C., and O. P. Ward (eds), Microbial Biotechnology Horticulture Enfield, NH.

Montet, M. P., E. Jamet, S. Ganet, M. Dizin, S. Miszczycha, L. Dunière, D. Thevenot, and C. Vernozy-Rozand. 2008. Growth and Survival of Acid-Resistant and Non-Acid-Resistant Shiga-Toxin-Producing *Escherichia coli* Strains during the Manufacture and Ripening of Camembert Cheese. *Int. J. Microbiol.* 2009:1-10.

Montet, D., R. C. Ray, and N. Z. Rozis. 2014. Lactic Acid Fermentation of Vegetables and Fruits, p. 109-135. In Microorganisms and Fermentation of Traditional Foods.

Nelson, N. J., 2006. Migrant studies aid the search for factors linked to breast cancer risk. *J. Natl. Cancer. Inst.* 98:436–438.

Neilsen, S. S. 1998. pH and Titratable acidity, p. 101-116. In Sadler G. D., and Murphy P. A. (2eds), Food Analysis. Aspen Publishers, Inc. Gaithersburg, MD

Nguyen, C., and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* 34:371–401.

Niksic, M., S. E. Niebuhr, J. S. Dickson, A. F. Mendonca, J. J. Koziczkowski, and J. L. E Ellingson. 2005. Survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7 during Sauerkraut Fermentation. *J. Food Prot.* 68:1367-1374.

Nout, M. J. R. 1994. Fermented foods and food safety. *Food Res. Int.* 27:291–298.

Oberman, H. 1985. Fermented milks, p. 167-195. In Wood B. J. B. (eds.), Microbiology of Fermented Foods. Elsevier Applied Science, London.

O'Hara, A. M., and F. Shanahan. 2006. The gut flora as a forgotten organ. *EMBO Rep.* 7:688–93.

Onilude, A. A., O. E. Fagade, M. M. Bello, and I. F. Fadahunsi. 2005. Inhibition of aflatoxin-producing aspergilli by lactic acid bacteria isolates from indigenously fermented cereal gruels. *Afr. J. Biotech.* 4:1404–1408.

Orgeron, R, A. Corbin, and B. Scott. 2016. Sauerkraut: A Probiotic Superfood. *Funct. Foods Health and Disease*. 6:536-543

Palumbo, S. A. 1989. Injury in emerging foodborne pathogens and their detection, p. 115-132. In: Ray, B. (ed.), Injured Index and Pathogenic Bacteria: Occurrence and Detection in Food, Water and Feeds. CRC Press, Inc., Boca Raton, FL.

Paramithiotis, S., A. I. Doulgeraki, I. Tsilikidis, G. J. E. Nychas, and E. H. Drosinos. 2012. Fate of *Listeria monocytogenes* and *Salmonella Typhimurium* during spontaneous cauliflower fermentation. *Food Control.* 27:178–183.

Park, K. J. Jeong, and Y. Lee. 2014. Health Benefits of Kimchi (Korean Fermented Vegetables) as a Probiotic *Food J. Med. Food.* 17:6-20.
Pederson, C. S., M. N. Albury, and R. S. Breed. 1954. *Pediococcus cerevisiae*, the beer sarcina. Wallerstein Labs. Communs.

Pederson, C. S., and M. N. Albury. 1969. The Sauerkraut Fermentation. Agricultural Gardner Station Bulletin 824, New York.

Peñas, E., J. Frias, R. Gomez, and C. V. Valverde. 2010. High hydrostatic pressure can improve the microbial quality of sauerkraut during storage. *Food Control.* 21:524–528.

Perez, A., J. Harwood, and A. Somwaru. 1995. Cabbage: An economic assessment of the feasibility of providing multiple-peril crop insurance. U.S. Department of Agriculture, Office of Risk Management. Available at: http://www.rma.usda.gov/pilots/ feasible/PDF/cabbage.pdf.

Pe´rez-Dı´az, I.M., F. Breidt, R. W. Buescher, F. N. Arroyo-Lo´ pez, R. Jime´nez-Dı´az, A. Garrido Ferna´ndez, J. Bautista Gallego, S. S. Yoon, and S. D. Johanningsmeier. 2013. Fermented and Acidified Vegetables. APHA Press. Available at: https://fbns.ncsu.edu/USDAARS/Acrobatpubs/P376-400/p390.pdf

Perez-Diaz, I. M., J. S.Hayes, E. Medinab, A. M.Webber, N. Butz, A. N. Dickey, Z. Luf and M. A. A. Peril. 2018. Assessment of the non-lactic acid bacteria microbiota in fresh cucumbers and commercially fermented cucumber pickles brined with 6% NaCl. *Food Microbio.* 77:10-20

Pereira, A. P., J. A. Pereira, A. Bento, and M. L. Estevinho. 2008. Microbiological characterization of table olives commercialized in Portugal in respect to safety aspects. *Food Chem. Toxicol.* 46:2845-2902.

Persson, T., B.O. Popescu, and A. Cedazo-Minguez. 2014. Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? *Oxid. Med. Cell. Longev.* doi: 10.1155/2014/427318.

Pisoschi, A.M., and Pop A., 2015. The role of antioxidants in the chemistry of oxidative stress: a review. *Eur. J. Med. Chem.* 97:55–74.

Plengvidhya, V., F. Breidt, and H. P. Fleming. 2004. Use of RAPD-PCR as a method to follow the progress of starter cultures in sauerkraut fermentations. *Int. J. Food Microbiol.* 93**:**287-296.

Plengvidhya, V., F. Breidt, Z. Lu, and H. P. Fleming. 2007. DNA fingerprinting of lactic acid bacteria in sauerkraut fermentations. *Appl. Environ. Microbiol.* 73(23):7697-702.

Podsędek, A. 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: a review. *LWT—Food Sci. Tech.* 40:1–11.

Prado, F. C., J. L. Parada, A. Pande**y**, and C. R. Soccol. 2008. Trends in non-dairy probiotic beverage. *Food Res. Int.* 41:111–123.

Prajapati, J. B., and B. M. Nair. 2008. The history of fermented foods, p. 1-22. In Farnworth E.R. (ed.), Handbook of Fermented Functional Foods. CRC Press, Taylor and Francis Group, LLC, Boca Raton, FL.

Quigley, E. M.M. 2013. Gut Bacteria in Health and Disease. *J. Gastroenterol. Hepatol.* 9(9): 560-569.

Raak, C., T. Ostermann, K. Boehm, and F. Molsberger. 2014. Regular Consumption of Sauerkraut and Its Effect on Human Health: A Bibliometric Analysis. *Glob. Adv. Health and Med.* 3:12-18.

Ray, B. 1989. Introduction, p.1-8. In Ray B. (ed.), Injured Index and Pathogenic Bacteria: Occurrence and Detection in Food, Water and Feeds. CRC Press, Inc., Boca Raton, FL.
Reddy, K. A., E. H. Marth. 1991. Reducing the sodium content of foods: A review. *J. Food Prot.* 54:138–150.

Roberts, J. S., and D. R. Kidd. 2005. Lactic acid fermentation of onions. *Food Sci. Tech.* 38:185–190.

Rosa, E., M. David, and M. Gomes. 2001. Glucose, fructose and sucrose content in broccoli, white cabbage and Portuguese cabbage grown in early and late seasons. *J. Sci. Food Agric.* 81:1145 - 1149.

Samish, Z., R. E. Tulczynska and M. Bick. 1963. The microflora within the tissue of fruits and vegetables. *J. Food Sci.* 28:259.

Sandra, C., W. F. Uva, and J. Roberts. 2006. Market Opportunities for New Sauerkraut Products. Department of Applied Economics and Management Cornell University. Ithaca, New York. Available from: https://www.researchgate.net/publication/45131638_Market_Opportunities_for_New_Sauerkraut Products/download

Sarowska, J., I. Choroszy-Król, B. Iregulska-Ilow, M. Frej-Mądrzak, and A. Jama-Kmiecik. The therapeutic effect of probiotic bacteria on gastrointestinal diseases. 2013. *Adv. Clin. Exp. Med.* 22:759-66.

Seow, A., J. M. Yuan, C. L. Sun, V. D. Berg, D., H. P. Lee, and M. C. Yu. 2002. Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese. *Health Study Carcinogenesis.* 23:2055–2061.

Sekirov, I., S. L. Russell, L.C. Antunes, and B. B. Finlay. 2010. Gut microbiota in health and disease. *Physiol. Rev.* 90(3):859-904.

Shintani, H. 2006. Importance of considering injured microorganisms in sterilization validation. *Biocontrol. Sci.* 11:91–106.

Smith, J. L., and S. A. Pablo. 1978. Injury to Staphylococcus aureus During Sausage Fermentation. *Appl. Environ. Food Microbiol.* 36:857-860.

Soomro, A.H., T. Masud and K. Anwaar. 2002. Role of Lactic Acid Bacteria (LAB) in Food Preservation and Human Health – A Review. *Pakistan J. Nutr.* 1:20-24.

Souci, S. W., W. Fatchmann, and H. Kraut (eds). 2000. Food Composition and Nutritional Tables, 6th Ed. MedPharm Scientific Publisher.

Stamer, J. R., B. O. Stoyla, and B. A. Dunkel. 1971. Growth rates and fermentation patterns of lactic acid bacteria associated with the sauerkraut fermentation. *J. Milk Food Tech.* 34:521–525.

Steinkraus, K. H. 1994. Nutritional significance of fermented foods. *Food Res. Int.* 27:259-267.
Steinkraus, K. H. 1997. Classification of fermented foods: Worldwide review of household fermentation techniques. *Food Control.* 8:311–7.

Steinkraus, K. H. 2002. Fermentations in world food processing. *Compr. Rev. Food. Sci.*1: 23–32.

Sugihara, T. F. 1985. Microbiology of Breadmaking. In Wood B.J.B. (eds.), Microbiology of Fermented Foods. Elsevier Applied Science Publishers, UK.

Takeuchi, K., and J. F. Frank. 2000. Penetration of E.coli O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *J. Food. Prot.* 63:434–440.

Tamang, J. P. 2015. Naturally fermented ethnic soybean foods of India. *J. Ethnic Foods*. 2:8–17.

Thakur, P. K., and J. Kabir. 2015. Effect of salt concentration on the quality of sauerkraut. 11: 46-48.

Thapa, N., and J. P. Tamang. 2015. Functionality and therapeutic values of fermented foods, p. 111-168. In Tamang J. P. (eds), Health Benefits of Fermented Foods. CRC Press, New York.

Trail, A. C., H. P. Fleming, C. T. Young, and R. F. McFeeters. 1996. Chemical and sensory characterization of commercial sauerkraut. *J. Food Qual*. 19:15–30.

Tse, G., and G. D. Eslick. 2014. Cruciferous vegetables and risk of colorectal neoplasms: a systematic review and meta-analysis. *Nutr. Cancer.* 66:128–139.

USDA. 2003. Vegetable and Melons Outlook 2003 Yearbook. Economic Research Service. Washington, D.C. Available at: http://usda.mannlib.cornell.edu/usda/ers/VGS/2000s/2004/VGS-08-19-2004.pdf.

USDA. 2005. Generally Recognized As Safe Notice; *Lactobacillus Acidophilus*. Available at: http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/ documents/document/ucm266351.pdf. Accessed 3 September 2015.

USDA. 2010. Dietary guidelines for Americans. : U.S. Government Printing Office. Washington, D.C. Available at: https://health.gov/dietaryguidelines/2015/resources/2015-2020_Dietary_Guidelines.pdf.

USDA. 2012. National Nutrient Database for Standard Reference. Available at: tps://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/usda-national-nutrient-database-for-standard-reference-dataset-for-what-we-eat-in-america-nhanes-survey-sr.

Vatansever, S., V. Anuradha, G. R. Julie and A.H. Clifford. 2016. The Effect of Fermentation on the Physicochemical Characteristics of Dry-Salted Vegetables. *J. Food Res.* 6 (5).

Verkerk, R., M. Schreiner, A. Krumbein, E. Ciska, B. Holst, I. Rowland, R. Hansen, M. Gerhäuser, C. Mithen, and M. Dekker. 2009. Glucosinolates in Brassica vegetables: the influence of the food supply chain on intake, bioavailability and human health. *Mol. Nutr. Food Res.* 53:219–265.

Viander, B., M. Mäki and A. Palva. 2003. Impact of low salt concentration, salt quality on natural large-scale sauerkraut fermentation. *Food Microbiol*. 20:391–395.

Wacher, C., G. Díaz-Ruiz, and J. P. Tamang. 2010. Fermented vegetable products, p. 151-190. In Tamang, J. P., K. Kailasapathi (eds.), Fermented Foods and Beverages of the World. CRC Press, Boca Raton, FL.

Wagner, E. Anika and G. Rimbach. 2009. Ascorbigen: chemistry, occurrence, and biologic properties. *Clin. Dermatol.* 27:217-224.

Wang, H. L., and C. W. Hesseltine. 1970. Sufu and Lao-chao. *J. Agric. Food Chem.* 18:572–575.

Wesche, A. M, J. B. Gurtler, B. P. Marks, and E. T. Ryser. 2009. Stress, Sublethal Injury, Resuscitation, and Virulence of Bacterial Foodborne Pathogens. *J. Food Prot.* 72:1121–1138

Wesche, A. M., and E. T. Ryser (ed.). 2014. The Cell injury and Method of Analysis. APHA Press. Washington, D. C.

Westlake, A. 2012. *E. coli* contaminated pickled cabbage kills 7, injures over 100 Japan Daily. Available at: http://www.foodsafetynews.com/2012/08/e-coli-in-pickled-cabbage-kills-7-in-japan/#.W1E6ydJKhPY.

White, D. 2007. The Physiology and Biochemistry of Prokaryotes. Oxford University Press, Inc. New York, NY.

Wiander, B., and E. L. Ryhänen. 2005. Laboratory and large-scale fermentation of white cabbage into sauerkraut and sauerkraut juice by using starters in combination with mineral salt with a low NaCl content. *Eur. Food Res. Tech.* 220:191-195

Wiander B., and H. J. T. Korhonen. 2015. Preliminary studies on using LAB strains isolated from spontaneous sauerkraut fermentation in combination with mineral salts, herbs and species in sauerkraut and sauerkraut juice fermentations. *Agric. Food Sci.* 20:176-182.

Wolkers-Rooijackers, J.C.M., S. M. Thomas, and M. J.R. Nout. 2013. Effects of sodium reduction scenarios on fermentation and quality of sauerkraut. *LWT—Food Sci. Tech*. 54: 383–388.

World Health Organization (WHO). 2007. Reducing salt intake in populations. Available from: http://www.who.int/dietphysicalactivity/Salt_Report_VC_april07.pdf

Wouters, D., S. Grosu-Tudor, M. Zamfir, and L. De Vuyst. 2013. Applicability of *Lactobacillus plantarum* IMDO 788 as a starter culture to control vegetable fermentations. *J Sci Food Agric.* 93: 3352- 3361.

Wu, V. C. H. 2008. A review of microbial injury and recovery methods in food. *Food Microbiol.* 25: 735-744.

Xiong, T., Li. Xiao, Q. Guan, F. Peng and M. Xie. 2014. Starter culture fermentation of Chinese sauerkraut: Growth, acidification and metabolic analyses. *Food Control*. 41:122-127.

Xiong, T., J. Li, F. Liang, Y. Wang, and Q. Guan. 2016. Effects of salt concentration on Chinese sauerkraut fermentation. *LWT- Food Sci. Tech.* 69:169-174.

Yamano, T., H. Iino, M. Takada, S. Blum, F. Rochat and Y. Fukushima. 2006. Improvement of the human intestinal flora by ingestion of the probiotic strain *Lactobacillus johnsonii*. *La1.Br. J. Nutr*. 95:303-312.

Yumani, M. I., F. G. A. Hammouth, M. A. Humeid, and R. K. Robinson. 1999. Production of fermented cucumbers and turnips with reduced levels of sodium chloride. *Trop. Sci*. 39:233–237.

Zhang, Q., G. Chen, W. Shen, Y. Wang, W. Zhang, and Y. Chi. 2016. Microbial safety and sensory quality of low-salt Chinese paocai. *Food Control.* 59:575-580.

Zhao, B., A. Seow, E. J. D. Lee, W. T. Poh, M. Teh, P. Eng, Y. T. Wang, W. C. Tan, M. C. Yu, and H. P. Lee. 2001. Dietary isothiocyanates, glutathione S-transferase -M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. *Cancer Epidemiol. Biomarkers. Prev.* 10:1063–1067.

BIOGRAPHY OF THE AUTHOR

Surbhi Khanna was born in Delhi, India on November 22, 1993. She was raised in Delhi, India and graduated from SRM University, Chennai with a Bachelor's degree in Biotechnology in 2016. She moved to Maine, USA to pursue her higher education in 2016 and entered the Food Science and Human Nutrition graduate program at The University of Maine School of Food and Agriculture. After receiving her Master's degree, Surbhi will be in working in food industry to grow further as a food professional. Surbhi is a candidate for the Masters of Science degree in Food Science and Human Nutrition from University of Maine in December 2018.