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Cheng, Yiqing. *Effects of Xanthan Gum Addition on the Survival of Salmonella sp. in Refrigerated Pie Dough*

**Abstract**

The purpose of this study was to determine the effect of xanthan gum addition (1% and 2% by weight of dry ingredient) on the survival of *Salmonella typhimurium* in sweet short pie dough systems refrigerated at 4 °C for 15 days. The study was conducted in two phases: pie dough formulation and a challenge study, during which pie dough samples were intentionally inoculated to simulate a post-processing *Salmonella* contamination in the product. Results indicated that neither of 1% xanthan gum and 2% xanthan gum addition exhibited any significant effects on the survival of *Salmonella* in pie dough refrigerated at 4 °C for 15 days.
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Table of Contents

Abstract ........................................................................................................................................... 2

List of Tables .................................................................................................................................. 6

List of Figures .................................................................................................................................. 8

Chapter I: Introduction .................................................................................................................... 9

  Statement of the Problem ............................................................................................................. 13

  Purpose of the Study .................................................................................................................... 13

  Assumptions of the Study ............................................................................................................. 14

  Definition of Terms ....................................................................................................................... 14

  Limitations of the Study .............................................................................................................. 15

Chapter II: Literature Review ....................................................................................................... 16

  Pastry ........................................................................................................................................... 16

  Pie .............................................................................................................................................. 19

  Outbreaks of Foodborne Pathogens in Dough ......................................................................... 37

  Xanthan Gum ............................................................................................................................. 53

Chapter III: Methodology ............................................................................................................. 61

  Pie Dough Preparation ............................................................................................................... 61

  Microbiology Study Preparation ............................................................................................... 62

  Salmonella Inoculum Preparation ............................................................................................. 63

  Sample Inoculation ..................................................................................................................... 64
21-Day Incubation at Refrigeration Temperature ............................................................. 64
Water Activity Measurement ............................................................................................ 64
Microbiological Identification .......................................................................................... 65
Statistical Analysis ............................................................................................................ 65

Chapter IV: Results ....................................................................................................................... 66

21-Day Incubation ............................................................................................................ 66
15-Day Incubation ............................................................................................................ 67
Water Activity ................................................................................................................... 68
Microbiological Identification .......................................................................................... 69

Chapter V: Discussion .................................................................................................................. 71
Discussion ........................................................................................................................... 71
Conclusions .......................................................................................................................... 74
Recommendations .................................................................................................................. 74

References ............................................................................................................................. 75
Appendix A: Pie Dough Formulation ..................................................................................... 85
List of Tables

Table 1: Protein Content of Wheat Flours (Percentages Are Approximate, as Every Brand of Flour Is Unique) ........................................................................................................................................................................23

Table 2: Amino Acid Composition of Wheat Flour Components (Number of Residues per 100 kg of Flour) ........................................................................................................................................................................27

Table 3: Estimated Annual Number of Domestically Acquired, Foodborne Illnesses, Hospitalizations, and Deaths Due To 31 Pathogens and Unspecified Agents Transmitted Through Food, United States ........................................................................................................................................................................44

Table 4: Top Five Pathogens Contributing to Domestically Acquired Foodborne Illnesses …..44

Table 5: Top Five Pathogens Contributing to Domestically Acquired Foodborne Illnesses Resulting in Hospitalization ........................................................................................................................................................................45

Table 6: Top Five Pathogens Contributing to Domestic Foodborne Illnesses Resulting in Death ........................................................................................................................................................................45

Table 7: Main Industrial Applications of Xanthan Gum ........................................................................................................................................................................56

Table 8: Common Spoilage Organisms and Their a_w Limits for Growth ........................................................................................................................................................................60

Table 9: Dry Ingredient Formulations of Pie Dough for Control and Treatment ........................................................................................................................................................................62

Table 10: Mean Log Reduction Values of Pie Dough Treatments with Increasing Levels of Xanthan Gum Concentrations (0%, 1%, and 2%) after 21 Days Incubation at Refrigeration Temperature 4 °C. Same Superscripts Are Not Significantly Different as Measured Using Single-Factor ANOVA ........................................................................................................................................................................67

Table 11: Mean Log Reduction Values of Pie Dough Treatments with Increasing Levels of Xanthan Gum Concentrations (0%, 1%, and 2%) after 15 Days Incubation at
Refrigeration Temperature 4 °C. Different Superscripts Are Significantly Different as Measured Using Single-factor ANOVA and Paired t-Tests

Table 12: Mean Water Activity Values of Pie Dough Treatments with Increasing Levels of Xanthan Gum Concentrations (0%, 1%, and 2%) after 10 Days Incubation at Refrigeration Temperature 4 °C. Different Superscripts Are Significantly Different as Measured Using Single-factor ANOVA and Paired t-Tests

Table 13: Microbiological Identification of Two Unknown Bacteria Appearing on XLD Agar Plates after 15 Days of Incubation at 4 °C

Table 14: Sheetability Determination of Different Pie Dough Formulas
List of Figures

Figure 1: Schematic Representation of Major Components Required to Make Shortcrust Pastry Pie Dough........................................................................................................................................................................22

Figure 2: Schematic Representation of the Model of Function Glutenin..............................................25

Figure 3: Schematic Representation of Gluten Network Formation through Disulfide Cross-
linkages between Gliadin and Glutenin.................................................................29

Figure 4: Scanning Electron Micrograph (SEM) of an Optimally Kneaded Dough with Starch Granules and Gas Pockets in Gluten Network..............................................30

Figure 5: Structure of the Amylose Molecule of Starch..........................................................31

Figure 6: Multistate Outbreak of *Salmonella* I 4,[5],12:i:- Infections Linked to Banquet® Pot Pies. Between January 1, 2007 and October 29, 2007, at Least 401 Isolates of *Salmonella* I 4,[5],12:i:- with an Indistinguishable Genetic Fingerprint Have been Collected from Ill Persons in 41 States. The Number of Ill Persons whose *Salmonella* Strain Has this Genetic Fingerprint Has been Reported from the Highlighted States. Their Ages Range from <1 to 89 Years with a Median Age of 18 Years; 51% of Ill Persons are Female. At Least 65 People Have been Hospitalized. No Deaths Have been Reported.................51

Figure 7: Schematic Illustration of One Xanthan Gum Monomer....................................................55

Figure 8: Surviving *Salmonella* Count (CFU/g) in Refrigerated Pie Dough with Varying Levels of Xanthan Gum (0% Control, 1%, and 2% w/dw) during 21-Day Storage Period at 4°C........................................................................................................................................................................66
Chapter I: Introduction

Pie is a popular and important food product with its origin tracking back to Egyptian Neolithic period (Olver, 2014; Pfister, n.d.). Pie eating is associated with many important holidays such as Thanksgiving and Christmas, especially in the United States. In the Oxford English Dictionary, pie is defined as “a baked dish of fruit, meat, fish or vegetables, covered with pastry (or a similar substance) and frequently also having a base and sides of pastry; also (chiefly North America) a baked open pastry case filled with fruit; a tart or flan” (Oxford Dictionaries online, 2014). According to International Markets Bureau (IMB) (2012), retail sales of total pastries in the U.S. stood at US$ 11.8 billion in 2006. Retail sales of total pastries increased to US$ 12 billion in 2010, with a compound annual growth rate (CAGR) of 0.2% over that period. Another 0.3% increase was observed in 2011. However, sales forecasting expects an annual decrease by 0.4% till 2016. Sales declines among unpackaged and artisanal products are expected to be responsible for the total decrease. These declines are primarily attributed to the fact that many consumer foodservice outlets such as Starbucks are offering a variety of low-calorie healthy pastries for all day long (IMB, 2012). The retail sales of pies in the U.S. were US$ 1.23 billion and US$ 1.25 billion in 2007 and 2010, respectively. Although CAGR of pie sales was only 0.2% between 2006 and 2010, a much stronger growth of 7.1% was observed in 2011. This could be attributed to the increasing consumer demand for comfort foods such as tarts during economic recession (IMB, 2012). Also, NPD Group’s National Eating Trend survey showed that consumption of pies accounted for approximately 8% of all eatings of sweet foods at home (IMB, 2012).

According to American Institute of Baking (AIB) (2013), a CAGR of 8.1% between 2006 and 2011 was observed in the refrigerated and frozen dough market. The growth took place
regardless of the recession. Competition from bakeries and restaurants is expected to increase as the economy recovers. Also, the dough market is confronted with new challenges. Customers are embracing more healthy lifestyles and eating-habits. Customers prefer natural ingredients over artificial ingredients. Clean labeling becomes increasingly important. Chemical preservatives are to be eliminated or replaced with natural preservatives such as organic acids and peptides fermented by bacteria (AIB, 2013; Mintel, 2012). To ensure market growth, dough manufacturers must adapt to customers’ need through product innovation. Therefore, there is an emerging need to develop dough products free of chemical preservatives without compromising the product quality and shelf-life.

As reported by United States Center for Disease Control and Prevention (CDC) (2014), *Salmonella* outbreaks have been linked to many food products such as poultry, beef, cheese, eggs, nuts, peanut butter, raw produce, wheat cereals, and pies. The sources of contamination vary in different outbreak cases. The CDC has not been able to determine the exact source of contamination in many outbreak cases (CDC, 2014). The presence of pathogenic microorganisms such as *Salmonella* in baked goods is generally attributed to pre-processing contamination, in-processing contamination, or post-processing contamination (Guy, 1981).

Pre-processing contamination, also known as ingredient contamination, occurs when the raw ingredients used are contaminated with foodborne pathogens such as *Salmonella* (Guy, 1981). Ingredient contamination is generally not an issue because proper industrial heat treatment should be more than sufficient to destroy all *Salmonella* serotypes. This is confirmed by the observation that *Salmonella* serotypes isolated from contaminated food products are different from the serotypes isolated from ingredients (Lowry n.d.). Although highly unlikely, heat processing failure in a manufacturing plant may also be responsible for *Salmonella* outbreak
(Guy, 1981). Certain food products such as pre-made pie dough, pie crust, and pies are not cooked or partially cooked by the manufacturers. If such food products contain contaminated ingredients, a potential outbreak can occur. This is because although adequate home cooking can kill *Salmonella*, many consumers may not read, misread, or misunderstand the cooking instructions printed on food package (CDC, 2007). Also, the popular habit of eating raw dough in the U.S. may significantly increase the risk of infections (CDC, 2007; Gilbert, Lake, Cressy, & King, 2010).

Post-contamination occurs when environmental foodborne pathogens such as *Salmonella* are introduced to the food products after the last step of processing (Lowry, n.d.). Post-contamination must not be overlooked because it can easily occur if no Good Manufacturing Practice (GMP) is implemented (AFIA, 2010; FDA, 2013). *Salmonella* are ubiquitous; they may be found and survive in most environments (AFIA, 2010). Most foods including pie dough can offer rich nutrient media for *Salmonella* growth (FDA, 2013). Pests including insects, rodents, and birds are vehicles by which *Salmonella* can be transmitted from outside or within the manufacturing plant (AFIA, 2010). Contaminated materials such as scrapings off boots, dust or dirt falling off clothing may also cause post-processing contamination (AFIA, 2010; Lowry, n.d.). Thus, employees handling the food products can serve as perfect vehicles of *Salmonella* contamination. As discussed earlier, raw pre-processing ingredients may be contaminated with *Salmonella*. The lack of a transition room between pre- and post-processing areas for employees to change clothing, as well as the lack of clear indication of pre- and post-processing utensils, conveyance equipment, and storage containers may also contribute to possible post-processing contamination (AFIA, 2010).
In 2007, a multistate outbreak of *Salmonella* infections linked to turkey pot pies occurred in the U.S (CDC, 2007). The Food and Drug Administration (FDA) identifies pie as combination products, which refer to products containing two distinct food systems. For example, turkey pot pies have two food systems: pie crust and meat filling. Interactions between two food systems increase the complexity of microbial ecology and thus result in less predictable pathogen behavior. Therefore, combination foods are recognized as potentially hazardous foods (FDA, 2013). Despite a thorough investigation of the *Salmonella* outbreak, the CDC was unable to determine the exact source of contamination. Both pre- and post-contamination were possible. The pie crust of these pot pies was not cooked in the manufacturing facility. Pie crust ingredients may be contaminated before or after entering the plant. It was also possible that finished pot pies came in contact with raw turkey pastes which often harbor *Salmonella* (CDC, 2007). No *Salmonella* outbreak linked to refrigerated pie dough has been reported by the CDC. However, the risk of *Salmonella* contamination in refrigerated pie dough, especially post-processing contamination, must not be overlooked because of the popular raw dough eating habit in the U.S (CDC, 2007; Gilbert et al., 2010).

Xanthan gum is a polysaccharide produced by bacteria *Xanthomonas campestris* via fermentation. It is an important additive in both food and non-food industries (Garcia-Ochoa, Santos, Casas, & Gomez, 2000). Xanthan gum is recognized by the FDA as Generally Recognized as Safe (GRAS). Typical use in baked goods is usually less than 2% (FDA, 2002). Xanthan gum addition may be needed to allow for enhanced mechanical properties of the dough, including sheeting. However, the effect of including xanthan gum into the formulations on the food safety aspects of products such as pie dough is largely unknown. Xanthan gum is famous for its water binding capacity. Although xanthan gum is not considered an antimicrobial, it is of
interest to see whether microbial population can be controlled by xanthan gum through water activity control (Garcia-Ochoa et al., 2000; Moncel, 2014). Typical growth temperature of *Salmonella* ranges from 7 to 48 °C (Lawley, 2013). If pie dough post-contaminated with *Salmonella* is subjected to abuse conditions such as 10, 12, and 25 °C or severe abuse conditions such as 35 °C, the growth of *Salmonella* in dough can be exponential (Guy, 1981). Since xanthan gum has no antimicrobial effects directly against *Salmonella* cells, it may not be practical to expect xanthan gum to inhibit *Salmonella* growth in dough stored at abuse conditions. It is, however, feasible to expect xanthan gum to control the number of *Salmonella* in dough stored at refrigeration temperature 4 °C.

The basis of this study was to investigate the efficacy of xanthan gum on the survival of *Salmonella* in refrigerated dough systems with different concentrations of xanthan gum. *Salmonella* population was monitored by the use of microbiological techniques such as incubation, serial dilution, plating, plate counting, and calculation.

**Statement of the Problem**

There is a growing trend to eliminate chemical preservatives in food products. Xanthan gum is generally added to foods as texture enhancer, stabilizer, and water binder. An ideal clean label solution to reduce or eliminate foodborne pathogens such as *Salmonella* would be to use a naturally fermented ingredient such as xanthan gum to impart both rheological and water-controlling properties. The effect of xanthan gum on the survival of *Salmonella* in refrigerated pie dough has not been previously studied.

**Purpose of the Study**

The purpose of this study was to determine the effect of xanthan gum addition (1% and 2% by weight of dry ingredient) on the survival of *Salmonella enterica* serotype *typhimurium*.
*Salmonella typhimurium* in pie dough systems incubated at refrigeration temperature 4 °C for a period of 15 days.

**Assumptions of the Study**

Inoculation of *Salmonella* onto the surface of pie dough was to simulate post-processing contamination that may occur during refrigerated plant or retail storage. It was assumed that acquired findings of this study were transferrable to real-world conditions.

XLD agar is an effective growth medium typically used for *Salmonella* in microbiological studies. XLD agar was assumed to be able to allow for the growth of both uninjured and injured cells of *Salmonella*, whether injured or not. Thus, it was assumed that colony counts on XLD agar were true representation of the actual *Salmonella* populations in the sample.

Due to limited space in the refrigerator, inoculated pie dough samples were stored at different locations in the refrigerator. It was assumed that there was no temperature difference within the various areas of the refrigerator.

**Definition of Terms**

**Gluten.** A composite protein, consisting of two fractions gliadin and glutenin, generally found in wheat grains. Gluten is not soluble in water and is responsible for wheat dough’s viscoelastic texture (The Free Dictionary by Farlex, n.d.c; Khan & Bushuk 1979).

**Foodborne pathogens.** Infectious agents which are carried by food, including viruses, bacteria, fungus, and parasites (The Free Dictionary by Farlex, n.d.b).

**Foodborne illness.** Any illness resulting from the consumption of food products that are contaminated with foodborne pathogens (CDC, 2012).
**Serotypes.** Distinct variations within a species of bacteria. Serotypes can be distinguished by their different antigenic specificity (Gilbert et al., 2010; WHO, 2013).

**Foodborne illness outbreak.** This is defined as a group of people consuming the same contaminated food or beverage and two or more of them becoming sick with the same illness (CDC, 2012).

**Xanthan gum.** A type of hydrocolloids; it is a heteropolysaccharide produced by the bacterium *Xanthomonas campestris* NRRL B-1459 via aerobic fermentation (Garcia-Ochoa et al., 2000; Moncel, 2014)

**Limitations of the Study**

During earlier dough formulation development, expertise in texture profile analysis was not available at UW-Stout. Therefore, no data on the rheological properties of pie dough was obtained.

Aseptic food manufacturing condition was not available in this study. Dough samples may have been contaminated with background bacteria. Contamination bacteria appearing on XLD agar plates were reducing the readability of those plates.

Pie dough samples are solid and thus very difficult to mix with 10 ml buffer solution to form a uniformly distributed suspension. Only 1 ml of the suspension was sampled, diluted, plated on XLD agar, and counted to determine the *Salmonella* population in dough samples. Due to the non-uniformity of the suspension, *Salmonella* population in 1 ml was not a true representation of the *Salmonella* population in the original 10 ml suspension.

This study was conducted by placing the samples at 4 °C following inoculation. Therefore the study results only apply to similar storage conditions. This study does not simulate pathogen growth at temperature abuse conditions.
Chapter II: Literature Review

In this chapter, pastry, pie, dough, foodborne pathogens and outbreaks, as well as xanthan gum are discussed to give an overview of past and current trends on the topic of this dissertation.

Pastry

**Definition.** Pastry, in general, is defined as a sweet baked food made of dough, especially the shortened paste used for pie crust and the like (The Free Dictionary by Farlex, n.d.d). Other examples of pastry goods include tarts, napoleons, and quiches. The term pastry also refers to the dough consisting primarily of flour, water, and shortening that is baked and often used a crust for pastry goods (Dictionary.com, n.d.).

**Origin.** The origin of pastry-making is often traced back to paper-thin multi-layered baklava and filo throughout the Mediterranean in ancient times (Olver, 2014). The plays of Aristophanes, written in the 5th century BC, mentioned small pastries filled with fruits. In ancient Roman baking, meat or poultry was enclosed in pastries made from flour, oil, and water to keep in the juice. Pastries served merely as shells and were not meant for consumption. Although the Romans and Greeks both had pastries in their baking traditions, food historians discovered strong evidence indicating that the Egyptians originally made pastry-like baked goods (Pfister, n.d.).

Returning crusaders brought these pastry recipes from the Mediterranean to medieval Europe where they were adopted and adapted differently over time in different European countries. French and Italian Renaissance chefs are credited for perfecting puff pastry and choux. Many new recipes, including brioche, Napoleons, cream puffs and éclairs, were introduced by the 17th and 18th century chefs. Chef Antonin Carême (1784–1833), who was the first to
incorporate art into pastry-making, is recognized by many culinary historians as the first great master of pastry making in modern times (Olver, 2014).

**Pastry vs. bread.** The major difference in ingredients between pastry and bread is that pastry has a higher fat content and much less water, resulting in a shortened product that is crumbly or flaky (Marston, 2013). Bread, however, uses a relatively large amount of water but a little or no fat. Yeast is usually added to yield a leavened product (Prichard, 2012). The greatest difference in processing is that pastry dough is gently mixed and worked as little as possible, whereas bread dough is mixed thoroughly and kneaded as much as possible. In addition to be fatty and flaky, a good pastry has to be strong and firm enough support the weight of the filling or topping (Marston, 2013). Good bread is generally light, airy, and soft. However, texture expectation of bread depends on the type of bread made, regional custom, and personal preference (Beranbaum, 2003).

**Types of pastry.** There are many types of pastry, but the following types are made most often: shortcrust pastry, sweet shortcrust pastry, flaky pastry, puff pastry, and choux pastry (Gough, B., Gough, J., & Toit, 2008; Pienaar, 2002). Pastry is used to make many different types of baked products. Thus, it would be very helpful to understand the major types of pastry and know when and how they should be used (Gough et al., 2008)

**Shortcrust pastry.** Shortcrust pastry is the simplest and most commonly made pastry. It’s generally made with four, fat, salt, and water. Two parts flour to one part fat is typical. Shortcrust pastry is expected to have a crisp, flaky, and short (melt-in-mouth) texture. It is used mainly in single crust pies and tarts. Other popular pastry dishes made with shortcrust pastry include quiches, sweet flans, and savory flans. The processes of making such pastry dough include
rubbing cold fat or shortening into flour first, adding water, and the gentle mixing. Shortcrust pastry dough is usually chilled and relaxed before rolling out (Gough et al., 2008; Pienaar, 2002).

**Sweetened shortcrust pastry.** Sweetened shortcrust pastry, sometimes called rich shortcrust pastry, is also known by its French name paté sucrée. Sweetened shortcrust pastry is closely related to shortcrust pastry except sugar and liquid egg yolk have been added instead of water to help bind the pastry. The sugar and egg yolk also make the pastry rich in texture and sweet in flavor, hence the name sweetened or rich shortcrust pastry. Such pastry is ideal for tartlets and can also be used for fruit tarts, flans, and pies (Gough et al., 2008; Pienaar, 2002).

**Puff pastry.** Similar to shortcrust pastry, puff pastry is made of flour, butter or shortening, salt, and water. However, one part flour one part fat is common when making such pastry. Puff pastry dough is made to have many very thin layers of flour and fat. When baked, the water trapped in the layers is heated and vaporized to puff the pastry, resulting in a multi-layered, light, flaky, and crispy texture. These layers are made by repeating the following process for up to six times: adding large rectangles of butter or shortening into flour, adding water, folding the dough, and refrigerating it. Puff pastry is generally used to make tarts, pie toppings, and other fine pastries (Gough et al., 2008; Pienaar, 2002).

**Flaky pastry.** In terms of ingredients and processing, flaky pastry is very similar to puff pastry except that the flaky pastry dough is not layered with large rectangles of fat. Instead, large lumps of fat are mixed into the flaky pastry dough, causing the dough to rise unevenly when baked. Therefore, flaky pastry is not as light and soft as puff pastry. Flaky pastry, however, still rises quite high and is still very flaky. It is usually used to make savory pastries such as sausage rolls. It also works great with pies that are meant to be served cold (Gough et al., 2008; Pienaar, 2002).
**Choux pastry.** The name choux is French, meaning cabbage, reflecting its somewhat cabbage-like shape after baking. Choux pastry is usually made of flour, butter, egg, milk, and water. The pastry is soft and liquid and can be piped into various shapes using a piping bag or spooned. In addition to its liquid state, choux pastry dough differs greatly from other pastries in terms of the way it is made. Unlike the other types of pastry, choux pastry is cooked twice. A saucepan pan is needed to bring the water or milk and butter to boil. Flour is added and mixed to form the dough. The dough is further enriched by beating eggs into the mixture. The mixture is cooked until a light and airy texture is achieved. The liquid mixture is then piped using a pastry piping bag onto a baking sheet in the shapes desired. Upon baking in the oven, the high content of water in the dough is heated and vaporized to make the pastry rise and expand. The pastry is further solidified when starch in the flour gelatinizes in presence of water and heat. After taking out of the oven, the pastry is punctured to release trapped steam, after which it is placed back in the oven to dry out. The final product is a light, hollow, and crispy pastry, which is often filled with different flavors of cream and topped with chocolate. Great examples of desserts made with choux pastry include chocolate éclair, croquembouche and profiterole (cream puff). Choux pastries can also be used to make savory appetizers by using fillings such as cheese, tuna, and chicken. (Gough et al., 2008; Pienaar, 2002).

**Pie**

**Definition.** Clarkson (2009) expressed her frustration of trying to define pie by quoting the famous American food journalist, Raymond Sokolov, “I may not be able to define a pie but I know it when I see it” (p. 1). Although difficult to define, Clarkson (2009) was able to point out two fundamental criteria for pie: the pie must be made from pastry dough and the pie must be baked, not fried, boiled, or steamed. According to Oxford English Dictionary, pie is defined as “a
baked dish of fruit, meat, fish or vegetables, covered with pastry (or a similar substance) and frequently also having a base and sides of pastry; also (chiefly North America) a baked open pastry case filled with fruit; a tart or flan” (Oxford Dictionaries online, 2014). Therefore, it is reasonable to define pie as a baked dish made of a pastry dough casing, which either covers or completely encloses a filling of different sweet or savory ingredients. Not all types of pastry can be used for making pies. Puff pastry and choux pastry are too light and puffy to support to support pie fillings or toppings. Flaky and shortcrust pastries are most commonly used for making pies. In the United States, shortcrust pastry is the most popular and is often used to make holiday desserts such American apple pies, pecan pie, and pumpkin pies (Gough et al., 2008; Pienaar, 2002).

**Types of pie.** Pies can be categorized by their crusts. For a bottom-crust pie, the baking dish is lined with pastry dough and the filling is placed on the top uncovered. Conversely, a top-crust pie has the filling at the bottom of the baking dish and sheeted pastry dough is used to cover the filling before baking. A double-crust crust pie completely contains the filling in the pastry shell (Lardman, 2010; Southern Utah University, n.d.). Other less commonly made types include lattice-top pies and crumble pies with a streusel topping (SUU, n.d.).

**Pie dough.** Dough is defined as a soft, thick mixture of dry ingredients, such as flour or meal, and liquid, such as water, that is kneaded, shaped, and baked, especially as bread or pastry (The Free Dictionary by Farlex, n.d.a). In baking industry, the physico-chemical properties of dough play a very important role. Dough is developed by the complex interactions between wheat constituents and water during mixing. Upon addition of water and initiation of mixing operation, the dry ingredients are hydrated and begin to develop into a sticky paste. Further mixing will increase the viscosity of the dough and decrease the sticky characteristics, resulting
in a viscoelastic mass possessing both elasticity and extensibility (Khatkar, n.d.). The dough at this stage would be ideal for bread-making purpose. However, such developed dough would result in a very tough and non-flaky pie crust if used for making pies. Pastry dough such as pie dough usually has a relatively high fat content and low water content. The mixing is minimized such that less gluten is developed and thus the pie dough is less elastic or extensible than bread dough (Gisslen, 2007). Such pie dough is often called “short” dough because the gluten strands are shortened by the presence of fat and the shortage of water and mixing (Khatkar, n.d.).

**Pie dough ingredients.** A typical shortcrust pie dough formulation includes the following ingredients: flour, fat, liquid, sugar, and salt. Flour, fat, and liquid must be included to form the dough (Mottershead & Woods, 2003). However, there are various selections for each component, such that different outcomes in taste, texture, and appearance can be achieved by manipulating ingredients (Figure 1) (Mottershead & Woods, 2003).

Flours used to make shortcrust pastry include plain white flour, self-raising white, wholemeal flour, and mix of wholemeal and white flour. Wholemeal flour can provide extra fiber and produce a pie crust with relatively dark color and firm texture. Lard, butter, margarine, and mix of lard and margarine are typical fats that are used to shorten the pie dough. While lard is white and thus has no contribution to the pie crust color, butter contributes to both color and flavor of the final product (Mottershead & Woods, 2003). Liquids that are usually added to pie dough include liquid whole egg, liquid egg yolk, milk, tomato juice, lemon juice, and most commonly cold water (Gough et al., 2008; Mottershead & Woods, 2003).
Figure 1. Schematic representation of major components required to make shortcrust pastry pie dough (Mottershead & Woods, 2003).

**Flour in pie dough.** Flour contains starch, protein, moisture, and a trace of fat. Usually, the trace amount of moisture and fat present in newly-opened dry flour can be neglected. Unlike protein, the textural properties of starch in flour do not change much during dough mixing. The primary functions of starch in pie dough are to act as a filler and to absorb and hold moisture (Kylie, 2009). Approximately 80% of wheat proteins are gliadin and glutenin, which are generally referred to as gluten proteins. The remainder is composed of albumen, globulin and
free amino acids (Mondelli, n.d.). The functionality of gluten proteins in wheat flour will be discussed below.

*Types of flour.* Rye, rice, corn, oat, buckwheat flours are made from grains other than wheat. These flours contain little to no wheat gluten and thus will certainly not be used to make pie dough. Whole wheat, bread, all-purpose, pastry, cake flours are wheat flours and they all contain gluten. The quantity of gluten protein varies depending on the type of wheat flour (Table 1) (Masibay, 2008).

Table 1

*Protein Content of Wheat Flours (Percentages are Approximate, as Every Brand of Flour is Unique)*

<table>
<thead>
<tr>
<th>Flour</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole wheat, bread, durum semolina</td>
<td>12-15</td>
</tr>
<tr>
<td>All-purpose</td>
<td>9-12</td>
</tr>
<tr>
<td>Pastry</td>
<td>8-9</td>
</tr>
<tr>
<td>Cake</td>
<td>7-8</td>
</tr>
</tbody>
</table>

(Masibay, 2008)

Although using a low protein flour can help achieve a tender pie crust, cake flour with protein content ranging from 7-8% lacks enough gluten to form a workable dough. Due to its high protein content, whole wheat and bread flour will react with water quickly and develop strong gluten network, which in turn result in a tough pie crust. Pastry flour with a protein content ranging from 8-9% is ideal for making pie dough. True to its name, all-purpose flour with protein content ranging from 9-12% works fine for most pies. The proper balance of starch
and protein in pastry flour and all-purpose flour permits desired amount of water absorption and gluten development. The result is a pie crust that is both flaky and tender (Masibay, 2008).

**Gluten.** Gluten is defined as the mixture of proteins, including gliadin and glutenin, found in wheat grains, which are not soluble in water and give wheat dough its elastic texture (The Free Dictionary by Farlex, n.d.c).

**Gliadin.** Approximately 35-40% of wheat flour proteins are gliadin. This protein is 70% soluble in aqueous ethanol. Gliadin is responsible for the viscous component of gluten’s viscoelastic properties (Khan & Bushuk 1979). A two-dimensional electrofocusing-electrophoresis technique is able to identify approximately 50 components of gliadin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) can be used to measure the molecular weights of these gliadin components, ranging from about 12,000 to 80,000 daltons. Most of these components have a molecular weight of approximately 36,000 daltons (Khan & Bushuk, 1979). It is worth noting that intra-polypeptide disulfide bonds are observed in majority of gliadin components (Khan & Bushuk, 1979).

**Glutenin.** Unlike gliadin, glutenin is insoluble in 70% aqueous ethanol. It is, however, soluble in dilute acid and alkali (Weiser, 2007). Wheat endosperm protein is generally comprised of 35-45% glutenin (Khan & Bushuk, 1979). Glutenin is responsible for the elastic component of gluten’s viscoelastic properties. During dough mixing or kneading, glutenin undergoes considerable rheological changes as opposed to gliadin. Thus, it is evident that glutenin is primarily responsible for the rheological properties of gluten (Tatham, Drake, & Shewry, 1990; Shewry, Tatham, Barro, Barcelo, & Lazzeri, 1995). The physical properties of glutenin, such as molecular weight, shape, and size, and chemical properties, such as amino acid composition, sequence, and tendency to aggregate impart glutenin its functional behaviour. Seventeen
polypeptide subunits ranging in molecular weight form 12,000 to 134,000 daltons have been identified in hexaploid wheat by using SDS-PAGE on reduced glutenin (Khan & Bushuk, 1979). These polypeptide units are connected to one another to form long concatenated structures or highly stable micelles through four different bonding mechanisms: intrapolypeptide disulfide bonding, interpolypeptide disulfide bonding, as well as secondary forces such as hydrophobic interactions and hydrogen bonding (Figure 2) (Bushuk, Khan, & McMaster, 1980; Khan & Bushuk, 1979; Lombardi et al., 2009).

Figure 2. Schematic representation of the model of function glutenin (Bushuk et al, 1980).

According to Cauvain and Young (2007), sulfur-containing amino acid cysteine residues present in glutenin play a critical role in both intro- and interploypeptide disulfide bond formations. Khan and Bushuk (1979) reported the amino acid composition of wheat flour
proteins and the number of residues of each amino acid within each component (Table 2). Glutenin
contains a very large number of glutamic acid residues, which are all present as glutamine. These
 glutamine residues supply abundant amide groups that are responsible for the formations of
intra- and interpolypeptide hydrogen bonds. Due to the addition of water, dough is considered an
aqueous environment, in which there are a polar side and a non-polar side of glutenin polypeptide
chains (Khan & Bushuk, 1979). Relatively high leucine content is also observed in glutenin (Table 2).
These leucine residues are hydrophobic and thus interact with one another at the non-polar side of
the chains. Although hydrogen bonds and hydrophobic interactions are rather weak bonding forces,
in large quantities, they facilitate the stabilization of glutenin aggregates (Khan & Bushuk, 1979).
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Gliadin</th>
<th>Glutenin</th>
<th>Gluten</th>
<th>Flour</th>
</tr>
</thead>
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<tr>
<td>Lysine</td>
<td>5</td>
<td>12.5</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Histidine</td>
<td>14.5</td>
<td>13</td>
<td>15</td>
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<tr>
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</tr>
<tr>
<td>Serine</td>
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<td>42</td>
</tr>
<tr>
<td>Glutamic acid</td>
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<tr>
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<td>114</td>
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<tr>
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</tr>
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<td>Cysteine</td>
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<tr>
<td>Valine</td>
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<tr>
<td>Methionine</td>
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<td>12</td>
<td>13</td>
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<tr>
<td>Isoleucine</td>
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<td>Tryptophan</td>
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<td>7</td>
</tr>
<tr>
<td>Amide</td>
<td>301</td>
<td>240</td>
<td>298</td>
<td>230</td>
</tr>
</tbody>
</table>

(Khan & Bushuk, 1979)
By using gel filtration technique, alkylated subunits of glutenin have been fractionated and divided into three subunit groups according to their molecular weights: lowest molecular weight with same mobility (35,000 daltons and 45,000 daltons), medium (68,000 to 12,000 daltons), and largest (134,000 to 60,000 daltons) (Khan & Bushuk, 1979). These three subunit groups also contributed greatly to glutenin’s unique properties, which in turn have a great impact on the overall functional properties of glutenin in gluten and the resultant dough (Khan & Bushuk, 1979).

*Gluten network.* The process of wetting the wheat proteins gliadin and glutenin is termed hydration. Upon mixing water and flour, the proteins are hydrated and begin to interact with each other (Crosby, 2012). Relatively strong chemical bonds such as disulfide bonds and relatively weak bonds such as hydrogen bonds and hydrophobic interactions begin to form within and between gliadin and glutenin. In other words, glutenin molecules cross-link the gliadin molecules through intermolecular chemical bonds. These bonds are called cross-linkages. Continued mixing or kneading encourages more cross-linkages to form between gliadin and glutenin until a large continuous proteinaceous network is formed, namely the gluten network (Figure 3) (Crockett, 2009; Crosby, 2012; Helmanstine, 2014).
Figure 3. Schematic representation of gluten network formation through disulfide cross-linkages between gliadin and glutenin (Crockett, 2009).

Upon further kneading, gluten networks combine to constitute sheets of gluten proteins. This is because kneading causes the disulfide bonds between adjacent polypeptide chains to break down, which are realigned in the direction of kneading to form gluten sheets (Crosby, 2012; Stauffer 1998). Kneading also incorporates more air. In presence of additional oxygen, stronger disulfide bonds can be formed. The final product resulting from the cross-linking of glutenin and gliadin is a very viscoelastic substance that is both elastic and extensible (Crosby, 2012).

**Functionality of gluten in pie dough.** Textural properties of dough depend primarily on the extent of its gluten development (Crosby, 2012). In a dough matrix, starch granules and fiber fragments are trapped within the gluten network (Figure 4) (Amend & Belitz, 1990). It is critical that gluten has an adequate balance between elasticity and extensibility, elasticity being commonly referred to as dough strength. Strong and elastic dough with fully developed gluten is ideal for making chewy baked goods such as bread and pizza. In order to make tender and flaky
pastry goods such as pies, the pie dough is intentionally made weaker and less elastic than bread dough by hindering gluten development through ingredients and processing manipulation (Beranbaum, 2003; Marston, 2013; Shewry et al. 1995).

Figure 4. Scanning electron micrograph (SEM) of an optimally kneaded dough with starch granules and gas pockets in gluten network (Amend & Belitz, 1990).

Functionality of water. Starch and protein are the two main components of the flour, starch being the most abundant component by weight and volume (Gisslen, 2008; Haegens, 2011). No reactions occur until flour is in contact with water. The process of absorbing water is called hydration. Upon hydration, different ingredients in the dough formulation react with water differently. These interactions are responsible for dough formation (Gisslen, 2008).

When water is added to flour, the outer layers of the flour particles are hydrated. Continued mixing strips away the hydrated outer surface layers and exposes new layers of flour particles to be hydrated. This process continues until all flour particles are hydrated (Arendt & Bello, 2011). The starch molecules in the flour are hydrated first before wheat proteins can absorb water and start to form gluten. During the mixing stage, it is worth noting that starch molecules absorb more water and absorb water faster than proteins (Kylie, 2009; Rosada, 2011).
**Starch and water.** Starch is a polysaccharide that is composed of a great number of glucose units joined via glycosidic bonds (Figure 5) (Abedon, 1997). Since starch molecules are large polymers of glucose, they generally do not dissolve in water (Gisslen, 2008). Each monomer or repeating unit of starch polymers contains three hydroxyl (-OH) groups that can help attract and bind with water molecules (Gisslen, 2008; Haegens, 2011). When starch molecules are hydrated, water molecules form a shell around starch granules by attaching to their surface (Gisslen, 2008). Sufficient time or heating is needed for the water molecules to get into the tightly packed starch granules (Gisslen, 2008; Haegens, 2011). It has been reported that wheat flours also contain pentosans, which are water soluble polysaccharides. They are essentially polymers of five carbon sugars which possess strong water binding ability (Haegens, 2011). At high temperatures starch gelatinize. During baking, the heat causes the hydrated starch to gelatinize, which help form the structure of baked goods. Gelatinization could not occur without adding water during mixing (Gisslen, 2008; Haegens, 2011).

![Structure of the amylose molecule of starch](image)

*Figure 5. Structure of the amylose molecule of starch (Abedon, 1997).*

**Gluten and water.** Wheat proteins glutenin and gliadin in dry flour are in the form of tight coils (Gisslen, 2008). Although neither protein is soluble in water, they can attract, bind, and interact with water molecules under the applied shear and tensile forces during mixing (Arendt & Bello, 2011; Gisslen, 2008). Upon hydration and mixing, glutenin and gliadin proteins begin to
partially unfold or uncoil. The partial uncoiling of these protein molecules facilitates hydrogen bonding, hydrophobic interactions, and disulfide cross-linking. As a result, straightened proteins stick together to form long gluten fibers. During continued mixing, these gluten fibers gradually stretch and become intertwined to form the viscoelastic gluten network. The formation of gluten is not possible in the absence of water (Arendt & Bello, 2011 & Gisslen, 2008). The firming or hardening of gluten proteins caused by heat is referred to as coagulation. Upon baking, the gluten proteins coagulate and solidify to form a firm structure (Gisslen, 2008).

**Other functions of water in dough.** Besides hydrating wheat proteins and starch, gluten formation, as well as starch gelatinization and gluten coagulation during baking, water has many other functions in dough (Arendt & Bello, 2011). Water is a solvent; it is necessary for solubilizing other ingredients such as salt and sugar (Haegens, 2011). Water helps homogenize all ingredients throughout the dough during mixing (Rosada, 2011). Water is also responsible for the production of steam and the distribution of heat during baking. Finally, water has an impact on the organoleptic properties of the baked goods (Haegens, 2011).

**Amount of water.** Water is essential for gluten development in pie dough. The amount of water in a formula can affect toughness or tenderness of the final baked pie crust. Therefore, by adding or withholding water from dough, one can encourage or prevent gluten development. (Gisslen, 2007; Gisslen, 2008; Masibay, 2008). Flaky and tender pastry dough is generally made with very little water to minimize gluten development. It is wise to dribble water into the dough because adding too much water can produce excessive gluten and result in a toughened pie crust (Masibay, 2008). Some gluten development is needed for pie dough. If not enough water is added, the gluten structure in pie dough will be too weak. The resultant pie dough will be too crumbly to sheet or fold and the final pie crust will fall apart (Gisslen, 2007). If way too much
water is added, the gluten structure will be weakened. This is because excessive water dilutes the proteins and thus restricts their interactions (Crosby, 2012; Gisslen, 2008; Masibay, 2008).

_Taste of water._ Regardless of its origin, water used in baking must be drinkable. Regular tap water is usually acceptable to be used to develop dough. However, unusually bad taste or off-odor can be identified after strong rainstorms or during the change of seasons. The flavor of the final baked product can be altered by such water. If necessary, additional filters can be purchased and installed on the water line to reduce the risk of getting off-flavor in the final baked goods (Mondelli, n.d.; Rosada, 2011).

_Hardness of water._ Water hardness refers to the mineral content of the water, the main ones being calcium, magnesium, and sodium (Gisslen, 2008; Rosada, 2011). These minerals are normally present in the water in the form of salts such as carbonates and bicarbonates (Mondelli, n.d.). Water having a high mineral content is known as hard water, whereas soft water contains a very low amount of minerals (Rosada, 2011). The textural properties of dough can be affected by the mineral content of the water. The presences of these mineral salts strengthen gluten by increasing water absorption by gluten proteins (Crosby, 2012; Mondelli, n.d.). If the water added is too hard, the dough will become very elastic and eventually stretches to lose elasticity. If the water is too soft, the dough made will be very sticky and slack (Gisslen, 2008; Mondelli, n.d.). To counteract these undesirable effects, water treatments or dough conditioners can be used (Gisslen, 2008). Certain bakeries are very concerned with their water hardness. Usually a hyper-filtration device called reverse osmosis system is installed to eliminate impurities and balance the mineral content. This also ensures a consistent quality of water (Rosada, 2011).

_pH of water._ The pH measures the water’s acidity or alkalinity, on a scale from 0 to 14. The mineral content of water increases its pH. The ideal pH range gluten development is from 5
to 6. Above or below this range weakens gluten strength and produces a pliable dough. The tenderness of a pie crust can be adjusted by adding an acidic liquid such as fruit juice or vinegar. This also allows the dough to be rolled out more easily (Crosby, 2012; Gisslen, 2008).

**Functionality of fat.** Fats are tenderizers (Gisslen, 2008). Fat is responsible for the tender, flaky, or crumbly texture of pie crusts. Fat can render flour particles water-resistant. When making pie dough, the fat is thoroughly worked into the flour before adding water. Once coated with fat, the starch granules can no longer absorb much water when liquid ingredients are added. Fat also weakens gluten. Fat coats glutenin and gliadin proteins, reduces hydration, and thus prevent the proteins from forming long and strong gluten fibers. (Masibay, 2008). In other words, the fat is to shorten the gluten fibers, hence the name shortening (Mottershead & Woods, 2003). With less and weaker gluten, the pie crust stays flaky, soft, and tender (Masibay, 2008; New Zealand Institute of Chemistry, 2008). The types of fat, the amount of fat, and the way it is added are the determining factors in affecting pie crust’s final texture (Warren, 2013).

**Types of fat.** The pie crust will develop a certain flavor depending on the type of fat used. Typical types of fat used in pastry baking include butter, lard, and vegetable shortening (Maranowski, 2012). These fats remain solid at room temperature and will harden when kept cold.

Among all of these fats, butter yields the best flavor in pie crust. However, it is more difficult to handle than shortening or lard because it has a lower melting point (Gisslen, 2007; Warren, 2013). The working range for butter is 58°F–68°F. This means the fat can be easily worked into flour without melting or being too firm within this temperature range (Gisslen, 2007; Maranowski, 2012). Butter is also more expensive, and is not often used in volume production of pie pastry (Gisslen, 2007; Warren, 2013).
Vegetable shortening, such as Crisco®, is a blend of partially and fully hydrogenated soybean and palm oils. The hydrogenation process renders the oil blend sold at room temperature. The working temperature range of vegetable shortening is 53°F–85°F (Maranowski, 2012). Due to such wide range, a workable dough can be easily made with vegetable shortening (Gisslen, 2007). Although vegetable shortenings hold their shape better than butter pie crusts, they lack richness in flavor (Warren, 2013). Nevertheless, vegetable shortening is the most popular fat used for making pie crusts (Gisslen, 2007). It has been reported that many customers eat only the filling and throw away the pie crust because they are not satisfied with the taste of pie crust made with vegetable shortening. If costs permit, it would be ideal to use a mixture of butter and vegetable shortening to make pie crusts so as to improve richness in flavor (Gisslen, 2007).

Lard contains 100% fat and 0% water and the working range for lard is 58°F–75°F (Maranowski, 2012; Warren, 2013). Lard yields the flakiest pie crust but have a flavor that some people dislike. Therefore, lard is generally not used in volume production of pie crusts (Gisslen, 2007; Warren, 2013).

Amount of fat. For making pie dough, two parts flour to one part fat is typical. However, if flours with relatively high protein content are used, the proportion of fat should be increased to ensure tenderness (Gisslen, 2007). Grade A and AA butters contain only 81% fat and 19% water. Thus, if all butter is used instead of shortening, the proportion of fat in the formula should be increased by approximately one-fourth. The water should be reduced accordingly (Gisslen, 2007; Warren, 2013). Low quality butters or reduced fat brands are not recommended for use when making pastry dough because these butters contain more water and will yield tough crust (Warren, 2013).
The way fat is added. The solid fat is generally rubbed into the flour with fingers or cut into the flour with a pastry blender. The fat remains in the dough in pieces or chunks. During baking, the pieces of fat melt to create voids in the crust, producing a flaky textures. Larger fat pieces result in flakier pie crust (Warren, 2013).

Functionality of salt. The main functions of salt include enhancing the flavors of the baked product, balancing the sweet tastes, counteracting bitterness, and concealing other off-flavors (Haegens, 2013; Kelly, 2014; Warren, 2013). Salt usage in dough generally ranges from 1.8% to 2.2% (Haegens, 2013). Salt also plays an important role in gluten formation. Salt strengthens the gluten and produces more elastic dough (Haegens, 2013; Kelly, 2014; Masibay, 2008). A typical dough has pH of approximately 5. The gluten fibers at such pH are positively charged. Since like charges repulse, the gluten fibers repel one another, forming a loosely interconnected structure. When salt, sodium chloride, is added to the dough, the negatively charged chlorine ions become attached to the positively charged site on gluten proteins, resulting in a neutralized overall charge. Thus, the neutrally charged gluten fibers can form a tightened strong gluten network (Glezer, 2014; Haegens, 2013; Kelly, 2014).

Functionality of sugar. Sugar is most commonly known as a sweetener. It is also a tenderizer that deters gluten development. Sugar is hygroscopic, which means it attracts and binds to water rapidly. Sugar promotes tenderness in pie crust by competing for available water that is essential for gluten formation. As a result, protein hydration is reduced and gluten development is hindered, and thus a tender crust is produced (Crosby, 2012; Gisslen, 2008; Kelly, 2014; Masibay, 2008; NZIC, 2008). The hygroscopic nature of sugar also helps render baked goods moist and fresh. (Warren, 2013). Sugar is also involved in a series of complex browning reactions above 160 °C, known as Maillard reactions. These reactions are chemical reactions
between amino acids and reducing sugars, producing a brown color and many desirable flavor compounds (Kelly, 2014; NZIC, 2008).

**Pie dough processing.** Mixing and temperature are the two main factors affecting pie dough processing (Gisslen, 2007).

**Mixing.** Mixing method, mixing time, and mixing speed together can be interpreted as total energy input (Cauvain & Young, 2007). To avoid tough and chewy crust, a pie dough is generally mixed and manipulated as little as possible to minimize gluten development. Hand mixing is strongly recommended for making small quantities of pie dough. For hand mixing, it is wise mix briefly with a light hand; for machine mixing, a pastry paddle and low speed should be used to encourage tenderness. Although most gluten proteins are coated with fat in a pie dough, overmixing can result in strong gluten structure and thus toughened crust (Gisslen, 2007; Masibay, 2008).

**Temperature.** Whether pie crust will become flaky and tender depends on not only the type of fat used, but the temperature at which it is added into the flour (Maranowski, 2012). Vegetable shortening has the best consistency when kept cool. If it is too warm, it melts and blends too rapidly with flour particles. If the shortening is too cold, it is too firm to work with. To limit gluten development and thus produce a tender crust, the liquid must be added cold, 4°C or colder. This also helps maintain a properly cold dough temperature. Gluten develops more slowly at cold temperatures than at warm room temperature. Thus, pie dough should be kept cool for relaxation at around 15.5 ºC (Gisslen, 2007; Gisslen, 2008).

**Outbreaks of Foodborne Pathogens in Dough**

**Foodborne pathogens.** In this section, definition of foodborne pathogens, top foodborne pathogens, and their epidemiology will be discussed.
**Definition.** Foodborne pathogens are defined as infectious agents which are carried by food, including viruses, bacteria, fungus, and parasites (The Free Dictionary by Farlex, n.d.b). According to the CDC (2012), foodborne illness, also known as foodborne disease, foodborne infection or food poisoning, is any illness resulting from the consumption of food products that are contaminated with foodborne pathogens. Depending on the cause of the illness, different foodborne illnesses have many different symptoms. However, gastrointestinal tract is the first place through which the microbes or toxins enter the human body. Reactions associated with the gastrointestinal tract such as nausea, vomiting, abdominal cramps, and diarrhea are typical first symptoms in many foodborne diseases (CDC, 2012).

**Top foodborne pathogens.** According to the FDA (2014), the top 14 foodborne pathogens include *Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Listeria monocytogenes, Norovirus, Pathogenic Escherichia coli (E. coli), Salmonella enteritidis, Salmonella typhimurium, Shigella, Staphylococcus aureus, Vibrio cholera, Vibrio parahaemolyticus, Vibrio vulnificus* and *Yersinia enterocolitica*. Pathogenic bacteria are the most common cause of foodborne illness. As the model organism for this study, *Salmonella* will be discussed in a separate section.

*Campylobacter jejuni. Campylobacter jejuni* is a bacterium that is the most common bacterial cause of diarrhea in the U.S. Other symptoms include stomach cramps, fever, muscle pain, headache, and nausea. Possible sources of contamination include raw milk, untreated water, raw and undercooked meat, poultry, or shellfish. Incubation can take 2 to 5 days after eating contaminated food. Illness can last 2 to 10 days. It is worth noting that children under age one and unborn babies and infants are especially vulnerable (FDA, 2014; ISU, 2010).
*Clostridium botulinum.* *Clostridium botulinum* is a bacterium can grow in moist, low-acid food. It produces a toxin that causes botulism disease. Initial symptoms include dry mouth, double vision followed by nausea, vomiting, and diarrhea. Later symptoms include constipation, weakness, muscle paralysis, and breathing problems. Botulism can be fatal and thus immediate medical help must be sought. Possible sources of contamination are home-canned and prepared foods, vacuum-packed and tightly wrapped food, meat products, seafood, and herbal cooking oils. Incubation takes 12 to 72 hours after eating contaminated food. Recovery can take from 1 week to a full year. It is worth noting that honey may contain *Clostridium botulinum* spores. Infant botulism is usually caused by consuming these spores. Therefore, one should not feed infant under age one any honey (FDA, 2014; ISU, 2010).

*Clostridium perfringens.* *Clostridium perfringens* is a bacterium that can produce heat-stable spores. These spores can thrive in undercooked food and foods that are left out at room temperature. Symptoms of *Clostridium perfringens* infection include abdominal pain, diarrhea, and sometimes nausea and vomiting. Meat and meat products are the most common sources of *Clostridium perfringens* contamination. Incubation can take 8 to 16 hours after consuming contaminated food. The illness usually lasts as short as 1 day or less (FDA, 2014; ISU, 2010).

*Listeria monocytogenes.* *Listeria monocytogenes* is a bacterium that can grow slowly at refrigeration temperatures. Symptoms of listeria infection include fever, headache, fatigue, muscle aches, nausea, vomiting, diarrhea, meningitis, and miscarriages. Refrigerated, ready-to-eat foods are the main sources of *Listeria monocytogenes* contamination. Such foods include meat, poultry, seafood, and unpasteurized milk products or foods made from unpasteurized milk. Incubation usually takes 9 to 48 hours after ingesting contaminated foods. Sometimes it may take as long as 6 weeks to develop after eating contaminated foods (FDA, 2014; ISU, 2010).
Norovirus. Norovirus, also known as Norwalk-like Virus, is an emerging foodborne pathogen, which may be responsible for a large percent of non-bacterial foodborne illness. Symptoms of such viral infection include diarrhea, nausea, vomiting, stomach cramps, headache, and fever. Possible sources of contamination include raw oysters, shellfish, coleslaw, salads, baked goods, frosting, contaminated water, and ice. Norovirus is also able to spread via person-to-person contact. Incubation generally takes 24 to 48 hours after ingestion. The symptoms can occur as early as 12 hours after exposure. Duration of illness varies from 1 to 3 days (FDA, 2014; ISU, 2010).

Pathogenic E. coli. Pathogenic E. coli is a group of bacteria that can produce a variety of deadly toxins. Symptoms of infection include severe stomach cramps, bloody diarrhea, and nausea. Non-bloody diarrhea and being symptomless are also possible. E. coli O157:H7, a serotype of E. coli, can cause permanent kidney failure which can lead to death in young children. Undercooked or raw meat, uncooked produce, raw milk, unpasteurized juice, and contaminated water are possible contamination sources. Incubation usually takes 3 to 4 days after ingestion. However, symptoms may occur as early as day 1 or as late as day 10 after eating contaminated food (FDA, 2014; ISU, 2010).

Shigella. Shigella is a bacterium that is only carried by human and can easily spread from person to person via food or as a result of poor hygiene, especially poor hand washing. Symptoms include diarrhea, fever, stomach cramps, vomiting, and bloody stools. Sources of contamination can include salads, milk and dairy products, raw oysters, ground beef, poultry, and unclean water. Incubation generally takes 1 to 2 days after ingestion. Duration of illness ranges from 5 to 7 days (FDA, 2014; ISU, 2010).
**Staphylococcus aureus.** *Staphylococcus aureus* is a bacterium that is carried on the skin and in the nasal passages of humans. Therefore, it can easily be transferred to food by a person as a result of poor hygiene, especially poor hand washing and sneezing. Symptoms of infection include nausea, stomach cramps, vomiting, and diarrhea. Possible sources of contamination are dairy products, salads, cream-filled pastries, high-protein foods such as cooked ham, raw meat and poultry, as well as humans via skin, infected cuts, pimples, noses, and throats. Incubation is usually very rapid. Symptoms can occur within 1 to 6 hours after consuming contaminated food. Duration of illness can last 1 to 2 days (FDA, 2014; ISU, 2010).

**Vibrio cholera.** *Vibrio cholera* is a bacterium that grows naturally in estuarine environments, where fresh water from rivers flows into salt water from the oceans. It causes cholera, a disease that can lead to death if untreated. Symptoms are often absent or mild. Some people may develop severe diarrhea, vomiting, and leg cramps. Loss of body fluids can cause dehydration and shock. Without proper treatment, death can occur within hours. Raw and undercooked seafood is the major source of contamination. Incubation may range from 6 hours to 5 days after eating contaminated food. Illness can last 3 to 7 days (FDA, 2014; ISU, 2010).

**Vibrio parahaemolyticus.** *Vibrio parahaemolyticus* is a bacterium that lives in saltwater and can cause gastrointestinal illness in humans. Symptoms of infection include diarrhea, stomach cramps, nausea, vomiting, headache, fever, and chills. Raw or undercooked fish and shellfish are the main source of contamination. Incubation ranges from 4 to 96 hours after ingesting contaminated food. Duration of illness varies from 2 to 5 days (FDA, 2014; ISU, 2010).

**Vibrio vulnificus.** *Vibrio vulnificus* is a bacterium that lives in warm seawater. It can not only infect people who eat contaminated seafood but those who have an open wound exposed to seawater. Symptoms of infection include diarrhea, stomach pain, nausea, vomiting, fever, and
sudden chills. Some victims may develop blister-like sores on their legs. Possible sources of contamination are raw fish and shellfish, especially raw oysters. Incubation takes 1 to 7 days after eating contaminated food or exposure to the bacteria. Illness can take 2 to 8 days (FDA, 2014; ISU, 2010).

*Yersinia enterocolitica.* *Yersinia enterocolitica* is a bacterium that causes yersiniosis, a disease characterized by fever, diarrhea, vomiting, and stomach pain. The symptoms may be more severe for children than adults. Possible sources of contamination include raw meat and seafood, dairy products, produce, and untreated water. Incubation usually takes 1 to 2 days after eating contaminated food and illness can last 1 to 3 weeks (FDA, 2014; ISU, 2010).

**Epidemiology.** Foodborne illness caused by foodborne pathogens contaminating the food supply frequently remind us of the fact that animal, plant, and microbial populations are linked with human by a complex food web (Behravesh, Williams, & Tauxe, 2012). Foodborne illness is common and costly public health problem (CDC, 2012). In less developed countries, foodborne pathogens are responsible for killing approximately 1.8 million people annually. In developed countries, foodborne pathogens result in millions of cases of infectious gastrointestinal diseases every year.

Foodborne pathogens are the leading causes of illness and death in less developed countries killing approximately 1.8 million people annually. In developed countries, millions of cases of infectious gastrointestinal diseases are caused by foodborne pathogens each year. Foodborne illness costs billions of dollars in medical care and lost productivity. (Fratamico, Bhunia, & Smith 2005).

The United States is famous for its extremely strict and safe food supply system. However, approximately 46 million cases of foodborne illness occur each year, along with about
250,000 hospitalizations and 3,000 deaths (Behravesh et al., 2012; ISU, 2010). Each year, for every six Americans consuming foods or beverages, one will get sick because the foods or beverages are contaminated (CDC, 2012). Every consumer is at risk. However, the most vulnerable populations are the very young, the elderly, and those with compromised immune systems (Behravesh et al., 2012).

There are many different foodborne diseases because many different types of pathogens can contaminate foods. More than 250 different foodborne diseases have been identified and described (CDC, 2012). According to CDC’s 2011 estimates for foodborne illness, the CDC was able to compute the estimates for two major groups of foodborne illnesses: known foodborne pathogens and unspecified agents (Table 3) (CDC, 2014). Known foodborne pathogens include 31 pathogens that have been identified as the cause of foodborne illnesses. These pathogens have been investigated by public health systems that can track diseases and outbreaks. Unspecified agents include agents with insufficient data, known agents not yet identified as pathogens, agents whose ability to cause illness is unproven, and agents not yet identified (CDC, 2014). The CDC estimated the number of illnesses caused by top five foodborne pathogens (Table 4). The CDC also estimated the number of hospitalizations and deaths caused by these illnesses (Table 5, Table 6).
Table 3

*Estimated Annual Number of Domestically Acquired, Foodborne Illnesses, Hospitalizations, and Deaths Due to 31 Pathogens and Unspecified Agents Transmitted Through Food, United States*

<table>
<thead>
<tr>
<th>Foodborne Agents</th>
<th>Estimated annual illnesses</th>
<th>%</th>
<th>Estimated annual hospitalizations</th>
<th>%</th>
<th>Estimated annual deaths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 Known</td>
<td>9.4 million</td>
<td>20</td>
<td>55,961</td>
<td>44</td>
<td>1,351</td>
<td>44</td>
</tr>
<tr>
<td>Unspecified agents</td>
<td>38.4 million</td>
<td>80</td>
<td>71,878</td>
<td>56</td>
<td>1,686</td>
<td>56</td>
</tr>
<tr>
<td>Total</td>
<td>47.8 million</td>
<td>100</td>
<td>127,839</td>
<td>100</td>
<td>3,037</td>
<td>100</td>
</tr>
</tbody>
</table>

(CDC, 2014)

Table 4

*Top Five Pathogens Contributing to Domestically Acquired Foodborne Illnesses*

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Estimated number of illnesses</th>
<th>90% Credible Interval</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norovirus</td>
<td>5,461,731</td>
<td>3,227,078–8,309,480</td>
<td>58</td>
</tr>
<tr>
<td><em>Salmonella</em>, nontyphoidal</td>
<td>1,027,561</td>
<td>644,786–1,679,667</td>
<td>11</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>965,958</td>
<td>192,316–2,483,309</td>
<td>10</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>845,024</td>
<td>337,031–1,611,083</td>
<td>9</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>241,148</td>
<td>72,341–529,417</td>
<td>3</td>
</tr>
<tr>
<td>Subtotal</td>
<td>91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(CDC, 2014)
Table 5

*Top Five Pathogens Contributing to Domestically Acquired Foodborne Illnesses Resulting in Hospitalization*

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Estimated number of hospitalizations</th>
<th>90% Credible Interval</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em>, nontyphoidal</td>
<td>19,336</td>
<td>8,545–37,490</td>
<td>35</td>
</tr>
<tr>
<td>Norovirus</td>
<td>14,663</td>
<td>8,097–23,323</td>
<td>26</td>
</tr>
<tr>
<td><em>Campylobacter spp.</em></td>
<td>8,463</td>
<td>4,300–15,227</td>
<td>15</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>4,428</td>
<td>3,060–7,146</td>
<td>8</td>
</tr>
<tr>
<td><em>E. coli</em> (STEC) O157</td>
<td>2,138</td>
<td>549–4,614</td>
<td>4</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td></td>
<td><strong>88</strong></td>
<td></td>
</tr>
</tbody>
</table>

(CDC, 2014)

Table 6

*Top Five Pathogens Contributing to Domestic Foodborne Illnesses Resulting in Death*

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Estimated deaths</th>
<th>90% Credible Interval</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em>, nontyphoidal</td>
<td>378</td>
<td>0–1,011</td>
<td>28</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>327</td>
<td>200–482</td>
<td>24</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>255</td>
<td>0–733</td>
<td>19</td>
</tr>
<tr>
<td>Norovirus</td>
<td>149</td>
<td>84–237</td>
<td>11</td>
</tr>
<tr>
<td><em>Campylobacter spp.</em></td>
<td>76</td>
<td>0–332</td>
<td>6</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td></td>
<td><strong>88</strong></td>
<td></td>
</tr>
</tbody>
</table>

(CDC, 2014)
Besides the top foodborne pathogens discussed above, new foodborne pathogens and foodborne illnesses are emerging. Driving factors include pathogen evolution, changes in agricultural and food manufacturing practices. In addition to food safety and quality concerns, food security concerns are growing. It is possible for terrorists to use foodborne pathogens to contaminate food and water supplies in attempts to infect thousands of people and thus to disrupt society stability and economic growth. Driven by these concerns, extensive research into the genomics, molecular biology and food microbiology of the most important foodborne pathogens has been done in recent years. (Fratamico et al., 2005)

**Salmonella as foodborne pathogens.** In this section, definition and nomenclature of *Salmonella*, symptoms and pathogenesis of Salmonellosis, as well as sources of *Salmonella* contamination will be discussed.

**Definition and nomenclature.** *Salmonella* is a genus of rod-shaped, Gram-negative bacteria. *Salmonella* are non-spore-forming enterobacteria with diameters around 0.7 to 1.5 µm and lengths from 2 to 5 µm. They are predominately motile with peritrichous flagella around the cell body. They are chemoorganotrophs, which means they can obtain energy from oxidation and reduction reactions using organic sources. *Salmonella* are facultative anaerobes; they can survive with or without oxygen. In the presence of oxygen, *Salmonella* can obtain energy via aerobic respiration. If oxygen is absent, they can switch to fermentation or anaerobic respiration (Fabrega & Vila, 2013).

This group of bacteria consists of two species: *Salmonella bongori* and *Salmonella enterica*. The latter is divided into 6 subspecies: *Salmonella enterica* subspecies *enterica*, *Salmonella enterica* subspecies *arizonae*, *Salmonella enterica* subspecies *diarizonae*, *Salmonella enterica* subspecies *houtenae*, *Salmonella enterica* subspecies *indica*, and *Salmonella enterica* subspecies *indica*. 
subspecies *salamae*. Each subspecies consists of numerous serotypes that differ by antigenic specificity. More than 2,500 different *Salmonella* serotypes have been identified for *Salmonella enterica* (Gilbert, Lake, Cressy, & King, 2010; WHO, 2013).

Most pathogenic *Salmonella* serotypes isolated from humans and other mammals belong to *Salmonella enterica* subspecies *enterica*. Other subspecies of *Salmonella enterica* and *Salmonella bongori* are more common in cold blooded animals. They are of much lower pathogenicity towards humans and livestock (Gilbert et al., 2010).

The *Salmonella* serotypes that are pathogenic to humans can be divided into two groups: typhoidal and nontyphoidal serotypes. Nontyphoidal serotypes are more common than typhoidal serotypes. Nontyphoidal serotypes are usually associated with foodborne illnesses, especially causing gastrointestinal diseases. They are zoonotic, which means they can infect a range of animals and can be transferred between humans and other animals. The most important and most studied nontyphoidal serotypes are *Salmonella enteritidis* and *Salmonella typhimurium* (Gilbert et al., 2010; WHO, 2013). Typhoidal serotypes are strictly adapted to invasion and survival in human tissues. They do not occur in other animals. Typhoidal serotypes can cause typhoid fever, a serious enteric fever. Typical typhoidal serotypes of *Salmonella* are *Salmonella typhi* and *Salmonella paratyphi* (Gilbert et al., 2010).

**Symptoms and pathogenesis.** Salmonellosis is an infection caused by the bacteria *Salmonella*. Typical symptoms include acute onset of fever, abdominal pain, diarrhea, nausea and sometimes vomiting. Infection usually occurs when people consume foods that are contaminated with a relatively high concentration of the bacteria. Infection can also occur when infants and young children ingest a small number of the bacteria because they are much more susceptible to *Salmonella* (FDA, 2014). The onset of symptoms usually occurs 12 to 72 hours
after ingestion of *Salmonella* contaminated food. Illness can last 2 to 7 days. Although in most cases, symptoms of salmonellosis are relatively mild and victims can recover without any treatments, the associated dehydration can lead to death in at-risk groups such as the young, the elderly, and people with compromised immune systems (FDA, 2014; WHO, 2013).

Certain proteins secreted by *Salmonella enterica* play an important role in its pathogenesis. *Salmonella* cells contain a considerable number of fimbrial and non-fimbrial adhesins. These adhesins can facilitate biofilm formation and attachment to host cells. Host cell invasion and intracellular proliferation, the two hallmarks of *Salmonella* pathogenesis, are mediated by the secreted proteins (Hensel, 2009).

**Sources of Salmonella contamination.** Transmission routes of *Salmonella* include person to person transmission, consumption of contaminated foods or beverages, animal contact, and exposure to a contaminated environment (Gilbert, et al., 2010).

**Human.** Person to person transmission of *Salmonella* is well recognized. Feces samples were taken from patients who are recovering from *Salmonella* infection. A high number of *Salmonella* per gram of feces (approximately 106 CFU/g) were observed and the high count persisted as long as 10 days after initial diagnosis. The counts of *Salmonella* in feces samples did not reduce to less than 100 CFU/g until 35 to 40 days after the initial diagnosis. Thus, it is not surprising that the fecal-oral route is the most common transmission routes of *Salmonella* (Gilbert, et al., 2010).

**Animal.** *Salmonella* can be found in a wide range of animals including mammals, fish, reptiles, amphibians, insects and birds. In most cases, *Salmonella* colonizations in animals produce no pathogenic symptoms. However, individuals in contact with infected animals or their feces can develop salmonellosis (Gilbert et al., 2010; WHO, 2013). Certain serotypes of
Salmonella are strictly confined to particular animal reservoirs. For example, Salmonella enterica serotype cholerae-suis is restricted to host pigs. Other serotypes such as Salmonella typhimurium are responsible for gastrointestinal infections in a wide range of phylogenetically unrelated species (Gilbert, et al., 2010; Swanson, Snider, & Braden, 2007). It is worth noting that domestic cats and pet rodents have also been shown to be sources of Salmonella infection (Swanson et al., 2007).

**Food.** Raw and undercooked eggs, raw meat, poultry, seafood, raw milk, dairy products, and produce are the most common sources of Salmonella contamination. However, a much wider variety of other foods have been associated with Salmonella outbreaks. Other foods that have been contaminated with Salmonella include nut products, cereal products, spices, oilseed products, chocolate, cocoa powder, dried yeast, and candies (Gilbert, et al., 2010).

**Environment.** Salmonella are ubiquitous and robust bacteria that can survive several weeks in dry environments and several months in moist environments (WHO, 2013). Pasture, soil, and water can be contaminated by Salmonella from sewage effluents and animal feces. They are capable of maintaining viable in soil for months. Handling and processing of infected animals may generate dust and aerosols that contain Salmonella (Gilbert, et al., 2010). It is worth noting that vacuum cleaner dust bags can serve as a reservoir of Salmonella (Hayson & Sharp, 2003).

**Foodborne illness outbreak.** In this section, definition of foodborne illness outbreak, outbreak detection, and outbreak involving Salmonella will be discussed.

**Definition.** According to the CDC (2012), an outbreak of foodborne illness occurs when a group of people consume the same contaminated food or beverage and two or more of them become sick with the same illness. For a foodborne illness outbreak to occur, a batch of food
must have been contaminated and then eaten by the victims. These outbreak victims may be a group of friends who ate dinner together at the same restaurant, or they can be complete strangers who happened to purchase and eat the same contaminated food from a grocery store (CDC, 2012). A typical series of events contributing to an outbreak include contaminated food being left out at room temperature for too long, bacterial proliferation, and insufficient cooking.

**Outbreak detection.** Outbreaks of foodborne illness can be detected in many ways. A person realizes that many people who went to the same catering event have become sick and the local health department is informed. A physician realizes unusually high number of patients show up with the same illness. A county health department receives an unusually large number of reports of certain illness. Outbreak spreading over a very large geographic region can be very difficult to detect. Surveillance reports at regional or national level are combined to search for unusual increases in infections of a specific serotype of pathogen. For example, state public health laboratories always determine the serotype of *Salmonella* isolated from patients. Emerging DNA fingerprinting technologies allow easier and faster detection of outbreaks. PulseNet, a molecular subtyping network has been used by state laboratories and the CDC to compare serotypes of pathogens from coast to coast to detect widespread outbreaks.

**Outbreak involving *Salmonella.*** *Salmonella* are responsible for many foodborne illness outbreaks. According to the CDC (2014), *Salmonella* outbreaks have been linked to many different types of foods, including poultry, beef, cheese, eggs, nuts, peanut butter, raw produce, wheat cereals, and pies.

*Salmonella* outbreak linked to pie. A *Salmonella* outbreak case was defined as infection with a *Salmonella* serotype I 4,5,12:i:- sharing the same pulsed-field gel electrophoresis (PFGE) pattern was detected by the Pennsylvania Department of Health and reported to PulseNet.
Subsequent investigation of the outbreak by local, state health departments and the CDC determined that 401 cases of salmonellosis were identified in 41 states during 2007 (Figure 6) (CDC, 2007). A multistate case-control study was conducted and the result showed that the outbreak was linked to consumption of Banquet® brand frozen, not-ready-to-eat pot pies (p<0.001). All meat ingredients in the contaminated pot pies were precooked before leaving the manufacturing plant. However, these pot pies had a raw flour pie crust, which was possibly the source of Salmonella contamination. Although an intensive investigation of the plant and its ingredient suppliers was conducted, the CDC (2007) was not able to determine the exact source of contamination.

Figure 6. Multistate outbreak of Salmonella I 4,[5],12:i:- infections linked to Banquet® pot pies. Between January 1, 2007 and October 29, 2007, at least 401 isolates of Salmonella I 4,[5],12:i:- with an indistinguishable genetic fingerprint have been collected from ill persons in 41 states.
The number of ill persons whose *Salmonella* strain has this genetic fingerprint has been reported from the highlighted states. Their ages range from <1 to 89 years with a median age of 18 years; 51% of ill persons are female. At least 65 people have been hospitalized. No deaths have been reported (CDC, 2007).

*Salmonella outbreak traced to flour.* Cereal grains can be easily contaminated with *Salmonella* from animal or human feces. Rodents and birds are the main sources of contaminations after harvest if the storage is inadequate. Although *Salmonella* does not grow in flours or on cereal grains owing to low water activity, *Salmonella* can remain viable for months. It has been reported that low water activity of cereal grain products can encourage heat resistance in *Salmonella* (Gilbert et al., 2010).

In developed countries, cereal grains and flours processing are confidently rendered *Salmonella*-free. Any residual risk is generally associated with consuming minimally processed, raw or underbaked dough, in which *Salmonella* can survive. Growth may even be possible when wet ingredients such as water or milk are added. Great examples are baking mixture to be baked at home such as cookie dough and pie dough. Consumption of raw flour dough is quite popular, especially during home baking or activities with homemade play-dough, in North American and New Zealand (Gilbert et al., 2010).

Over 30 years ago, Australian, European, and U.S. researchers detected *Salmonella* in soy, rye, and wheat flours at low levels (Aydin, Paulsen, & Smulders, 2009; Phyllis, 2008). In recent years, according to researchers in Australia and North America, the prevalence of *Salmonella* contamination in wheat flour was as low as 0.0-1.3% (Gilbert et al., 2010). There is almost no information on the prevalence of *Salmonella* contamination in other flours or on other cereal grains. Despite the fact that little information is available on the concentration of
Salmonella in flours or on cereal grains, the concentration is suspected to be very low (Gilbert et al., 2010).

The New Zealand Food Safety Authority detected a Salmonella outbreak that occurred between October 13th 2008 and January 2th 2009. A case-control study was conducted and the results indicated that the Salmonella outbreak was linked to wheat flour. A total of 75 outbreak cases associated with Salmonella enterica serotype typhimurium phage type 42 were identified. Molecular analysis results showed that 67 isolates of the 75 were the same strain. About 24% cases were not older than age 4 and 76% of cases were females. Twelve cases were hospitalized and there were no fatalities (Gilbert et al., 2010; Phyllis, 2008). Interviewing outbreak victims showed that many victims ate uncooked flour mixture such as cookie dough.

The flour samples analyzed in this outbreak investigation contained low counts of Salmonella, pointing out the possibility that the risk of infection can still be high when relatively few cells are consumed. It is also possible that the distribution of contamination was not homogenous and thus the concentration of Salmonella in the actual flour batch was much higher. Therefore, although the risk of human salmonellosis due to contaminated flour can be classified as low, Salmonella outbreak linked to flour contamination has the potential to affect a great number of people, especially those who desire ingestion of raw baking mixture (Gilbert et al., 2010; Phyllis, 2008).

Xanthan Gum

Definition. Xanthan gum is a natural polysaccharide that belongs to a group of substances called hydrocolloids. Xanthan gum is an important food additive; it is also of great importance in many other non-food products (Garcia et al., 2000; Moncel, 2014). In the 1950s, xanthan gum was discovered by researchers at the Northern Regional Research Laboratories
Extensive research on xanthan gum was conducted during the 1960s and large-scale commercial production started in 1964 (Garcia-Ochoa et al., 2000). However, it was not approved for use as a food additive in the U.S. and Europe until 1968 (Weingarten, 2010).

Xanthan gum is produced by the bacterium *Xanthomonas campestris* NRRL B-1459 via aerobic fermentation (Garcia-Ochoa et al., 2000; Moncel, 2014). As illustrated in Figure 7 (Sharma, Naresh, Dhuldhoya, & Merchant, 2006), xanthan gum is a heteropolysaccharide, a polymer consisting of repeated pentasaccharide monomers. One pentasaccharide monomer is comprised of 5 sugar units: two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.8: 2.0: 2.0. The backbone chain of xanthan gum is made of b-D-glucose units linked at the 1 and 4 positions, the chemical structure of which is identical to that of cellulose. Trisaccharide side chains, consisting of a D-glucuronic acid unit between two D-mannose units, are linked to every other glucose unit in the backbone chain at the O-3 position. A pyruvic acid residue is observed in about 50% of the terminal D-mannose. However, the exact distribution can vary depending on the substrain of *Xanthomonas campestris* used and the conditions of fermentation (Cargill, 2014; Garcia-Ochoa et al., 2000). All D-mannose units linked to the backbone chain contain an acetyl group at the position O-6 (Garcia-Ochoa et al., 2000; Whitcomb & Macosko, 1978). Xanthan gum is an anionic polysaccharide with highly negative charge due to the presence of glucuronic and pyruvic acid residues (Cargill, 2014; Garcia-Ochoa et al., 2000). The molecular weight of xanthan gum can range from $2 \times 10^6$ to $20 \times 10^6$ Daltons. This molecular weight distributions depends primarily on the interactions between different polymer chains and the formation of aggregates, which is in turn determined by the variations of fermentation conditions (Garcia-Ochoa et al., 2000).
Functionalities and applications. Xanthan gum is widely used in food and non-food industries owing to its rheological properties, stability, and compatibility (Table 7) (Garcia-Ochoa et al., 2000). Cargill (2014) recognized xanthan gum as one of the most successful hydrocolloids, especially in harsh environments such as acid, base, high salt, high temperature, low temperature, and high shear stress. Xanthan gum is acid and base stable and it can dissolve in most acids and bases. The stability of xanthan gum solution is generally not affected by changes in pH. Xanthan gum is salt tolerant, such that addition of large amounts of salt does not affect the viscosity and stability of xanthan gum solutions. Xanthan gum also exhibits good heat stability and freeze/thaw stability (Cargill, 2014).
Table 7

*Main Industrial Applications of Xanthan Gum*

<table>
<thead>
<tr>
<th>Application</th>
<th>Concentration (% w/w)</th>
<th>Functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salad dressings</td>
<td>0.1 - 0.5</td>
<td>Emulsion stabilizer; suspending agent, dispersant</td>
</tr>
<tr>
<td>Dry mixes</td>
<td>0.05 - 0.2</td>
<td>Eases dispersion in hot or cold water</td>
</tr>
<tr>
<td>Syrups, toppings, relishes, sauces</td>
<td>0.05 - 0.2</td>
<td>Thickener; heat stability and uniform viscosity</td>
</tr>
<tr>
<td>Beverages</td>
<td>0.05 - 0.2</td>
<td>Stabilizer</td>
</tr>
<tr>
<td>(fruit and non-fat dry milk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy products</td>
<td>0.05 - 0.2</td>
<td>Stabilizer; viscosity control of mix</td>
</tr>
<tr>
<td>Baked goods</td>
<td>0.1 - 0.4</td>
<td>Stabilizer; facilitates pumping</td>
</tr>
<tr>
<td>Frozen foods</td>
<td>0.05 - 0.2</td>
<td>Improves freeze ±thaw stability</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>0.1 - 1</td>
<td>Emulsion stabilizer; uniformity in dosage formulations</td>
</tr>
<tr>
<td>(creams and suspensions)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cosmetic (denture cleaners, shampoos, lotions)</td>
<td>0.2 - 1</td>
<td>Thickener and stabilizer</td>
</tr>
<tr>
<td>Agriculture (additive in animal feed and pesticide formulations)</td>
<td>0.03 - 0.4</td>
<td>Suspension stabilizer; improved sprayability, reduced drift, increased cling and permanence</td>
</tr>
<tr>
<td>Textile printing and dyeing</td>
<td>0.2 - 0.5</td>
<td>Control of rheological properties of paste; preventing dye migration</td>
</tr>
</tbody>
</table>
Ceramic glazes 0.3 - 0.5 Prevents agglomeration during grinding

Slurry explosives 0.3 - 1.0 Thickens formulations; improves heat stability (in combination with guar gum)

Petroleum production 0.1 - 0.4 Lubricant or friction reduction in drill-hole

Enhanced oil recovery 0.05 - 0.2 Reduces water mobility by increasing viscosity and decreasing permeability

(Garcia-Ochoa et al., 2000)

Xanthan gum is generally compatible with other hydrocolloids such as locust bean gum and guar gum, exhibiting synergistic effects in food applications (Cargill, 2014). For example, when xanthan gum is mixed with locust bean or guar gum, the viscosity of the resultant mixture is much more than that when either one is used alone. Due to such synergistic increase in viscosity, less amount of each hydrocolloid can be used (Cargill, 2014; Garcia-Ochoa et al., 2000; Weingarten, 2010).

**Thickening agent.** The two most important properties of xanthan gum are its thickening and stabilizing abilities. As an effective thickening agent, xanthan gum is able to increase the viscosity of a liquid dramatically by adding at low concentrations, 0.05-2%. The thickening power of xanthan gum is primarily owing to its high molecular weights and branched polymer structure (Cargill, 2014; Garcia-Ochoa et al., 2000; Weingarten, 2010). Food applications of xanthan gum as a thickening agent include syrups, toppings, relishes, and sauces (Table 7) (Garcia-Ochoa et al., 2000). Many fat-free salad dressings use xanthan gum as a thickening agent to maintain viscosity (Weingarten, 2010). The shear forces generated by shaking and mixing can decrease the viscosity of the salad dressing thickened with xanthan gum. As a result, the salad dress can be easily poured. Once the shaking is stopped and shear forces removed, the salad
dressing will turn thick again. This property of xanthan gum is referred to as shear thinning or pseudoplasticity (Cargill, 2014; Garcia-Ochoa et al., 2000; Weingarten, 2010). Xanthan gum addition does not alter the color or flavor of foods or beverage at low concentration. Therefore, it is a perfect choice of thickening agent for those with swallowing disorders (Logsdon, 2014).

**Stabilizing agent.** Although not strictly an emulsifier, xanthan can help to prevent oil separation by stabilizing the emulsion. Typical food applications are salad dressings and sauces. In suspension systems, xanthan gum can help suspend the solid particles such as spices (Garcia-Ochoa et al., 2000). In ice creams, xanthan gum helps to stabilize and prevent ice crystal formations, ensuring a pleasant and smooth texture. Xanthan gum is also added to thicken and stabilize commercial egg substitutes (Logsdon, 2014).

**Gluten substitute.** Xanthan gum can form high-viscosity pseudoplastic material. This property of xanthan gum plays an important role in developing gluten free products, especially during dough preparation. When combined with water, hydrocolloids such as xanthan gum can mimic rheological effect of gluten in gluten-free systems. Thus, xanthan gum is often used as a gluten substitute in gluten-free products (Ahlborn, Pike, Hendrix, Hess, & Huber, 2005).

**Pharmacological and biological activities.** It has been reported that xanthan gum is non-toxic to three studied animals, rat, cat, and dog (Khan, & Abourashed, 2011). Although no complete toxicity or safety data for humans is available, xanthan gum is non-sensitizing and is not a skin or eye irritant (Garcia-Ochoa et al., 2000; Khan, & Abourashed, 2011). Xanthan gum has been reported to be synergistically active with 5-fluorouracil or bleomycin on inhibiting the growth of Ehrlich ascites tumor and S-180 in mice (Khan, & Abourashed, 2011). Enamel saving effect was observed for xanthan gum. Addition of xanthan gum to black currant soft drinks
Antimicrobial activities and water binding capacity. Little information is available on the antimicrobial activity of xanthan gum. Xanthan is non-toxic and will not suppress microbial growth directly (Garcia-Ochoa et al., 2000). According to Cargill (2014), xanthan gum offers superior water binding properties. Sánchez, Bartholomai, and Pilosof (1995) measured the water binding capacity of several food gums including xanthan gum, guar gum, sodium alginate, propylene glycol alginate, and locust bean gum. The results indicated that xanthan gum was by far the most effective water binder (232 ml/g) and the second best water binder was guar gum (40 ml/g).

Whether a microbe grows or not is determined by water activity. Microbial growth can be controlled by controlling water activity (AquaLab, 2014). In food science, water activity or $a_w$ is defined as the partial vapor pressure of water in a food product divided by the standard state partial vapor pressure of pure water at the same temperature. It can also be defined as the equilibrium relative humidity (ERH) divided by 100. Common foodborne pathogens and spoilage organisms and their water activity limits for growth are listed in Table 8 (AquaLab 2014).
Table 8

*Common Spoilage Organisms and Their $a_w$ Limits for Growth*

<table>
<thead>
<tr>
<th>Microbial Group</th>
<th>Example</th>
<th>$a_w$</th>
<th>Products Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal bacteria</td>
<td><em>Salmonella</em> species</td>
<td>0.91</td>
<td>Fresh meat, milk</td>
</tr>
<tr>
<td></td>
<td><em>Clostridium botulinum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal yeast</td>
<td>Torulopsis species</td>
<td>0.88</td>
<td>Fruit juice concentrate</td>
</tr>
<tr>
<td>Normal molds</td>
<td><em>Aspergillus flavus</em></td>
<td>0.80</td>
<td>Jams, jellies</td>
</tr>
<tr>
<td>Halophilic bacteria</td>
<td><em>Wallemia sebi</em></td>
<td>0.75</td>
<td>Honey</td>
</tr>
<tr>
<td>Xerophilic molds</td>
<td><em>Aspergillus echinulatas</em></td>
<td>0.65</td>
<td>Flour</td>
</tr>
<tr>
<td>Osmophilic yeast</td>
<td><em>Saccharomyces bisporus</em></td>
<td>0.60</td>
<td>Dried fruits</td>
</tr>
</tbody>
</table>

(AquaLab, 2014)
Chapter III: Methodology

Pie Dough Preparation

**Ingredients and additives.** The pie dough formula was developed through extensive researches on the ingredient functionalities and dough sheetability (Appendix A). Pie dough was made with unbleached organic all-purpose flour (Gold Medal, Minneapolis, MN), granulated white cane sugar (C&H, Yonkers, NY), salt (Morton, Chicago, IL), unsalted butter (Great Value, Bentonville, AR), and water (tap water, UW-Stout, Menomonie, WI). The variable hydrocolloid added to this sweet short pie dough was xanthan gum obtained from Ener-G (Seattle, WA).

**Formulation and dough making process.** Dry ingredients, unbleached organic flour, sugar, and salt, were mixed together in a large bowl. Xanthan gum was added to the dry mixture at 0% by total dry weight for the control, and 1% and 2% for treatment 1 (T1) and treatment 2 (T2), respectively. A summary of dry ingredient formulations for control and both treatments were listed (Table 9). Unsalted butter, kept very cold, was diced into ½-inch cubes and sprinkled over the dry ingredients mix. Pastry cutter was used to cut the butter into the dry mixture until the butter pieces were the size of tiny peas and distributed evenly. Ice cold water was prepared beforehand and 180 ml of ice cold water was drizzled over the butter and flour mixture. A rubber spatula was used to mix the dry and wet ingredients together to form premature dough. The dough was then gently kneaded by hand and relaxed for 2 hours at 4 °C.
Table 9

Dry Ingredient Formulations of Pie Dough for Control and Treatments

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control (%)</th>
<th>T1 (%)</th>
<th>T2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt</td>
<td>0.89</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>Sugar</td>
<td>2.68</td>
<td>2.68</td>
<td>2.68</td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Unsalted Butter</td>
<td>40.18</td>
<td>40.18</td>
<td>40.18</td>
</tr>
<tr>
<td>Flour</td>
<td>56.25</td>
<td>55.25</td>
<td>54.25</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Sample preparation. Eighty-four sterile 50 ml centrifuge tubes were divided into 3 different groups and labeled accordingly, “C” for control, “1%” for 1% xanthan gum addition, and “2%” for 2% xanthan gum addition. About 10 gram of pie dough was weighed out and placed into the centrifuge tube. Pie dough sample was packed tightly into the bottom of the tube by using a sterile disposable 10 ml pipette.

Microbiology Study Preparation

Tryptic soy yeast extract broth (TSYEB). In 1000 ml of deionized (DI) water, 36 grams of TSYEB was added. The suspension was frequently agitated and boiled to dissolve completely. The mixture was then sterilized by autoclaving at 121 °C for 15 minutes. The final pH should be within 7.3±0.2.

Buffered peptone water (BPW). BPW was prepared by adding 10 grams of BPW powder to 500 ml of DI water. The mixture was shaken to dissolve completely and sterilized by
autoclaving at 121 °C for 15 minutes. Serial dilution blanks were prepared by pipetting 9 ml of BPW into sterile test tubes.

**Xylose lysine deoxycholate agar (XLD agar).** In 1000 ml of DI water, 55 grams of XLD agar powder was added. The suspension was frequently agitated and boiled to dissolve completely. The mixture was then divided into 2 500 ml Pyrex® agar bottles and sterilized by autoclaving at 121 °C for 15 minutes. XLD agar plates were prepared by pouring the hot agar onto petri dishes. XLD agar plates were ready as the agar solidified after cooling down. Extra plates were stored at refrigeration temperature.

**Salmonella Inoculum Preparation**

**Initial culture.** Three different strains of *Salmonella enterica* serotype *typhimurium* were used in this study. Their American Type Culture Collection (ATCC) numbers were 25241, 14028, and 13311. Each strain was previously streaked for isolation and thus available in the form of colonies on a petri dish. The initial culture for each strain was prepared by mixing 1-2 colonies with 10 ml of TSYEB. The mixture was then incubated at 37 °C for 24 hours.

**Subculture.** The subculture for each strain was prepared by pipetting 100 µl of initial culture into a fresh 25 ml of TSYEB. The mixture was vortexed to mix well and then incubated at 37 °C for 24 hours.

**Inoculum cocktail.** The 25 ml subculture of each strain was centrifuged at 3500 rpm for 15 mins. The pellet was retained. The supernatant was sterilized by autoclaving and then discarded. The pellet was washed with 0.85% saline solution and the solution discarded following autoclaving. Exactly 10 ml of 0.85% saline solution was pipetted into the tube and vortexed to resuspend the pellet. The inoculum cocktail was prepared by exactly pipetting 1 ml of each strain into 27 ml of 0.85% saline solution. The mixture was vortexed to mix thoroughly.
Sample Inoculation

**Inoculation.** Samples were inoculated by pipetting exactly 100 µl of inoculum onto the 10 grams of tightly packed pie dough at the bottom of the centrifuge tube. All samples were inoculated except two which were uninoculated to serve as negative control.

**Determination of day 0 Salmonella population.** After inoculation, 10 ml of BPW was pipetted into the sample tube. The tube was vortexed vigorously to obtain a well-mixed suspension. Serial dilution with 5 dilution blanks was conducted. Diluted samples with appropriate dilution factors were plated by pipetting 100 µl of sample onto XLD agar plates. A sterile cell spreader was used to evenly distribute bacterial cells on the agar plates. The XLD agar plates were then incubated at 37 °C for 24 hours. The plates were counted and the original *Salmonella* population in the original sample tube was calculated.

**21-Day Incubation at Refrigeration Temperature**

Pie dough samples were incubated at 4 °C for a period of 21 days. Pie dough samples were analyzed in triplicates for each treatment every 3 days on day 3, 6, 9, 12, 15, 18, and 21. Serial dilution, plating, incubation, plate counting, and calculation were performed to determine the *Salmonella* population of each day’s samples.

**Water Activity Measurement**

Water activity measurements were taken at day 10. Water activity measurement samples were prepared in triplicates by placing approximately 1 gram of pie dough sample onto the center of a water activity meter sample cup. The sample cup was placed inside an AquaLab water activity meter (AquaLab Series 3TE) to take measurements at 25 °C.
Microbiological Identification

Microbiological identification procedures were performed to identify unknown contamination bacteria appearing on XLD agar plates.

**Colony morphology.** Visual inspection of different colonies on XLD agar plates was performed. Colony morphological parameters such as size, shape, color, and opacity were recorded.

**Gram staining and microscopic examination.** Gram staining and microscope were used to determine the shape of unknown bacteria and whether they were gram positive or gram negative.

**Oxidase strip test.** Oxidase strip (Oxoid Ltd, Hants, UK) test was performed to determine whether the unknown bacteria were oxidase positive or oxidase negative.

Statistical Analysis

Significant differences in mean log reduction of *Salmonella* population among treatments (0% xanthan gum control, 1% xanthan gum, and 2% xanthan gum) were analyzed by using single-factor Analysis of Variance (ANOVA). Significant pairwise difference among treatment means was analyzed by using paired t-tests assuming equal variance. All statistical analyses were done at a significance level of 0.05 (α=0.05). All statistical procedures were computed using Excel 2010 version 14.0.4760.1000 (Microsoft Corporation, Redmond, Washington).
Chapter IV: Results

The purpose of this challenge study was to investigate the efficacy of xanthan gum against *Salmonella* survival in refrigerated pie dough. Population of *Salmonella* in pie dough was measured on inoculation day (day 0) and was measured every 3 days for a period of 21 days at refrigeration temperature 4 °C.

21-Day Incubation

**Change of *Salmonella* population.** The *Salmonella* population is shown as $\log_{10}$ (CFU/g), where CFU/g is the number of colony forming unit per gram of pie dough sample. Base 10, also known as common base, is widely used in microbiological science. A column chart was used to show the change of *Salmonella* population over a period of 21 days and also to illustrate comparison among different treatments. A generally decreasing trend with storage time was observed in *Salmonella* population for all treatment groups (Figure 8).

![Figure 8. Surviving *Salmonella* count (CFU/g) in refrigerated pie dough with varying levels of xanthan gum (0% control, 1%, and 2% w/dw) during 21-day storage period at 4 °C.](image-url)
**Log reduction.** Log reduction is the bacterial population reduction calculated in log. It was calculated by subtracting day-21 population in log from day-0 population in log. The mean 21-day log reductions were reported and the results obtained by performing a single-factor ANOVA demonstrated that there was no significant difference (p>0.05) in log reduction between control, 1%, and 2% xanthan gum treatment groups (Table 10).

Table 10

*Mean Log Reduction Values of Pie Dough Treatments with Increasing Levels of Xanthan Gum Concentrations (0%, 1%, and 2%) after 21 Days Incubation at Refrigeration Temperature 4 °C. Same Superscripts Are Not Significantly Different as Measured Using Single-Factor ANOVA*

<table>
<thead>
<tr>
<th>Xanthan Gum</th>
<th>21-Day Mean Log Reduction</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0%</td>
<td>0.599&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00827</td>
</tr>
<tr>
<td>1%</td>
<td>0.657&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04322</td>
</tr>
<tr>
<td>2%</td>
<td>0.675&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05798</td>
</tr>
</tbody>
</table>

F (2, 6)=0.88, p>0.05

**15-Day Incubation**

*Log reduction.* Log reduction was calculated by subtracting day-15 population in log from day-0 population in log. The mean 15-day log reductions were reported and the results obtained by performing a single-factor ANOVA demonstrated that there was significant difference (p<0.05) in log reduction between control, 1%, and 2% xanthan gum treatment groups. Follow up tests, paired t-tests assuming equal variance, were conducted to evaluate pairwise difference among the means. The results showed that 0% xanthan gum control group had significantly greater log reduction than 1% and 2% xanthan gum groups (both p<0.05,
respectively). However, there was no significant difference in log reduction between 1% and 2% xanthan gum groups (p>0.05) (Table 11).

Table 11

Mean Log Reduction Values of Pie Dough Treatments with Increasing Levels of Xanthan Gum Concentrations (0%, 1%, and 2%) After 15 Days Incubation at Refrigeration Temperature 4 °C. Different Superscripts Are Significantly Different as Measured Using Single-Factor ANOVA and Paired t-Tests

<table>
<thead>
<tr>
<th>Xanthan Gum</th>
<th>15-Day Mean Log Reduction</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0%</td>
<td>0.683&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0502</td>
</tr>
<tr>
<td>1%</td>
<td>0.449&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0363</td>
</tr>
<tr>
<td>2%</td>
<td>0.405&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0233</td>
</tr>
</tbody>
</table>

F (2, 6)=15.22, p<0.05

Water Activity

The mean water activity measurements were reported and the results obtained by performing a single-factor ANOVA demonstrated that there was significant difference (p<0.05) in water activity between control, 1%, and 2% xanthan gum treatment groups. Follow up tests, paired t-tests assuming equal variance, were conducted to evaluate pairwise difference among the means. The results showed that 0% xanthan gum control group had significantly greater water activity than 1% and 2% xanthan gum groups (both p<0.05, respectively). However, there was no significant difference in water activity between 1% and 2% xanthan gum groups (p>0.05) (Table 12).
Table 12

Mean Water Activity Values of Pie Dough Treatments with Increasing Levels of Xanthan Gum Concentrations (0%, 1%, and 2%) After 10 Days Incubation at Refrigeration Temperature 4 °C. Different Superscripts Are Significantly Different as Measured Using Single-Factor ANOVA and Paired t-Tests

<table>
<thead>
<tr>
<th>Xanthan Gum</th>
<th>Water Activity</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0%</td>
<td>0.957&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00338</td>
</tr>
<tr>
<td>1%</td>
<td>0.948&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00033</td>
</tr>
<tr>
<td>2%</td>
<td>0.943&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00088</td>
</tr>
</tbody>
</table>

F (2, 6)=15.50, p<0.05

Microbiological Identification

The pie dough samples were only inoculated with Salmonella. However, two unknown bacterial appeared on XLD agar plates after 15 days of incubation at refrigeration temperature. Results obtained by performing a series of microbiological identification procedures showed that the two unknown were most likely Escherichia coli and Pseudomonas aeruginosa (Table 13).
Table 13

Microbiological Identification of Two Unknown Bacteria Appearing on XLD Agar Plates after 15 Days of Incubation at 4 °C

<table>
<thead>
<tr>
<th>Microbiological Parameters</th>
<th>Unknown Bacteria #1</th>
<th>Unknown Bacteria #1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony size</td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td>Colony shape</td>
<td>Round, flat</td>
<td>Round, spherical</td>
</tr>
<tr>
<td>Colony color</td>
<td>Yellow, whitish yellow</td>
<td>White</td>
</tr>
<tr>
<td>Colony opacity</td>
<td>Opaque</td>
<td>Translucent</td>
</tr>
<tr>
<td>Gram staining</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Bacterial morphology</td>
<td>Coccobacillus</td>
<td>Bacillus</td>
</tr>
<tr>
<td>Oxidase strip test</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Possible Match</td>
<td><em>Escherichia coli</em></td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
</tbody>
</table>
Chapter V: Discussion

Discussion

Effect of xanthan gum addition on the survival of *Salmonella* after 21 days. The pie dough samples were incubated at 4 °C to mimic commercial storage temperature of pre-made refrigerated pie dough. It can be seen from Figure 8 that *Salmonella* population in pie dough samples stored at refrigeration temperature (4 °C) remained somewhat constant from day 0 to day 9 for all treatment groups. The population started to slightly decrease after 9 days. This was expected because *Salmonella* should be inhibited by low temperature during refrigeration. Most *Salmonella* serotypes grow over the temperature range from 7 to 48 °C. Growth is slow around temperature 10 °C (Lawley, 2013). Andreoletti and Budka (2009) reported that *Salmonella* in eggs stored at 4 °C didn’t multiply as opposed to 10 °C or higher. It is worth noting that although growth is inhibited at 4 °C, the chilling does not kill *Salmonella*. In fact, *Salmonella* can survive in refrigerated and frozen foods for a long period of time (Andreoletti & Budka, 2009; Lawley, 2013). The *Salmonella* population is expected to remain unchanged during refrigerated storage; the decline in population may be attributed to limited growth medium and competition within and between species.

According to Table 10, the mean log *Salmonella* reduction after 21 days of the 2% xanthan gum treatment (0.675) was higher than that of the 1% xanthan gum treatment (0.657), which was in turn higher than 0% xanthan gum control (0.599). However, statistical analysis indicated that the differences among treatment groups were not significant (p>0.05). Based on the obtained data, it can be concluded that the xanthan gum addition had no significant impact on the *Salmonella* population in pie dough system after a period of 21 days incubation at refrigeration temperature 4 °C.
In food microbiological studies, XLD agar is a selective growth medium typically used for the isolation of *Salmonella* from food samples. *Salmonella* colonies on XLD agar plates are characterized by the formation of black centers. After examining each plate, it is worth noting that two types of unknown colonies started to appear on XLD agar plates plated after 15 days. On day 18, only a few plates were contaminated with unknown colonies. On day 21, about half of the plates were taken over by the unknown colonies. The reduction in *Salmonella* population was possibly due to competing with these unknown bacteria for growth medium. The log reduction data for *Salmonella* obtained on day 18 and day 21 might not be accurate. Therefore, data obtained after 15 days were eliminated and statistical significance was reanalyzed.

**Effect of xanthan gum addition on the survival of *Salmonella* after 15 days.**

According to Table 11, 0% xanthan gum control had a mean log reduction (0.683) significantly greater than 1% xanthan gum (p<0.05) and 2% xanthan gum (p<0.05) treatment groups, respectively. The difference between the two xanthan gum treatment groups was not significant (p>0.05). Greater log reduction means greater efficacy against the survival of *Salmonella*.

*Salmonella* needs water activity values of at least 0.94 in order to grow in foods (Lawley, 2013). As indicated in Table 12, water activity values of the control, 1% xanthan gum, and 2% xanthan gum treatment groups were 0.957, 0.948, and 0.943, respectively. Minimal growth water activity for *Salmonella* was achieved for all treatment groups. Water activity of dough made with xanthan gum was significantly lower than the control (both p<0.05). This was expected because xanthan gum is capable of lowering the water activity by binding with the water. However, increasing the xanthan gum concentration from 1% to 2% did not result in significant reduction in water activity (p>0.05).
It is rather surprising to see that 0% xanthan gum control group with a higher water activity value actually had greater log reduction than the two xanthan gum treatment groups. The xanthan gum added to dough did not have any efficacy against *Salmonella* population. The microbiology of the dough system was actually worsened by the addition of xanthan gum. This could be explained by the fact that the dough samples were contaminated with other bacteria. The reduction in *Salmonella* population was dependent on the extent of competition between *Salmonella* and the contamination bacteria, which rendered any possible xanthan gum efficacy insignificant. All microbiological procedures and analysis were performed steriley. However, the pie dough was made in food science pilot plant, a non-sterile environment. The countertop, chopping board, knife, pastry blender, spatula, parchment paper, plastic wrap, and water were not sterile and possible contamination could occur.

**Microbiological identification of the contamination bacteria.** As shown in Table 13, unknown bacteria #1 had large, round, flat, yellow, and opaque colonies on XLD agar plates. Based on gram staining, microscopic examination, and oxidase strip test, unknown bacteria #1 is gram-negative, oxidase-negative coccobacillus. Cross-referencing with literature findings, unknown bacteria #1 is most likely a strain of *Escherichia coli* (SIFIN, 2009). Unknown bacteria #2 had small, round, spherical, white, and translucent colonies on XLD agar plates. Based on gram staining, microscopic examination, and oxidase strip test, unknown bacteria #2 is gram-negative, oxidase-positive bacillus. Cross-referencing with literature findings, unknown bacteria #2 is most likely a strain of *Pseudomonas aeruginosa* (SIFIN, 2009).

Although the above investigation was still insufficient to confirm the identities of the unknown bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* are the most promising candidates. Both *Escherichia coli* and *Pseudomonas aeruginosa* are ubiquitous bacteria and thus
contamination can occur easily due to the lack of aseptic food preparation conditions. The growth temperature range of *Escherichia coli* is between 4 and 45 °C. According to Andreoletti and Budka (2009), *Pseudomonas aeruginosa* is able to grow at 4 °C as well and at both 4 °C and 37 °C, *Salmonella* is unable to compete with *Pseudomonas aeruginosa* in eggs.

**Conclusions**

This study revealed that neither of 1% xanthan gum and 2% xanthan gum addition had any antibacterial effects on the survival of *Salmonella enterica* serotype *typhimurium* in pie dough refrigerated at 4 °C for 15 days.

**Recommendations**

Future studies should be conducted to investigate the effect of xanthan gum addition on the survival or growth of *Salmonella* in refrigerated pie dough stored at abuse temperature such as 10°C, 12°C, and 25 °C and severe abuse temperatures such as 35 °C.

Future studies can be conducted to distinguish the pathogenic ecology and behavior between pre- and post-processing contaminated pie dough.

Microscopic examination studies can be conducted to analyze the dough structure, especially the interface between water, fat, and flour.
References


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cat-kelly.wikispaces.com/file/view/Chemistry+of+Baking.docx


Washington, DC: American Chemical Society.


http://www.foodsafetywatch.org/factsheets/Salmonella/


Appendix A: Pie Dough Formulation

Original formulation of pie dough for this study was obtained from a private recipe provider. The original formulation contains absolutely no water. Dough made with such formulation was very crumbly and not able to be sheeted to pie crust. The original formulation was adjusted by using different types of fat, different egg ingredients, and adding small amounts of water (Table 14). However, no sheetable dough was produced.

Table 14

Sheetability Determination of Different Pie Dough Formulas

<table>
<thead>
<tr>
<th>Formula</th>
<th>Flour</th>
<th>Fat</th>
<th>Egg</th>
<th>Water</th>
<th>Sugar</th>
<th>Salt</th>
<th>Sheetability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>20#*</td>
<td>10# Butter</td>
<td>6# Egg yolk</td>
<td>0</td>
<td>6#</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>1</td>
<td>20#</td>
<td>10# Butter</td>
<td>6# Whole egg</td>
<td>0</td>
<td>6#</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>20#</td>
<td>10#</td>
<td>6# Whole egg</td>
<td>0</td>
<td>6#</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soybean oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20#</td>
<td>10# Butter</td>
<td>6# Whole egg</td>
<td>1#</td>
<td>6#</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>20#</td>
<td>10# Butter</td>
<td>6# Whole egg</td>
<td>2#</td>
<td>6#</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>Actual</td>
<td>20#</td>
<td>14.2# Butter</td>
<td>0</td>
<td>11.4#</td>
<td>0.95#</td>
<td>0.32#</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* #=parts, for example, in original formula, for every 20 parts of flour, 10 parts of butter was added

With no or small amounts of water added to flour, there was no or very weak gluten network developed in the dough. The resulting dough lacked viscoelasticity and was very crumbly. Thus, it was not able to be shaped to a disk or sheeted to a flat pie crust. Xanthan gum has been reported to possess rheological properties similar to gluten (Ahlborn et al., 2005). In order for xanthan gum to manifest its rheological properties, it must be hydrated as well. With no
water interacting with xanthan gum, it was not surprising to see dough made with 1% or 2% xanthan gum could not be sheeted to a pie crust. Therefore, the original no water formula was abandoned.

The actual formulation used for this study had a dry ingredient to wet ingredient ratio of 3.1:1.0. The amount of ingredients used in this formula was converted to compare with previous formulations (Table 14). Dough made in all treatment groups can be easily shaped into a disk or sheeted to a pie crust. The dough was sheeted by hand and there was no obvious difference in sheetability observed between control and xanthan gum treatment groups.