EFFECT OF RINSING-, WASHING-TIME AND WATER TEMPERATURE ON REMOVAL OF PEANUT ALLERGEN FROM DAIRY PROCESSING EQUIPMENT

By

Jiacheng Zhang

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Food Science – Master of Science

2018

ABSTRACT

EFFECT OF RINSING-, WASHING-TIME AND WATER TEMPERATURE ON REMOVAL OF PEANUT ALLERGEN FROM DAIRY PROCESSING EQUIPMENT

By

Jiacheng Zhang

The prevalence of food allergy is increasing in recent years, and the best way to protect consumers with food allergy from eliciting allergic reaction is to avoid exposure. However, cross contamination occurs commonly during food production in the food industry that produce food with different food allergens. Effective cleaning of the equipment is important to prevent food allergen cross contamination. In this study, it was hypothesized that higher water temperature and longer rinsing-, washing-time resulted in lower peanut allergen left on stainless steel surface. Ice cream Buckeye Blitz, which contains peanut and soy, was used in this study. Thawed ice cream was filled in a stainless steel pipe for 1.5 hour and then rinsed or rinsed and washed in a simulated clean-in-place system. The effect of three times (10, 20 and 30 seconds) and five water temperatures (20, 30, 40, 50 and 60°C) on removal of peanut allergen on stainless steel pipe was investigated by swabbing the stainless steel surface and testing for the peanut allergen Ara h1. When equipment was only rinsed, concentrations of peanut allergen residue left on the pipe ranged from 207 ppm to 63 ppm. The overall trend suggested that higher water temperature and longer rinsing time resulted in lower peanut allergen concentration on the equipment. When equipment was rinsed and then washed, concentrations of peanut allergen residue ranged from 1.43 ppm to 0.015 ppm. The overall trend suggested that time showed a less important effect as temperature on allergen removal in this study. Effective cleaning can reduce the chance of cross contamination as well as save time and money for the food industry. Understanding the principle of rinsing and washing is essential for effective allergen removal.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Zeynep Ustunol for her continuous guidance, expertise and support during my time at Michigan State University. Thank you for helping me to be admitted in this program and being my advisor, I could not make it so far without your help. I would also like to thank Dr. Leslie D. Bourquin and Dr. Venugopal Gangur for serving on my guidance committee. Thank you for providing valuable advice and very helpful suggestions for my research.

I would like to also thank Dr. John Partridge, Dr. Jeff Swada, Rodney Clark, Josh Hall and Gary Smith for providing support and assistance for my research. Chun-Lung Lee helped me to analyze the data. My research could not be completed without their selfless help.

Last but not the least, I would like to thank my parents for providing me an opportunity to study in the USA. It is an excellent and unforgettable experience in my life. Lastly, I want to thank Sitong for her patience and love.

TABLE OF CONTENTS

LIST OF TABLES vi

LIST OF FIGURES vii

CHAPTER 1 1
INTRODUCTION 1

CHAPTER 2 3
LITERATURE REVIEW 3
2.1. Food Allergy and Food Allergens 3
2.2. Mechanism of Food Allergies 3
2.3. Peanut Allergy and Peanut Allergens 4
2.5. Allergen Management in the Food Industries 9
2.6. Equipment Cleaning in Food Industry 10
2.6.1. Types of Soils in Food Industries 10
2.6.2. Food Processing Surface 11
2.6.3. Detergents 12
2.6.4. Cleaning Method----Wet Cleaning 13
2.6.4.1. Clean out of Place (COP) and Clean in Place (CIP) 14
2.6.5. Cleaning Studies on Removal of Allergens 15
2.7. Methods to Detect Allergens 17
2.7.1. Polymerase Chain Reaction (PCR) 17
2.7.2. Immunoassay 18
2.7.2.1. Enzyme Linked Immunosorbent Assay (ELISA) 18
2.7.2.2. Lateral Flow Strips (LFS) 20
2.8. Significance and Justification 20

CHAPTER 3 22
MATERIALS AND METHODS 22
3.1. Ice Cream and Inoculation 22
3.2. Verification of Clean out of Place (COP) Washing 22
3.3. Investigating Effectiveness of Temperature and Time on Simulated CIP Rinse 23
3.4. Investigating Effectiveness of Temperature and Time on Simulated CIP Wash 27
3.5. Verification of CIP Washing System in MSU Dairy Plant 28
3.6. Statistical Analysis 29

CHAPTER 4 30
RESULTS AND DISCUSSION 30
4.1. Preliminary Tests 30
4.2. Clean Out of Place (COP) Washing 30
4.3. Investigating Effect of Time and Temperature on Clean-In-Place (CIP) Rinse 32
4.4. Investigating Effect of Time and Temperature on Clean-In-Place (CIP) Wash 40
4.5. Verification of CIP Washing System in MSU Dairy Plant 48
4.6. Recommendations on current CIP system in the MSU Dairy Plant 49

CHAPTER 5 50
CONCLUSIONS AND LIMITATIONS 50
5.1. Conclusions 50
5.2. Limitations 51
5.3. Future Studies 51

APPENDICES 52
APPENDIX A: NUTRITION FACTS AND INGREDIENTS LIST 53
APPENDIX B: ITEMS USED IN THIS STUDY 54
APPENDIX C: PAIRWISE COMPARISION OF RESULTS 59

REFERENCES 69

LIST OF TABLES

Table 1. Protein family and molecular weights of peanut allergen proteins. 7

Table 2. Different countries require different foods to be labelled. 8

Table 3. Specific water temperature and time combinations in each treatment. 26

Table 4. Concentrations of peanut allergen protein Ara h1 residue left on the stainless steel pipe after rinsing. 34

Table 5. ANOVA table for rinsing time, rinsing temperature and rinsing time and temperature interaction. 35

Table 6. Concentrations of peanut allergen protein Ara h1 residue left on the stainless steel pipe after rinsing then washing. 42

Table 7. ANOVA table for rinsing time, rinsing temperature and rinsing time and temperature interaction. 43

Table C1. Pairwise comparison of rinsed data using least square means method. 59

Table C2. Pairwise comparison of rinsed then washed data using least square mean method. ... 64

LIST OF FIGURES

Figure 1. Diagram of the equipment used in clean-in-place (CIP) rinsing experiment. Rinsing water was in Tank A. Tank A is a double-wall tank, and steam source came behind the tank to provide heat for rinsing water. After rinsing, water was drained. 25

Figure 2. Diagram of the equipment used in clean-in-place (CIP) washing experiment. During rinsing the pipe, the valve A was turned on and the valve B was turned off. The detachable pipe can rotate, so that the rinse water can go into the drain. During washing the pipe, the valve A was turned off and valve B was turned on, the detachable pipe can rotate back to the position shown above to recirculate the washing solution. 28

Figure 3. Standard curve for COP washing. It was used for samples obtained from 69.8°F/21°C and 132.8°F/56°C manual washing. Dilution ratio was 1:1. 31

Figure 4. Standard curve for rising. It was used for rinsed combinations from 9 to 15. Dilution ratio was 1:500. 33

Figure 5. Standard curve for rinsing. It was used for rinsed combinations from 1 to 8. Dilution ratio was 1:2500. 33

Figure 6. Concentrations of peanut allergen protein Ara h1 left on the stainless steel pipe after rinsing. a to e, different letters within the same time are significantly different. x to z, different letter within the same temperature are significantly different. Significance level is 0.05. 34

Figure 7. Overall time effect of rinsing on removing peanut allergen Ara h1 on stainless steel pipe. For letter a to c, different letter indicates there was significant difference (p-value<0.05).37

Figure 8. Results for peanut allergen Ara h1 concentrations left on stainless steel equipment after rinsing in a contour graph. Darker area means higher peanut allergen concentration. 38

Figure 9. Results for peanut allergen Ara h1 concentrations left on stainless steel equipment after rinsing in a three-dimensional graph for rinsed results. 39

Figure 10. Standard curve for rinsed then washed. It was used for combination 1-2, 4-6, 9 and 11-15. Dilution ratio was 1:10 for 1-2, 6 and 11-12. Dilution ratio was 1:1 for 4, 5, 9, 13-15. . 41

Figure 11. Standard curve for rinsed then washed. It was used for combinations 3, 7, 8 and 10. Dilution ratio was 1:10 for 3, 7 and 8. Dilution ratio was 1:1 for 10. 41

Figure 12. Concentrations of peanut allergen protein Ara h1 left on the stainless steel pipe after rinsing then washing. a to d, different letters within the same time are significantly different. x to z, different letter within the same temperature are significantly different. The significant level is 0.05..... 42

Figure 13. Overall effect of rinsing then washing time on removing peanut allergen Ara h1 on stainless steel pipe. For letter a and b, different letter indicates there was significant difference (p-value<0.05)..... 46

Figure 14. Overall effect of rinsing then washing temperature on removing peanut allergen Ara h1 on stainless steel pipe. For letter a to d, different letter indicates there was significant difference (p-value<0.05). 46

Figure 15. Results for peanut allergen Ara h1 concentrations left on stainless steel equipment after rinsing and washing in a contour graph. Darker area means higher peanut allergen concentration..... 47

Figure 16. Results for peanut allergen Ara h1 concentrations left on stainless steel equipment after rinsing and washing in a three-dimensional graph for rinsed then washed..... 48

Figure A1. Nutrition Facts label and ingredients list for Buckeye Blitz ice cream, which was used in this study..... 53

Figure B1. Stainless steel pipe and pan were used in this study..... 54

Figure B2. Brushes were used in this study. White brush was used for washing inside surface of the stainless steel pipe. Red brush was used for washing outside surface of the stainless steel pipe, the pan and two covers..... 55

Figure B3. Neogen BioKits Allergen Swabbing Kit (Neogen, East Lansing, MI, USA)..... 56

Figure B4. Neogen BioKits Peanut Assay Kit (Neogen, East Lansing, MI, USA). 57

Figure B5. The simulated CIP system was used for CIP experiments in this study..... 58

CHAPTER 1

INTRODUCTION

Food allergies have evolved from being a problem for the people who have food allergies to a significant public health issue (Gupta and others 2013). Most allergic reactions are mild and cause minor symptoms, but some are severe and can cause death. Several studies have found an increase in peanut allergies over the last two decades (Grundy and others 2002; Nwaru and others 2014; Venter and others 2010; Sicherer and others 2010). Increasing prevalence of allergies may result in increasing awareness among people. Up to 25% of parents believe that they or their children suffer from a food allergy, but in fact only 6-8% of children are afflicted by a food allergy in their first three years of life and the prevalence decreases over the first decade of life (Sampson 2005). To protect allergic consumers, the U.S. Food and Drug Administration (FDA) summarized "Big Eight" food allergens, which account for approximately 90% of food allergic reactions in the U.S., foods which contain or may contain these big eight food allergen(s) need to be labeled to state separately to warn consumers. Consumers are solely relying on food labels to avoid food allergens, since there is no cure for food allergies so far, and avoidance is the best way to prevent reactions from occurring.

However, food containing undeclared allergens due to cross contamination was the biggest problem during the last five years, and dairy was one of the leading causes (FDA 2016). Food producers are responsible for developing and implementing food allergen control plans to minimize cross contamination during production. Equipment cleaning is one of the common components in allergen control plans. Cleaning equipment involves cleaning methods, cleaning tools and chemicals. Cleaning methods and chemicals need to be chosen based on types of surface and residues. Currently, many food manufacturers utilize clean-in-place (CIP) system

equipment in their plants. Cleaning in the CIP system equipment proceeds without dismantling the equipment, and it is controlled by a computer program. The program may be designed to clean potential residues in the equipment, but it may not be effective for all plants and food residues due to the differences between plant situation and complexity of food matrix. Therefore, validation of the cleaning process for each plant is essential.

In the cleaning process, there are some important factors to be considered, such as contact time between washing solution and equipment surface, temperature and water pressure (turbulence). Different combinations of these factors affect the cleanliness of the equipment and effectiveness of CIP system. In this study, different combinations of contact time and water temperature were investigated.

Therefore, the hypotheses of this research were that higher water temperature and longer rinsing-, washing-time resulted in lower concentration of peanut allergen left on stainless steel dairy processing equipment, and both the clean out of place and clean-in-place system in the Michigan State University (MSU) are effective to remove peanut allergen. The specific objectives were as follows:

1) Verify the effectiveness of Clean out of place (COP) washing in the MSU Dairy Plant in removal of peanut allergen.

2) Investigate the effectiveness of different combinations of rinse-, and wash-time and water temperature in removal of peanut allergen in a simulated clean in place (CIP) system

3) Verify the effectiveness of current CIP washing system in the MSU Dairy Plant on removing peanut allergen.

4) Make recommendations to the MSU Dairy Plant on cleaning practices for more effective removal of peanut allergens if needed.

CHAPTER 2

LITERATURE REVIEW

2.1. Food Allergy and Food Allergens

Food allergy has been defined by the National Institute of Allergy and Infectious Diseases (NIAID) as "An adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food" (Boyce and others 2010). The prevalence of food allergy was estimated to affect about 5% and up to 10% of the population globally (Sicherer and Sampson 2018). Food allergy affects 1-5% in the United States (Renz and others 2018), and it is more common on children than on adults (Tang and others 2017, Sicherer and Sampson 2018). Increasing of prevalence of food allergy in the last 2 to 3 decades may be explained by hygiene hypothesis, allergen avoidance hypothesis, dual allergen exposure hypothesis and nutritional immunomodulation hypothesis (Sicherer and Sampson 2018).

The FDA identified eight food groups as the major food allergens, which account for about 90% of food allergy reactions in the U.S. They are milk, eggs, fish, crustacean shellfish, tree nuts, peanut, wheat and soybeans. Milk, shellfish, peanut and tree nut allergy are the most reported among these eight food groups (McGowan and Keet 2013). Among tree nuts, prevalence of walnut and cashew allergy are the highest in adults and children in the US (McWilliam and others 2015).

2.2. Mechanism of Food Allergies

The increasing occurrence of food allergies among the general population requires more knowledge on the mechanisms. Generally, adverse reactions to food can be divided by toxic or nontoxic (Valenta and others 2015). Nontoxic adverse food reactions can be further divided to immune-mediated and nonimmune-mediated reactions. Food allergy falls into immune-mediated

reactions, including those caused by adaptive and innate immune system. Gell and Coombs (1963) defined four different types of immune-mediated hypersensitivity reactions, and most of the food allergies belong to Type I, which is IgE-mediated.

Food allergens are defined as those specific components of food or ingredients within food (typically proteins, but sometimes also chemical haptens) that are recognized by allergen-specific immune cells and elicit specific immunologic reactions, resulting in characteristic symptoms (Boyce and others 2010). The IgE-mediated allergic process starts with exposure to food allergen without producing clinical symptoms, then the allergen promotes B cells to mature into the plasma cells and produce IgE corresponding to the epitopes within the allergen. This IgE antibody can bind to mast cells in the skin and basophils in the blood circulation, via high affinity IgE receptors. Upon re-exposure to the same allergen, IgE binds on the surface of mast cells and basophils, which results in the secretion of inflammatory mediators in our bodies. These inflammation mediators are responsible for allergic symptoms. The primary mediator of acute-phase allergic reactions is histamine. The effects caused by histamine are hives and flushing, wheezing and pain, emesis, and hypertension. A secondary mediator is tryptase, which is found mainly in mast cells, and which plays a role in bronchospasm (Kim and Burks 2015).

2.3. Peanut Allergy and Peanut Allergens

The prevalence of peanut allergy in the USA in children increased from 0.4% in 1997 to 1.4% in 2008 and to 2% in 2013 (Zhou and others 2013, National Academies of Sciences and others 2016). Peanut allergy sometimes can be life-threatening and persist throughout life time, and thus greatly reduce the quality of life of the people with peanut allergy (King and other 2009).

Peanut allergen is one of the most studied food allergens among big eight food allergens. Seventeen allergens are identified in peanut (**Table 1**) (WHO/IUIS Allergen Nomenclature Sub-Committee 2018). The major allergens are Ara h 1, Ara h 2, Ara h 3 and Ara h 6, Ara h 1 and Ara h 3 are seed storage proteins (Mueller and other 2016). Generally, a peanut seed contains about 29% of protein, in which 12-16% is Ara h 1 and 5.9-9.3% is Ara h 2 (Koppelman 2001). Ara h 2 and Ara h 6 can bind more strongly with IgE from peanut allergic patients and more efficiently release mediator from basophils, which is consistent that these two allergens are more potent *in vitro* and *in vivo* studies, although they are less abundant than Ara h 1 and Ara h 3 (Jayasena and others 2015). Ara h 2 is the most important and recognizable *in vitro, ex vivo* and *in vivo* assays among Ara h 1, Ara h 2 and Ara h 3 (Koppelman and others 2004).

Many allergens such as peanut allergen Ara h 1 and Ara h 2 can lead to allergic reactions by the first contact and had clinical symptoms upon secondary contact. These allergens are stable when exposed to heat and gastrointestinal digestion. The thermal stability of proteins is due to intramolecular disulphide bonds, ion-binding, protein oligomerization, and chemical modification (Lorenz and others 2015). But processing like frying and boiling can reduce IgE-binding intensity as well as soluble peanut allergen Ara h 2 content to decrease the allergenicity compared with that in roasting (Beyer and others 2001, Comstock and others 2016). During frying, content of α-helices decreases and contents of its β-sheets, β-turn and random coil increases dramatically, and thus altering Ara h 2 epitopes to reduce its allergenicity (Zhang and others 2016). In terms of boiling, boiling induces a partial loss of secondary structure which then become a complex with reduced IgE-binding capacity due to decreased epitope availability (Blanc and others 2011). It was also showed that processing can decrease the level of extract proteins, and lead to underestimate the content of allergen protein Ara h1 and Ara h 2 in a biscuit

model (Montserrat and others 2015). These authors also suggested that direct competitive ELISA was more sensitive for Ara h 2 compared to sandwich ELISA in their model.

One of the biggest problems in food allergy diagnosis and detection is allergen cross-reactivity. Cross-reactivity IgE to multiple nuts and or legumes can be often observed on peanut allergic individuals' specific IgE test. About 50% of peanut-allergic patients show positive results in skin prick test to other legumes, but in fact only less than 5% are clinically symptomatic upon ingestion of legumes (Mueller and other 2016).

Table 1. Protein family and molecular weights of peanut allergen proteins.

Allergen	Biochemical name	Molecular weight (SDS-PAGE) (kDa)
Ara h 1	Cupin	64
Ara h 2	Conglutin (2S albumin)	17
Ara h 3	Cupin	60
Ara h 4	Rename to Ara h 3.02	N/A
Ara h 5	Profilin	15
Ara h 6	Conglutin (2S albumin)	15
Ara h 7	Conglutin (2S albumin)	15
Ara h 8	Pathogenesis-related protein, PR-10, Bet v 1 family member	17
Ara h 9	Nonspecific lipid-transfer protein type 1	9.8
Ara h 10	16 kDa oleosin	16
Ara h 11	16 kDa oleosin	14
Ara h 12	Defensin	8
Ara h 13	Defensin	8
Ara h 14	Oleosin	17.5
Ara h 15	Oleosin	17
Ara h 16	non-specific Lipid Transfer Protein 2	8.5
Ara h 17	non-specific Lipid Transfer Protein 2	11

Many scientists tried to determine the threshold for food allergens. However, different sensitivity of individuals and food matrix increase the difficulty in determinations (Taylor and other 2014). In the report of the Voluntary Incidental Trace Allergen Labeling (VITAL) expert panel, reference doses were established for 11 allergenic foods via reviewing previous clinical challenges of food-allergic subjects (Taylor and other 2014). Reference dose by the definition of the Environmental Protection Agency (EPA) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (EPA 2018). Reference doses range from 0.03 mg for egg protein to 10 mg for shrimp protein, and reference dose for peanut is 0.2 mg (Taylor and other 2014).

Table 2. Different countries require different foods to be labelled.

Country	Allergic foods
United States	Wheat, egg, milk, peanut, fish, crustaceans, soy, tree nuts
European Union	Wheat, egg, milk, peanut, fish, crustaceans, soy, tree nuts, sesame, shellfish/mollusks, mustard, celery, lupine
Canada	Wheat, egg, milk, peanut, fish, crustaceans, soy, tree nuts, sesame, shellfish/mollusks, mustard
Australia/New Zealand	Wheat, egg, milk, peanut, fish, crustaceans, soy, tree nuts, sesame
China	Wheat, egg, milk, peanut, fish, crustaceans, soy, tree nuts
Japan	Wheat, egg, milk, peanut, crustaceans

2.5. Allergen Management in the Food Industries.

Although people realize food allergy is a public health issue, and Food Allergen Labeling and Consumer Protection Act (FALCPA) is also enacted to protect people with food allergy, the incidences of recalls and adverse reaction related to undeclared allergens continue to happen with high frequency and the rate is still increasing from 2009 to 2014 (FDA 2016). Undeclared allergen hazard has accounted for nearly 45% or 412 out of 949 total primary entries in the FDA Reportable Food Registry for all five years (2009-2014) and dairy was the third leading entry after bakery and chocolate/confections/candy (FDA 2016). Undeclared allergens can accidently be in a food product via different ways, such as incorrect labelling of the product, improper handling, cross contamination and insufficient cleaning and sanitation (Jackson and others 2008). Cross contamination is a big concern at any stage during food production. A survey from the Food and Drug Administration indicated that there were nearly 90% or 416 out of 463 facilities using at least two major food allergens, and milk was in the majority of the most frequent combinations of major food allergens in these facilities (Gendel and other 2013). The Dairy industry is a good example for producing products which contain different food allergens especially the dairy industry producing ice cream. Since producing ice cream needs to use many kinds of ingredients to make different flavors. One of the most common ways for cross-contact occur is transferring allergenic protein during processing and handling, especially when shared equipment is used to produce different foods with different allergens (Jackson and others 2008). Ice cream freezer is a good example of shared equipment. The survey also suggested that nearly 80% or 359 out of 463 of the facilities used shared equipment and about 10% or 32 out of 359 of them didn’t clean the equipment between allergen and non-allergen products (Gendel and other 2013).

To reduce the incidence of such problems, it is essential to understand what allergen control practices are currently used by food industry. In the early 1990s, food industries started to develop allergen control plans to prevent unintended presence of allergens in the products (Jackson and other 2008). An allergen control plan ensures that cross contact is prevented through manufacturing controls and now also includes all major food allergens intended in the products are declared via label controls (Gendel and others 2013). In a survey conducted by the Institute of Food Technologists (IFT) in 2005 suggested that more than 94% of the food companies had allergen control plans and more than 77% of these companies included cleaning and sanitation in their allergen control plan (Taylor and others 2006). Water is the most powerful tool to remove food allergens on the processing line and thus water is considered to be the first defense line against cross-contact on shared equipment in the food industries (Jackson and others 2008).

2.6. Equipment Cleaning in Food Industry

2.6.1. Types of Soils in Food Industries

The best way to protect consumers from food allergy reactions is to avoid contact with food allergens. Developing a suitable cleaning plan is essential for a food industry. Before a suitable cleaning plan is developed, it is necessary to understand the nature of soils that need to be cleaned. The composition of soils varies due to complexity of food processed in plant, surface oil or dust, insoluble cleaner components and insoluble hard-water salts. Soils can be classified based on solubility in water, acid, and alkali (Schmidt 2014). There are four types of soils commonly found on the food processing surface: protein-based soils, carbohydrate-based soils, mineral salt-based soils and fat-based soils. Fat-based and protein-based can be removed by alkaline detergent. Protein-based soils are the most difficult one to remove among the four

types of soils, especially heat-denatured proteins; a highly alkaline detergent needs to be used (Schmidt 2014). Protein-based soils are the most common ones in dairy industry, such as whey protein from milk and food allergen protein from other ingredients. Carbohydrate-based soils can be removed by just warm water or using mild detergent, since they are readily water soluble. Mineral salt-based soils are usually removed by acidic detergent. Sequestering agents like phosphates or chelating agents are often used in acidic detergents to help to remove mineral salts (Schmidt 2014).

2.6.2. Food Processing Surface

Food processing surface is another important factor that needs to be considered. Although there are different types of equipment with different functions made from different materials, there is one rule in common, they must be able to be easily cleaned (Holah 2014). The types of surfaces found in food processing plants include stainless steel, various plastics (polyethylene UHMW, polycarbonate, PVC, vinyl) and rubber (Lopez 2011). Large food contact surface materials should have a surface finish equal to or less than R_a=0.8 μm, especially the surface in CIP system, or a prove is made to demonstrate the cleanability can be acquired by a cleaning protocol. R_a is a parameter for surface roughness. Higher R_a means the surface is rougher, which makes it more difficult to be cleaned. Generally, stainless steel is required for constructing processing equipment. It gained its popularity due to resistance to corrosion by food, ease of cleaning, and malleability. AISI 316 stainless steel is suitable for higher level of chlorides (0.015-0.05%) at moderate temperatures (<60°C); while AISI 304 stainless steel is widely used in many food industries, particularly in the food processing industries producing food with low level of chlorine ions (Lewan and Partington 2014). In the dairy industry, AISI 304 is used to build the majority of the processing equipment.

In addition to stainless steel, plastic is another material that is used commonly as a food contact surface. They mainly are used for protection of tools, implements from metal-to-metal contact as guides and covers, or for hoses due to their plasticity and corrosion resistance. Types of plastics must be approved before using as a food contact material, since some plastics are porous and can absorb product constituents and even diffuse into food (Lewan and Partington 2014). A list of plastics that are easy to clean and are used in hygienic design include: polypropylene, polyvinylchloride, acetal copolymer, polycarbonate and high-density polyethylene. It is important to note that plastics may degrade in some chemical environments and this process can be accelerated by mechanical stress (Lewan and Partington 2014). Rubber is another widely-used material in food industry because of their elasticity. It is generally used for gaskets, caps, and hoses. Rubber is composed of several ingredients, such as elastomers, mineral fillers, plasticizers. Elastomer, which is composed of long repetitive molecular chains of various origins, mainly affects plastics' properties (Lewan and Partington 2014).

2.6.3. Detergents

Cleaning and sanitizing are important aspects of food processing in food industry, and it is crucial that they are done properly and efficiently. A clean and sanitized environment can minimize the potential of transferring dirt, microorganisms or food allergens to food products. Detergents are one strategy for maintaining a cleaned and sanitized environment. Detergents are usually composed of two kinds of ingredients: physically active ingredients and chemically active ingredients. Physically active ingredients alter physical characteristics such as solubility or colloidal stability, while chemically active ingredients modify soil components to make them more soluble (Schmidt 2014).

The primary physically-active ingredients are surfactants. They help to wet the surface thoroughly, then suspend and dissolve solids, so that solids can be more easily removed by water. Physical energy may need to apply for effective cleaning (Maddox 1994). Surfactants are usually organic compounds and are amphiphilic. The molecules in surfactants promote physical cleaning actions through being emulsified by hydrophilic heads, penetration, spreading, foaming, and wetting by disrupting water's hydrogen bonding by polar heads (Holah 2014, Schmidt 2014).

Chemically active ingredients include alkalis, acids, and sequestering agents. Highly alkaline detergents use sodium/potassium hydroxide and moderately alkaline detergents use sodium, potassium, or ammonium salts of phosphates, silicates, or carbonates (Schmidt 2014). They help to break down protein through action of hydroxyl ions, saponify fats and may be bactericidal (at high concentrations) (Holah 2014). Acid detergents include inorganic acids such as phosphoric acid, nitric acid, sulfamic acid, sodium acid sulfate, and hydrochloric acid as well as organic acids, such as hydroxyacetic acid, citric acid, and gluconic acid (Schmidt 2014). They have low detergency but are useful in dissolving carbonate and mineral scales. They are only periodically used for cleaning and not as frequently as alkalis (Holah 2014). Sequestering agents are usually chelating agents that are primarily used to control water hardness by forming soluble complexes with Ca^{2+} and Mg^{2+} (Schmidt 2014).

2.6.4. Cleaning Method----Wet Cleaning

Wet-cleaning can be divided into four categories: clean out of place (COP), clean in place (CIP), foam or gel cleaning, and manual or hand cleaning (Jackson and other 2008). COP requires partially disassembling of equipment and cleaning. CIP requires minimal or no disassembly of equipment and is automated. Foam and gel cleaning is where chemical is

sprayed onto equipment as a foam or gel. The benefit of this method is that it can increase contact time with soil. Manual and cleaned by hand is to disassemble equipment fully and clean by hand according to the protocol developed by the facilities. The choice of cleaning methods is based on the characteristics of food product on the line and types of equipment used.

2.6.4.1. Clean out of Place (COP) and Clean in Place (CIP)

Clean out of place (COP) generally is used when clean in place cannot be used. COP is one of the leading methods for wet cleaning (Keener 2005). Advantages of COP are that visual observation can be used to check the effectiveness of cleaning and low initial cost. Disadvantages are that it requires dissembly and assembly of equipment before and after cleaning and possibility of contamination after cleaning.

COP consists of 4 steps: pre-rinse, wash, rinse and sanitization. In these four steps, water temperature, type of detergent used, concentration of detergent, washing time and force or agitation on the surface play important roles (Keener 2005).

Clean in place (CIP) is cleaning the entire piece of equipment in the plant and/or pipe line without dissembling the equipment (Romney 1990). CIP has been widely used in dairy, brewery, food and wine processing for over 55 years, and there are several advantages for using CIP (Stewart and Seiberling 1996): CIP can provide highly repeatable results. CIP can lower operating costs, especially labor costs. Using CIP can improve safety, since CIP doesn't require workers to dismantle equipment and touch chemicals. Disadvantages of CIP are the initial cost of CIP is high, and a trained worker is required to operate the professional cleaning program.

CIP can be simply classified into three categories: total loss CIP, partial recovery CIP and total recovery CIP. In total loss CIP, used solutions are drained off and no recovery is carried out. Partial recovery CIP recovers both concentration and volume of cleaning solutions by

monitoring conductivity. Total recovery CIP system re-uses the final rinse from previous as pre-wash in the subsequent cleaning, and it also recovers concentration and volume of cleaning solution (Stanga 2010). Although CIP has three different treatments for used cleaning solution, it has general procedures in all three categories, which includes pre-washing, alkaline cleaning, rinse, acid cleaning, rinse disinfection, and final rinse (Stanga 2010).

During cleaning, some factors play important roles: contact time, temperature, turbulence and resulting shear forces acting on deposits, type of soil, and concentration of cleaning chemicals (Australian Standards 2001). During contact time, cleaning chemical diffuses into soil layer, swells the soil, and transfers into liquid then flush. High temperature enhances the effectiveness in cleaning, by accelerating diffusion and reaction rate, and a temperature above 40°C is recommended. However, extremely high temperature may denature protein in fresh residues and affect amount of soil deposited and its composition, which can increase the difficulty of cleaning. A water temperature below 65°C is recommended for fresh contaminants (Stanga 2010). Flow velocity and flow rate are another two important parameters in CIP. When flow velocity increases, the thickness of the boundary of soil decreases. The minimum of flow rate should be 1.5 m s^{-1}, and 2 m s^{-1} is optimal (Stanga 2010). The force acted on the tank wall is provided by the pressure and volume of the solution. Water pressure needs to exceed 1 bar, such that the constantly descending flow on the wall is turbulence (Stanga 2010).

2.6.5. Cleaning Studies on Removal of Allergens

Röder and others (2008) conducted experiments about cleaning equipment after producing hazelnut cookie dough at pilot scale. They compared effectiveness of manual scraping, manual scraping and cleaning with hot water, and manual scraping and cleaning with hot water and detergent. They used hazelnut ELISA to test dough samples drawn from the

follow-up dough/product after various cleaning treatment at each device from dough kneading to baking equipment. The results showed that manual scraping & hot water can reduce hazelnut residual in subsequently non-hazelnut cookies effectively, while the addition of detergent had similar results as without detergent.

Courtney (2016) studied removal of milk soil from various food processing surfaces. Four cleaning chemicals were used in her experiment: a commercial caustic (Exelerate CIP, Ecolab MN), a commodity caustic prepared from sodium hydroxide, an acid cleaner (Envirocid Plus), and an oxidizing sanitizer (Vortexx). She found out that the caustic solution can easily remove milk soil, while acid and sanitizing solution left a soiled surface as expected. The commercial caustic solution has greater soil suspendibility compared to the commodity caustic solution.

Stephan and others conducted (2004) an experiment on cleaning peanut slurry. Slurry preparation equipment underwent rinsing, alkaline cleaning, rinsing, acidic cleaning, and rinsing. Rinsing water was collected and tested via peanut ELISA. They concluded that peanut residue can be removed effectively after alkaline cleaning.

Wang and others (2010) conducted an experiment on investigating removal of gliadin on equipment after processing wheat-battered chicken on industrial scale. Cleaning methods included water rinsing (104-122°F/40-50°C), foam and rinsing (foam comprised NaOH, NaOCl and a surfactant), and sanitize and rinsing (sanitizer: 1% Sterbac). Samples were collected by swabbing equipment surfaces after each cleaning method and tested by gliadin ELISA. The results showed that concentration of gliadin decreased to about 1ppm after just water rinsing; both foam and rinse, and sanitize and rinse have lower concentration than just water rinse, while there was no big difference between foam and rinse and sanitize and rinse.

Jackson and others (2004) investigated efficacy of water, chlorinated alkali cleaner and acid detergent cleaner on removal of milk soil and peanut butter soil from various food contact materials at ambient temperature (20-23°C), 62.8°C and 73.8°C for 30 min. They found that water alone was not effective to remove milk soil from stainless steel plates, but was effective to remove peanut butter soil from most of the surface at 62.8°C and 73.8°C. Chlorinated alkali cleaner was able to remove both hot and cold milk soil at these three temperatures. Both chlorinated alkali cleaner and acid detergent cleaner was able to remove peanut butter soil from the food contact surface at 62.8°C.

Perry and other (2004) studied the removal of peanut allergen Ara h 1 from various household and school environments. They found that Ara h 1 can be easily cleaned by hand washing with liquid soap, bar soap and commercial wipes. Plain water was unable to fully remove Ara h 1. Common household cleaning agents, besides dish-washing liquid was effective to remove Ara h 1.

2.7. Methods to Detect Allergens

Many analytical methods have been developed over the past 20 years to detect food allergens (Poms and others 2004), and these methods have been dominated by two analytical methodologies. The Polymerase chain reaction (PCR) detects the presence of DNA sequence from food allergenic ingredients. Immunoassays use antibodies to detect food allergens from allergic foods (Johnson and others 2011).

2.7.1. Polymerase Chain Reaction (PCR)

PCR is a technique to amplify a single copy or a few copies of DNA sequence through several thermal cycles of duplication and then generating many copies of that copy of DNA sequence. Generally, the thermal cycles contain three steps and can be controlled by controlling

temperature: melting of double stranded DNA, annealing of the primers, and extension of the primers by the polymerase (Poms and others 2004). In recent years, PCR has been used to detect food allergens. PCR is a DNA-based method to detect food allergens. It needs to be noted that the presence of allergen DNA doesn't necessarily mean the food sample contains the food allergen protein produced from the allergen DNA.

There are some advantages to PCR. Firstly, PCR makes a selective assay for a food stuff, which is simple and inexpensive. Secondly, amplifying DNA can be extremely sensitive, since theoretically only one segment of DNA can be detected, although detection limits depend on reaction conditions. Thirdly, DNA is more stable than protein when both of them are extracted from a harsh environment. Fourth, DNA can resist geographical and seasonal variations, which may affect protein compositions (Poms and others 2004).

There are drawbacks to PCR. Extraction of pure DNA requires special training. Heat and enzymes may affect fragmentation of DNA (Baumgartner and others 2007). Acidic pH and PCR-inhibitors from a food matrix can also affect the PCR method (Allmann 1993). Food processing may separate proteins and DNA, which can give erroneous results regarding the presence of allergens in the product (Poms and other 2004; Johnson and others 2011).

2.7.2. Immunoassay

2.7.2.1. Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a method based on specific binding of an antigen to an antibody in the wells of a microtiter plate. A color change is measured at a specific wavelength of light (depends on enzyme and substrate used) in a spectrophotometer and quantified by calculating it by using a standard curve, which is established with several known concentrations of antigen. It was developed in 1971 (Engvall and Perlmann 1971) and became

popular in the 1980s and has been widely used in food-related application since then, due to its specificity, sensitivity, and high screening capacity (Baumgartner and others 2007). A survey conducted in 2006 (Taylor and others 2006) suggested that ELISA was the second most common allergen cleaning verification method after visual inspection among food companies. Food companies used ELISA to test finished products, ingredients, equipment surface, water rinse and push-through, while testing finished products and ingredients were much less common.

The advantages of ELISA techniques include high automation of the test procedure, the resulting extensive screening potential, high sample throughput, and the easy operation (Baumgartner and others 2007). However, there are some problems related to ELISA. Before conducting an ELISA, the allergen needs to be extracted in solution. It takes a long time to extract, and measurements of allergen concentration can be affected by manufacturer of the kit, food matrix interference and type of processing food undergo (van Hengel 2007; Taylor and others 2009). Additionally, ELISA cannot detect hydrolyzed protein residuals altered by heat processing (Taylor and Baumert 2015).

Commercial ELISA kits have been developed for most of the "big eight" allergens, except fish and a few tree nuts (i.e. macadamia nut and pine nut) (Taylor and Baumert 2015). However, results from different commercial ELISA kits lack comparability since different measurands, extraction procedures, antibodies, detection systems and calibrants are used by different companies (Zeleny and Schimmel 2010). A recent study (Jayasena and others 2015) investigated six commercial peanut ELISA kits and found that four of them underestimated the protein content in standard reference material (SRM) for peanut (2387) developed by the National Institute of Standards and Technology (NIST), although kit manufacturers have optimized extraction procedures in the instructions. They also suggested that the recognition of

peanut major protein (Ara h 1, 2, 3 and 6) also differed among the different methods, and 5 of them were most sensitive to Ara h 3. It is important to understand which kind of protein will be studied and demonstrate the extraction method and kit used when communicating the results.

2.7.2.2. Lateral Flow Strips (LFS)

LFS, also known as lateral flow immunochromatographic assay (LFA) or dipsticks, is widely used by food industries to assess the cleanliness of shared processing surfaces after sanitation (Taylor and Baumert 2015). LFS was first developed in 1997 (Mills and others 1997) based on strip tests for detecting pregnancy (Leuvering and others 1980). LFS is a type of qualitative or semi-quantitative immunoassay relying on specific antibody-antigen recognition. It contains zones where antibodies are affixed to the solid pads. Prepared samples wicked on the strip can produce visible lines where antigens and antibodies interact. A new labeling material—superparamagnetic nanoparticle (SPMNP) can replace traditional labeling materials, such as colloidal gold and latex (Wang and others 2009). SPMNP are quite stable and allow LFS to be used as a quantitative method by measuring signal intensity. Advantages of LFS are that it is sensitive, fast, portable and inexpensive (Schubert-Ullrich and others 2009; Koczula and Gallotta 2016). Disadvantages of LFS are that its reproducibility varies from lot to lot, and pretreatment of samples is required (Bahadır and Sezgintürk 2016; Sajid and others 2014).

2.8. Significance and Justification

It was estimated that overall economic cost of food allergy was $24.8 billion annually ($4184 per year per children) (Gupta and others 2013). Prevalence of peanut allergy was found to be the highest among big eight food allergens in children (Gupta and other 2011). The consequence of consuming peanut by people who have peanut allergy is also severe, it can lead to anaphylaxes and even death. Additionally, ice cream recalls due to undeclared allergens in the

last two years (2015-2017) from the FDA recall history, undeclared peanut got the first place (FDA 2017).

Cross contamination is one of the reasons for undeclared allergens. Cleaning is the best way to reduce the chance of cross contamination on shared equipment. With the increasing popularity of CIP systems used in the food industry, understanding the principle of cleaning in CIP system is essential for developing a cleaning protocol in an allergen control plan. Time and temperature are two important parameters in a CIP system. Study on how cleaning time and water temperature change in CIP system affect the removal of peanut allergen is rare. In terms of detection method, ELISA for peanut allergen Ara h 1 was chosen based on the quantity of Ara h 1 in peanut.

On the other hand, CIP program from the CIP system manufacturers may not be suitable for all food industries due to difference of processing environment and food matrix. It is necessary to verify effectiveness of CIP program in each food industry.

The MSU dairy plant produces over 20 different flavors of ice cream in total and produces at least two flavors in one production day. Although the dairy plant has an allergen control plan to avoid allergen cross contamination in one production day, properly and completely cleaning allergens in the flavor tank is critical to eliminate cross contamination between each production day. There is only one continuous ice cream freezer in the MSU Dairy Plant, which can be considered as shared equipment to produce different ice creams containing different allergens. Furthermore, whether the CIP program in the dairy plant can remove peanut allergen completely has not been verified. Verification of cleanliness can provide food safety and more confidence that the products from the dairy plant are clean and without cross contamination by other allergens.

CHAPTER 3

MATERIALS AND METHODS

3.1. Ice Cream and Inoculation

Buckeye Blitz ice cream which contains peanut and soy allergens, was obtained from the MSU Dairy Plant. The list of ingredients and nutritional facts label can be found in Appendix A. The concentration of peanut allergen protein Ara h 1 in the thawed ice cream was estimated that approximately 6000 ppm based on the information from the ice cream formulation and Pomes and others (2013). Buckeye Blitz ice cream was thawed at 40°F/4.4°C overnight and turned into liquid state to simulate the condition in flavor tank in the MSU Dairy Plant. This liquid state ice cream is called mix in the following text. A stainless steel pipe type 304 (2.2 cm inside diameter, 21.5 cm length) was filled up with the mix and both ends were covered by two stainless steel plates. This configuration is shown in Appendix B **Figure B1**. After filling up with the mix, the stainless steel pipe stood vertically for 90 mins at 38°F /3.3°C before cleaning treatments.

3.2. Verification of Clean out of Place (COP) Washing

To verify the COP washing method, the stainless steel pipe was filled with the mix as described in section 3.1. and was rinsed with a hose (water pressure 90 psi, flow rate 0.29 L/s, water temperature 104°F/40°C) until all residue was removed. Then it was washed manually by following the COP washing protocol of the MSU Dairy Plant. It was washed with chlorinated alkaline (NaOH and Na_2CO_3) detergent solution for 10 seconds using brushes (figure shown in Appendix B **Figure B2)** followed by a tap water rinse (122°F/50°C) with the hose for 5 seconds. The chlorinated alkaline water was prepared from 30 grams of Ecolab HC-10 Chlorinated Kleer-Mor (Saint Paul, MN) chlorinated alkaline powder per liter of hot water (132.8°F/56°C), total 15

liters of washing solution. The pipe was washed with the detergent solution at 132.8°F/56°C or with the solution cooled down to room temperature at 69.8°F/21°C. After manual washing procedure, the entire inside surface of the pipe was swabbed by BioKits Allergen Swabbing Kit (figure shown in Appendix B **Figure B3)** from Neogen (East Lansing, MI). The samples from the Allergen Swabbing Kit were analyzed by BioKits Peanut Allergen Assay Kit (figure shown in Appendix B **Figure B5)** from Neogen following the procedure in the manual. Absorbance was measured by using µQuant™ Microplate Spectrophotometer from BioTek Instruments, Inc. (Winooski, VT). Concentration of peanut allergen (Ara h 1) was calculated from the standard curve developed from standard solutions. By following the manual of BioKits, when an absorbance value falls within the quantifiable range (0-20 ppm), a conversion factor x10 was used to convert the concentration to ng/mL (e.g. a swab sample (no dilution) giving an absorbance that extrapolates to 2 ppm on the peanut standard curve contains 20 ng/mL peanut allergen protein). Each treatment was done in duplicate with the same stainless steel pipe.

3.3. Investigating Effectiveness of Temperature and Time on Simulated CIP Rinse

The stainless steel pipe filled with the mix for 90 minutes was attached on equipment shown in **Figure 1** (Appendix B **Figure B5** shows the actual equipment) and rinsed with combinations of water temperature and time shown in **Table 1.** The equipment was assembled with type 304 stainless steel pipes and a Reliance® Duty Master® A-C Motor Type P Design E pump from ABB (Cary, NC), water pressure was 70 psi, flow rate was 1.53L/s. In the rinsing treatment, there were five temperatures and three time periods. Therefore, it had total 15 combinations. Tank A was filled with tap water for rinsing. After rinsing, the pipe was detached and set vertically for 3 minutes to drain the water. Samples were obtained from the pipe via BioKits Allergen Swabbing Kit from Neogen, then the samples were tested for concentration of

peanut allergen (Ara h 1) by Neogen BioKits Peanut Allergen Assay Kit. All the inside surface of the pipe was swabbed. Absorbance was measured by using µQuant™ Microplate Spectrophotometer from BioTek Instruments, Inc. (Winooski, VT). Concentration of peanut allergen (Ara h 1) was calculated from the standard curve developed from standard solutions. The pipe was manually washed via the method mentioned in section 3.2. after swabbing by the test kit and dried for the next mix inoculation. Each combination of water and temperature was tested twice with the same stainless steel pipe.

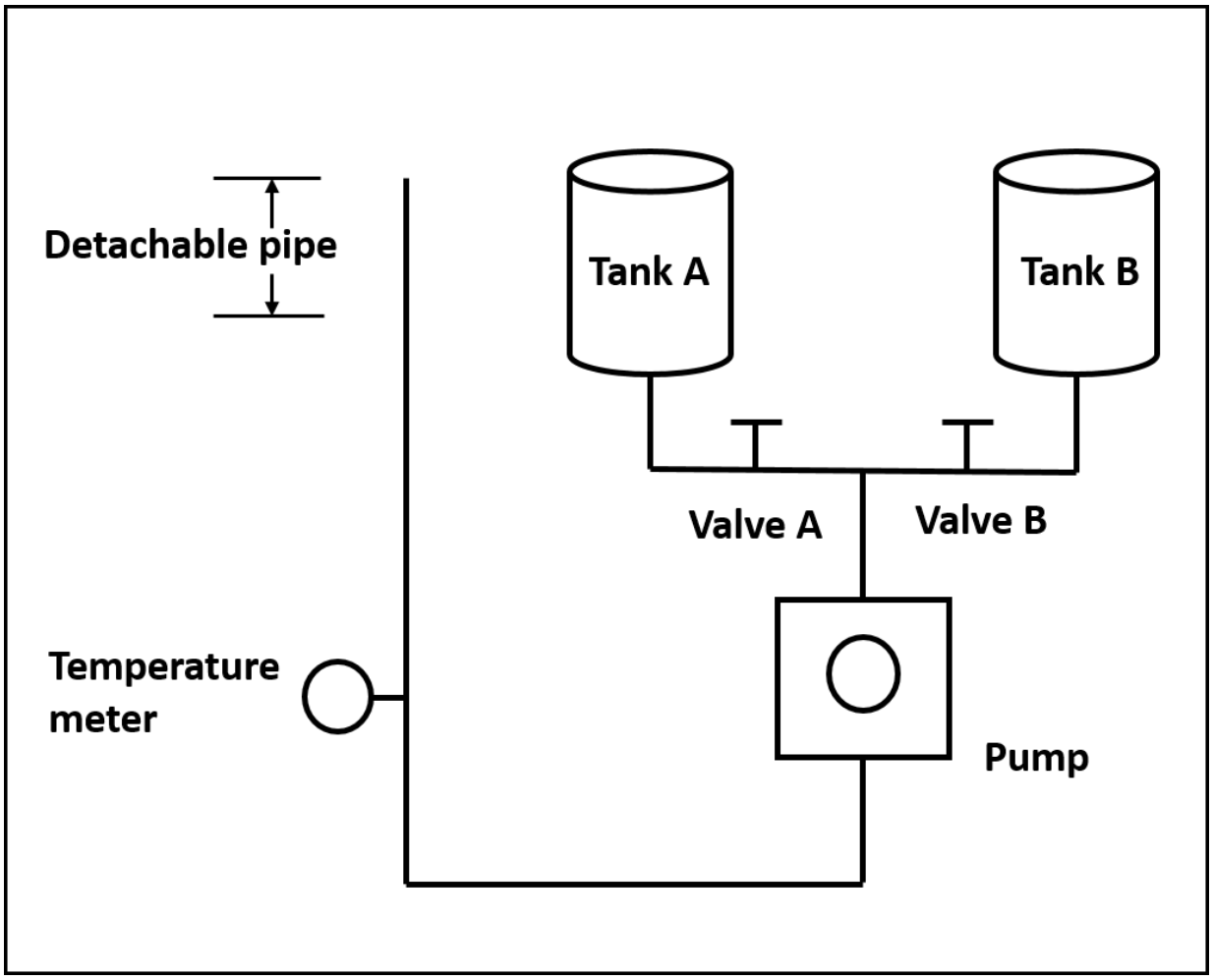

Figure 1. Diagram of the equipment used in clean-in-place (CIP) rinsing experiment. Rinsing water was in Tank A. Tank A is a double-wall tank, and steam source came behind the tank to provide heat for rinsing water. After rinsing, water was drained.

Table 3. Specific water temperature and time combinations in each treatment.

Combination number	Water temperature (°F/°C)	Time (seconds)
1	68/20	10
2	68/20	20
3	68/20	30
4	86/30	10
5	86/30	20
6	86/30	30
7	104/40	10
8	104/40	20
9	104/40	30
10	122/50	10
11	122/50	20
12	122/50	30
13	140/60	10
14	140/60	20
15	140/60	30

3.4. Investigating Effectiveness of Temperature and Time on Simulated CIP Wash

Stainless steel pipe filled with the mix and held for 90 minutes as described in section 3.1. was attached to the equipment shown in **Figure 2.** It was the same equipment as shown in **Figure 1,** but with extending the detachable pipe to the position between Tank A and Tank B. Tank A was filled with rinse water and Tank B was filled with detergent solution. The pipe was rinsed with rinse water first with the combinations of water temperature and time shown in **Table 1,** then was washed with the recirculating detergent solution in Tank B with same temperature and time as rinsing. Washing solution was prepared according to the direction: 7.81mL of Principal™ (NaOH and NaOCl) from Ecolab (Saint Paul, MN) per liter of water. After washing, the pipe was rinsed again with the water in Tank A for 5 seconds. For example, the pipe was rinsed with water at 68°F/20°C for 10 seconds, and then it was washed with the recirculated detergent solution at 68°F/20°C for 10 seconds, then rinsed again with the water at 68°F/20°C for 5 seconds. The pipe was detached and set vertically for 3 minutes to drain the water. Samples were obtained via BioKits Allergen Swabbing Kit from Neogen and analyzed by BioKits Peanut Allergen Assay Kit. Absorbance was measured by using µQuant™ Microplate Spectrophotometer from BioTek Instruments, Inc. (Winooski, VT). Concentration of peanut allergen (Ara h 1) was calculated from the standard curve developed from standard solutions. The pipe was manually washed via the method mentioned in section 3.2. after swabbing by the test kit and dried for the next mix inoculation. Each combination was tested twice with the same stainless steel pipe.

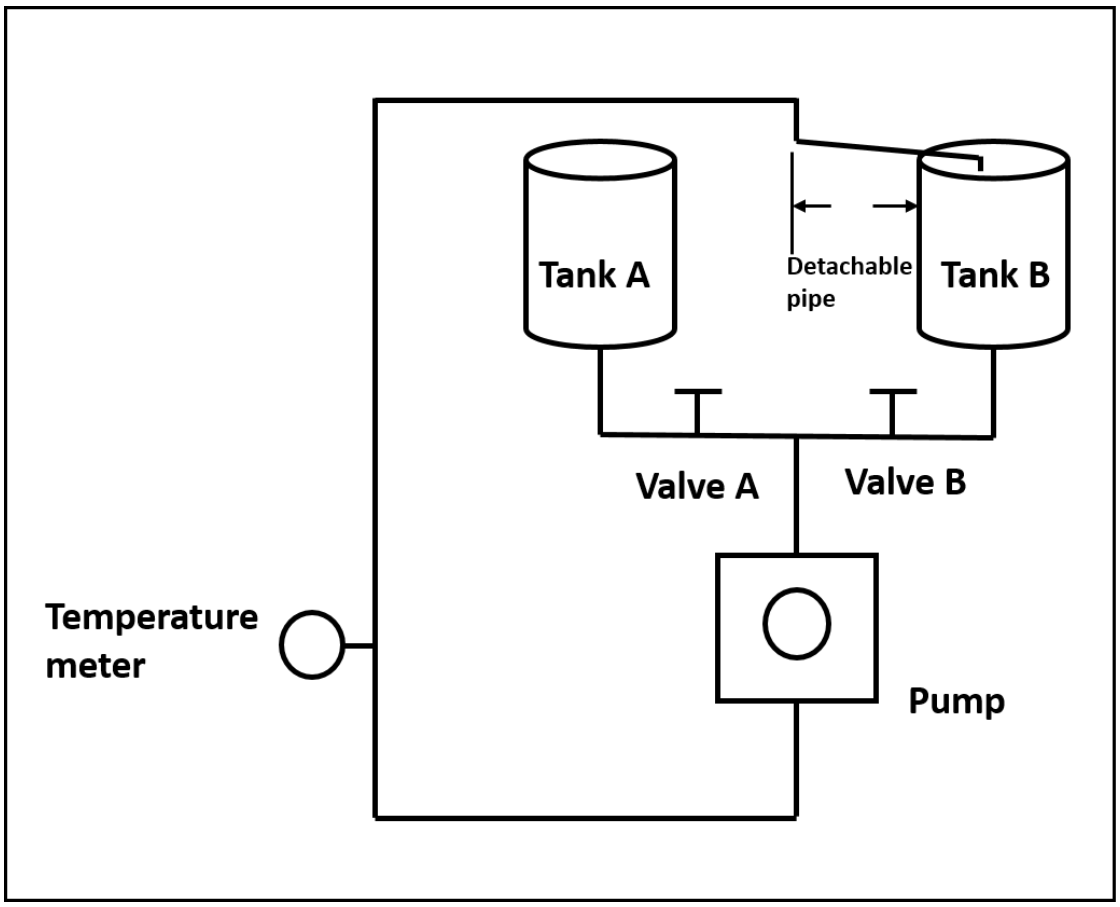

Figure 2. Diagram of the equipment used in clean-in-place (CIP) washing experiment. During rinsing the pipe, the valve A was turned on and the valve B was turned off. The detachable pipe can rotate, so that the rinse water can go into the drain. During washing the pipe, the valve A was turned off and valve B was turned on, the detachable pipe can rotate back to the position shown above to recirculate the washing solution.

3.5. Verification of CIP Washing System in MSU Dairy Plant

On a production day of Buckeye Blitz ice cream February 28th, 2018, after the cleaning program in the Dairy Plant was completed, two samples were collected via Neogen BioKits Allergen Swabbing Kit in the flavor tank used to produce Buckeye Blitz ice cream and tested with Neogen BioKits Peanut Assay Kit. One sample was collected from the middle of the inside

wall on the left, and the other was collected from the inside surface of the pipe just connected to the flavor tank.

3.6. Statistical Analysis

Peanut allergen concentrations left on the stainless steel pipe after manual washing clean out of place (COP) at 69.8°F/21°C and 132.8°F/56°C were compared by using student t-test at the significance level of 0.05.

Peanut allergen concentrations left on the stainless steel pipe after rinsing or rinsing then washing were analyzed by a general linear mixed modeling (GLMM) approach conducted by Statistical Analysis Software (SAS) (Cary, NC) Version 9.4 (Littell and others 2006, Milliken and Johnson 2009). The mixed modeling estimation was performed by the method of Restricted Maximum Likelihood (REML). The statistical inference was based on mixed effects Analysis of Variance (ANOVA) with Kenward-Roger Degrees of Freedom Approximation. The main and interaction effects (e.g., overall time effect, temperature effect, time and temperature interaction) were estimated via Least Squares Means (LS-Means), and the simple effects (e.g., a marginal effect of temperature given a temperature) were examined using a slicing approach with Fisher's Least Significant Difference (LSD) for multiple comparisons at the significance level of 0.05. In all statistical analyses, the assumptions of normality of statistical errors and homogeneity of variances were checked and met for avoiding bias from inappropriate assumption violation and thus for improving the generalizability and reproducibility of findings in this study (Melakeberhan and others 2018).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Preliminary Tests

Two preliminary tests were conducted to test the presence of peanut allergen left on the stainless steel pipe after just water rinsing. For the first one, two stainless steel pipes were filled with Buckeye Blitz ice cream mix as described in the section 3.1. After that, the two pipes were rinsed with a hose (water pressure 90 psi, flow rate 0.29 L/s) for 30 seconds at water temperature 59°F/15°C or 104°F/40°C. These two pipes then tested with the Neogen (East Lansing, MI) Reveal 3D peanut test kit, the one rinsed at 59°F/15°C showed a positive result and the one rinsed at 104°F/40°C showed a negative result. For the second preliminary test, same treatment was used to treat three stainless steel pipes, but they were rinsed with a pump (water pressure 70 psi, flow rate 1.53 L/s, water temperature 82.4°F/28°C) for 20s. The same test kit was used to test same amount of area on these three pipes, and all of them showed positive results. These two preliminary tests suggest that peanut allergen can attach on the surface of stainless steel pipe. Further experiment is reasonable to be conducted to investigate the effect of time and temperature on removing peanut allergen on stainless steel pipe.

4.2. Clean Out of Place (COP) Washing

For the verification of COP washing, the stainless steel pipe after ice cream treatment was washed manually following manual washing protocol in MSU Dairy Plant. The pipe was washed with the detergent solution at 132.8°F/56°C or with the detergent solution cooled down to room temperature 69.8°F/21°C. The standard curve used in this section is shown in **Figure 3.** When the pipe was washed at 132.8°F/56°C detergent solution, the concentration ± standard

deviation was 0.0072 ± 0.0056 ppm. When the pipe was washed at room temperature (69.8°F/21°C), concentration ± standard deviation of peanut allergen was 0.0020 ± 0.0014 ppm.

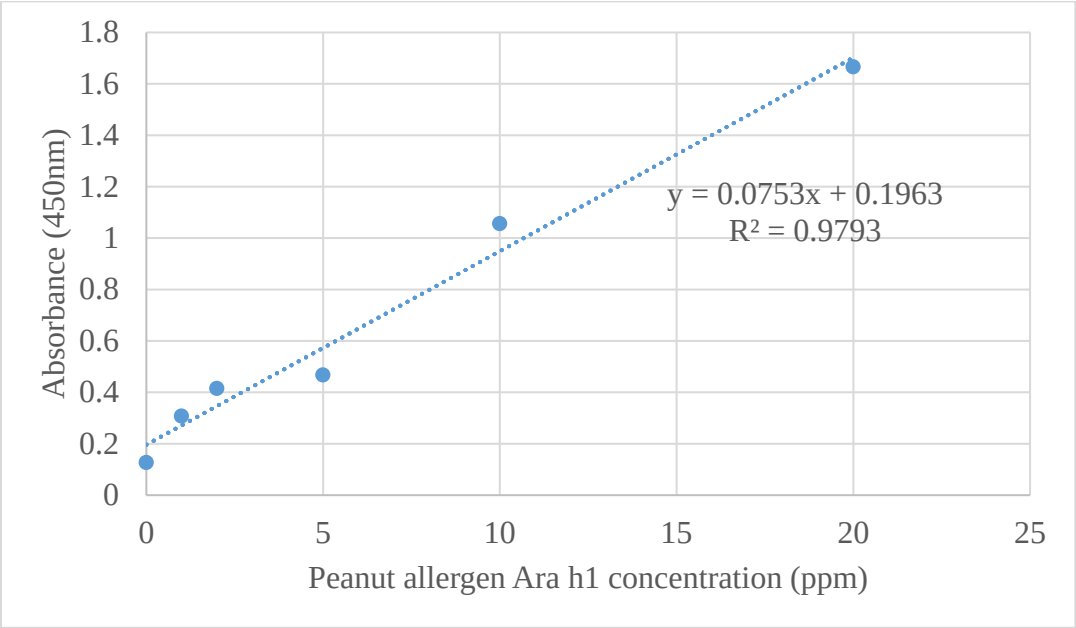

Figure 3. Standard curve for COP washing. It was used for samples obtained from 69.8°F/21°C and 132.8°F/56°C manual washing. Dilution ratio was 1:1.

Although washing at 133°F/56°C had a relatively higher concentration left on the pipe, there is no significant difference ($p<0.05$) when ran student t-test. According to the direction of HC-10 Chlorinated Kleer-Mor, the powder should dissolve in the water at about 120°F/48.8°C. Therefore, it is recommended to wash at 120°F/48.8°C. But in actual working condition, the detergent solution is made with 120°F/48.8°C water and used at once as well as used after it cools down to room temperature. This was the reason for testing the detergent solution at room temperature. Fortunately, this kind of detergent solution is still able to work normally at room temperature. On the other hand, this result also suggested that temperature didn't play an

important role on removing peanut allergen when using this detergent solution. Alkaline detergent solution is very effective for cleaning protein residue (Holah 2014). The reference dose of peanut allergen for human is 0.2 mg (Taylor and other 2014), manual washing is effective to remove most of the peanut allergen on the stainless steel pipe. The amount of peanut allergen left on the pipe is unlikely to trigger allergic reactions.

4.3. Investigating Effect of Time and Temperature on Clean-In-Place (CIP) Rinse

In this study, three times (10, 20 and 30 seconds) and five temperatures (20, 30, 40, 50 and 60°C) were compared in the CIP rinse. The stainless steel pipe was rinsed at different water temperatures and times after thawed ice cream treatment on the equipment shown in **Figure 1.** Standard curves used for calculating concentrations in this experiment are shown in **Figure 4.** and **Figure 5.** The results are shown in **Table 2.** Concentrations of peanut allergen residue on the stainless steel pipe ranged from 207.4 ppm to 63.0 ppm. The ANOVA table for rinsing data is shown in **Table 3.** Time, temperature and time*temperature interaction all had significant effect ($p<0.05$). Main effect had significant difference means at least one level of time or temperature had a different mean value of concentration compared to the other levels. Interaction effect had significant difference means relationship between one effect and peanut allergen concentration based on the other effect. The results of pairwise comparison for each combination using least square means method is shown in Appendix **Table C1**.

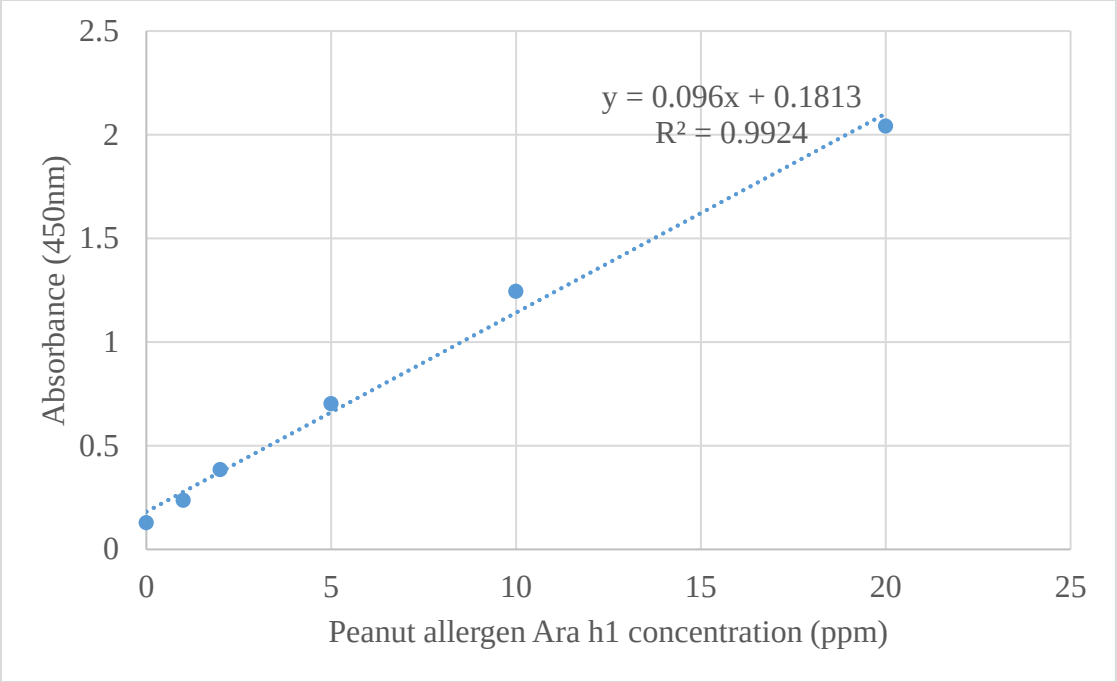

Figure 4. Standard curve for rising. It was used for rinsed combinations from 9 to 15. Dilution ratio was 1:500.

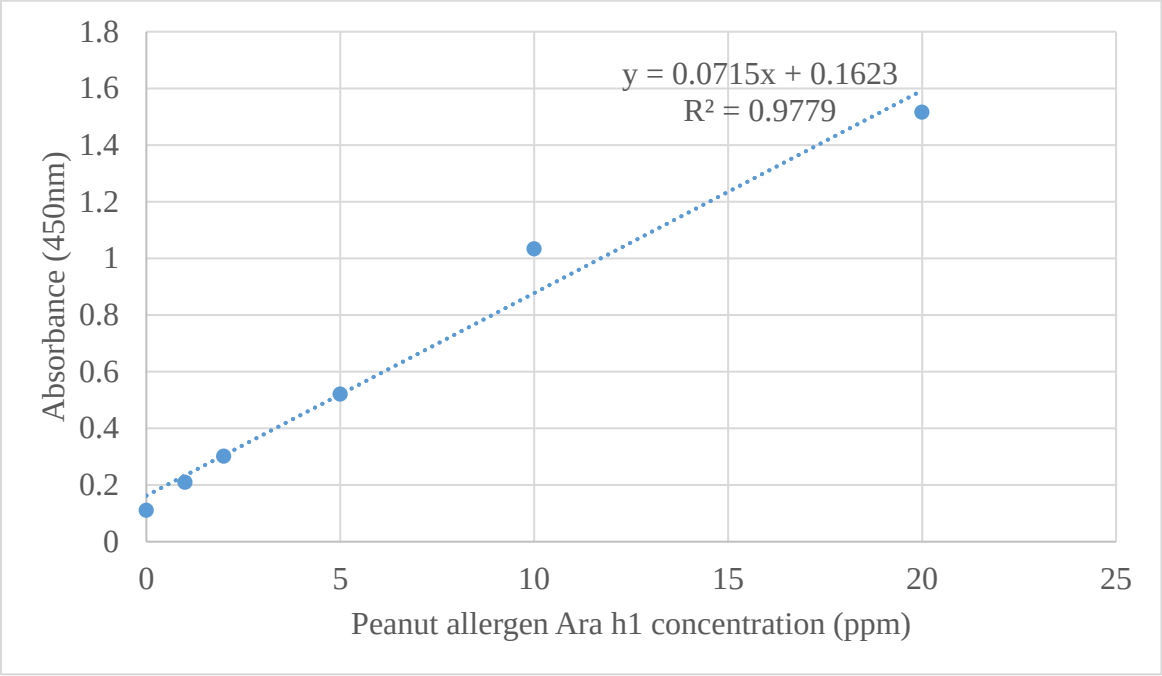

Figure 5. Standard curve for rinsing. It was used for rinsed combinations from 1 to 8. Dilution ratio was 1:2500.

Table 4. Concentrations of peanut allergen protein Ara h1 residue left on the stainless steel pipe after rinsing.

Time (s)	Peanut allergen Ara h1 concentrations (ppm)				
	20°C	30°C	40°C	50°C	60°C
10	163.4±3.4^{cx}	207.4±3.4^{ax}	187.7±3.4^{bx}	145.3±3.4^{dx}	101.0±3.4^{ex}
20	140.5±3.4^{ay}	128.5±3.4^{by}	105.4±3.4^{cy}	73.8±3.4^{dy}	81.6±3.4^{dy}
30	84.4±3.4^{az}	90.9±3.4^{az}	88.5±3.4^{az}	59.2±3.4^{bz}	63.0±3.4^{bz}

Mean of duplicate samples ± standard error. $^{a, b, c, d, e}$ different letter within the same row are significantly different. $^{x, y, z}$ different letter within the same column are significantly different. The significance level is 0.05.

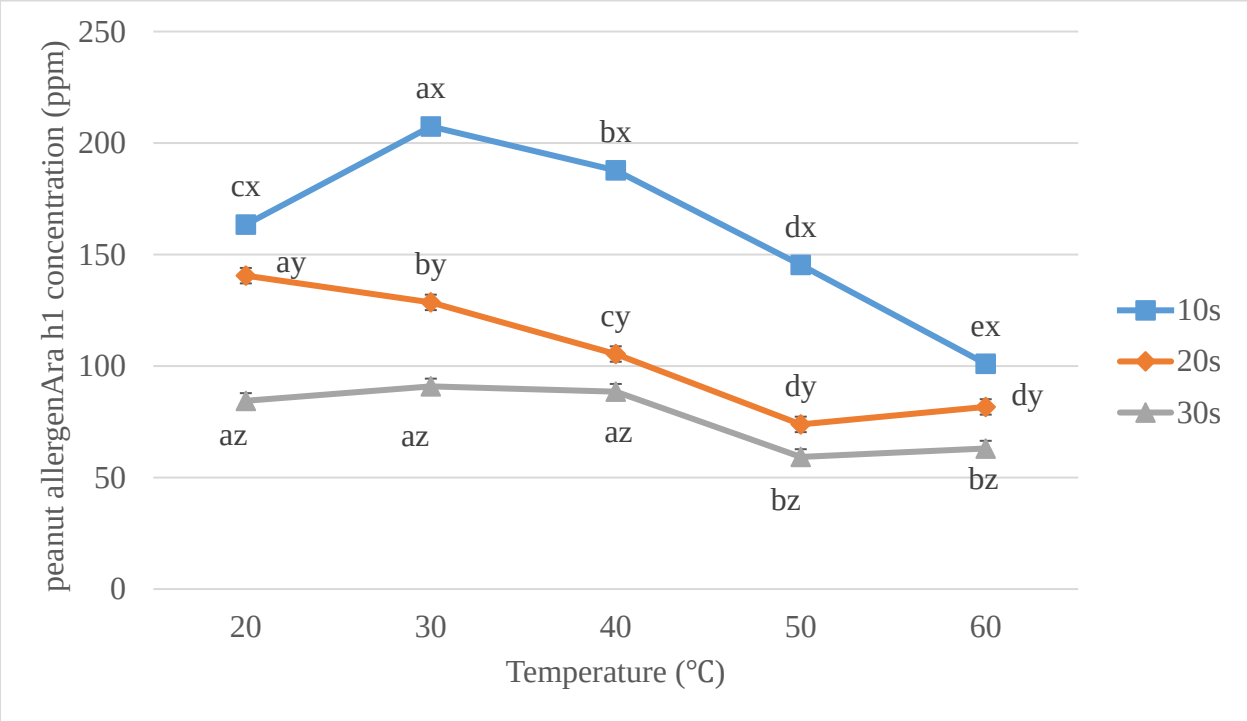

Figure 6. Concentrations of peanut allergen protein Ara h1 left on the stainless steel pipe after rinsing. a to e, different letters within the same time are significantly different. x to z, different letter within the same temperature are significantly different. Significance level is 0.05.

Table 5. ANOVA table for rinsing time, rinsing temperature and rinsing time and temperature interaction.

Source	DF	Sum of Square	Mean of Square	Error DF	F value	Pr > F
Time	2	36239	18119	15	757	< .0001
Temp.	4	16140	4035	15	169	< .0001
Time*Temp.	8	5730	716	15	30	< .0001
Residual	15	359	24			

Note. ANOVA is based on a two-way factorial analysis of variance model with an interaction.

There were two standard curves used in this section. In the first measurement, samples were all diluted with the ratio 1:500. However, results from combination 1 to 8 didn't fall within the standard curve, and they had a concentration higher than 20 ppm. Further dilution needed to be done for these samples. The Sample from combination 1 to 8 were diluted to 1:2500. Results obtained were within the standard curve under this dilution ratio.

Comparing this result with the previous concentration of peanut allergen in the mix, very effective effect of rinsing on removing peanut allergen on the stainless steel pipe can be observed. Over 90% of the allergen residue was removed by just rinsing, although it cannot be considered as safe. From **Figure 6,** three rinsing time at five temperatures all were statistically significant ($p<0.05$) from each other. This means increasing rinsing time from 10 seconds to 30 seconds can significantly reduce peanut allergen residue on the stainless steel pipe. Since the denaturation temperature for peanut allergen Ara h 1, which was detected in the ELISA in this study, is 179.6-194°F/82-90°C (Montserrat and others 2013), the reduction of peanut allergen concentration can be considered by rinsing.

Rinsing for a longer time can reduce more residue. However, the impact of rinsing time on removing residue decreased as the time increases. As can be seen in the **Figure 6,** the slope

for rinsing 10 seconds and rinsing 20 seconds is larger than the slope for rinsing 20 seconds and rinsing 30 seconds. The deposits were mainly removed at the beginning of the rinsing (Jurado-Alameda and others 2011), and the mechanical force played an important role in this process (Weidemann and others 2013). Increasing rinsing time alone may not affect the effective of removing peanut allergen, since some strongly bonded protein maybe hard to remove by water rinse alone.

When rinsing the pipe for ten seconds, water temperature at 86°F/30°C had the highest peanut allergen residue, and it was significantly different from water temperature at 68°F/20°C and 104°F/40°C ($p<0.05$). A previous study from Kulkarni and others (1975) about investigating the effect of rinsing water temperature on removing whey protein found that rinsed with cold water (50 to 59°F/10 to 15°C) had similar effect as rinsed with hot water (167 to 176°F/75 to 80°C). The plot for peanut allergen residue temperature vs concentration for a very short time rinse maybe a "n" shape curve. The maximum residue concentration level maybe around 86°F/30°C. But more and wider range of temperature points need to be further studied. When rinsing the pipe for 20 seconds, higher temperatures had a better removal of peanut allergen, but there was no significant difference between 122°F/50°C and 140°F/60°C ($p<0.05$). This observation was consistent with previous studies on milk soil removal by rinsing (Fan and others 2015, Xin and others 2004). These two studies indicated that the rinsing effect of temperature on removing whey protein was limited when the temperature exceed 113°F/45°C and 131°F/55°C respectively. Gillham (1999) suggested that there was limited impact of temperature on removing deposits when it exceeded 122°F/50°C. When rinsing the pipe for 30 seconds, there was no significant difference among 68°F/20°C, 86°F/30°C and 104°F/40°C and between 122°F/50°C and 140°F/60°C ($p<0.05$). Higher temperature tends to increase the solubility as

well as accelerate the diffusion rate (Stanga 2010) and thus may help to remove the residue. In this study, rinsing for 30 seconds had more water to dissolve peanut allergen and made the effect of temperature on increasing solubility less obvious. But when the water temperature was increased to 122°F/50°C and 140°F/60°C, the solubility of peanut allergen may have increased and thus resulting in significant difference from temperature at 68°F/20°C, 86°F/30°C and 104°F/40°C.

Figure 8 and **Figure 9** show effect of time and temperature on removing peanut allergen visually. Both time and temperature play an important role in removing peanut allergen.

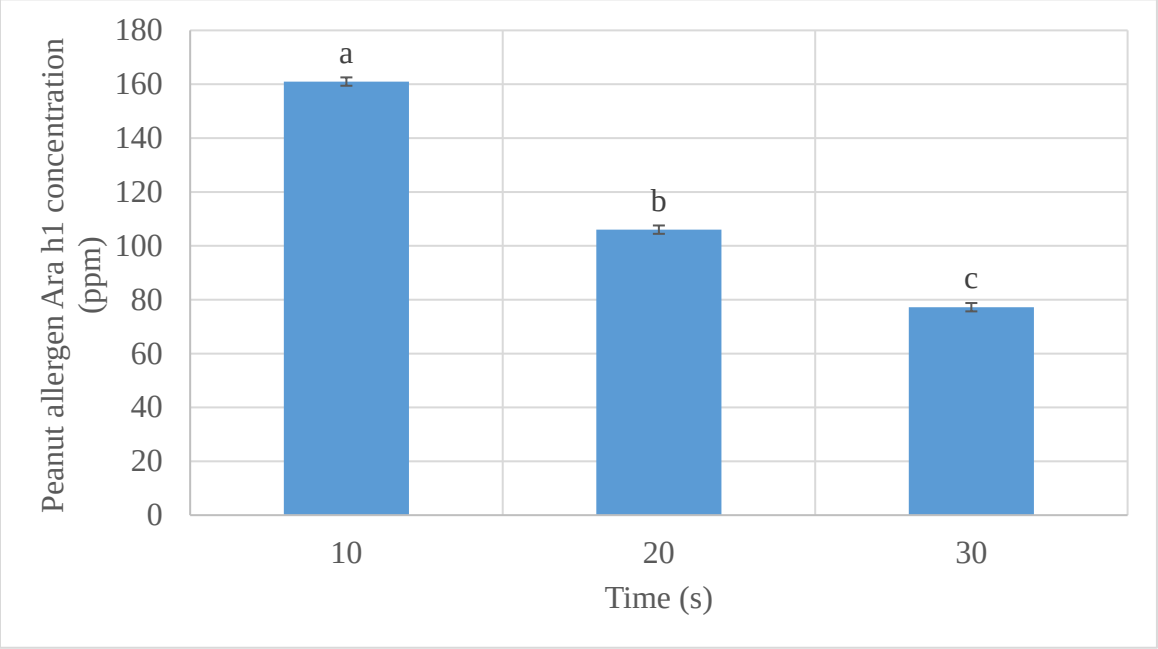

Figure 7. Overall time effect of rinsing on removing peanut allergen Ara h1 on stainless steel pipe. For letter a to c, different letter indicates there was significant difference (p-value<0.05).

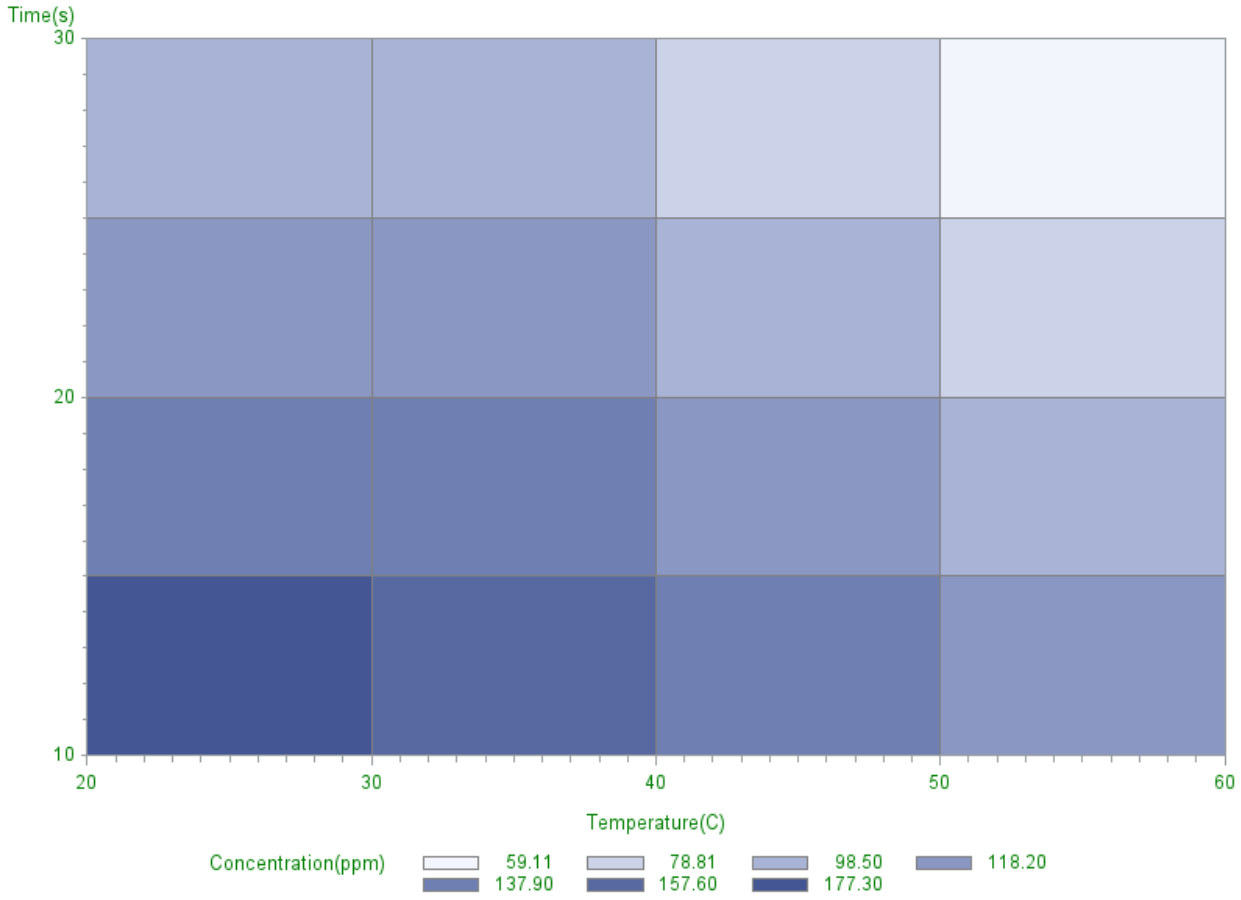

Figure 8. Results for peanut allergen Ara h1 concentrations left on stainless steel equipment after rinsing in a contour graph. Darker area means higher peanut allergen concentration.

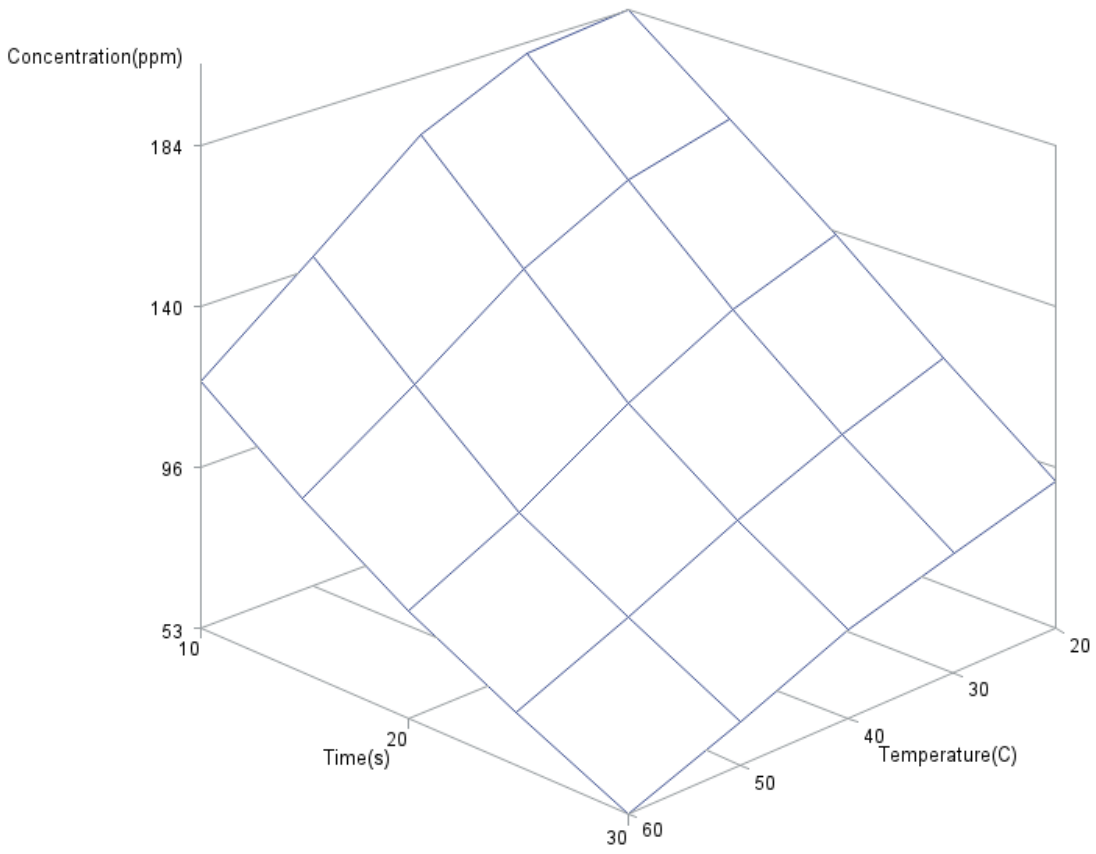

Figure 9. Results for peanut allergen Ara h1 concentrations left on stainless steel equipment after rinsing in a three-dimensional graph for rinsed results.

4.4. Investigating Effect of Time and Temperature on Clean-In-Place (CIP) Wash

In this study, when investigating time and temperature effect on removing peanut allergen on stainless steel pipe, three times (10, 20 and 30 seconds) and five temperatures (20, 30, 40, 50 and 60°C) were used. The stainless steel pipe was rinsed then washed with detergent at different water temperature and time after thawed ice cream treatment on the equipment shown in **Figure 2.** After that, the pipe was rinsed again for 5 seconds. The standard curves used in this experiment are shown in **Figure 10** and **Figure 11.** Concentrations of peanut allergen residue left on the pipe after rinsing then washing ranged from 1.4325 ppm to 0.0149 ppm. The ANOVA table for rinsing then washing data is shown in **Table 5.** As can be seen in the ANOVA table, time, temperature and time*temperature interaction all had significant effect ($p<0.05$). Main effect had significant difference means at least one level of time or temperature had a different mean value of concentration compared to the other levels. Interaction effect had significant difference means relationship between one effect and peanut allergen concentration based on the other effect. The results of pairwise comparison for each combination using least square means method is shown in Appendix **Table C2**.

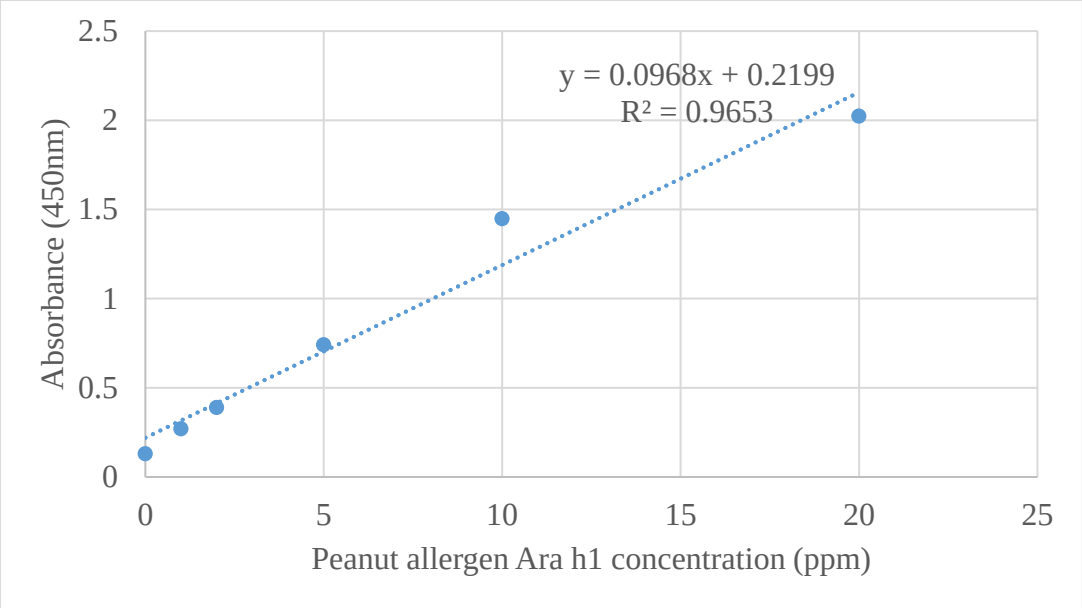

Figure 10. Standard curve for rinsed then washed. It was used for combination 1-2, 4-6, 9 and 11-15. Dilution ratio was 1:10 for 1-2, 6 and 11-12. Dilution ratio was 1:1 for 4, 5, 9, 13-15.

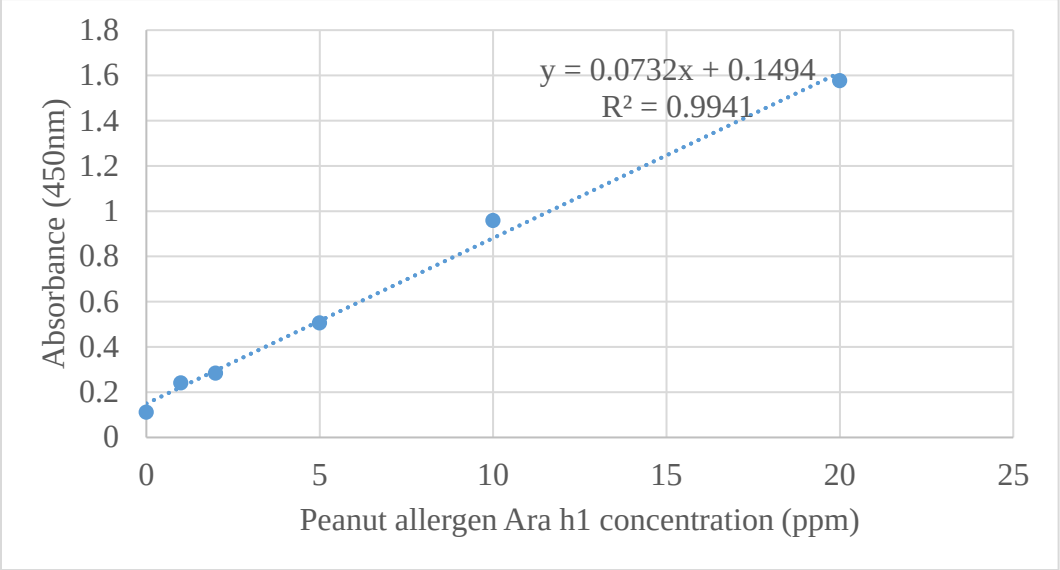

Figure 11. Standard curve for rinsed then washed. It was used for combinations 3, 7, 8 and 10. Dilution ratio was 1:10 for 3, 7 and 8. Dilution ratio was 1:1 for 10.

Table 6. Concentrations of peanut allergen protein Ara h1 residue left on the stainless steel pipe after rinsing then washing.

Time (s)	Peanut allergen Ara h1 concentrations (ppm)				
	20°C	30°C	40°C	50°C	60°C
10	1.29 ± 0.05^{ax}	1.27 ± 0.05^{ax}	0.38 ± 0.05^{by}	0.07 ± 0.05^{cx}	0.09 ± 0.05^{cx}
20	1.43 ± 0.05^{ax}	0.87 ± 0.05^{by}	0.69 ± 0.05^{cx}	0.03 ± 0.05^{dx}	0.02 ± 0.05^{dx}
30	1.36 ± 0.05^{ax}	0.81 ± 0.05^{by}	0.12 ± 0.05^{cz}	0.02 ± 0.05^{cx}	0.01 ± 0.05^{cx}

Mean of duplicate samples ± standard error. $^{a, b, c, d}$ different letter within the same row are significantly different. $^{x, y, z}$ different letters within the same column are significantly different at the significant level of 0.05.

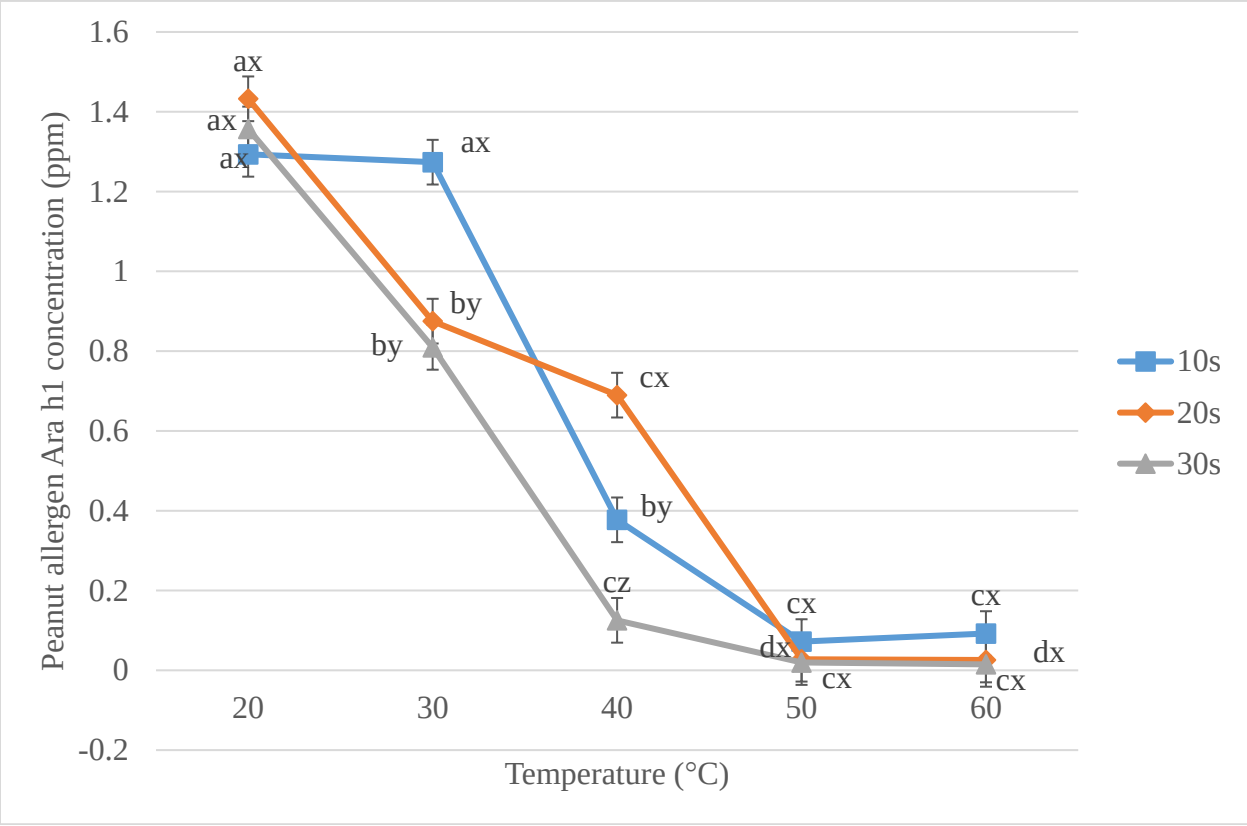

Figure 12. Concentrations of peanut allergen protein Ara h1 left on the stainless steel pipe after rinsing then washing. a to d, different letters within the same time are significantly different. x to z, different letter within the same temperature are significantly different. The significant level is 0.05.

Table 7. ANOVA table for rinsing time, rinsing temperature and rinsing time and temperature interaction.

Source	DF	Sum of Squares	Mean Square	Error DF	F value	Pr > F
Time	2	0.1521	0.076	15	12.24	0.0007
Temp.	4	8.3147	2.0787	15	334.69	<.0001
Time*Temp	8	0.4496	0.0562	15	9.05	0.0002
Residual	15	0.09316	0.006211			

Note. ANOVA is based on a two-way factorial analysis of variance model with an interaction.

Two standard curves were used in this section too. In the first measurement, the samples obtained from rinsing then washing at 68°F/20°C and 86°F/30°C were diluted to 1:10, the others were 1:1. However, combination 3 and 8 need to be further diluted since the concentrations were higher than 20 ppm. The reason for re-testing combination 7 and 10 was to lower the standard deviation of these two samples.

The peanut residue concentrations decreased about 100 times compared to the concentration obtained from just rinsing. These results agreed with previous study (Stephan and others 2004) about using alkaline detergent to clean peanut slurry, alkaline detergent was very effective on removing proteinaceous residue as expected. However, these results were lack of agreement with another study (Wang and others 2010). They found that gliadin residue can be removed to 1 ppm by just rinsing while in this study, peanut allergen residue was removed to 1 ppm by rinsing then washing. This difference may result from different study object, peanut vs gliadin. Although the recommended temperature for the detergent used in this study was 140-150.8°F/60-66°C, it can function even at as low as 68°F/20°C, which shows the effectiveness of alkaline solution for removing proteinaceous residue.

For the overall time effect on removing peanut allergen, there was no significant difference between 10 seconds and 20 seconds ($p<0.05$), but there was significant difference between these two times and 30 seconds as shown in **Figure 13** ($p<0.05$)**.** Similar trend was reported by Fan and others (2015) and Xin and others (2004) on removal of whey protein. When the pipe was washed at 68°F/20°C, there was no significant difference among three times ($p<0.05$), and 10 seconds had a relatively lower concentration. When was washed at 86°F/30°C, there was no significant difference between 20 seconds and 30 seconds ($p<0.05$). But washing for 10 seconds had significant difference with these two ($p<0.05$). Similar pattern can be seen in the data obtained from just rinsing. The maximum residue concentration for rinsing then washing for 10 seconds maybe around 86°F/30°C. But it also needs to be further studied with more temperature points to confirm these observations. When washed at 104°F/40°C, mean concentration of peanut allergen residue when washed for 10 seconds was lower than washing for 20 seconds and higher than washing for 30 seconds, and they were significantly different from each other ($p<0.05$). It is important to note that when stainless steel pipe was rinsed then washed for 20 seconds, it had the highest concentration of peanut allergen. When comparing the overall trends of 20 seconds and 30 seconds in **Figure 12**, both trends were very similar from 68°F/20°C to 140°F/60°C except at 104°F/40°C. Contamination of peanut allergen from other samples nearby may occur in this sample or during ELISA testing. Although the concentration was slightly higher than expected, it still can be considered as safe. There was no significantly difference when washed at 122°F/50°C and 140°F/60°C ($p<0.05$), although washing longer had a relatively lower peanut allergen residue concentration. One possible reason for this is the detergent may function similarly at 122°F/50°C and 140°F/60°C. Tank B (Shown in **Figure 2**) didn't have a heat source and it just had a single wall. To maintain the washing solution above

testing temperature (e.g. 122°F/50°C or 140°F/60°C) during the cleaning cycle, the washing solution generally was 35.6°F/2°C higher than the testing temperature when it was decanted into Tank B. The recommended temperature for the detergent used in this study was 140-150.8°F/60-66°C. The detergent may partially activate at about 122°F/50°C. To the best of author's knowledge, there is no study comparing the effect of temperature at 122°F/50°C and 140°F/60°C on removing peanut allergen.

The overall temperature effect on CIP wash is shown on **Figure 14.** Higher temperature had lower peanut allergen residue concentration as expected. Higher temperature helps to accelerate the diffusion rate as well as the chemical reaction (Stanga 2010). Hot alkaline solution can also break disulphide bonds between protein molecules, and thus decrease protein fragment size, which can increase the cleaning efficiency (Xin and others 2002). It needs to be noted that there was no significant difference between 50°C and 60°C when rinsed then washed for different times ($p<0.05$). Explanation for this condition is the volume of cleaning solution used in this study. At least 4 gallons of solution is needed to allow the system function properly. Therefore, the volume of cleaning solution in this study was 4 gallons. Comparing the ratio of volume of cleaning solution to the length of the pipe, the ratio in this study was about two to three times larger than the ratio in the MSU Dairy Plant. From the mean of square in ANOVA table, the contour graph in **Figure 15** and three-dimensional graph in **Figure 16**, it can be concluded that the effect of temperature on removing peanut allergen was more important than the effect of time.

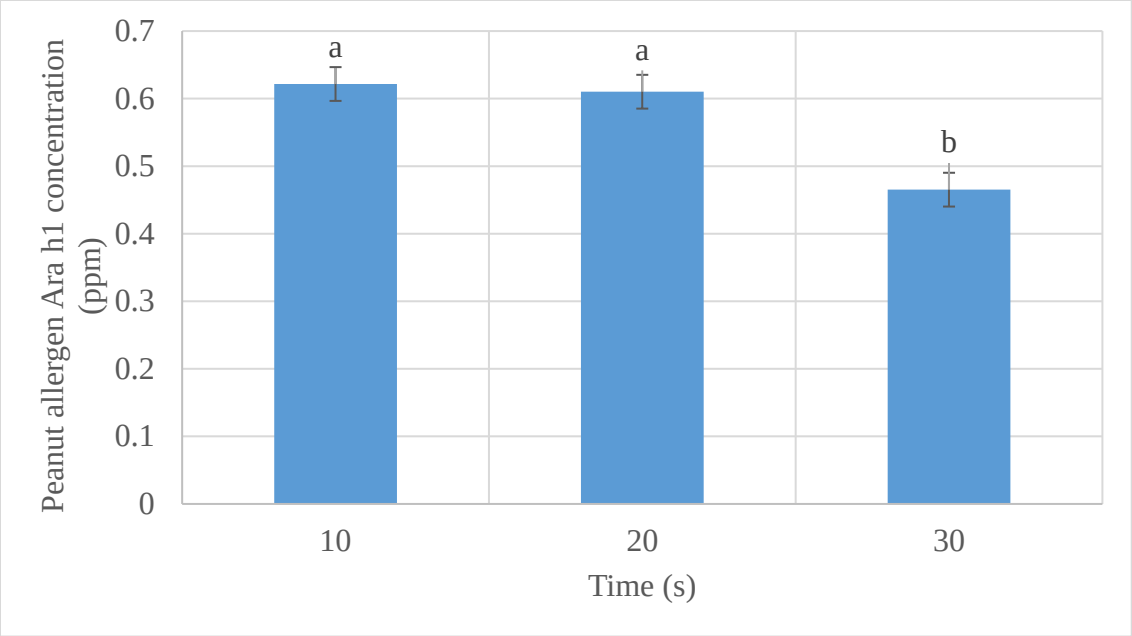

Figure 13. Overall effect of rinsing then washing time on removing peanut allergen Ara h1 on stainless steel pipe. For letter a and b, different letter indicates there was significant difference (p-value<0.05).

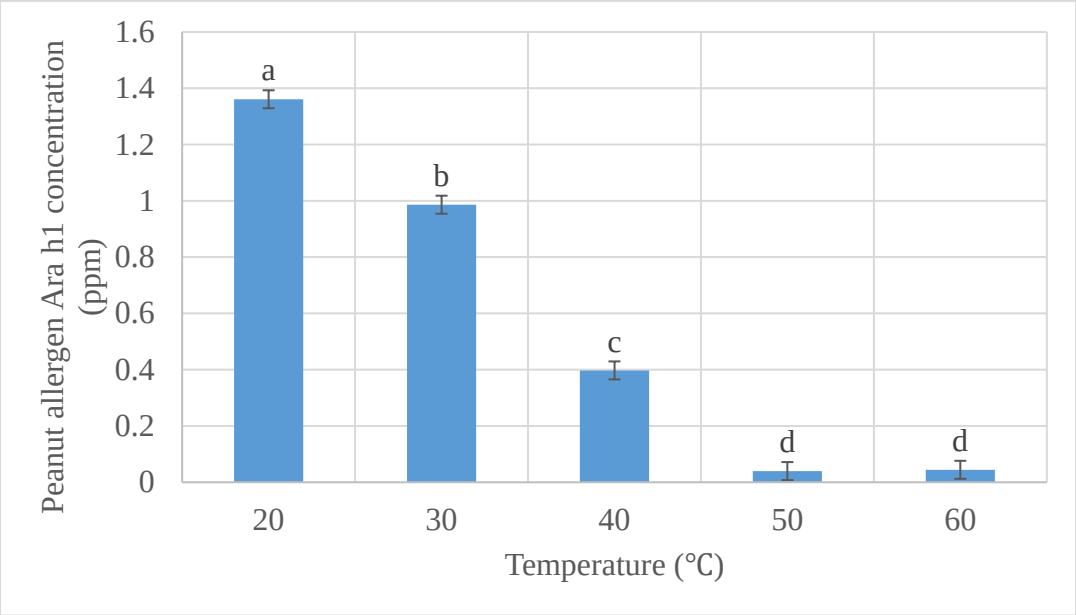

Figure 14. Overall effect of rinsing then washing temperature on removing peanut allergen Ara h1 on stainless steel pipe. For letter a to d, different letter indicates there was significant difference (p-value<0.05).

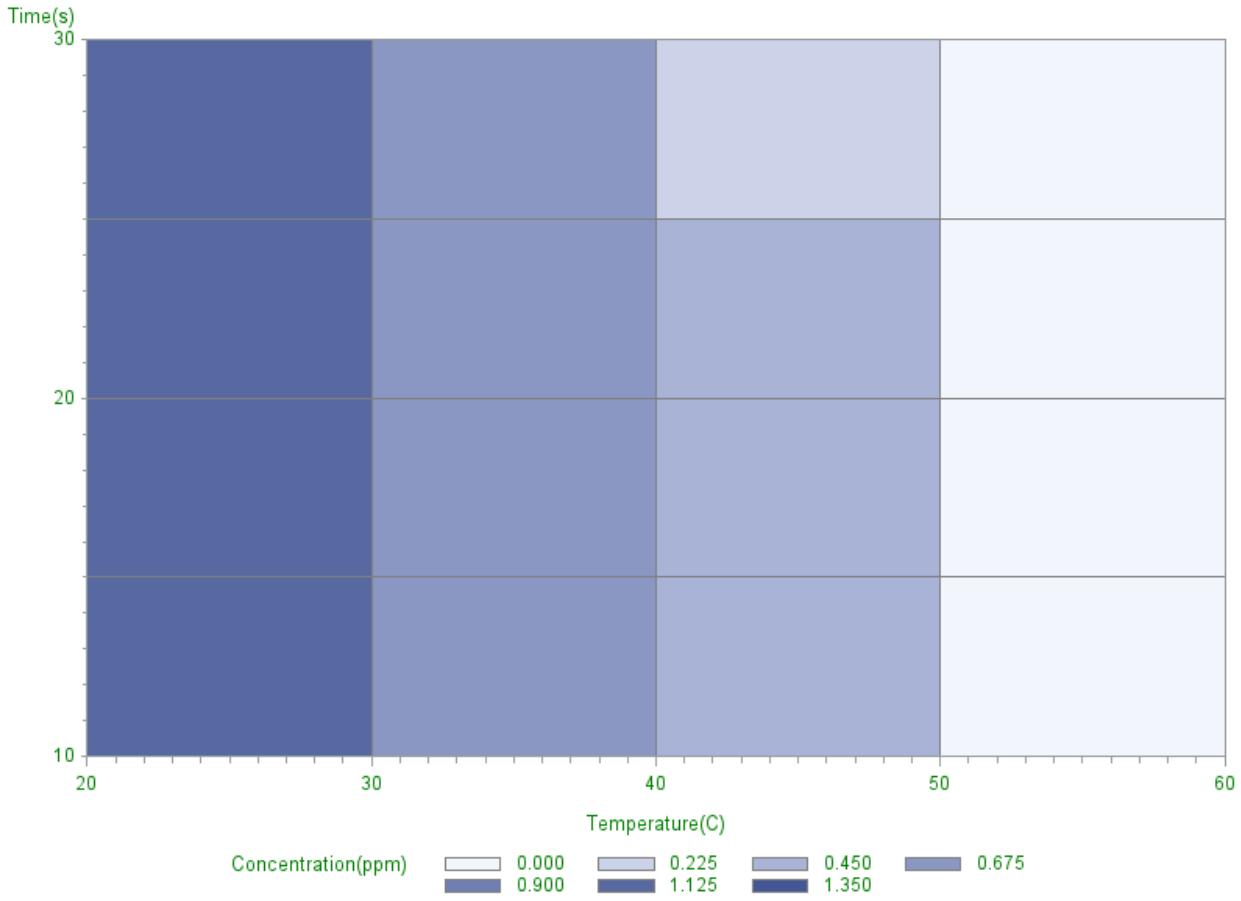

Figure 15. Results for peanut allergen Ara h1 concentrations left on stainless steel equipment after rinsing and washing in a contour graph. Darker area means higher peanut allergen concentration.

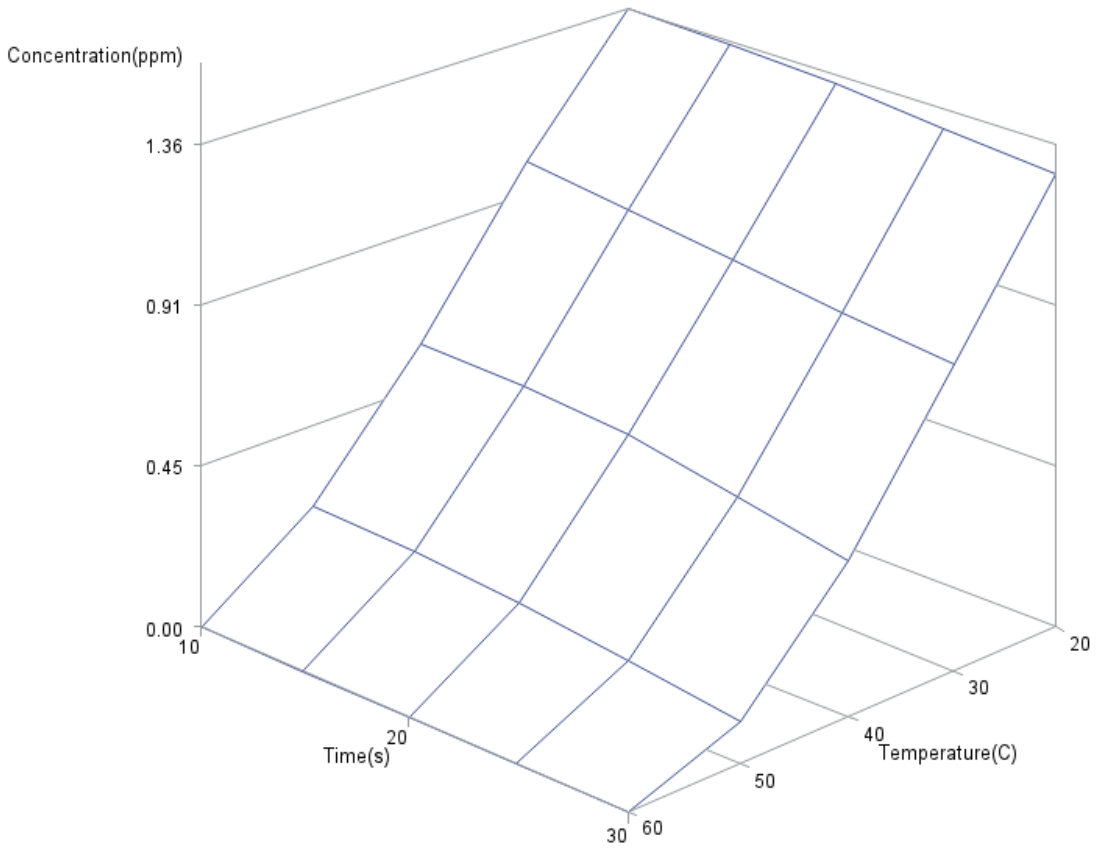

Figure 16. Results for peanut allergen Ara h1 concentrations left on stainless steel equipment after rinsing and washing in a three-dimensional graph for rinsed then washed.

4.5. Verification of CIP Washing System in MSU Dairy Plant

Two samples were collected after producing Buckeye Blitz ice cream from the flavor tank. The concentrations for the sample obtained from the inside wall was 0.005±0.000007 ppm, from the pipe just connected to the flavor tank was 0.051±0.0004 ppm. The results were recorded by mean ± standard deviation. Although the pipe had higher peanut allergen residue concentration, both locations can still be considered as safe.

4.6. Recommendations on current CIP system in the MSU Dairy Plant

When considering applying the findings in this study to the current CIP system, it is important to note that the flow velocity in this study was different from the flow velocity in the Dairy Plant. The flow velocity in this study was 4 m/s and the flow velocity in the Dairy Plant is estimated 12 m/s. Higher flow velocity can provide higher shear force, and shear force from the water flow is the primary mechanism for removing viscous deposits as well as a key factor affecting cleaning (Yeckel and Middleman 1987; Gillham and others 2000). In addition to shear force, higher flow velocity also provides higher mass transfer rate, which explains why higher flow velocity has better cleaning ability (Plett 1985). Therefore, it is recommended to reduce the rinsing time in the current CIP system. Current rinsing time is about one minute, this can be reduced to 30 seconds for peanut allergen. For the washing part, it takes about 8 minutes to reach the washing temperature 142°F/61.1°C and then washing for about 20 mins in current CIP system. Since the effect of washing at 122°F/50°C was similar as washing at 140°F/60°C in this study, the temperature can be lowered to 140°F/50°C. Washing time can also be reduced to 6+10 minutes, 6 minutes for heating water to 122°F/50°C and 10 minutes for washing. The reason why ten minutes is recommended is that considering the water volume per meter of stainless steel pipe in this study was larger than in the Dairy Plant and other ingredients in the Buckeye Blitz ice cream weren't investigated. However, it is important to note that only peanut allergen removal was investigated in this study. Buckeye Blitz ice cream also contains other allergen like soy. Other food allergens still need to be studied if the current CIP system is adjusted based on the findings from this study. Further studies for verification and validation are still needed to examine other scenarios in washing and rinsing processes for finding optimal cleaning time and water temperature in the current CIP system.

CHAPTER 5

CONCLUSIONS AND LIMITATIONS

5.1. Conclusions

For verification of COP washing in the MSU Dairy Plant, peanut allergen was effectively removed by following the protocol of COP washing. Although the recommended water temperature is 120°F/48.8°C, the cleaning solution was still effective to remove peanut allergen as low as 69.8°F/21°C. It was suggested that the cleaning solution still can be used after cooling down to room temperature.

For investigation of CIP rinse, rinsing can remove over 90% of peanut allergen residue on the stainless steel pipe, although there was still over 100 ppm of peanut allergen left on the pipe. Longer rinsing time and higher rinsing temperature resulted in a better removal of peanut allergen as expected. But the trends were different for rinsing for 10 seconds, 20 seconds and 30 seconds. For example, rinsing for 10 seconds had a peak at 86°F/30°C, which showed a statistical difference than rinsing for 20 or 30 seconds ($p<0.05$). Thus, the optimization cleaning of time and temperatures need to be further investigated in the future studies. The trends of rinsing for 20 and 30 seconds indicated when temperature exceeds 122°F/50°C, the effectiveness of removal of peanut allergen slightly decreases somehow. Both time and temperature played an important role in removing peanut allergen.

For investigation of CIP wash, alkaline detergent solution had a potent power on removing peanut allergen residue on the stainless steel pipe. It provided about 100 times more reduction in allergen after washing comparing to that after rinsing. Longer washing time and higher washing temperature also resulted in a better removal of peanut allergen. However, washing time had no significant difference when washing temperature at 122°F/50°C and

140°F/60°C ($p<0.05$). Since the effect of temperature on cleaning decreases when temperature exceeds 122°F/50°C. It was observed that the effect of temperature was more important than the effect of time on washing. Functional temperature of the detergent and the volume of washing solution used in this study are likely the reasons.

For the verification of current CIP system in the MSU Dairy Plant, both the wall of the flavor tank and the pipe attached to the flavor tank were tested. Both locations showed very low concentrations of peanut allergen residue and can be considered as safe, which indicated that current CIP system is effective enough to remove peanut allergen.

5.2. Limitations

MSU Dairy Plant is a food processing facility producing cheese and ice cream with multiply food allergens. Especially the ice cream continuous freezer, it produces ice creams containing six food allergens. Recommendations provided in this study in the previous section is suitable only for after producing Buckeye Blitz ice cream. The food matrix interference also need to be considered. On the other hand, Buckeye Blitz is an ice cream containing soy, peanut and milk. Only peanut was investigated in this study. Removal of soy and milk wasn't investigated. Another important parameter for the CIP system flow velocity wasn't investigated in this study either. Flow velocity was difficult to control and adjusted with in current equipment.

5.3. Future Studies

Studies can be conducted to investigate whether there is a difference on the difficulty of removing different kinds of food allergens on dairy processing equipment. Different structures of allergen protein may affect the effectiveness. On the other hand, studies on how flow velocity affect effectiveness of removal of allergen can also be investigated.

APPENDICES

APPENDIX A: NUTRITION FACTS AND INGREDIENTS LIST

Buckeye Blitz

Revised: February of 2018

Nutrition Facts

Serving Size 1/2 cup (76g)

Amount Per Serving	
Calories 230	Calories from Fat 140
	% Daily Value*
Total Fat 17g	**26%**
Saturated Fat 6g	**30%**
Trans Fat 0g	
Cholesterol 25mg	**8%**
Sodium 75mg	**3%**
Total Carbohydrate 21g	**7%**
Dietary Fiber 1g	**4%**
Sugars 18g	
Protein 4g	
Vitamin A 4% • Vitamin C 2%	
Calcium 8% • Iron 4%	

*Percent Daily Values are based on a 2,000 calorie diet.

Ingredients: CREAM, SUGAR, THICK FUDGE RIBBON (PEANUT OIL, SUGAR, COCOA (PROCESSED WITH ALKALI), WHEY, SALT, SOY LECITHIN), PEANUT BUTTER BASE (PEANUTS, CANOLA OIL, FIGH FRUCTOSE CORN SYRUP, SALT, MONO AND DIGLYCERIDES), SKIM MILK, MILK CHOCOLATE PEANUT BUTTER BUCKEYES (SUGAR, PEANUT BUTTER (PEANUTS, SALT), COCONUT OIL, PARTIALLY DEFATTED PEANUT FLOUR, NONFAT MILK, WHOLE MILK, COCOA (PROCESSED WITH ALKALI), PALM KERNEL OIL, SOY LECITHIN, SALT NATURAL FLAVORS), MONO & DIGLYCERIDES, LOCUST BEAN GUM, GUAR GUM, CARRAGEENAN.

Contains Milk, Peanut, Soy. (Manufactured in a plant that processes or uses peanuts, tree nuts, eggs, soy, wheat and dairy.)

Figure A1. Nutrition Facts label and ingredients list for Buckeye Blitz ice cream, which was used in this study.

APPENDIX B: ITEMS USED IN THIS STUDY

Figure B1. Stainless steel pipe and pan were used in this study.

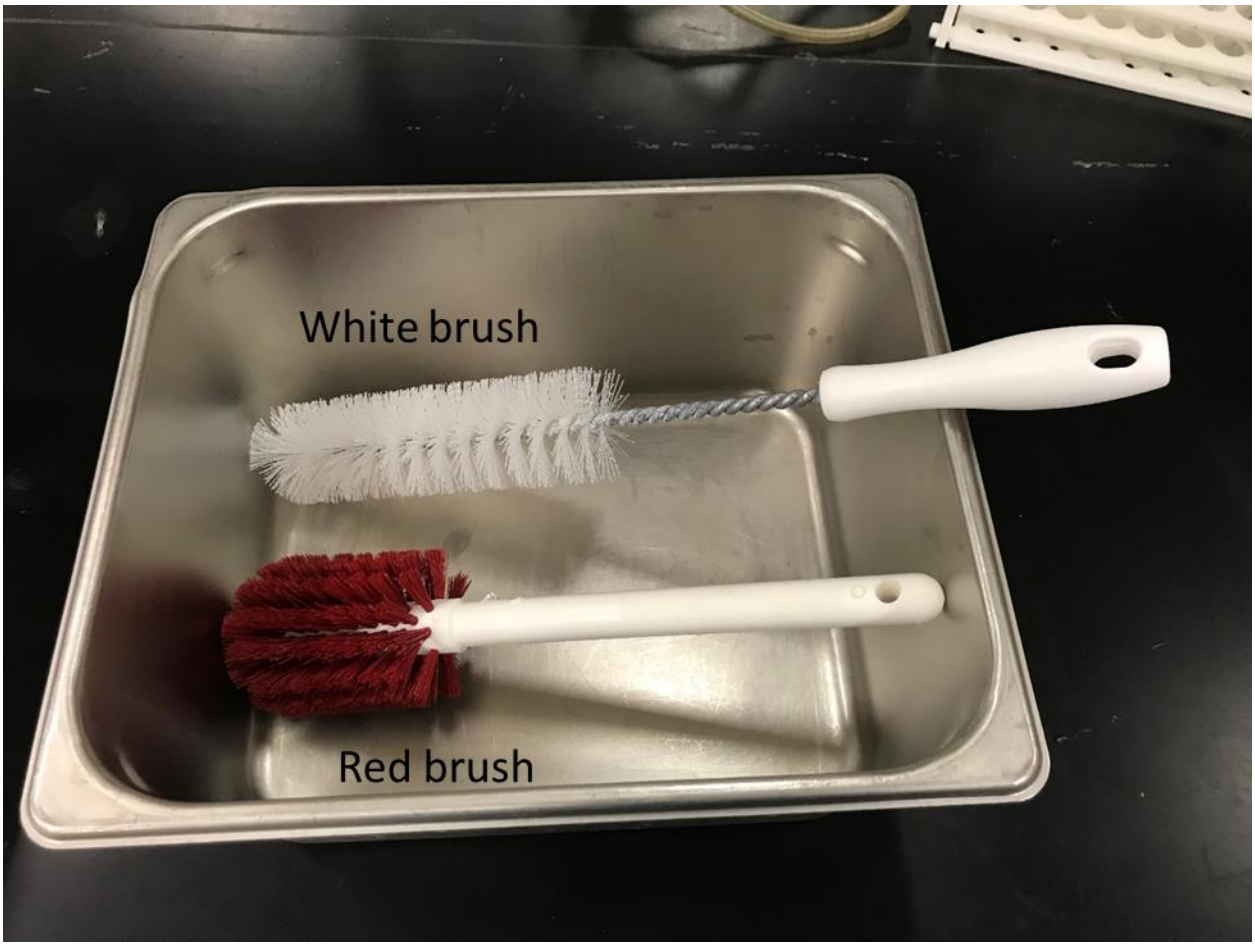

Figure B2. Brushes were used in this study. White brush was used for washing inside surface of the stainless steel pipe. Red brush was used for washing outside surface of the stainless steel pipe, the pan and two covers.

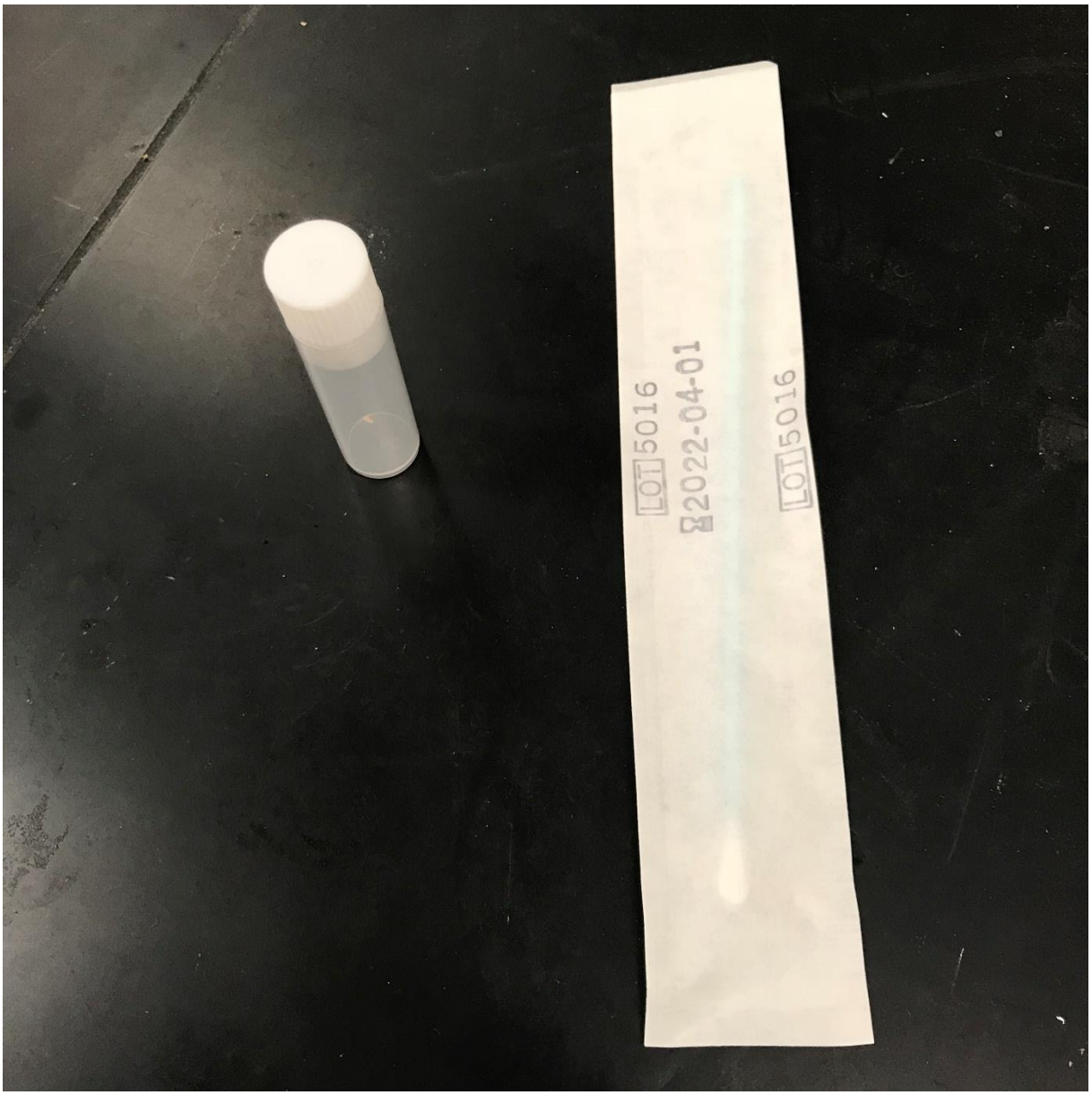

Figure B3. Neogen BioKits Allergen Swabbing Kit (Neogen, East Lansing, MI, USA).

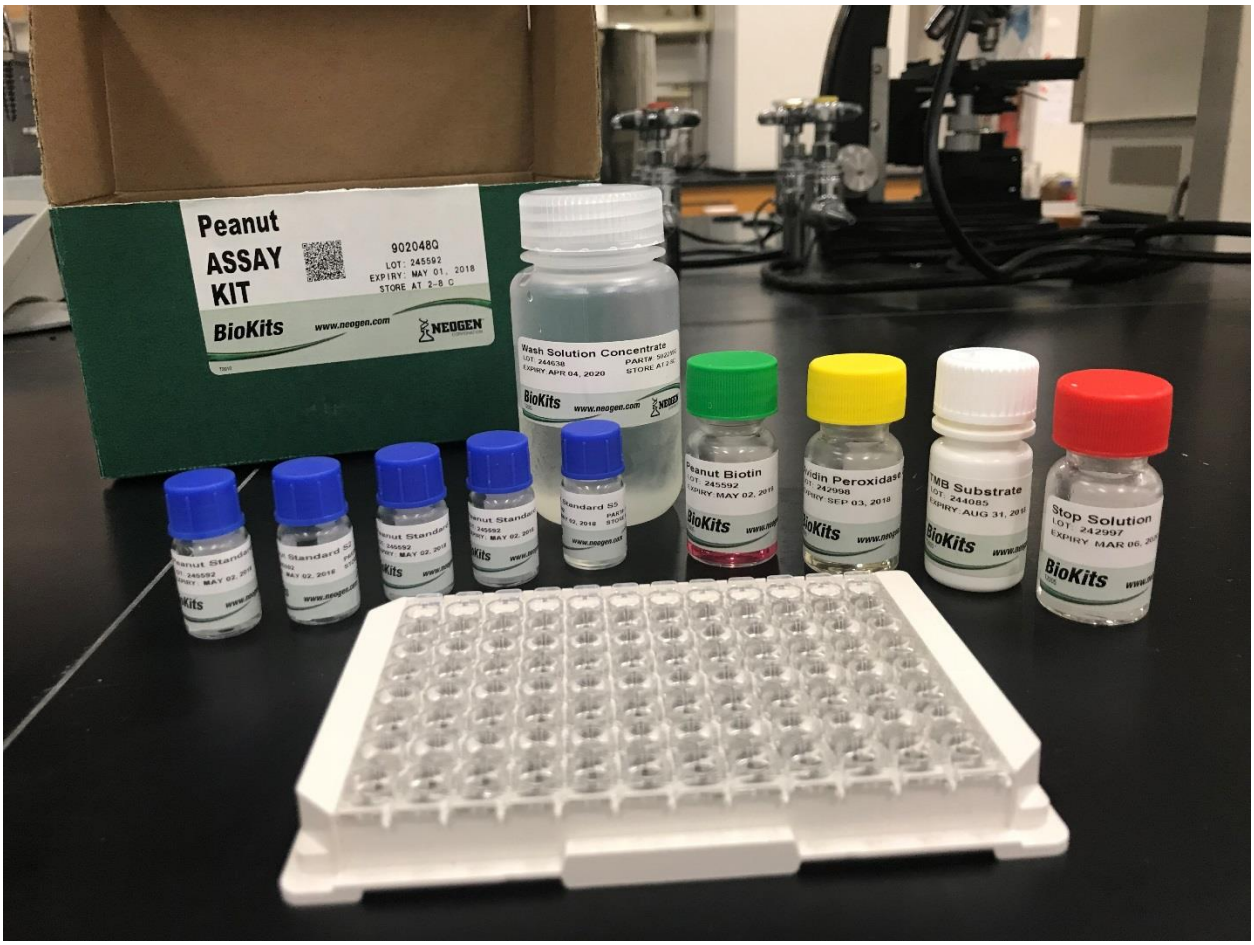

Figure B4. Neogen BioKits Peanut Assay Kit (Neogen, East Lansing, MI, USA).

Figure B5. The simulated CIP system was used for CIP experiments in this study.

APPENDIX C: PAIRWISE COMPARISION OF RESULTS

Table C1. Pairwise comparison of rinsed data using least square means method.

Differences of time*temp Least Squares Means									
time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
10	**20**	**10**	**30**	-43.9700	4.8911	14.39	-8.99	<.0001	0.05
10	**20**	**10**	**40**	-24.3000	4.8911	14.39	-4.97	0.0002	0.05
10	**20**	**10**	**50**	18.0950	4.8911	14.39	3.70	0.0023	0.05
10	**20**	**10**	**60**	62.4150	4.8911	14.39	12.76	<.0001	0.05
10	**20**	**20**	**20**	22.9050	4.8911	14.39	4.68	0.0003	0.05
10	**20**	**20**	**30**	34.8800	4.8911	14.39	7.13	<.0001	0.05
10	**20**	**20**	**40**	58.0450	4.8911	14.39	11.87	<.0001	0.05
10	**20**	**20**	**50**	89.6050	4.8911	14.39	18.32	<.0001	0.05
10	**20**	**20**	**60**	81.7550	4.8911	14.39	16.72	<.0001	0.05
10	**20**	**30**	**20**	79.0450	4.8911	14.39	16.16	<.0001	0.05
10	**20**	**30**	**30**	72.5500	4.8911	14.39	14.83	<.0001	0.05
10	**20**	**30**	**40**	74.9200	4.8911	14.39	15.32	<.0001	0.05
10	**20**	**30**	**50**	104.19	4.8911	14.39	21.30	<.0001	0.05
10	**20**	**30**	**60**	100.43	4.8911	14.39	20.53	<.0001	0.05
10	**30**	**10**	**40**	19.6700	4.8911	14.39	4.02	0.0012	0.05
10	**30**	**10**	**50**	62.0650	4.8911	14.39	12.69	<.0001	0.05
10	**30**	**10**	**60**	106.39	4.8911	14.39	21.75	<.0001	0.05
10	**30**	**20**	**20**	66.8750	4.8911	14.39	13.67	<.0001	0.05
10	**30**	**20**	**30**	78.8500	4.8911	14.39	16.12	<.0001	0.05
10	**30**	**20**	**40**	102.02	4.8911	14.39	20.86	<.0001	0.05
10	**30**	**20**	**50**	133.58	4.8911	14.39	27.31	<.0001	0.05
10	**30**	**20**	**60**	125.73	4.8911	14.39	25.70	<.0001	0.05
10	**30**	**30**	**20**	123.02	4.8911	14.39	25.15	<.0001	0.05
10	**30**	**30**	**30**	116.52	4.8911	14.39	23.82	<.0001	0.05
10	**30**	**30**	**40**	118.89	4.8911	14.39	24.31	<.0001	0.05

Table C1 (cont'd)

Differences of time*temp Least Squares Means									
time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
10	**30**	**30**	**50**	148.16	4.8911	14.39	30.29	<.0001	0.05
10	**30**	**30**	**60**	144.40	4.8911	14.39	29.52	<.0001	0.05
10	**40**	**10**	**50**	42.3950	4.8911	14.39	8.67	<.0001	0.05
10	**40**	**10**	**60**	86.7150	4.8911	14.39	17.73	<.0001	0.05
10	**40**	**20**	**20**	47.2050	4.8911	14.39	9.65	<.0001	0.05
10	**40**	**20**	**30**	59.1800	4.8911	14.39	12.10	<.0001	0.05
10	**40**	**20**	**40**	82.3450	4.8911	14.39	16.84	<.0001	0.05
10	**40**	**20**	**50**	113.91	4.8911	14.39	23.29	<.0001	0.05
10	**40**	**20**	**60**	106.06	4.8911	14.39	21.68	<.0001	0.05
10	**40**	**30**	**20**	103.35	4.8911	14.39	21.13	<.0001	0.05
10	**40**	**30**	**30**	96.8500	4.8911	14.39	19.80	<.0001	0.05
10	**40**	**30**	**40**	99.2200	4.8911	14.39	20.29	<.0001	0.05
10	**40**	**30**	**50**	128.49	4.8911	14.39	26.27	<.0001	0.05
10	**40**	**30**	**60**	124.73	4.8911	14.39	25.50	<.0001	0.05
10	**50**	**10**	**60**	44.3200	4.8911	14.39	9.06	<.0001	0.05
10	**50**	**20**	**20**	4.8100	4.8911	14.39	0.98	0.3416	0.05
10	**50**	**20**	**30**	16.7850	4.8911	14.39	3.43	0.0039	0.05
10	**50**	**20**	**40**	39.9500	4.8911	14.39	8.17	<.0001	0.05
10	**50**	**20**	**50**	71.5100	4.8911	14.39	14.62	<.0001	0.05
10	**50**	**20**	**60**	63.6600	4.8911	14.39	13.02	<.0001	0.05
10	**50**	**30**	**20**	60.9500	4.8911	14.39	12.46	<.0001	0.05
10	**50**	**30**	**30**	54.4550	4.8911	14.39	11.13	<.0001	0.05
10	**50**	**30**	**40**	56.8250	4.8911	14.39	11.62	<.0001	0.05
10	**50**	**30**	**50**	86.0950	4.8911	14.39	17.60	<.0001	0.05
10	**50**	**30**	**60**	82.3350	4.8911	14.39	16.83	<.0001	0.05
10	**60**	**20**	**20**	-39.5100	4.8911	14.39	-8.08	<.0001	0.05

Table C1 (cont'd)

Differences of time*temp Least Squares Means									
Time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
10	**60**	**20**	**30**	-27.5350	4.8911	14.39	-5.63	<.0001	0.05
10	**60**	**20**	**40**	-4.3700	4.8911	14.39	-0.89	0.3863	0.05
10	**60**	**20**	**50**	27.1900	4.8911	14.39	5.56	<.0001	0.05
10	**60**	**20**	**60**	19.3400	4.8911	14.39	3.95	0.0014	0.05
10	**60**	**30**	**20**	16.6300	4.8911	14.39	3.40	0.0042	0.05
10	**60**	**30**	**30**	10.1350	4.8911	14.39	2.07	0.0567	0.05
10	**60**	**30**	**40**	12.5050	4.8911	14.39	2.56	0.0224	0.05
10	**60**	**30**	**50**	41.7750	4.8911	14.39	8.54	<.0001	0.05
10	**60**	**30**	**60**	38.0150	4.8911	14.39	7.77	<.0001	0.05
20	**20**	**20**	**30**	11.9750	4.8911	14.39	2.45	0.0277	0.05
20	**20**	**20**	**40**	35.1400	4.8911	14.39	7.18	<.0001	0.05
20	**20**	**20**	**50**	66.7000	4.8911	14.39	13.64	<.0001	0.05
20	**20**	**20**	**60**	58.8500	4.8911	14.39	12.03	<.0001	0.05
20	**20**	**30**	**20**	56.1400	4.8911	14.39	11.48	<.0001	0.05
20	**20**	**30**	**30**	49.6450	4.8911	14.39	10.15	<.0001	0.05
20	**20**	**30**	**40**	52.0150	4.8911	14.39	10.63	<.0001	0.05
20	**20**	**30**	**50**	81.2850	4.8911	14.39	16.62	<.0001	0.05
20	**20**	**30**	**60**	77.5250	4.8911	14.39	15.85	<.0001	0.05
20	**30**	**20**	**40**	23.1650	4.8911	14.39	4.74	0.0003	0.05
20	**30**	**20**	**50**	54.7250	4.8911	14.39	11.19	<.0001	0.05
20	**30**	**20**	**60**	46.8750	4.8911	14.39	9.58	<.0001	0.05
20	**30**	**30**	**20**	44.1650	4.8911	14.39	9.03	<.0001	0.05
20	**30**	**30**	**30**	37.6700	4.8911	14.39	7.70	<.0001	0.05
20	**30**	**30**	**40**	40.0400	4.8911	14.39	8.19	<.0001	0.05
20	**30**	**30**	**50**	69.3100	4.8911	14.39	14.17	<.0001	0.05
20	**30**	**30**	**60**	65.5500	4.8911	14.39	13.40	<.0001	0.05

Table C1 (cont'd)

Differences of time*temp Least Squares Means									
Time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
20	**40**	**20**	**50**	31.5600	4.8911	14.39	6.45	<.0001	0.05
20	**40**	**20**	**60**	23.7100	4.8911	14.39	4.85	0.0002	0.05
20	**40**	**30**	**20**	21.0000	4.8911	14.39	4.29	0.0007	0.05
20	**40**	**30**	**30**	14.5050	4.8911	14.39	2.97	0.0100	0.05
20	**40**	**30**	**40**	16.8750	4.8911	14.39	3.45	0.0038	0.05
20	**40**	**30**	**50**	46.1450	4.8911	14.39	9.43	<.0001	0.05
20	**40**	**30**	**60**	42.3850	4.8911	14.39	8.67	<.0001	0.05
20	**50**	**20**	**60**	-7.8500	4.8911	14.39	-1.60	0.1302	0.05
20	**50**	**30**	**20**	-10.5600	4.8911	14.39	-2.16	0.0482	0.05
20	**50**	**30**	**30**	-17.0550	4.8911	14.39	-3.49	0.0035	0.05
20	**50**	**30**	**40**	-14.6850	4.8911	14.39	-3.00	0.0093	0.05
20	**50**	**30**	**50**	14.5850	4.8911	14.39	2.98	0.0097	0.05
20	**50**	**30**	**60**	10.8250	4.8911	14.39	2.21	0.0435	0.05
20	**60**	**30**	**20**	-2.7100	4.8911	14.39	-0.55	0.5880	0.05
20	**60**	**30**	**30**	-9.2050	4.8911	14.39	-1.88	0.0802	0.05
20	**60**	**30**	**40**	-6.8350	4.8911	14.39	-1.40	0.1835	0.05
20	**60**	**30**	**50**	22.4350	4.8911	14.39	4.59	0.0004	0.05
20	**60**	**30**	**60**	18.6750	4.8911	14.39	3.82	0.0018	0.05
30	**20**	**30**	**30**	-6.4950	4.8911	14.39	-1.33	0.2049	0.05
30	**20**	**30**	**40**	-4.1250	4.8911	14.39	-0.84	0.4128	0.05
30	**20**	**30**	**50**	25.1450	4.8911	14.39	5.14	0.0001	0.05
30	**20**	**30**	**60**	21.3850	4.8911	14.39	4.37	0.0006	0.05
30	**30**	**30**	**40**	2.3700	4.8911	14.39	0.48	0.6353	0.05
30	**30**	**30**	**50**	31.6400	4.8911	14.39	6.47	<.0001	0.05
30	**30**	**30**	**60**	27.8800	4.8911	14.39	5.70	<.0001	0.05
30	**40**	**30**	**50**	29.2700	4.8911	14.39	5.98	<.0001	0.05

Table C1 (cont'd)

Differences of time*temp Least Squares Means									
Time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
30	**40**	**30**	**60**	**25.5100**	**4.8911**	**14.39**	**5.22**	**0.0001**	**0.05**
30	**50**	**30**	**60**	**-3.7600**	**4.8911**	**14.39**	**-0.77**	**0.4545**	**0.05**

Table C2. Pairwise comparison of rinsed then washed data using least square mean method.

Differences of time*temp Least Squares Means									
time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
10	**20**	**10**	**30**	0.01965	0.07881	14.39	0.25	0.8066	0.05
10	**20**	**10**	**40**	0.9161	0.07881	14.39	11.62	<.0001	0.05
10	**20**	**10**	**50**	1.2213	0.07881	14.39	15.50	<.0001	0.05
10	**20**	**10**	**60**	1.2012	0.07881	14.39	15.24	<.0001	0.05
10	**20**	**20**	**20**	-0.1392	0.07881	14.39	-1.77	0.0985	0.05
10	**20**	**20**	**30**	0.4181	0.07881	14.39	5.31	0.0001	0.05
10	**20**	**20**	**40**	0.6036	0.07881	14.39	7.66	<.0001	0.05
10	**20**	**20**	**50**	1.2655	0.07881	14.39	16.06	<.0001	0.05
10	**20**	**20**	**60**	1.2674	0.07881	14.39	16.08	<.0001	0.05
10	**20**	**30**	**20**	-0.06355	0.07881	14.39	-0.81	0.4331	0.05
10	**20**	**30**	**30**	0.4838	0.07881	14.39	6.14	<.0001	0.05
10	**20**	**30**	**40**	1.1680	0.07881	14.39	14.82	<.0001	0.05
10	**20**	**30**	**50**	1.2738	0.07881	14.39	16.16	<.0001	0.05
10	**20**	**30**	**60**	1.2783	0.07881	14.39	16.22	<.0001	0.05
10	**30**	**10**	**40**	0.8964	0.07881	14.39	11.37	<.0001	0.05
10	**30**	**10**	**50**	1.2017	0.07881	14.39	15.25	<.0001	0.05
10	**30**	**10**	**60**	1.1815	0.07881	14.39	14.99	<.0001	0.05
10	**30**	**20**	**20**	-0.1588	0.07881	14.39	-2.02	0.0629	0.05
10	**30**	**20**	**30**	0.3985	0.07881	14.39	5.06	0.0002	0.05
10	**30**	**20**	**40**	0.5839	0.07881	14.39	7.41	<.0001	0.05
10	**30**	**20**	**50**	1.2459	0.07881	14.39	15.81	<.0001	0.05
10	**30**	**20**	**60**	1.2477	0.07881	14.39	15.83	<.0001	0.05
10	**30**	**30**	**20**	-0.08320	0.07881	14.39	-1.06	0.3085	0.05
10	**30**	**30**	**30**	0.4641	0.07881	14.39	5.89	<.0001	0.05
10	**30**	**30**	**40**	1.1484	0.07881	14.39	14.57	<.0001	0.05
10	**30**	**30**	**50**	1.2541	0.07881	14.39	15.91	<.0001	0.05

Table C2 (cont'd)

Differences of time*temp Least Squares Means									
Time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
10	**30**	**30**	**60**	1.2587	0.07881	14.39	15.97	<.0001	0.05
10	**40**	**10**	**50**	0.3052	0.07881	14.39	3.87	0.0016	0.05
10	**40**	**10**	**60**	0.2851	0.07881	14.39	3.62	0.0027	0.05
10	**40**	**20**	**20**	-1.0553	0.07881	14.39	-13.39	<.0001	0.05
10	**40**	**20**	**30**	-0.4980	0.07881	14.39	-6.32	<.0001	0.05
10	**40**	**20**	**40**	-0.3125	0.07881	14.39	-3.97	0.0013	0.05
10	**40**	**20**	**50**	0.3494	0.07881	14.39	4.43	0.0005	0.05
10	**40**	**20**	**60**	0.3513	0.07881	14.39	4.46	0.0005	0.05
10	**40**	**30**	**20**	-0.9796	0.07881	14.39	-12.43	<.0001	0.05
10	**40**	**30**	**30**	-0.4323	0.07881	14.39	-5.49	<.0001	0.05
10	**40**	**30**	**40**	0.2520	0.07881	14.39	3.20	0.0063	0.05
10	**40**	**30**	**50**	0.3577	0.07881	14.39	4.54	0.0004	0.05
10	**40**	**30**	**60**	0.3623	0.07881	14.39	4.60	0.0004	0.05
10	**50**	**10**	**60**	-0.02015	0.07881	14.39	-0.26	0.8018	0.05
10	**50**	**20**	**20**	-1.3605	0.07881	14.39	-17.26	<.0001	0.05
10	**50**	**20**	**30**	-0.8032	0.07881	14.39	-10.19	<.0001	0.05
10	**50**	**20**	**40**	-0.6178	0.07881	14.39	-7.84	<.0001	0.05
10	**50**	**20**	**50**	0.04420	0.07881	14.39	0.56	0.5835	0.05
10	**50**	**20**	**60**	0.04605	0.07881	14.39	0.58	0.5680	0.05
10	**50**	**30**	**20**	-1.2849	0.07881	14.39	-16.30	<.0001	0.05
10	**50**	**30**	**30**	-0.7375	0.07881	14.39	-9.36	<.0001	0.05
10	**50**	**30**	**40**	-0.05330	0.07881	14.39	-0.68	0.5096	0.05
10	**50**	**30**	**50**	0.05245	0.07881	14.39	0.67	0.5162	0.05
10	**50**	**30**	**60**	0.05705	0.07881	14.39	0.72	0.4807	0.05
10	**60**	**20**	**20**	-1.3404	0.07881	14.39	-17.01	<.0001	0.05
10	**60**	**20**	**30**	-0.7831	0.07881	14.39	-9.94	<.0001	0.05

Table C2 (cont'd)

Differences of time*temp Least Squares Means									
Time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
10	**60**	**20**	**40**	-0.5976	0.07881	14.39	-7.58	<.0001	0.05
10	**60**	**20**	**50**	0.06435	0.07881	14.39	0.82	0.4275	0.05
10	**60**	**20**	**60**	0.06620	0.07881	14.39	0.84	0.4146	0.05
10	**60**	**30**	**20**	-1.2647	0.07881	14.39	-16.05	<.0001	0.05
10	**60**	**30**	**30**	-0.7174	0.07881	14.39	-9.10	<.0001	0.05
10	**60**	**30**	**40**	-0.03315	0.07881	14.39	-0.42	0.6802	0.05
10	**60**	**30**	**50**	0.07260	0.07881	14.39	0.92	0.3721	0.05
10	**60**	**30**	**60**	0.07720	0.07881	14.39	0.98	0.3435	0.05
20	**20**	**20**	**30**	0.5573	0.07881	14.39	7.07	<.0001	0.05
20	**20**	**20**	**40**	0.7428	0.07881	14.39	9.42	<.0001	0.05
20	**20**	**20**	**50**	1.4047	0.07881	14.39	17.82	<.0001	0.05
20	**20**	**20**	**60**	1.4066	0.07881	14.39	17.85	<.0001	0.05
20	**20**	**30**	**20**	0.07565	0.07881	14.39	0.96	0.3529	0.05
20	**20**	**30**	**30**	0.6230	0.07881	14.39	7.90	<.0001	0.05
20	**20**	**30**	**40**	1.3072	0.07881	14.39	16.59	<.0001	0.05
20	**20**	**30**	**50**	1.4130	0.07881	14.39	17.93	<.0001	0.05
20	**20**	**30**	**60**	1.4175	0.07881	14.39	17.99	<.0001	0.05
20	**30**	**20**	**40**	0.1855	0.07881	14.39	2.35	0.0333	0.05
20	**30**	**20**	**50**	0.8474	0.07881	14.39	10.75	<.0001	0.05
20	**30**	**20**	**60**	0.8493	0.07881	14.39	10.78	<.0001	0.05
20	**30**	**30**	**20**	-0.4817	0.07881	14.39	-6.11	<.0001	0.05
20	**30**	**30**	**30**	0.06565	0.07881	14.39	0.83	0.4184	0.05
20	**30**	**30**	**40**	0.7499	0.07881	14.39	9.52	<.0001	0.05
20	**30**	**30**	**50**	0.8557	0.07881	14.39	10.86	<.0001	0.05
20	**30**	**30**	**60**	0.8602	0.07881	14.39	10.92	<.0001	0.05
20	**40**	**20**	**50**	0.6620	0.07881	14.39	8.40	<.0001	0.05

Table C2 (cont'd)

Differences of time*temp Least Squares Means									
Time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
20	**40**	**20**	**60**	0.6638	0.07881	14.39	8.42	<.0001	0.05
20	**40**	**30**	**20**	-0.6671	0.07881	14.39	-8.46	<.0001	0.05
20	**40**	**30**	**30**	-0.1198	0.07881	14.39	-1.52	0.1501	0.05
20	**40**	**30**	**40**	0.5645	0.07881	14.39	7.16	<.0001	0.05
20	**40**	**30**	**50**	0.6702	0.07881	14.39	8.50	<.0001	0.05
20	**40**	**30**	**60**	0.6748	0.07881	14.39	8.56	<.0001	0.05
20	**50**	**20**	**60**	0.001850	0.07881	14.39	0.02	0.9816	0.05
20	**50**	**30**	**20**	-1.3291	0.07881	14.39	-16.86	<.0001	0.05
20	**50**	**30**	**30**	-0.7817	0.07881	14.39	-9.92	<.0001	0.05
20	**50**	**30**	**40**	-0.09750	0.07881	14.39	-1.24	0.2358	0.05
20	**50**	**30**	**50**	0.008250	0.07881	14.39	0.10	0.9181	0.05
20	**50**	**30**	**60**	0.01285	0.07881	14.39	0.16	0.8727	0.05
20	**60**	**30**	**20**	-1.3309	0.07881	14.39	-16.89	<.0001	0.05
20	**60**	**30**	**30**	-0.7836	0.07881	14.39	-9.94	<.0001	0.05
20	**60**	**30**	**40**	-0.09935	0.07881	14.39	-1.26	0.2275	0.05
20	**60**	**30**	**50**	0.006400	0.07881	14.39	0.08	0.9364	0.05
20	**60**	**30**	**60**	0.01100	0.07881	14.39	0.14	0.8909	0.05
30	**20**	**30**	**30**	0.5473	0.07881	14.39	6.94	<.0001	0.05
30	**20**	**30**	**40**	1.2316	0.07881	14.39	15.63	<.0001	0.05
30	**20**	**30**	**50**	1.3373	0.07881	14.39	16.97	<.0001	0.05
30	**20**	**30**	**60**	1.3419	0.07881	14.39	17.03	<.0001	0.05
30	**30**	**30**	**40**	0.6842	0.07881	14.39	8.68	<.0001	0.05
30	**30**	**30**	**50**	0.7900	0.07881	14.39	10.02	<.0001	0.05
30	**30**	**30**	**60**	0.7946	0.07881	14.39	10.08	<.0001	0.05
30	**40**	**30**	**50**	0.1057	0.07881	14.39	1.34	0.2004	0.05
30	**40**	**30**	**60**	0.1103	0.07881	14.39	1.40	0.1826	0.05

Table C2 (cont'd)

Differences of time*temp Least Squares Means									
Time	**temp**	**_time**	**_temp**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr >|t|**	**Alpha**
30	**50**	**30**	**60**	0.004600	0.07881	14.39	0.06	0.9543	0.05

REFERENCES

REFERENCES

Allmann M, Candrian U, Höfelein C, Lüthy J. 1993. Polymerase chain reaction (PCR): a possible alternative to immunochemical methods assuring safety and quality of food detection of wheat contamination in non-wheat food products. Z Lebensm Unters Forsch 196(3):248-51.

Australian Standards, AS. 2001. Guide to cleaning and sanitizing of plant and equipment in the food industry - AS 4709-2001.

Bahadır EB and Sezgintürk MK. 2016. Lateral flow assays: Principles, designs and labels. TrAC Trends in Analytical Chemistry 82:289-306.

Baumgartner S, Krska R and Welzig E. 2007. Detecting allergens in foods. In: Millers C, Wicher H and Hoffmann-Sommergruber K. Managing allergens in food. Cambridge, England: CRC Press.

Beyer K, Morrow E, Li X, Bardina L, Bannon GA, Burks AW and Sampson HA. 2001. Effects of cooking methods on peanut allergenicity. Journal of allergy and clinical immunology 107(6):1077-1081.

Blanc F, Vissers YM, Adel-Patient Nm and others. 2011. Boiling peanut Ara h 1 results in the formation of aggregates with reduced allergenicity. Mol. Nutr. Food Res. 55:1887-1894

Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, … Schwaninger JM. 2010. Guidelines for the Diagnosis and Management of Food allergies in the United States: Report of the NIAID-Sponsored Expert Panel. The Journal of Allergy and Clinical Immunology, 126(60), S1–58. Available from: https://www. ncbi. nlm. nih. gov/pmc/articles/PMC4241964/ Access date: Jan 12th 2017

Comstock SS, Maleki SJ and Teuber SS. 2016. Boiling and Frying Peanuts Decreases Soluble Peanut (Arachis Hypogaea) Allergens Ara h 1 and Ara h 2 But Does Not Generate Hypoallergenic Peanuts. PloS ONE 11(6):e0157849.

Courtney RC. 2016. Evaluation of Qualitative Food Allergen Detection Methods and Cleaning Validation Approaches. DigitalCommons@University of Nebraska.

Engvall E and Perlmann P. 1971. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. Immunochemistry 8(9):871-874.

Environmental Protection Agency. 2018. Vocabulary Catelog: Integrated Risk Information System (IRIS) Glossary. Available from: https://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkey

wordlists/search.do?details=&glossaryName=IRIS%20Glossary Accessed Date: Feb 17th 2018.

Fan M, Phinney DM and Heldman DR. 2015. Effectiveness of Rinse Water during In-Place Cleaning of Stainless Steel Pipe Lines. Journal of food science 80(7): E1490-7.

Food and Drug Administration. 2004. Food Allergen Labeling and Consumer Protection Act of 2004. Available from: http://www. fda. gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106187. htm Access date: January 10th, 2017.

Food and Drug Administration. 2006. Food Allergen Labeling And Consumer Protection Act of 2004 Questions and Answers. Available from: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106890.htm Access date: April 2nd 2018.

Food and Drug Administration. 2016. The reportable food registry: A five year overview of targeting inspection resources and identifying patterns of adulteration. Available from: https://www.fda.gov/downloads/Food/ComplianceEnforcement/RFR/UCM502117.pdf Accessed Date: Feb 17th 2018.

Food and Drug Administration. 2017. Archive for Recalls, Market Withdrawals & Safety Alerts. Available from: https://www.fda.gov/Safety/Recalls/ArchiveRecalls/default.htm Access date: April 2nd 2018.

Gell PGH and Coombs RRA. 1963. Clinical Aspects of Immunology. Oxfird, England: Blackwell.

Gendel SM. 2012. Comparison of international food allergen labeling regulations. Regulatory Toxicology and Pharmacology 63(2):279-285.

Gendel SM, Khan N and Yajnik M. 2013. A survey of food allergen control practices in the U.S. food industry. Journal of food protection; 76:302-306.

Gillham CR, Fryer PJ, Hasting AP and Wilson DI. 1999. Cleaning-in-place of whey protein fouling deposits: mechanisms controlling cleaning. Food and bioproducts processing 77(2):127-136.

Gillham CR, Fryer PJ, Hasting APM, Wilson DI. 2000. Enhanced cleaning of whey protein soils using pulsed flows. J Food Eng 46(3):199–209.

Grundy J, Matthews S, Bateman B, Dean T and Arshad SH. 2002. Rising prevalence of allergy to peanut in children: data from two sequential cohorts. J Allergy Clin Immunol 110:784-789.

Gupta R, Holdford D, Bilaver LDyer A, Holl JL and Meltzer D. 2013. The Economic Impact of Childhood Food Allergy in the United States. JAMA Pediatr. 2013;167(11):1026–1031.

Gupta RS, Springston EE, Warrier MR, Smith B, Kumar R, Pongracic J and Holl JL. 2011. The prevalence, severity, and distribution of childhood food allergy in the United States. Pediatrics. 128: e9–e17.

Hefle S. 2006. Methods for detecting peanuts in food. Detecting allergens in food: The nature of food allergy. pp. 185-200. Woodhead Publishing: Cambridge, England.

Holah JT. 2014. Cleaning and disinfection practices in food processing. In: Lelieveld HLM, Holah J, Napper D. Hygiene in food processing, second edition. Woodhead publishing Limited. p 259-304

Jackson LS, Al-Taher FM, Moorman M, DeVries JW, Tippett R, Swanson KMJ, Fu TJ, Salter R, Dunaif G, Estes S, Albillos S, and Gendel SM. 2008. Cleaning and other control and validation strategies to prevent allergen cross-contact in food-processing operations. J. Food Prot 71:445-458

Jackson LS, Schlesser JE, Beacham-Bowden T, Fu TJ, Gendel SM and Moorman M. 2004. Effects of cleaning on removal of peanut allergens from food-contact surfaces, 49I-5. Abstr. Annu. Meet. Inst. Food Technol. 2004. Institute of Food Technologists, Chicago, Ill.

Jayasena S, Smits M, Fiechter D, de Jong A, Nordlee J, Baumert J, Taylor SL, Pieters RH and Koppelman SJ. 2015. Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J. Agric. Food. Chem 63:1849-1855.

Johnson PE, Sancho AI, Crevel REW and Mills ENC. 2011. Detection of allergens in foods. In: Nollet L and Hengel A. Food allergens analysis instrumentation and methods. Florida: CRC Press. p 13-28.

Jurad-Alameda E, Bravo-Rodriguez V, Bailon-Moreno R, Nunez-Olea J, Vaz DA. 2011. Fatty soils removal from hard surfaces in a Clean In Place system. J Food Process Eng 34(4):1053–70.

Keener L. 2005. 28 – Improving cleaning-out-of-place (COP). In: Handbook of Hygiene Control in the Food Industry. pp 445-467.

Kim EH and Burks W. 2015. Immunological Basic of Food allergies (IgE-Mediated, Non-IgE-Mediated, and Tolerance). Chem Immunol Allergy 101: 8-17.

King Rm, Knibb RC and Hourihane JO. 2009. Impact of peanut allergy on quality of life, stress and anxiety in the family. Allergy 64(3):461-468.

Koczula KM and Gallotta A. 2016. Lateral flow assays. Essays in biochemistry 60 (1):111-

120.

Koppelman SJ, Wensing M, Ertmann M, Knulst AC and Knol EF. 2004. Relevance of Ara h1, Ara h2 and Ara h3 in peanut‐allergic patients, as determined by immunoglobulin E Western blotting, basophil–histamine release and intracutaneous testing: Ara h2 is the most important peanut allergen. Clinical & Experimental allergy 34(4):583-590.

Koppelman SJ, Vlooswijk RA, Knippels LM, Hessing M, Knol EF, van Reijsen FC and Bruijnzeel-Koomen CA. 2001. Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia, and Valencia, bred in different parts of the world. Allergy 56(2):132-7.

Kulkarni SM, Arnold RG and Maxcy RB. 1975. Reuse limits and regeneration of solutions for cleaning dairy equipment. Journal of Dairy Science 58(8):1095-1100.

Leuvering JHW, Thal PJHM, Van Der Waart M, Schuurs AHWM. 1980. Sol Particle Immunoassay (SPIA). J Immunoassay 1:77

Lewan, M, and Partington E. 2014. 5 - food processing equipment construction materials. p142-154. In: Lelieveld HLM, Holah JT, and Napper D, Hygiene in food processing, 2nd Ed. Woodhead Publishing, Cambridge.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. 2006. SAS for Mixed Models. Cary, NC: SAS Institute.

Lopez, S. 2011. Allergen cleaning validation. AIB Update. July/August:10-13

Lorenz AR, Scheurer S, Vieths S. 2015. Food allergens: molecular and immunological aspects, allergen databases and cross-reactivity. Chem Immunol Allergy 101:18-29.

Maddox IS. 1994. Cleaning and sanitizing chemicals. In: Ian S. Maddox. Practical sanitation in food industry. Gordon and Breach Science Publishers. p 63-87

McGowan EC and Keet CA. 2013. Prevalence of self-reported food allergy in the National Health and Nutrition Examination Survey (NHANES) 2007–2010. J Allergy Clin Immunol 2013 Nov; 132(5): 10.1016/j.jaci.2013.07.018.

McWilliam V, Koplin J, Lodge C, Tang M,Dharmage S and Allen K. 2015. The Prevalence of Tree Nut Allergy: A Systematic Review. Current Allergy and Asthma Reports 15:54.

Melakeberhan, H., Maung, Z., Lee, C. L., Poindexter, S., and Stewart, J. 2018. Soil type-driven variable effects on cover-and rotation-crops, nematodes and soil food web in sugar beet fields reveal a roadmap for developing healthy soils. European Journal of Soil Biology, 85, 53-63.

Montserrat M, Mayayo C, Sanchez L, Calvo M and Perez MD. 2013. Study of the

thermoresistance of the allergenic Ara h1 protein from peanut (*Arachis hypogaea*). J. Agric. Food Chem 61:3335-3340.

Montserrat M, Sanz D, Juan T, Herrero A and others. 2015. Detection of peanut (*Arachis hypogaea*) allergens in processed foods by immunoassay: Influence of selected target protein and ELISA format applied. Food control 54:300-307.

Milliken GA. and Johnson DE. 2009. Analysis of messy data volume 1: designed experiments (Vol. 1). CRC Press.

Mills ENC, Potts A, Plumb GW, Lambert N, Morgan MRA. 1997. Development of a rapid dipstick immunoassay for the detection of peanut contamination of food. Food Agric Immunol 9:37

Mueller GA, Maleki SJ, Pedersen LC. 2016. The molecular basis of peanut allergy. Curr Allergy Asthma Rep 14(5):429.

National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Food and Nutrition Board; Committee on Food Allergies: Global Burden, Causes, Treatment, Prevention, and Public Policy; Oria MP, Stallings VA, editors. 2016. 3 Prevalence. In: Finding a Path to Safety in Food Allergy: Assessment of the Global Burden, Causes, Prevention, Management, and Public Policy. National Academies Press (US).

Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, Dubois AEJ, Halken S, Hoffmann-Sommergruber K, Poulsen LK, Roberts G, Van Ree R, Vlieg-Boerstra BJ, Sheikh A. 2014. The epidemiology of food allergies in Europe: a systematic review and meta-analysis. Allergy 69: 62-75.

Perry TT, Conover-Walker MK, Pomes A, Chapman MD and Wood RA. 2004. Distribution of peanut allergen in the environment. J Allergy Clin Immunol 113:973-976.

Plett EA. 1985. Relevant mass transfer mechanisms during rinsing. Fouling and cleaning in Food processing. Madison: Univ. of Wisconsin. p 395–409.

Pomes A, Helm RM, Bannon GA, Burks AW, Tsay A and Chapman MD. 2003. Monitoring peanut allergen in food products by measuring Ara h 1. J Allergy Clin Immunol 111(3):640-5.

Poms RE, Klein CI and Anklam E. 2004. Methods for allergen analysis in food: a review. Food Additives Contaminants 21:1-31.

Renz H, Allen KJ, Sicherer SH, Sampson HA, Lack G, Beyer K and Oettgen HC. 2018. Food Allergy. Nature Reviews Disease Primers. doi:10.1038/nrdp.2017.98

Röder M, Ibach A, Baltruweit I, Gruyters H, Janise A, Suwelack C, Matissek R, Vieths S,

and Holzhauser T. 2008. Pilot plant investigations on cleaning efficiencies to reduce hazelnut cross contamination in industrial manufacture of cookies. J. Food Prot 71:2263-2271.

Romney AJD. 1990. CIP: Cleaning in Place. In: Romney AJD. The Society of Dairy Technology, Huntingdon UK.

Sampson HA. 2005. Food allergies: accurately identifying clinical reactivity. Allergy 60:19-24.

Sajid M, Kawde AN, Daud M. 2014. Designs, formats and applications of lateral flow assay: A literature review. Journal of Saudi Chemical Society 19 (6):689-705.

Schmidt RH. 2014. Basic elements of equipment cleaning and sanitizing in food processing and handling operations. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences. Available at:http://edis. ifas. ufl. edu/fs077

Schubert-Ullrich P, Rudolf J, Ansari P, Galler B, Führer M, Molinelli A and Baumgartner S. 2009. Commercialized rapid immunoanalytical tests for determination of allergenic food proteins: An overview. Anal. Bioanal. Chem 395:69-81.

Sicherer SH, Munoz-Furlong A, Godbold JH, Sampson HA. 2010. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. J of allergy and clinical immunology 125:1322-1326.

Sicherer SH and Sampson HA. 2018. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. Journal of Allergy and Clinical Immunology 141(1):41-58.

Stanga M. 2010. CIP (Cleaning in place). In: Stanga M. Sanitation: Cleaning and Disinfection in the Food Industry. p 301-363.

Stephan O, Weisz N, Vieths S, Weiser T, Rabe B, and Vatterott W. 2004. Protein quantification, sandwich elisa, and real-time pcr used to monitor industrial cleaning procedures for contamination with peanut and celery allergens. J. AOAC Int. 87:1448-1457.

Stewart JC and Seiberling DA. 1996. Clean in Place. Chemical engineering 103: 72-79.

Tang MLK and Mullins RJ. 2017. Food allergy: is prevalence increasing? International medicine journal 47(3):256-264.

Taylor SL and Baumert JL. 2015. Worldwide food allergies labeling and detection of allergens in processed foods. In: Ebisawa M, Ballmer-weber BK, Vieths S and Wood RA. Food allergies: Molecular Basis and Clinical Practice. Chem Immunol Allergy. Basel Karger 101:227-234

Taylor SL, Baumert JL, Kruizinga AG, Remington BC, Crevel WR, Brooke-Taylor S, Allen

KJ and Houben G. 2014. Establishment of reference doses for residues of allergenic foods: Report of the VITAL expert panel. Food Chem. Toxicol. 63:9-17.

Taylor, S. L., S. L. Hefle, K. Farnum, S. W. Rizk, J. Yeung, M. E. Barnett, F. Busta, F. R. Shank, R. Newsome, S. Davis, and C. M. Bryant. 2006. Analysis and evaluation of food manufacturing practices used to address allergen concerns. Compr. Rev. Food Sci. Food Saf. 5:138-157.

Taylor SL, Nordlee JA, Niemann LM, Lambrecht. 2009. Allergen immunoassays – consideration for use of naturally incurred standards. Analytical and Bioanalytical Chemistry; 395:83-92.

Valenta R, Hochwallner H, Linhart B and Phar S. 2015. Food Allergies: The Basics. Gastroenterology; 148(6):1120-1131.

van Hengel AJ. 2007. Food allergen detection methods and the challenge to protect food-allergic consumers. Analytical and Bioanalytical Chemistry; 389:111-118.

Venter C, Arshad SH, Grundy J, Pereira B, Clayton CB, Voigt K, Higgins B, Dean T. 2010. Time trends in the prevalence of peanut allergy: three cohorts of children from the same geographical location in the UK. Allergy 65:103-108.

Wang Y, Xu H, Wei M, Gu H, Xu Q and Zhu W. 2009. Study of superparamagnetic nanoparticles as labels in the quantitative lateral flow immunoassay. Materials science and engineering 29 (3):714-718.

Wang X, Young OA and Karl DP. 2010. Evaluation of cleaning procedures for allergen control in a food industry environment. J. Food Sci. 75: T149-T155

Weidemann C, Stahl S, Nirschl H. 2013. Development of a qualitative test method for the cleanability of polymer woven filter media. Food and Bioprod Process 91(4):515–24.

WHO/IUIS Allergen Nomenclature Sub-Committee. 2018. *Arachis hypogaea* (Peanut, groundnut). Available from: http://www.allergen.org/search.php?allergensource=peanut&searchsource=Search Accessed date: April 28, 2018.

Xin H, Chen X and Ozkan N. 2002. Cleaning Rate in the Uniform Cleaning Stage for Whey Protein Gel Deposits. Food and bioproducts processing 80(4):240-246.

Xin H, Chen X and Ozkan N. 2004. Removal of a model protein foulant from metal surfaces. American institute of chemical engineers 50(8):1961-73.

Yeckel A, Middleman S. 1987. Removal of a viscous film from a rigid plane surface by an impinging liquid jet. Chem Eng Commun 50(1-6):165–75.

Zeleny R and Schimmel H. 2010. Towards comparability of ELISA results for peanut

proteins in food: A feasibility study. Food Chem 123:1343−1351.

Zhang w, Zhu q, Zhang T, Cai Q and Chen Q. 2016. Thermal processing effects on peanut allergen Ara h 2 allergenicity in mice and its antigenic epitope structure. Food chem 212:657-662.

Zhou Y, Wang J, Yang X, Lin D, Gao Y, Su Y, Yang S, Zhang Y and Zheng J. 2013. Peanut Allergy, Allergen Composition, and Methods of Reducing Allergenicity: A Review. International Journal of Food Science, vol. 2013, Article ID 909140, 8 pages, 2013.