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Food safety in the retail environment

Ana Lorena Monge

Iowa State University

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Food safety in the retail environment

by

Ana Lorena Monge Brenes

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Food Science and Technology

Program of Study Committee:
Angela Shaw, Co-major Professor
Keith Vorst, Co-major Professor
Joey Talbert
James Dickson
Susan Wohlsdorf-Arendt
Kurt Rosentrater

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2019

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DEDICATION

Dedicated to my son and daughter, Santiago and Luisa, for the love, support, and patience that made this effort possible. To my mom for everything I am, and my brother JC for his unconditional support.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>viii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ix</td>
</tr>
<tr>
<td>CHAPTER 1. GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>References</td>
<td>3</td>
</tr>
<tr>
<td>CHAPTER 2. LITERATURE REVIEW</td>
<td>6</td>
</tr>
<tr>
<td>Microbiological Safety of Produce</td>
<td>7</td>
</tr>
<tr>
<td>Produce Safety at the Retail Level</td>
<td>9</td>
</tr>
<tr>
<td>Packaging in Microbiological Safety and Shelf-life of Produce</td>
<td>11</td>
</tr>
<tr>
<td>Transportation, Storage, and Display Conditions of Produce</td>
<td>12</td>
</tr>
<tr>
<td>Display Cases in Retail Stores</td>
<td>13</td>
</tr>
<tr>
<td>Consumers and Display Cases</td>
<td>15</td>
</tr>
<tr>
<td>The Role of Retail Employees in Maintaining Food Safety at the Retail Level</td>
<td>16</td>
</tr>
<tr>
<td>Training retail food handlers</td>
<td>17</td>
</tr>
<tr>
<td>Retail Produce Stockers in the US</td>
<td>18</td>
</tr>
<tr>
<td>The Food Safety Culture</td>
<td>19</td>
</tr>
<tr>
<td>Food Packaging as a Food Safety Risk</td>
<td>20</td>
</tr>
<tr>
<td>Per- and Polyfluorinated Alkyl Substances (PFAS) in Paper Packaging</td>
<td>20</td>
</tr>
<tr>
<td>Toxicity and Health Effects of PFAS</td>
<td>22</td>
</tr>
<tr>
<td>Food Contact Paper Packaging</td>
<td>23</td>
</tr>
<tr>
<td>References</td>
<td>24</td>
</tr>
<tr>
<td>CHAPTER 3. TEMPERATURE PROFILING OF OPEN- AND CLOSED-DOORED PRODUCE CASES IN RETAIL GROCERY STORES</td>
<td>34</td>
</tr>
<tr>
<td>Highlights</td>
<td>34</td>
</tr>
<tr>
<td>Abstract</td>
<td>34</td>
</tr>
<tr>
<td>Keywords</td>
<td>35</td>
</tr>
<tr>
<td>Introduction</td>
<td>35</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>38</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>39</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>40</td>
</tr>
<tr>
<td>Conclusions</td>
<td>47</td>
</tr>
<tr>
<td>Conflicts of Interest</td>
<td>48</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>48</td>
</tr>
<tr>
<td>References</td>
<td>48</td>
</tr>
</tbody>
</table>
APPENDIX B

CHAPTER 5. PFOA AND PFOS LEVELS IN MICROWAVE PAPER PACKAGING BETWEEN 2005 AND 2018

Abstract ......................................................................................... 69
Introduction .................................................................................. 70
Materials and Methods ................................................................. 73
  Sample Preparation ..................................................................... 73
  Focused Ultrasonic Liquid Extraction ......................................... 74
  LC-MS Conditions ..................................................................... 75
Results ......................................................................................... 77
  LOD and LOQ ........................................................................... 77
  Measurement Results ................................................................. 79
Discussion .................................................................................... 81
Conclusions ................................................................................ 84
Funding ....................................................................................... 85
Citation ....................................................................................... 86
References .................................................................................. 86

CHAPTER 6. GENERAL CONCLUSIONS ................................................. 89

APPENDIX A. RETAIL OBSERVATIONS ............................................. 92
  Employee Health and Hygiene .................................................... 92
  Handwashing ........................................................................... 92
  Cross-contamination ................................................................ 92
  Stocking and Rotation .............................................................. 93
  Handling of Fallen Produce ..................................................... 94
  Refrigeration and Temperature Control ................................... 94
  Display Case Cleaning ............................................................. 95
  Maintenance ............................................................................. 95
  Customer Behavior ................................................................. 95

APPENDIX B. TRAINING MATERIALS ............................................... 97
  Handwashing - Flipchart ........................................................... 98
  Stocking and Rotation - Flipchart ............................................. 111
Samples previously ground using an IKA-A11 mill are ground to a voluminous consistency with no evident intact pieces of the original paper; the sample has been completely separated into fibers, as seen in the following picture.

Internal Standard solution (IS)

Spike solution

Spiked sample preparation

Sample extraction

Sample concentration and reconstitution

SAS Code

APPENDIX E. NO IRB REQUIRED STATEMENT
LIST OF FIGURES

Figure 1 Side view diagram of a vertical self-service display case showing sensor locations. ........................................... 39

Figure 2 Calibration curve for PFOA .................................................................................................................. 78

Figure 3 Calibration curve for PFOS .................................................................................................................. 78

Figure 4 Poster of food safety in retail stores covering topics related to display case cleaning, refrigeration, stocking, handwashing, cross-contamination, and handling of fallen produce ......................... 97

Figure 5 Poster of display cases good practices covering topics related to refrigeration, display case cleaning, stocking, and maintenance ................................................................. 97

Figure 6 Handwashing flipchart explains the ways in which employees can get cross-contaminated and the importance of handwashing, following CDC’s recommended steps of proper handwashing technique ............................................. 98

Figure 7 Stocking and Rotation flipchart that addresses recommended best practices and correct food handling procedures related to stocking of fruits and vegetables in retail stores ................................................................. 111

Figure 8 Employee health and personal hygiene flipchart for employees based on FDA's handbook, covering information on symptoms related to foodborne illness ....... 118

Figure 9 Employee health and personal hygiene extension publication for managers based on FDA's handbook, covering information on the decisions related to symptoms of foodborne illness ................................................................. 125

Figure 10 Display case cleaning video that shows the general best practices to clean refrigerated display cases .................................................................................................................. 126

Figure 11 Ground samples of paper packaging .................................................................................................. 154

Figure 12 Packaging to be spiked and enough ethyl acetate to fully cover the sample ...................... 155

Figure 13 Weighed packaging sample in test tube ............................................................................................. 156

Figure 14 Sample in ice bath for sonication ....................................................................................................... 157

Figure 15 Extracted samples in scintilllation vials in the process of evaporation ............................... 157


**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Mean values for temperature and performance parameters of display cases for the main effects of before and after retrofitting with doors.</td>
<td>42</td>
</tr>
<tr>
<td>Table 2</td>
<td>Mean values for performance parameters of display cases compared by retailer, before and after retrofitting with doors. There was no significant retailer by sensor position interaction.</td>
<td>43</td>
</tr>
<tr>
<td>Table 3</td>
<td>Means values for performance parameters of display cases compared by sensor (datalogger) position, before and after retrofitting with doors. There was no significant retailer by sensor position interaction.</td>
<td>44</td>
</tr>
<tr>
<td>Table 6</td>
<td>Mean (SE) of the 1st, 5th, 95th, and 99th relative humidity (%) percentiles before and after doors were installed on the display cases. The means represent an overall average for the display cases in which they were measured as not all positions within the case were measured.</td>
<td>46</td>
</tr>
<tr>
<td>Table 7</td>
<td>Values for performance parameters of display cases compared by grouping sensors according to shelf (Top=TB, TF, Middle=MB, MF, Bottom=BB, BF, and Under=UB, UF), before and after retrofitting with doors.</td>
<td>47</td>
</tr>
<tr>
<td>Table 8</td>
<td>PFOA and PFOS concentrations on samples.</td>
<td>80</td>
</tr>
<tr>
<td>Table 9</td>
<td>PFOA and PFOS concentrations in paper packaging materials analyzed and spike recoveries.</td>
<td>81</td>
</tr>
<tr>
<td>Table 10</td>
<td>PFOA and PFOS concentrations in paper packaging per surface area.</td>
<td>81</td>
</tr>
<tr>
<td>Table 11</td>
<td>PFOA and PFOS concentration in paper packaging per bag for samples analyzed.</td>
<td>83</td>
</tr>
<tr>
<td>Table 12</td>
<td>PFOA and PFOS concentration (ng dm⁻²) in microwave popcorn bags from studies between 2005 and 2018, including this study (Zabaleta, et al. 2016).</td>
<td>84</td>
</tr>
</tbody>
</table>
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Retail establishments have the responsibility to deliver safe food to consumers. This research focuses on food safety in the retail environment from three different perspectives: refrigeration of fruits and vegetables, food handling behavior of produce stockers, and migration of harmful chemicals from packaging into food. The recent information provided gives an updated perspective to food safety issues such as produce refrigeration in retail establishments, food handler behavior, and perfluorinated compound migration in microwave popcorn. Twenty-five open and closed display cases stocked with produce from four retailers in five states of the United States (US) were monitored in eight positions for temperature and relative humidity (RH) conditions. Significant factors were doors, retailer, and sensor positions. Abuse time and variability in temperature and RH conditions were reduced in closed display cases. Employee behavior and food handling practices were observed in a casual way in retail stores in four states of the US over a period of 12 months. Observations were recorded and classified into topics for handwashing, stocking and rotation, handling of fallen produce, display case cleaning, customer behavior, refrigeration and temperature control, maintenance, cross-contamination, and employee health and hygiene. The developed reinforcement training material for retail produce stockers includes two posters, three flipcharts, one extension publication, and one training video. The proposed implementation was described considering the development or maintenance of a food safety culture. Perfluoro octanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) used to be added to food contact paper packaging and can be harmful to human health. Quantification of PFOA and PFOS on seven popcorn bags and three snack and sandwich bags show that two popcorn bags contain PFOA above the method’s limit of quantitation and all
samples were below the limits of detection for PFOS. Studies from 2005 to 2018 suggest a reduction in PFOA and PFOS levels over time in this type of packaging.
CHAPTER 1. GENERAL INTRODUCTION

With an increasing world population, the food industry is facing major challenges to make enough and safe food available for people (Food and Agriculture Organization, 2018; United Nations, 2019). An estimated 1.3 billion tons of food are wasted every year (Food and Agriculture Organization, 2018). Food safety and food waste are closely related (U.S. Food and Drug Administration, 2019). Food safety recalls increased significantly from 2004 to 2013 and have an impact on the food industry and the world’s resources (Page, 2018; U.S. Food and Drug Administration, 2019). Recalls can occur when there is a potential risk due to biological, chemical, or physical contaminants (National Seafood HACCP Alliance, 2001). This dissertation provides updated information on temperature abuse that may lead to potential increase in food loss and/or waste and possible increased microbiological risks on produce, possible microbiological and chemical risks associated to incorrect produce handling, and chemical risks associated to packaging migration of contaminants into microwave popcorn.

Approximately 360 million tons of food are lost every year due to lack of proper refrigeration (Chaomuang, Flick, & Laguerre, 2017). The United States Food and Drug Administration (FDA) is dedicated to ensuring a safe food supply and regulates retail establishments through the Food Code. Temperature controlled for safety (TCS) foods such as cut leafy greens must be stored at temperatures below 41 °F, or 5 °C (U.S. Food and Drug Administration, 2010). Some produce is displayed in refrigerated cases to comply with FDA, extend shelf life, and improve food safety. At low refrigeration temperatures, microbial and enzymatic activity is reduced, therefore extending shelf-life (Francis & O’Beirne, 2001). Produce is usually displayed in open cases or closed cases with glass doors. Studies have shown that closed display cases can improve temperature control in closed display cases (Atilio de Frias,
Luo, Kou, Zhou, & Wang, 2015; Kou, Luo, Ingram, Yan, & Jurick, 2014; Luo, He, & McEvoy, 2010). Zeng et al. (2014) and Luo et al. (2009, 2010) studied microbial growth at proper refrigeration conditions and abuse conditions, observing pathogenic growth of up to 3 log under abuse conditions. Based on these studies, the current research monitored open and closed retail display cases for temperature and RH conditions and performance was compared with and without recloseable doors.

Retail employees also play an important role in maintaining food that is safe for customers (Ellis, Arendt, Strohbehn, Meyer, & Paez, 2010). Proper food safety training and retention of the knowledge acquired through training is critical to establish safe food handling practices and behavior (Reynolds & Dolasinski, 2019). Establishing a strong food safety culture can help improve food safety behavior (Yiannas, 2009). In this dissertation, retail produce stockers were observed in their normal activities, and their food handling practices that could lead to food safety risks were recorded. Reinforcement food safety materials were developed on the topics observed that were not compliant with FDA’s Food Code 2017. The purpose of the reinforcement training implementation is to promote an improved food safety culture.

Another food safety risk in the retail environment comes from packaging materials. They are a source of chemical contaminant exposure to humans which are usually underestimated (Muncke, 2013). Voluntary recalls have occurred linked to the presence of residual compounds in approved FDA food contact materials (FCM) due to the presence of chemical safety risks (Lunder, Andrews, & Houlihan, 2010). Chemical migration from packaging is so common that it has been described as ubiquitous but little attention is drawn to this food safety risk (Muncke, 2013; Seltenrich, 2015). Paper packaging in microwave popcorn bags was treated with PFOA and PFOS to impart barrier properties and migration into food has been determined to be one of
the routes of human ingestion, with negative consequences to human health (European Food Safety Authority, 2008). For this dissertation, unused samples of paper packaging commercially used in retail to contain microwave popcorn products and snack and sandwich bags were analyzed to determine the content of PFOA and PFOS. Results were compared with studies from 2005 through 2018 to determine if there are evident trends in PFOA and PFOS content in microwave popcorn paper packaging.

Food safety in the retail level is critical in ensuring safe supply of food to consumers. Scientists have researched different aspects of food safety in retail. Microbiological safety of produce, employee food handling behavior, and packaging as a food safety risk will be covered in more depth in the following chapter, as it relates to food safety in the retail environment.

References


CHAPTER 2. LITERATURE REVIEW

Food safety can be associated to biological, physical and/or chemical hazards (World Health Organization, 2019). Biological hazards are those associated with living organisms or substances produced by them and include bacteria, viruses, or parasites. Chemical hazards relate to compounds present in food that can cause adverse health effects due to immediate or long-term exposure. Physical hazards are extraneous objects present in food that can harm and individual when eaten. Physical hazards can include glass, metal or plastic fragments (National Seafood HACCP Alliance, 2001; World Health Organization, 2019).

Food can become unsafe at any point from the farm to the plate, including processing facilities and retail establishments (Beuchat, 1996). Food safety programs such as Hazard Analysis Critical Control Points (HACCP), Current Good Manufacturing Practices (CGMP) or the Risk-Based Preventive Controls for Human Food under the Food Safety Modernization Act (FSMA) aim to deliver safe foods to customers, preventing foodborne illness (U.S. Food and Drug Administration, 2019). With the implementation of FSMA, the FDA shifted the approach from corrective actions after outbreaks to a preventive system (U.S. Food and Drug Administration, 2019).

Retailers enter the complex food distribution system almost at the end but play a very important role in maintaining food safety, delivering safe food to customers. Retailers are regulated by FDA through the Food Code to ensure safe products are reaching the consumers (U.S. Department of Health and Human Services, 2017). Some retail stores have voluntarily decided to certify their operations under the Global Food Safety Initiative (GFSI) in an attempt to standardize their food safety standards, which also includes their training programs (Shinbaum, Crandall, & O’Bryan, 2016). Updated information on critical food safety issues
such as refrigeration, employee food handling practices, and packaging migration are very useful to the food industry in general, as continuous improvements are made and the relevance may shift.

**Microbiological Safety of Produce**

Fruits and vegetables sold at the retail level can be contaminated with bacteria from the environment (Beuchat, 1996). Microorganisms can be on the surface or they can also be found inside the plant as they enter through the root system, stomata, or wounds. Most of these microorganisms are harmless to humans, but occasionally, human pathogens can be found (Lopez-Velasco, Welbaum, Boyer, Mane, & Ponder, 2011; Söderqvist, 2017). The occurrence of pathogens in fresh cut produce in retail stores have been detected and although contamination of fresh-cut produce may be rare, it still poses an unacceptable food safety risk because of the large amounts consumed (Denis, Zhang, Leroux, Trudel, & Bietlot, 2016; Miller & Painter, 2013). Pathogens such as *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7, *Campylobacter*, *Shigella*, Hepatitis A, Norovirus, *Shigella*, and *Cyclospora cayetanensis* have been reported on fruits and vegetables and are responsible for nearly half of all foodborne illnesses in the United States (Beuchat, 1996; CDC, 2019a; Denis et al., 2016).

Each year, more than 9 million foodborne illnesses from major pathogens occur in the United States. In 2013, the CDC published a comprehensive report of foodborne illnesses in the US from 1998 to 2008, estimated by food type to determine which foods cause more illnesses, hospitalizations, and deaths. More comprehensive reports will be published in order to evaluate trends and effectiveness of legislation in the future (Miller & Painter, 2013). In this CDC report, produce was identified as fruits, nuts, and five categories of vegetables (fungi, leafy vegetables, root, sprout, and vine-stalk) was responsible for 46% of all illnesses, where 23% of all illnesses were attributable to leafy vegetables. In addition, produce was responsible for 38% of
hospitalizations and 23% of deaths. The CDC explained that this is not because produce is particularly unsafe. The cause of the high incidence of illnesses attributable to produce is because of the large amounts consumed, and recommends to keep consuming fruits and vegetables as they have been linked to lower risk of heart attacks, strokes, and cancer (Miller & Painter, 2013). As of October 30, 2019, 14 outbreaks have been or are under investigation by the CDC. Out of those, three are related to produce (fresh basil, papayas, and pre-cut melon) with a total of 459 reported cases, 71 hospitalizations, and zero deaths. Two of the outbreaks were due to *Salmonella* infections and the other was due to *Cyclospora* (CDC, 2019d, 2019c, 2019b, 2019e).

These pathogens contaminate fresh produce through manure, insects, water and soil in the field. Produce can also be contaminated from further stages such as harvest, washing, cutting, packaging, transportation, or handling (Beuchat, 1996; Mir et al., 2018). Fresh cut vegetables have high moisture content and the rough surfaces can harbor microorganisms that are not easily eliminated through washing (Zeng et al., 2014). Fruits and vegetables have been associated with outbreaks in the United States and other countries (Centers for Disease Control and Prevention, 2019; Denis et al., 2016; Oliveira et al., 2010). Some produce is consumed minimally processed, ready to eat (RTE), fresh-cut, or raw, which increases the risk to human health. Minimal processing of fruits and vegetables may not ensure microbial safety and there is an inherent risk of having psychotropic bacteria and other pathogens that can cause outbreaks (Lianou & Sofos, 2007). Some outbreaks are caused by single produce items and others are caused by mixed produce. Among the most common vehicles of transmission are leafy greens, mixed fruits, and mixed vegetables. Other very common single vehicles of transmission are lettuce, spinach,
melon, sprouts, juices, berries, green onions, and carrots, among others (Centers for Disease Control and Prevention, 2019; Murray, Wu, Shi, Jun Xue, & Warriner, 2017).

Produce Safety at the Retail Level

Retailers use their produce departments to attract customers and influence customers’ purchase decisions (Zind, 1989). Produce is generally located towards the front of the store to sell them quickly (California Department of Public Health, 2011). A study in Japan on products that attract customers’ purchases, called “store magnets” and their location in the floor layout, found that all 64 store layouts studied had the fruits and vegetables section at the front of the store (Ohta & Higuchi, 2013). Some studies point out the importance of the general atmosphere given by the display of merchandise and the arrangement of the store when customers choose their preferred retail stores. Customers’ buying behavior can be influenced by modifying the store layout, color, lighting, and general atmosphere (Singh, Katiyar, & Verma, 2014). The emotive response and preference of customers is directly related to the physical aspects within the store (Lin Thang & Tan, 2003). Product display can have a great impact on customer spending behavior (Grewal, Roggeveen, & Nordfält, 2017). Retail stores display approximately half of all their food products in refrigerated display cases (Bertrand, 1993; Chaomuang, Flick, & Laguerre, 2017). Refrigeration in retail stores is a common way to extend shelf life of food and improve food safety.

Fruits and vegetables are susceptible to microbial contamination and have caused numerous outbreaks in the past. Bacteria can have different growth and death behavior, depending on the type of produce, temperature and time of storage, and strain of bacteria. *Listeria monocytogenes* and *Escherichia coli* O157:H7 have caused some of these outbreaks and are of great concern with numerous studies evidencing growth under refrigeration conditions (Evans, Scarcelli, & Swain, 2007; Kou, Luo, Ingram, Yan, & Jurick, 2014; Nunes et al., 2009;
Although the growth of bacteria is slow under refrigeration, food safety is still a major concern (Zeng et al., 2014). *L. monocytogenes* is a very persistent strain that can survive and grow for many years (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017; Francis & O’Beirne, 2001a; Walker, Archer, & Banks, 1990). Walker et al. (1990) studied three strains of *L. monocytogenes* and found generation times as low as 13 hours at 5 °C and up to 131 hours at 0 °C (Walker et al., 1990). Francis et al. (2001) also found that both *L. monocytogenes* and *E. coli* O157:H7 can increase up to 2.5 log CFU/g over a 12 day storage period on shredded lettuce, depending on the strain (Francis & O’Beirne, 2001a). Zeng et al. (2014) also observed up to 3 log CFU/g growth in *E. coli* and *L. monocytogenes* on romaine lettuce when temperature abuse was up to 16 °C and no pathogen growth when kept under 4 °C (Zeng et al., 2014). Overall, lower temperatures reduce microbial growth (Francis & O’Beirne, 2001a; Zeng et al., 2014).

In addition to pathogenic microbial contamination, spoilage bacteria and mold can also be challenging. Temperature abuse of fresh fruits and vegetables is one of the main causes of spoilage. Any wounded area on the fruit or vegetable will provide nutrients, favoring microbial growth. Storage at low temperatures results in a reduction of the respiration of the product and reduced metabolic rate and growth of microorganisms that extend shelf-life (Ragaert, Devlieghere, & Debevere, 2007). Brackett (1987) found that vegetables have a natural predominant microflora of bacteria, while fruits have a natural microflora of fungi and lactic acid bacteria. Storage temperature and relative humidity (RH) variations can have a negative impact on product quality due to shrinkage and spoilage. During periods of time with temperature and RH humidity fluctuation, water droplets may form, favoring bacterial growth (Brackett, 1987). Even at high moisture levels, the low pH of fruits and vegetables can give molds a competitive advantage over bacteria and spoilage due to mold growth can occur (Moss, 2008).
Packaging in Microbiological Safety and Shelf-life of Produce

Fruits and vegetables are sometimes packed in semipermeable sealed packages and sold refrigerated at retail stores (Cutter, 2002; Farber et al., 2003; Oliveira et al., 2010). Fresh produce can be packaged in flexible packages or rigid containers modifying the gas content inside the package for preservation and control of microbial growth (Cutter, 2002). This modification of the internal atmosphere of the package is commonly known as modified atmosphere packaging (MAP) or controlled atmosphere packaging, depending on the method by which the gas is modified. In MAP, oxygen levels are maintained low (2-5% approximately), while carbon dioxide is increased from 0.03% to 3-10%, and this is done by flushing and introducing the desired gases at the moment of packaging. Oxygen gas (O₂), carbon dioxide gas (CO₂), and nitrogen gas (N₂) are the most commonly used gases in MAP packaging. (Farber et al., 2003). In CAP, sachets and selective barrier films are used to remove water or gases such as ethylene and oxygen. These sachets can also release desired gases such as carbon dioxide. CAP will ensure that the atmosphere inside the package remains constant in time (Zahra et al., 2016). This slows aerobic microbial growth and together with refrigeration, the shelf-life of packaged produce is extended (Francis & O’Beirne, 2001b). Although MAP and CAP can extend the shelf-life of fresh-cut fruits and vegetables and should slow microbial growth, some studies have shown that these processes can produce off-flavors, affecting product quality (Farber et al., 2003). Recent studies have shown that L. monocytogenes and E. coli can survive and grow under reduced oxygen conditions (Farber et al., 2003; Oliveira et al., 2010). Oliveira et al. studied the effect of the packaging atmosphere on Salmonella spp., E. coli, and Listeria monocytogenes survival and growth and found that it has no significant effect in controlling pathogen presence and growth (Oliveira et al., 2010). Other packaging methods include active packaging in which antimicrobials, scavengers, inhibitors, or other additives have been added to the polymer used as
packaging. Active packaging has the capacity to add or remove compounds over time and therefore control for loss of quality or microbial growth in the food product (Majid, Ahmad Nayik, Mohammad Dar, & Nanda, 2018). Product inventory management such as the “first in, first out” principle for packaged product may prevent high levels of microorganisms by reducing storage time and therefore reduce the risk (Lianou & Sofos, 2007).

**Transportation, Storage, and Display Conditions of Produce**

In order to maintain a constant supply of fruits and vegetables for consumers, these are imported and distributed internationally. In locations where the harvest season is short due to weather, there may be extended storage and transportation times. During transportation, these products may be subject to temperature abuse for extended periods of time, depending on the distance travelled, which may increase the food safety risk (Mercier, Villeneuve, Mondor, & Uysal, 2017; Zeng et al., 2014). Sometimes, fresh produce that should be refrigerated is transported with produce that doesn’t require refrigeration. Mixed loads are common, and this may cause microbial growth and damage of the produce. The shelf life of produce transported under adverse conditions may be reduced and in some extreme cases, the entire load can be lost even before display (Nunes, Emond, Rauth, Dea, & Chau, 2009).

Low product-specific temperatures cause chilling or freezing damage, and high product-specific temperatures can potentially increase enzymatic and microbial activity (Badia-Melis, McCarthy, Ruiz-Garcia, Garcia-Hierro, & Robla Villalba, 2018). The U.S. Food and Drug Administration (FDA) Food Code states that temperature controlled for safety (TCS) foods, such as fresh fruits and vegetables, should be kept under 5°C (U.S. Department of Health and Human Services, 2017). Improper temperature control can result in food waste and therefore financial losses for retail companies and customers (Badia-Melis et al., 2018). A study in Sweden showed
that 85% of the mass base food waste from retail stores comes from fruits and vegetables, and this accounts for 49% of the total wastage carbon footprint (Scholz, Eriksson, & Strid, 2015).

Control of relative humidity conditions should be done according to the evaporative surface of produce as this will determine the rate at which it loses water, and therefore quality. Produce stored at low product-specific relative humidity will lose weight, wilt, and wrinkle. On the other hand, produce stored at low relative humidity will result in low quality products and food loss. It has been suggested that relative humidity storage conditions for fruits and vegetables should be above 90% (Nunes et al., 2009) to avoid financial losses.

In retail establishments, produce is displayed in refrigerated display cases that control temperature and relative humidity. Some display cases are closed with glass doors while others are open and have air curtains to form a barrier and maintain internal conditions. Various studies have shown that the front positions have higher temperatures than the back positions and temperatures vary inside open display cases from -1 to 19.3 °C (Evans et al., 2007; Kou et al., 2014; Nunes et al., 2009; Zeng et al., 2014). Atilio de Frias et al. compared the quality of baby spinach in open and closed cases with and without doors, showing that under the same conditions in a controlled environment, the temperature fluctuation in cases with doors was reduced (Atilio de Frias, Luo, Kou, Zhou, & Wang, 2015). The quality of baby spinach in cases with doors significantly improved after 4 days of shelf life and the decay rates were also reduced due to better temperature control and reduced temperature abuse conditions (Atilio de Frias et al., 2015). Display case manufacturers and retail stores are aware of all these challenges and the hazards associated with incorrect produce temperature control.

**Display Cases in Retail Stores**

Refrigeration systems have to run continuously, and they can account for up to 50% of the energy costs of the establishment (Fricke & Becker, 2011). Infiltration from the environment
of warm air into the display cases causes products in the front row to have higher temperatures. This infiltration of convective heat can account for up to 70% of the heat load of the refrigeration system (Atilio de Frias et al., 2015; Fricke & Becker, 2011). Compressors and condensers use approximately 60 – 70% of the energy consumption and the rest is consumed by the fans, lights, defrosting system, and anti-sweat heaters (avoid condensation on doors and exterior surfaces) (Baxter, 2002). Other causes of temperature variability inside display cases include defrost cycles, proximity to lights, and interruptions in the air flow (Mercier et al., 2017).

Refrigeration systems have been improved in recent years to reduce energy consumption, enhance refrigeration while protecting the environment, boost temperature control systems, and comply with legislation. Supermarkets contribute significantly to the emission of greenhouse gases due to the indirect CO2 emissions from energy production and direct refrigerant leakage from refrigeration systems (James & James, 2010). One percent of the world’s CO2 emissions can be attributed to the cold-chain (Mota-Babiloni et al., 2015; Tassou, Ge, Hadawey, & Marriott, 2011). Some of the drivers for technological improvement for the refrigeration industry are the Ozone Depletion Montreal Protocol, Kyoto Protocol on Global Warming, HFC Kigali Amendment (2016), and Department of Energy (DOE) 2017 (Navigant Consulting Inc., 2013; Ozone Secretariat, 2018; Patenaude, 2018). Based on international agreements to reduce greenhouse gasses and improve energy efficiency, the US Environmental Protection Agency (EPA) and the DOE have revised refrigeration technologies and issued a regulation that impacts refrigerated display cases in retail, as well as other systems. Manufacturers are responsible for implementing these regulations for new cases that are sold. Along with the energy efficiency improvements, modifications also include the de-listing of refrigerants that are harmful for the environment (Shebik, 2015; United States Environmental Protection Agency (EPA), 2018b).
Manufactures have made design changes on new display cases to include light-emitting diodes (LED) lights, energy efficient motors, and glass doors to avoid infiltration, among others. The DOE’s Better Building Alliance (BBA) has issued a guide on best practices to retrofit open display cases with doors in order to achieve higher energy efficiency.

Previous work has shown that retrofitting existing open display cases with doors is potentially more cost-effective than complete replacement with new closed cases (Navigant Consulting Inc., 2013). Newer display cases with glass doors decrease the energy consumption to 77% that of new open display cases per unit of length (Fricke & Becker, 2011). Faramarzi et al. (2002) also studied the impact of retrofitting doors to open display cases and estimated a reduction in the refrigeration load by 68%, reducing overall temperature in food products by 6 °C, under controlled laboratory conditions (Faramarzi, Coburn, & Sarhadian, 2002). Some of the aspects considered when retrofitting with doors are the control valve sizes, riser and piping sizes, and rack considerations (Patenaude, 2018).

**Consumers and Display Cases**

Behavioral issues and perceptions have also limited the willingness of retail stores to retrofit open display cases, as they may consider that the barrier between the shopper and food product may have a negative impact on sales (Foster, Hammond, Bown, Evans, & Maidment, 2018). Recently, studies have shown that customers understand the benefits of having doors and have a positive perception (Lindberg, Salomonson, Sundström, & Wendin, 2018). Although the benefits of doors on display cases have been demonstrated in previous studies, consumer perception and impact on the shopping experience must be considered in retail stores, as the return of the investment of retrofitting with doors can take up to 5 years (Atilio de Frias et al., 2019). Senses such as vision, smell, and touch can influence consumer perception of freshness and willingness to buy. Customer experience is important for shopping behavior and inducing
spending (Ebster, 2011; Sachdeva & Goel, 2015). Some customer may make informed decisions but others may be spontaneous, often encouraged by visual presentations (Grewal et al., 2017). Clean transparent doors have a positive effect on consumers, while door handles and heavy packages inside closed display cases may lead to avoidance by some customers (Lindberg et al., 2018). However, a previous study on the impact of open versus closed display cases shows no significant overall impact on sales (Fricke & Becker, 2011). Even though the advantages of closed display cases have been proven, open display cases are very common at retail stores because they offer a sensory rich and convenient experience for customers (Atilio de Frias et al., 2019). Due to discrepancies in these findings, more research should be conducted to better understand consumer perception and purchasing behavior regarding open and closed display cases.

Chapter 3 of this dissertation provides data on the current status of refrigeration conditions in open versus closed display cases in the US, after the DOE 2017 rule changes. The research provides current multi-state information and highlights the importance of retailer control and self-verification. More research on improving refrigeration technology to avoid temperature abuse conditions is required. Food safety, food waste, and food loss are critical parameters to follow in future research.

The Role of Retail Employees in Maintaining Food Safety at the Retail Level

The World Health Organization emphasizes the importance of having access to enough safe food, free from biological, chemical and physical contaminants that can cause foodborne illness if consumed (World Health Organization, 2019). Employees from the food retail business play a major role in preventing foodborne illness (U.S. Department of Health and Human Services, 2017). In the year 2017, 12.5% of the employees of the Grocery Stores Industry group were stock clerks and order fillers. The average salary for this job position was less than half of
the national average salary, making it a low wage salary position (Data USA, 2019; Salary.com, 2019). The education required for the position is generally a high school degree but there may be employees that do not hold a high school diploma. Their specific tasks include filling up shelves, cases, and displays with products to be exhibited, receiving merchandise and reporting defects, pulling out undesired product from the exhibition, safe and sanitary handling of food, and orienting customers to where products are located within the store (Job Description Hub, 2018; Salary.com, 2019). In addition, retail employees have a high voluntary turnover in grocery stores (Lewis, 2019).

Training retail food handlers

The application of safe food handling procedures by employees requires food safety training and this training should be reinforced so that it translates into safe food handling practices (Reynolds & Dolasinski, 2019). Food safety knowledge and proper food handling training have been addressed in numerous studies (Reynolds & Dolasinski, 2019). A study by Arendt, Strohben, and Jun on motivators and barriers to safe food handling revealed eight motivators and six barriers to safe food handling. Employees reported being motivated to follow safe food handling behavior by the following: in order to avoid bacterial growth and cross-contamination; not harm customers; by having the proper knowledge and training; acknowledging that it is required by law, regulations, and procedures; having good practices and/or habits; being motivated by internal rewards; responding to the culture of the workplace; and the drive to satisfy customers. The main barriers to safe food handling behavior were found to be: forgetfulness and/or lack of habit, being too busy, lack of knowledge, negative consequences of following safe food handling practices, availability and use of resources, and the standards or culture of the workplace (Arendt, Strohbehn, & Jun, 2015). Regular employee training can increase the knowledge in food safety and improve proper food handling skills.
Literature has shown that acquiring knowledge does not always translate into improved correct food handling practices and there could be other factors that affect handling behavior (Arendt et al., 2015).

According to the Food Marketing Foundation (FMI), retail food training has traditionally been based on gaining knowledge about the correct food handling recommended practices and avoiding foodborne illnesses, correct food practices when preparing, handling and storing food, food safety regulations that apply to their sector, and the impact of food handling on the community and the company. Training sessions used to be very theoretical and focused on the overview of food safety, leaving trained managers with a lot of information and the responsibility of implementing it with their teams, but no true way of how to make it effective (Neal, 2014). While food safety training is recognized as a practical method to deliver knowledge, training methods have not been standardized.

**Retail Produce Stockers in the US**

Retail employees that stock produce in U.S. grocery stores average between 36 and 37 years old (Data USA, 2019). Transferring knowledge to retail stockers can be challenging. Adult learning, conceptualized by Merriam (2002) as andragogy, has been described through five major factors. Adults are independent and self-motivated individuals, capable of directing their own learning, have past life experience that can be used as a relatable resource in learning, have learning needs liked to their social roles, are interested in problem centered and applicable knowledge, and are driven by internal factors that motivate learning (Merriam, 2001). Food handlers in the U.S. have very diverse ethnicities, educational level, and have different cultural backgrounds, increasing training challenges (Howton et al., 2016; Reynolds & Dolasinski, 2019). Age and generational differences can also affect the factors that drive individual motivation to have safe food handling practices (Ellis, Arendt, Strohbehn, Meyer, & Paez, 2010). Howton et al.
(2016) studied the effectiveness of food safety programs using the Customizable Tool for Online Training Evaluation. They observed that basic language (middle school reading level) and easy to follow instructions were the best training principles (Howton et al., 2016). Adult trainees prefer shorter sessions and hand-on activities, with which they can relate (Merriam, 2001).

**The Food Safety Culture**

Food safety culture has been identified as a condition that once established in an organization, it will permeate into daily activities and be learned by newer employees (Neal, 2014; Yiannas, 2009). In order to build a food safety culture, management commitment and participation is extremely important. Employees want to see that principles are consistent in the organization and management is involved (Neal, 2014; Yiannas, 2009). Egan et al. reported that in some cases, management is not properly trained to supervise and therefore lack the knowledge to assess risks (Egan et al., 2007). Research shows that work environments and safety culture determine employee’s food handling practices (Abidin, Arendt, & Strohbehn, 2013). A company’s profitability depends on workers performance. A marked increase in worker and business performance can be observed when all employees in a company are aligned with the company’s business goals and objectives. Employees should know what is expected of them and how they align with the companies goals (SAP, 2019)

Chapter 4 of this dissertation aims to help fill in the current gaps observed in retail produce stockers’ food handling behavior. This study provides useful information related to the knowledge and/or behavioral gaps that might pose a food safety risk in the retail industry. The training materials developed are specific to the current observations and more topics should be developed to help retailers maintain safe food handling practices.
Food Packaging as a Food Safety Risk

Food packaging has several functions that can be identified in general as containment, convenience, protection, and communication. Some packages can even become so distinct that they themselves become part of the brand (Robertson, 2012; Yam & Lee, 2012). Food packaging has evolved in some cases by consumer’s lifestyle or ecological concerns. In other cases, it has been driven by cost and profits, or by food safety and regulations. In all cases, food packaging evolves to improve the sustainability of the food supply chain (Yam & Lee, 2012). With the recent globalization of food markets and consumers’ demands for convenient, safe, and minimally processed foods with enhanced shelf life, food packaging has evolved in order to satisfy this changing market (Majid et al., 2018).

Generally, food packaging can be considered an integral component of food products that will extend shelf-life, maintain quality, and improve food safety. Unfortunately, this is not always the case. Food packaging can be a source of chemical food contaminants that can migrate into food, having negative consequences on human health. Paper and board or plastics are packaging materials that can contain substances on their surface or in their structure that migrate into food. Contaminants can migrate from the surface or across the packaging layer and into the food matrix. Inks are examples of substances that migrate across the packaging material and into the food sample (Muncke, 2013). Per- and polyfluorinated compounds in coated paper packaging can also migrate into the food by direct food contact (Still, Schlummer, Gruber, Fiedler, & Wolz, 2013).

**Per- and Polyfluorinated Alkyl Substances (PFAS) in Paper Packaging**

PFAS are organic substances with a hydrocarbon backbone in which fluorine has substituted all the hydrogens. All PFAS are synthetic and very stable compounds (Giesy & Kannan, 2002). They are resistant to biological, chemical, and thermal degradation. Due to their
stability, toxicity, bioaccumulation and long half-lives in mammals they have been classified as persistent organic pollutants (Lindstrom, Strynar, & Libelo, 2011; Schaider et al., 2017; Stahl, Mattern, & Brunn, 2011).

PFAS were first produced in the 1940’s and 1950’s to be used in grease and oil resistant coatings, surfactants, and firefighting foams (European Food Safety Authority, 2008; OECD, 2013). New and novel applications in semiconductors, mechanical parts, wetting agents, mist suppressants, among others provide benefits for industries such as food, aerospace, photographic imaging, semiconductors, automotive, electronics, and aviation, among others (Lindstrom et al., 2011).

Long chained molecules containing six or more carbons in their backbone are called legacy PFAS. New short chain molecules with five or less carbons in their backbone are referred to as emerging PFAS. Emerging PFAS also include a family of molecules used in the manufacturing process of fluoropolymers commonly referred to as GenX. Even though most long chain PFAS have been regulated and removed from the market, short chain and GenX PFAS continue to be used (Interstate Technology Regulatory Council, 2018). They are used in food packaging, paints, cleaning products, non-stick coatings, outdoor fabrics, and firefighting foam, among other applications (United States Environmental Protection Agency (EPA), 2018a). Over 4500 different PFAS have been identified (Lim, 2019) but most research has been on only a few of these molecules, mainly perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) and a few other molecules (European Food Safety Authority, 2008; Giesy & Kannan, 2002; Jogsten et al., 2009; Moreta & Tena, 2013, 2014; Newton et al., 2017; OECD, 2013). Most PFAS molecules haven’t been studied and are not regulated (Interstate Technology Regulatory Council, 2018).
Many PFAS degrade to form perfluorocarboxilic acids (PFCA) and perfluorosulfonic acids (PFSA). PFOA and PFOS are some of these degradation end products for C8 precursor PFAS, making these compounds of great interest for researchers (Giesy & Kannan, 2002; Newton et al., 2017). Studies have shown that they are widely spread in the environment, are contaminants in our food supply, and some have adverse health effects on humans (European Food Safety Authority, 2008; Lindstrom et al., 2011; Martínez-Moral & Tena, 2012; OECD, 2013; Stahl et al., 2011). They contaminate our water supply and pass on to different elements in our food chain (Giesy & Kannan, 2002; Newton et al., 2017). They enter our food supply through animal protein, fruits, vegetables, processed food, packaging, and water. Environmental sources such as indoor dust also contribute to human exposure. The distribution and actual intake mechanisms are still ambiguous and continue to be studied (Domingo, 2012; OECD, 2013; Yolanda, Marinella, Llorca, & Damià, 2011).

**Toxicity and Health Effects of PFAS**

PFAS are mobile, bioaccumulate, and don’t degrade, or degrade slowly in the environment (Interstate Technology Regulatory Council, 2018). PFOA and PFOS have been detected in the environment: water, soil, plants, and animals. They can be detected in high concentrations near manufacturing facilities that use PFAS in their products but surprisingly, PFOA and PFOS are detectable even in the Arctic region, far away from any noticeable source (Giesy & Kannan, 2002; Lindstrom et al., 2011; Newton et al., 2017).

PFOA and PFOS have significant bioaccumulation and elimination differences. In general, there are differences between species and between sexes within species. In humans, studies have shown an increase in triglycerides and other health effects, with great controversy. Some epidemiological studies in humans show contradicting results from workers exposed to PFOA and PFOS. More recent epidemiological studies have shown high incidence of testicular,
liver, pancreatic and breast cancers linked to PFOA and PFOS exposure (Pierozan, Jerneren, & Karlsson, 2018). PFOA has been identified as a thyroid hormone disruptor and has been associated with reduced birth weight. Animal studies in monkeys and rodents have shown that both PFOA and PFOS increase cancer risk, reduced childbirth weight and reduce gestational age, affect hormonal activity, and metabolism, among many other health effects that continue to be studied. The Scientific Panel on Contaminants in the Food Chain (CONTAM) has established that the Tolerable Daily Intake (TDI) for PFOS is 150 ng/kg b.w. per day and 1.5 µg/kg b.w. per day for PFOA (European Food Safety Authority, 2008) Geueke, 2016).

Even though research has demonstrated the toxicity of PFAS and regulatory agencies in some countries have tried to legislate against their production, other countries have picked up production to fill in the need. The EPA worked with 3M and DuPont to agree upon a voluntary phase-out from the production of PFOS and related compound use (Lindstrom et al., 2011). After the year 2000, PFOA and PFOS production in Japan, Western Europe and the U.S. dropped, but China, India, Poland and Russia have increased their production levels (Geueke, 2016).

International efforts to stop the production and use of PFOA and PFOS have included them in the Stockholm Convention as persistent pollutants (Newton et al., 2017). Short chain and GenX PFAS continue to be used in increasing applications but little is known about their persistence, potential bioaccumulation and toxicity (Interstate Technology Regulatory Council, 2018; Lim, 2019).

**Food Contact Paper Packaging**

Some consumers like paper packaging and perceive it as sustainable and environmentally friendly (Magnier & Crié, 2015). Paper packaging is hydro and lipophilic so packaging manufacturers coat it with PFAS for water, oil, and grease repellency (Lindstrom et al., 2011; Martínez-Moral & Tena, 2012). Studies evaluating PFAS content in food paper packaging
materials have found that migration can occur into food and/or food simulants, therefore introducing a chemical food safety hazard (Begley, Hsu, Noonan, & Diachenko, 2008; Moreta & Tena, 2014; Zafeiraki, Costopoulou, Vassiliadou, Bakeas, & Leondiadis, 2014).

Paper packaging has been analyzed with good recovery rates using liquid extraction with solvents, followed by liquid chromatography. Studies report using various types of ultrasound and high pressure assisted techniques of extraction, as well as several types of liquid chromatography coupled with different detection methods for quantitation. Some of these studies have identified PFOA and/or PFOS in their analysis; others have not been able to detect them. It is an analytical challenge to quantify these compounds in food contact paper packaging materials and the development of LC-MS/MS, LC-(QqQ)MS/MS, and LC-(QTOF)MS/MS methods have proven to be useful reading PFCs at low levels (Jogsten et al., 2009; Moreta & Tena, 2013, 2014; Schaider et al., 2017; Zafeiraki et al., 2014).

Chapter 5 of this dissertation quantifies the presence of PFOA and PFOS in food contact paper packaging. This study provides relevant updated information for the paper packaging and the microwave popcorn industry. Negative information linking these industries to PFOA and PFOS is no longer relevant and a new evaluation of the risks associated to this type of packaging for this application is needed.

References


CHAPTER 3. TEMPERATURE PROFILING OF OPEN- AND CLOSED-DOORED PRODUCE CASES IN RETAIL GROCERY STORES

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Highlights

• Significant differences between retailers were observed in the performance of their display cases.

• Display cases retrofitted with recloseable doors experienced improved temperature and relative humidity control.

• Food safety evaluations of display cases provide useful and needed information to the retail business and scientific community to better understand the current cold chain.

Abstract

Temperature control of produce in the retail environment is essential to reduce food safety risks, maintain quality, and reduce food waste. Previous studies have demonstrated that retrofitting or replacing open display cases to recloseable doors better control temperature and humidity. However, there are no studies to date that comprehensively evaluated temperature profile in cases with and without doors in the actual retail store environment. Twenty-five open
and closed refrigerated display cases in ten retail stores in five states were monitored for temperature and humidity over 9 months. Sensors recorded data every 2 minutes in eight positions (top, middle, bottom and under the bottom shelves, in the front and back locations of each shelf). Results of this study found significant differences between open and closed cases, retailers, and sensor position in display cases (p<0.0001). Seven display cases were retrofitted with doors and, as a result, temperature variations were significantly minimized (p-value <0.0001). Cases with doors held temperatures significantly lower (4.7°C, p-value <0.0001), with the top front position exhibiting the highest temperature (5.7°C) and abuse due to high temperature (>5°C) for the longest duration (35.7% of total time observed). Temperatures and abuse conditions above 5°C were not significantly different between front and back positions in the cases. Further, the range of temperature and RH variability was reduced following door installation. With changes in display case technology over the past five years, this study provides updated data on operational temperatures in display cases before and after retrofitting with doors. It also provides evidence of the importance of temperature monitoring within display cases to ensure abuse conditions do not persist.

Keywords
Refrigerated display cases, doors retrofitting, retail, temperature abuse, food safety, produce.

Introduction
Approximately half of all food products in retail stores are held in refrigerated display cases (Bertrand, 1993; Chaomuang, Flick, & Laguerre, 2017). As of 2010, based on the United States Department of Agriculture’s (USDA)’s Economic Research Service estimates, 31% of food waste comes from retail and consumer environments (United States Department of Agriculture, n.d.-a; USDA Office of the Chief Economist, n.d.). In October 2018, the USDA,
U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drugs Administration (FDA) signed the Winning on Reducing Food Waste Initiative, an interagency agreement to reduce 50% of food loss and waste by 2030 (United States Department of Agriculture, n.d.-b).

The use of refrigerated display cases to hold food in retail stores is a common way to extend the shelf-life of food and improve food safety. Retail stores are currently considering the cost: benefit of converting their open refrigerated display cases to closed display cases to enhance food quality through improved temperature and humidity control. A study conducted in 2010 on the impact of open vs. closed display cases showed no significant overall impact on sales (Fricke & Becker, 2011). Another study in 2017 showed that customers understand the benefits of having doors and have a positive perception of having food in closed display cases (Lindberg, Salomonson, Sundström, & Wendin, 2018).

The FDA’s Food Code states that temperature controlled for safety (TCS) foods must be maintained at temperatures of 41ºF (5ºC) or less during cold storage and display (U.S. Department of Health and Human Services, 2017). Studies showed that there was a significant increase in food waste, loss of quality, shelf-life reduction, and increased food safety risk in produce kept at temperatures above 5ºC due to increase enzymatic and microbial activity (de Frias et al., 2018; Evans, Scarcelli, & Swain, 2007; Kou, Luo, Ingram, Yan, & Jurick, 2014; Luo, He, & McEvoy, 2010; Luo, He, Mcevoy, & Conway, 2009; Nunes, Emond, Rauth, Dea, & Chau, 2009; Zeng et al., 2014). Temperature monitoring studies in the United States, United Kingdom, and France also showed that food displayed in open refrigerated display cases was subjected to temperature abuse, with temperatures ranging from -1 to 16 ºC (Derens, Palagos, & Guilpart, 2006; Evans et al., 2007). Luo et al. (2009) observed a 1 log CFU Escherichia coli O157:H7 increase when keeping commercial packages of baby spinach at 12 ºC after 3 days with an
increased quality decay. In a later study, Luo et al. (2010) also observed that bagged lettuce salads stored at 12 °C for 3 days promoted E. coli O157:H7 growth by more than 2 log CFU, while visual quality was still acceptable. E. coli in the bagged lettuce salads below 5 °C survived but had limited growth. Improper product-specific temperature control, both low and high, can result in increased food safety risk and monetary losses for retail companies and customers (Badia-Melis, Mc Carthy, Ruiz-Garcia, Garcia-Hierro, & Robla Villalba, 2018).

Low product-specific storage temperatures for produce, after harvest and throughout the cold chain, will extend shelf-life (Brecht et al., 2003), but extremely low temperatures can cause chilling injury or freezing damage (Badia-Melis et al., 2018). A recommended minimum low temperature of 0 °C in display cases holding temperate fruits and vegetables will maintain quality while maximizing shelf-life (Gast, 2008; Mercier, Villeneuve, Mondor, & Uysal, 2017; U.S. Department of Health and Human Services, 2017). Relative humidity should also be controlled as produce stored at too low of a RH will lose weight, wilt, and shrivel. This will inevitably render it inedible, increasing food waste and causing economic losses to retail businesses. The optimum relative humidity values for fresh fruits and vegetables is usually recommended as above 90% (Nunes et al., 2009).

At the retail level, temperature and humidity storage conditions for produce are controlled by displaying produce in either closed display cases with doors or open display cases with air curtains. Even though cases are set to appropriate temperatures, actual temperatures inside the display cases may vary and therefore product temperatures can vary. Infiltration of warm air into the display cases causes product at the front of the cases to have a higher temperature. Display case product temperature is also affected by defrost cycles, proximity to lights, and interruptions in air flow (Mercier et al., 2017). Faramarzi et al. studied the impact of
retrofitting doors to open display cases and estimated a reduction in refrigeration load of 68% while reducing overall temperature in food products by 6°C (Faramarzi, Coburn, & Sarhadian, 2002).

Since this study, refrigeration systems have been improved in recent years by manufacturers to reduce energy consumption, increase temperature control, and comply with legislation. Retailers faced the decommissioning of certain refrigerants and refrigeration systems forced by manufacturers’ compliance with the rule. (Navigant Consulting Inc., 2013; Shebik, 2015). Work conducted by Navigant Consulting Inc. in collaboration with the U.S. Department Of Energy (DOE), the Better Buildings Alliance, and members of industry found that retrofitting existing open display cases with doors was potentially more cost-effective than completely replacing the cases with closed-door units (Navigant Consulting Inc., 2013).

The objectives of this research were to determine the temperature and relative humidity (RH) profiles in refrigerated fresh-produce, open vs. closed display cases located in multiple states and with multiple retailers. Twenty-five display cases of ten retail stores representing four retailers located in five states were monitored. Seven of the 25 display cases were retrofitted with doors and data were collected over a 9-month period, both before and after modification.

**Materials and Methods**

This study was conducted in ten retail stores representing four major retailers in five states: California, Florida, New York, Iowa, and Nebraska. Each retailer provided two or three store locations for monitoring based on future plans to retrofit the display cases with doors during the study. Seven stores of 3 retailers retrofitted their display cases with clear glass doors, to convert from open to closed case. Between one and four display cases were tagged per store based on where the fresh fruits and vegetables were displayed (i.e., leafy greens, fresh-cut carrots, containers of fresh-cut fruit, etc.). Display cases were different across retailers and
within retailers. This study didn’t control for display case or store design, thus showing the current status of display case equipment in retailers across the U. S. The vertical refrigerated self-service display cases, both open and fitted with a recloseable door, were tagged at 8 locations: at the front and back of the top, middle, and bottom shelves, and under the bottom rack (Figure 1).

Figure 1 Side view diagram of a vertical self-service display case showing sensor locations.

Display case temperature and relative humidity (RH) were measured using FlashLink BLE (Bluetooth Low Energy) reusable temperature or temperature and RH data loggers (models 40900 and 40901, respectively, DeltaTrak, Pleasanton, CA). All data loggers had an accuracy of ± 0.4°C from -10°C to 60°C and a resolution of 0.01 °C. The loggers with RH function had an accuracy of ± 6% RH max (between 20% to 80% RH - non-condensing from -10°C to 60°C), ± 4% RH typical (over recommended range), with a resolution of 0.03% RH. The sensors were installed under or on the side of the shelves using double-sided tape and were set to record temperature and humidity values at 2-minute intervals.

**Statistical Analysis**

Sensors were identified with the position, case, city, and restart code. Data were analyzed before and after retrofitting with doors, for temperature, RH, percent of time above 5 °C
(abuse due to high temperature), and percent of time below 0 °C (abuse due to low temperature) using SAS ver. 9.4 (SAS Institute, Cary, NC). Analysis of variance was also used to establish differences between open and closed display cases by position, and retailer with the case effect nested within retailer. Data were paired and compared for before and after retrofitting with doors using Tukey’s HSD and Bonferroni’s Correction for retailer and sensor location effects. Data for temperature, RH, and percent abuse due to high temperature (> 5 °C) met assumptions of ANOVA, and comparisons were made using the differences of least square means with corresponding standard errors. Data for percent abuse due to low temperature (< 0 °C) were log transformed in order to meet assumptions of ANOVA. and then back transformed median values were reported. In order to assess the range of temperature and RH values, the 1st, 5th, 95th, and 99th percentiles were computed rather than the 0 and 100th percentiles before and after doors were retrofitted. These values were more representative of an expected range because the outliers, especially the maximum values were artificially high due to situations (e.g. maintenance) not related to the normal operations of the cases.

**Results and Discussion**

Avoiding temperature abuse, both high (>5 °C) and low (<0 °C), while maintaining a high humidity level (above 90%) during retail display is critical to maintain food safety and food quality. The temperature for display cases retrofitted with doors decreased by 4.7 °C compared to open display cases (Table 1), resulting in a beneficial temperature below the FDA recommended upper storage limit of 5 °C. Abuse due to high temperature was also reduced by 34.7% when the display cases were fitted with recloseable doors. It is imperative to keep produce under constant low temperature as Listeria monocytogenes and Escherichia coli O157:H7 have caused many produce-related health problems and research has shown that these pathogens have the ability to grow under refrigerated conditions (Evans et al., 2007; Kou et al.,
2014; Nunes et al., 2009; Zeng et al., 2014). Walker et al. studied three strains of L. monocytogenes and found generation times as low as 13 hours at 5 °C and up to 131 hours at 0 °C (Walker, Archer, & Banks, 1990). Francis et al. also found that both L. monocytogenes and E. coli O157:H7 can increase up to 2.5 log CFU/g on shredded lettuce over a 12-day storage period, depending on the strain (Francis & O’Beirne, 2001). Zeng et al. observed up to 3 log CFU/g growth of E. coli O157:H7 and L. monocytogenes on romaine lettuce when temperature abuse was up to 16 °C, but no pathogen growth when the lettuce was kept below 4 °C (Zeng et al., 2014).

In contrast to the reduction in abuse at temperatures >5 °C, abuse due to low temperature increased 0.018% when display cases were retrofitted with doors (Table 1). Lower temperatures reduce microbial growth (Francis & O’Beirne, 2001; Zeng et al., 2014), however, may cause chilling or freezing damage to specific products (Badia-Melis et al., 2018). Temperatures below 0 °C may lead to freezing injury, breaking plant cells, and accelerated spoilage of leafy greens (Steele, 2004). The reported highest freezing points of Bibb, Boston, Iceberg and Romaine lettuce range from just –0.39 to –0.17°C (Whiteman, 1957). Physical freezing conditions with leafy-green packaged products were observed by researchers while collecting data.

RH increased by 16.2% to 95.7% after the display cases were retrofitted, compared to the detrimental 79.5% RH for cases without doors (Table 1). The observed RH is significantly higher in cases with doors, which could represent an improvement in quality and shelf-life of unpacked produce displayed in cases with doors.
Table 1 Mean values for temperature and performance parameters of display cases for the main effects of before and after retrofitting with doors.

<table>
<thead>
<tr>
<th>Doors</th>
<th>Temperature (°C)</th>
<th>Percentage of Time &gt; 5 °C (%)</th>
<th>Percentage of Time &lt; 0 °C (%)</th>
<th>Relative Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Door</td>
<td>7.3</td>
<td>41.0</td>
<td>0.001</td>
<td>79.5</td>
</tr>
<tr>
<td>With Door</td>
<td>2.6</td>
<td>6.3</td>
<td>0.019</td>
<td>95.7</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.2</td>
<td>1.6</td>
<td>N/A</td>
<td>0.8</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Retailers have different designs and technologies incorporated in their display cases and corresponding refrigeration systems, and maintenance can affect the performance of these systems (Mota-Babiloni et al., 2015). There were significant differences according to ANOVA with the Bonferroni correction observed between retailers in the performance of their display cases (Table 2). The display cases maintained by retailer 4 had a significantly higher temperature, % abuse due to high temperature, and lower relative humidity, before and after retrofitting with doors, compared to retailers 2 and 3. Retailer 4 failed to maintain TCS food products at temperatures < 5 °C more than 84% of the time before retrofitting, but reacted proactively immediately retrofitting and achieving a significant reduction to 14.2% when doors were installed. The temperature and RH characteristics of display cases maintained by retailers 2 and 3 were also significantly improved when the cases were retrofitted. This provides evidence that if a retailer has a display case that is showing abuse conditions, retrofitting may be a solution to temperature control.

The display cases of retailers 2, 3, and 4 decreased in temperature and percentage of high temperature abuse (> 5°C) when retrofitted with doors (Table 2). In contrast, the percentage of time product experienced abuse due to low temperature (<0 °C) increased for retailers 2 and 4.
but decreased for retailer 3 after retrofitting. RH increased in all cases when doors were installed.

Table 2 Mean values for performance parameters of display cases compared by retailer, before and after retrofitting with doors. There was no significant retailer by sensor position interaction.

<table>
<thead>
<tr>
<th>Retailer</th>
<th>Doors</th>
<th>Temperature(°C)</th>
<th>Percentage of Time &gt; 5 °C</th>
<th>Percentage of Time &lt; 0 °C</th>
<th>Relative Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retailer 2</td>
<td>No Door</td>
<td>3.8</td>
<td>17.7</td>
<td>0.000</td>
<td>94.2</td>
</tr>
<tr>
<td></td>
<td>Door</td>
<td>1.7</td>
<td>1.5</td>
<td>0.142</td>
<td>99.7</td>
</tr>
<tr>
<td></td>
<td>Std Error</td>
<td>0.4</td>
<td>2.9</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Retailer 3</td>
<td>No Door</td>
<td>3.7</td>
<td>21.2</td>
<td>0.008</td>
<td>93.5</td>
</tr>
<tr>
<td></td>
<td>Door</td>
<td>2.7</td>
<td>3.1</td>
<td>0.002</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td>Std Error</td>
<td>0.3</td>
<td>2.4</td>
<td></td>
<td>1.3</td>
</tr>
<tr>
<td>Retailer 4</td>
<td>No Door</td>
<td>14.5</td>
<td>84.1</td>
<td>0.000</td>
<td>50.7</td>
</tr>
<tr>
<td></td>
<td>Door</td>
<td>3.4</td>
<td>14.2</td>
<td>0.018</td>
<td>87.8</td>
</tr>
<tr>
<td></td>
<td>Std Error</td>
<td>0.4</td>
<td>2.9</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*Standard error for percent time < 0 °C is not reported because the statistical model was log transformed to meet ANOVA assumptions, and therefore this value was not calculated.

This study indicates that better temperature control can be obtained in refrigerated display cases retrofitted with doors. Other studies have also found that products displayed in open refrigerated display cases may be subjected to abusive temperatures and that closed display cases have more homogeneous temperature profiles (Atilio de Frias, Luo, Kou, Zhou, & Wang, 2015; de Frias et al., 2018; Lindberg et al., 2018; Mercier et al., 2017). Faramarzi et al. found that open refrigerated display cases are more vulnerable to infiltration of warm air from the environment (Faramarzi et al., 2002).
Table 3 Means values for performance parameters of display cases compared by sensor (datalogger) position, before and after retrofitting with doors. There was no significant retailer by sensor position interaction.

<table>
<thead>
<tr>
<th>Positions</th>
<th>Temperature (°C)</th>
<th>Percentage of Time &gt; 5 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Door</td>
<td>Door</td>
</tr>
<tr>
<td>Bottom Back – BB</td>
<td>6.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Bottom Front – BF</td>
<td>7.6</td>
<td>2.4</td>
</tr>
<tr>
<td>Middle Back – MB</td>
<td>6.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Middle Front – MF</td>
<td>7.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Top Back – TB</td>
<td>6.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Top Front – TF</td>
<td>8.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Under Back – UB</td>
<td>7.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Under Front – UF</td>
<td>8.6</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Standard Error: 0.3, 0.3, 3.1, 3.1
p-value: <0.0001, <0.0001

There were significant differences in temperature among sensor positions within the display cases, with significant overall decrease in temperature after the cases were retrofitted (Table 3). The average temperature reduction by position was 4.7°C, with the top front position experiencing less improvement, reducing only 2.4°C. High temperature abuse was reduced on average 34.7% after retrofitting, with the top front position having the lowest reduction of 9.6%. The top front position experienced the highest temperatures, highest percentage of time at temperatures above 5 °C, and showed little improvement when the cases were retrofitted with doors.
Table 4 Mean (SE) of the 1st, 5th, 95th, and 99th temperature (°C) percentiles for display cases compared by sensor (datalogger) position, before and after retrofitting with doors. The ‘difference’ between before and after display case doors were installed is also presented.

<table>
<thead>
<tr>
<th>Sensor location</th>
<th>Temperature (°C) Percentiles</th>
<th>1</th>
<th>99</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(SE)</td>
<td>(SE)</td>
</tr>
<tr>
<td>Top Front - TF</td>
<td></td>
<td>2.46</td>
<td>16.58</td>
</tr>
<tr>
<td>No Doors</td>
<td></td>
<td>(0.37)</td>
<td>(3.58)</td>
</tr>
<tr>
<td>Doors</td>
<td></td>
<td>2.63</td>
<td>9.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.99)</td>
<td>(1.95)</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>0.18</td>
<td>-7.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.13)</td>
<td>(3.35)</td>
</tr>
<tr>
<td>Top Back - TB</td>
<td></td>
<td>0.78</td>
<td>16.23</td>
</tr>
<tr>
<td>No Doors</td>
<td></td>
<td>(0.45)</td>
<td>(3.60)</td>
</tr>
<tr>
<td>Doors</td>
<td></td>
<td>0.06</td>
<td>5.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.32)</td>
<td>(0.24)</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>-0.72</td>
<td>-10.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.58)</td>
<td>(3.41)</td>
</tr>
<tr>
<td>Middle Front - MF</td>
<td></td>
<td>2.43</td>
<td>15.59</td>
</tr>
<tr>
<td>No Doors</td>
<td></td>
<td>(0.33)</td>
<td>(3.70)</td>
</tr>
<tr>
<td>Doors</td>
<td></td>
<td>1.54</td>
<td>5.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.36)</td>
<td>(0.25)</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>-0.89</td>
<td>-10.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.56)</td>
<td>(3.56)</td>
</tr>
<tr>
<td>Middle Back - MB</td>
<td></td>
<td>1.28</td>
<td>15.79</td>
</tr>
<tr>
<td>No Doors</td>
<td></td>
<td>(0.48)</td>
<td>(3.63)</td>
</tr>
<tr>
<td>Doors</td>
<td></td>
<td>0.58</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.36)</td>
<td>(0.24)</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>-0.69</td>
<td>-10.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.68)</td>
<td>(3.46)</td>
</tr>
<tr>
<td>Bottom Front - BF</td>
<td></td>
<td>2.23</td>
<td>15.20</td>
</tr>
<tr>
<td>No Doors</td>
<td></td>
<td>(0.4)</td>
<td>(3.57)</td>
</tr>
<tr>
<td>Doors</td>
<td></td>
<td>1.38</td>
<td>5.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.37)</td>
<td>(0.23)</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>-0.85</td>
<td>-10.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.99)</td>
<td>(3.42)</td>
</tr>
<tr>
<td>Bottom Back - BB</td>
<td></td>
<td>1.11</td>
<td>15.70</td>
</tr>
<tr>
<td>No Doors</td>
<td></td>
<td>(0.51)</td>
<td>(3.76)</td>
</tr>
<tr>
<td>Doors</td>
<td></td>
<td>0.49</td>
<td>5.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.38)</td>
<td>(0.20)</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>-0.61</td>
<td>-10.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.72)</td>
<td>(3.56)</td>
</tr>
<tr>
<td>Under Front - UF</td>
<td></td>
<td>3.32</td>
<td>16.62</td>
</tr>
<tr>
<td>No Doors</td>
<td></td>
<td>(0.39)</td>
<td>(3.29)</td>
</tr>
<tr>
<td>Doors</td>
<td></td>
<td>1.13</td>
<td>5.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.34)</td>
<td>(0.24)</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>-2.19</td>
<td>-10.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.57)</td>
<td>(3.09)</td>
</tr>
<tr>
<td>Under Back - UB</td>
<td></td>
<td>1.95</td>
<td>16.38</td>
</tr>
<tr>
<td>No Doors</td>
<td></td>
<td>(0.26)</td>
<td>(3.56)</td>
</tr>
<tr>
<td>Doors</td>
<td></td>
<td>0.79</td>
<td>5.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.41)</td>
<td>(0.25)</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>-1.16</td>
<td>-10.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.31)</td>
<td>(3.33)</td>
</tr>
</tbody>
</table>

Analyses of temperature, time abuse due to high and due to low temperatures found differences when comparing the average of all front positions (TF, MF, BF, UF) with those of the back positions (TB, MB, BB, UB) in the cases but were not significantly different (p-values > 0.05). However, studies by Laguerre et al., Evans et al., Zeng et al., de Frias et al., and Kou et al. found significant differences between the front and back locations of retail display cases (Atilio de Frias et al., 2015; Evans et al., 2007; Kou et al., 2014; Laguerre, Hoang, Osswald, & Flick, 2012; Zeng et al., 2014). There was no effect of position on the duration when temperature fell below 0 °C for open or closed display cases (p-value = 0.8997). The range of temperatures
showed a non-significant decrease of 0.87 °C and 1.14 °C over all the sensor positions for the 1st and 5th percentiles after retrofitting the doors (Table 4). However, temperature ranges decreased significantly 9.14 °C and 9.97 °C at the 95th and 99th percentiles after retrofitting. There was no significant difference among sensor positions for the difference between the 1st and 99th percentiles. However, there was a 9.10 (+2.02) °C difference between these percentiles, which was significantly different from zero, and was indicative of reduced variability after door installation. A similar pattern was observed for the 5th to 95th percentile differences of 8.0 (+1.92) °C. Since RH was measured at only three positions per display case (TF- top front, MF- middle front, and MB- middle back), an analysis by position was not performed for this factor.

The RH ranges increased significantly after door retrofitting 18.9% and 16.1% for the 1st and 5th percentiles, respectively, and a non-significant increase of 5.9% for both the 95th and 99th percentile (Table 5). The RH varied 22.4% between the 1st and 99th percentiles before retrofitting and 9.4% after retrofitting, a difference of 13% which was indicative of reduced variability in RH after retrofitting.

Table 4 Mean (SE) of the 1st, 5th, 95th, and 99th relative humidity (%) percentiles before and after doors were installed on the display cases. The means represent an overall average for the display cases in which they were measured as not all positions within the case were measured.

<table>
<thead>
<tr>
<th>Relative Humidity (%) Percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doors</td>
</tr>
<tr>
<td>No Doors</td>
</tr>
<tr>
<td>Doors</td>
</tr>
<tr>
<td>Difference</td>
</tr>
</tbody>
</table>

Differences among shelves were significant for temperature and abuse due to high temperature (p-values < 0.0001) but not significant due to low temperature (p-value = 0.9980). In open display cases, under the bottom rack has higher temperature and abuse due to high
temperature (>5 °C), but once the cases were retrofitted with recloseable doors, the top shelf has higher temperature and abuse due to high temperature (Table 6).

Table 5 Values for performance parameters of display cases compared by grouping sensors according to shelf (Top=TB, TF, Middle=MB, MF, Bottom=BB, BF, and Under=UB, UF), before and after retrofitting with doors.

<table>
<thead>
<tr>
<th>Shelf</th>
<th>No Door</th>
<th>Door</th>
<th>No Door</th>
<th>Door</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom</td>
<td>7.0</td>
<td>2.1</td>
<td>37.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Middle</td>
<td>7.1</td>
<td>2.2</td>
<td>37.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Top</td>
<td>7.3</td>
<td>3.7</td>
<td>40.0</td>
<td>19.0</td>
</tr>
<tr>
<td>Under</td>
<td>7.9</td>
<td>2.3</td>
<td>49.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard Error</th>
<th>0.3</th>
<th>0.3</th>
<th>2.4</th>
<th>2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Evans et al. (2007) found that there are significant technological differences between display case designs that impact performance and therefore temperature control (Evans et al., 2007). Technological modifications from display case manufacturers in order to comply with the Department of Energy and EPA refrigerant regulations (DOE, 2017) caused changes in refrigeration systems associated to the de-listing of refrigerants (Shebik, 2015). Retailer specific technological modifications to improve control and reduce energy costs, may have affected overall case performance, but this would have to be evaluated in future research.

Conclusions

Retrofitting open refrigerated display cases with doors improved case ambient conditions, reducing case temperatures, increasing case RH, and reducing temperature and RH variability within the cases. However, the benefits of retrofitting display cases with doors varied among the retailers. All retailers experienced a decrease in case temperature, less time that product experienced temperatures >5 °C, higher case RH, and less fluctuation in temperature and RH when cases were retrofitted with recloseable doors. Food safety evaluations of display cases such
as this study provide useful and needed information to the retail business and scientific community to better understand the current status of the cold chain and the impact of regulatory driven technological changes. Additional data on overall energy consumption and food waste generated in open vs closed display cases at retail stores will provide additional useful information to continue to improve case performance.

Conflicts of Interest

All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

Acknowledgements

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References


CHAPTER 4. REINFORCING FOOD SAFETY BASED ON OBSERVED RETAIL EMPLOYEES’ BEHAVIOR

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Modified from a manuscript to be submitted to Iowa State Extension Store

Abstract

Fresh produce sold in retail stores can be contaminated with pathogenic microorganisms from previous steps in the food production system or they can also be contaminated at the retail level by improper handling. Fruits and vegetables are ready-to-eat foods that can be eaten raw and have the potential to cause foodborne illness if previously contaminated. Produce stockers are trained in safe food handling practices, acquiring valuable knowledge on proper produce handling to avoid food safety risks. Unfortunately, food safety knowledge doesn’t always translate into safe food handling behavior. A strong positive food safety culture has been studied and reported to have a strong relation with safe food handling behavior. Produce stockers in retail stores in four (4) states were casually observed for food safety practices that could lead to increased food safety risks. Observations were reported and classified into nine (9) topics: employee health and hygiene, handwashing, cross-contamination, stocking and rotation, handling of fallen produce, refrigeration and temperature control, display case cleaning, maintenance, and customer behavior. These topics were addressed in training materials developed to reinforce knowledge gaps in the training, and include two (2) posters, three (3) flipcharts, one (1) extension publication, and one (1) video. The implementation of the training
reinforcement program was not developed but has been stated. Periodic monthly observations and knowledge evaluations are suggested. The information collected will be shared within the company, and food safety discussions between employees and management will provide an open communication space. The aim of the reinforcement food safety training program is to build a strong food safety culture that will bring improved food safety behavior and handling practices.

**Introduction**

Consumption of fruits and vegetables has been associated with the prevention of heart disease, cancer, diabetes, and obesity, among other chronic diseases (Callejón et al., 2015; Centers for Disease Control and Prevention, 2019a). Fruits and vegetables sold in retail stores can be contaminated with bacteria from the environment (Beuchat, 1996). Produce can be contaminated at any point from the field to the consumer, including improper food handling (Mir et al., 2018; Rowell, Binkley, Alvarado, Thompson, & Burris, 2013).

Acquiring and applying safe food handling procedures by employees requires food safety training, and this training should be reinforced so that it translates into safe food handling practices (Reynolds & Dolasinski, 2019). Food safety knowledge and proper food handling training have been addressed in numerous studies (Abdelmassih et al., 2016; Arendt, Strohbehn, & Jun, 2015; Reynolds & Dolasinski, 2019). A study by Arendt, Strohben, and Jun on motivators and barriers to safe food handling revealed eight motivators and six barriers to safe food handling. Employees reported being motivated to have safe food handling behavior in order to avoid bacterial growth and cross-contamination; avoid harming customers; having the proper knowledge and training; acknowledging that it is required by law, regulations, and procedures; having good practices and/or habits; being motivated by internal rewards; responding to the culture of the workplace; and the drive to satisfy customers (Arendt et al., 2015). The main barriers to safe food handling behavior are forgetfulness and/or lack of habit, being too busy,
lack of knowledge, negative consequences of following safe food handling practices, availability and use of resources, and the standards or culture of the workplace (Arendt et al., 2015). Regular employee training can provide food safety knowledge and improve proper food handling skills. Literature has shown that acquiring knowledge doesn’t always translate into improved correct food handling practices, even when the skills and concepts are clear, showing that there could be other factors that affect handling behavior (Arendt et al., 2015).

According to the Food Marketing Foundation (FMI), retail food training has focused on gaining knowledge about the correct food handling recommended practices and avoiding foodborne illnesses, correct food handling practices when preparing, handling and storing food, food safety regulations that apply to their sector, and the impact of food handling on the community and the company. Trainings were theoretical and focused on the overview of food safety, transferring a lot of information to managers. Managers were left with the responsibility of passing this knowledge on to their employees, but with no real effective and practical way of doing so (Neal, 2014). Food safety training is recognized as a practical method to deliver knowledge, but training methods have not been standardized. Adult learning was called andragogy by Merriam (2001) and describes adult learning by establishing that unlike younger students, adults are independent and self-motivated individuals. They are capable of directing their own learning and have past life experience that can be used as a relatable resource in learning. Their learning needs are linked to their social roles, and they are interested in acquiring knowledge that is problem-centered and applicable. They are driven by internal factors to learn (Merriam, 2001). In addition, adult students want respect (Neal, 2014).

Produce stockers in 14 retail stores were observed while performing their routine activities in the produce sections in four (4) states: Florida, New York, Iowa, and Nebraska.
Small and large retail stores were observed, and the behavioral observations were recorded for food handling behavior that might result in food safety risks to consumers. These observations were used to develop reinforcement training materials. While training activities haven’t been implemented, they have been developed and are described later in this study.

**Materials and Methods**

Retail stores in four states in the United States including Florida, New York, Iowa, and Nebraska were visited to casually observe employees that stock produce and customer behavior that might pose or lead to food safety risks for humans. Observations were collected using the Food Code 2017 as the guideline of safe food handling behavior and violations to the legal requirements were recorded as observations. Observations were recorded in a total of 14 stores for a period of 12 months and used to establish training topics that should be reinforced to retail personnel. The observations were grouped together by requirements for safe handling of TCS foods, retail food employees’ and managers’ responsibilities, and general food safety recommended practices. Observations were further grouped into employee health and hygiene, handwashing, cross-contamination, stocking and rotation of produce, handling of fallen produce, refrigeration and temperature control, display case cleaning, maintenance, and customer behavior. Three (3) flipcharts, two (2) posters, one (1) extension publication, and one (1) video were developed for retail employee and / or management personnel retraining. Even though the training was not implemented, the suggested implementation process is described based on recent studies’ recommendations.

**Results and Discussion**

Observations of employees that stock produce and customer behavior that might pose or lead to food safety risks were recorded and divided into topics. Recommended or required safe food handling practices corresponding to the grouped observations were researched, and training
materials directly address the observed and closely related situations. Recommended proper behavior for employee health and hygiene, handwashing, cross-contamination, handling of fallen produce, and refrigeration and temperature control were taken from the FDA Food Code (2017) and the Employee Health and Hygiene Handbook (U.S. Department of Health and Human Services, 2017; U.S. Food and Drug Administration, 2012). Stocking and rotation of produce, display case cleaning, and maintenance were taken from industry standards and best practices (EcoChill, 2017; Foster, Hammond, Bown, Evans, & Maidment, 2018; Steel, 2004; Hillphoenix, n.d.; Lakicevic, Nastasijevic, & Raseta, 2015; Lianou & Sofos, 2007; Mercier, Villeneuve, Mondor, & Uysal, 2017; U.S. Department of Health and Human Services, 2017; US Fresh Fruit Basic Training, n.d.). Customer behavior is out of the retail store’s control and these observations were used to reinforce concepts of handwashing, health and hygiene, and handling of fallen produce. These topics were then combined or covered separately in four (4) flipcharts, two (2) posters, one (1) extension publication, and one (1) training video. Customer behavior is a topic to be explored in future research. These training materials address common situations that employees can relate to and the formats vary so that the learning session is short, uses basic language, and can be used in different settings.

Posters

**Food Safety in the Supermarket**

This poster is a quick reminder on food safety for employees and correct behavior when working with display cases. The topics covered include cleaning display cases frequently to remove filth and harmful bacteria, and frequent cleaning of shopping carts to avoid cross-contamination of the store (Hillphoenix, n.d.; Lakicevic et al., 2015; Lianou & Sofos, 2007). It also recommends keeping refrigeration temperature inside the display cases at or below 41 °F or 5 °C (U.S. Department of Health and Human Services, 2017). When stocking the cases with
food, employees should place the boxes or containers on carts, crates or other elevated surfaces to avoid contact with the floor and therefore avoid cross-contamination (U.S. Department of Health and Human Services, 2017). Fallen produce should not be placed back on display as this food may be contaminated with harmful bacteria (U.S. Department of Health and Human Services, 2017). Finally, important employee health and hygiene practices such as not working when sick and frequent handwashing, especially after using the bathroom are also included in the recommended practices to maintaining food safety in the supermarket (U.S. Department of Health and Human Services, 2017; U.S. Food and Drug Administration, 2012). The poster has been included in Annex B of this document.

**Display Cases**

This poster addresses good practices and key points to keep in mind when working with display cases. Open display cases create a barrier between the warm ambient air and the cool air circulating inside the display case by blowing an air curtain from the top of the case, through the honeycomb, and taking the air in the bottom through the return air grill. Air flow should not be interrupted as this will increase temperatures inside the case and therefore the products (Atilio de Frias, Luo, Kou, Zhou, & Wang, 2015; Kou, Luo, Ingram, Yan, & Jurick, 2014). Products kept in display cases should remain below 41 °F or 5 °C(U.S. Department of Health and Human Services, 2017). Product stocked should already be at this temperature and overstocking should be avoided as this can affect product temperature. General good practices include: cleaning the honeycomb, not covering the return grill or vent, cleaning frequently, and maintaining the air curtain flow (Foster et al., 2018; Hillphoenix, n.d.). Display cases should be cleaned regularly to remove filth and microorganisms (EcoChill, 2017; Hillphoenix, n.d.; Lakicevic et al., 2015; Lianou & Sofos, 2007). Stocking of produce usually follows the first in first out (FIFO) method,
unless otherwise stated by management on specific occasions, and removing bruised and damaged products must be done regularly to avoid spoilage and maintain attractive displays (Mercier et al., 2017; U.S. Department of Health and Human Services, 2017; US Fresh Fruit Basic Training, n.d.). This training material has been included in Annex B of this document.

**Flipcharts**

Flipcharts have been developed for face-to-face training. Employees will be trained by the person in charge (PIC) or manager of the store. This type of training addresses visual aids while the person is reading the information. Despite the use of the flipchart, it is important for the employee to have enough time to ask questions during the training session. Hands-on activities have been included in some of the materials to encourage the application of learned concepts and/or behavior. Hands-on activities help employees have a clear understanding of what and when certain behavior is expected of them (Egan et al., 2007) and actively include managers into the food safety culture (Yiannas, 2009).

**Handwashing**

The handwashing flipchart focuses on sources of cross-contamination, integrating customer behavior that was observed along with activities to have the employee and the manager discuss sources of contamination and conditions that could be improved at the store. It has a step-by-step handwashing procedure with a hands-on activity based on the CDC’s handwashing procedure (Centers for Disease Control and Prevention, 2019b). Another topic covered in this material is the “no bare hands” contact with ready-to-eat (RTE) food (U.S. Department of Health and Human Services, 2017; U.S. Food and Drug Administration, 2012). This training material has been included in Annex B of this document.
Stocking and Rotation

This flipchart is based on industry best practices and the observations of the study. The topic covered is organizing produce displays in a clean, orderly, and attractive manner. Bruised or damaged fruits and vegetables should be removed, as they can contaminate other produce. Stocking produce displays, both refrigerated and not refrigerated should be done following the First-In-First-Out (FIFO) principle so that produce on display remains fresh (US Fresh Fruit Basic Training, n.d.).

Employee Health and Hygiene for Employees

This publication is based on the FDA’s Employee Health and Hygiene Handbook. In this training, the concept of an outbreak is defined as two or more confirmed cases of similar illness that result from having a common food. The main symptoms that employees should always be aware of and should report immediately to their PIC or manager, because they can be linked to foodborne illness, include vomiting, diarrhea, jaundice (yellow skin or eyes), and sore throat with fever. Cuts or wounds on hands or wrists should also be reported to the PIC or manager. The wounds should also be covered with an impermeable bandage and the employee should wear a single use glove over it at all times if they go back to work. This training material goes through the responsibilities of employees on reporting when having foodborne illness symptoms (U.S. Food and Drug Administration, 2012). It has been included in Annex B of this document.

Extension Publication on Employee Health and Hygiene for Managers

This flipchart is very similar to the Employee Health and Hygiene for Employees flipchart. It is also based on the FDA’s Employee Health and Hygiene Handbook. In this training, the concepts of outbreak, and symptoms associated with foodborne illness are also explained. Since this flipchart is aimed at managers’ responsibilities, decision trees for the symptoms and exposures reported by employees are presented so that the manager can easily act
(U.S. Food and Drug Administration, 2012). This training material has been included in Annex B of this document.

**Display Case Cleaning - Video**

The format of the video is a PowerPoint slide deck that can run by itself like a video on regular computers, tablets, or smartphones. This slide deck can also be printed and made available in cases where the technology is not available or in cases where hard copies are preferred. The purpose is to illustrate retail employees and managers on the general procedure and best practices to clean display cases. This training material is based on technical recommendations from manufacturers, studies of pathogens harbored within refrigerated cases, and the observations obtained (EcoChill, 2017; Hillphoenix, n.d.; Lakicevic et al., 2015; Lianou & Sofos, 2007). Given that different display case manufacturers may use different materials and technology, in case any step or suggestion in this material has a conflict with the manufacturer’s recommendations, employees should follow the manufacturer’s recommendations to avoid damage to the equipment. The main benefits that can be obtained from cleaning regularly the display cases are the reduction in energy and maintenance costs, and the decreased risk of food spoilage and contamination (EcoChill, 2017; Hillphoenix, n.d.; Lakicevic et al., 2015; Lianou & Sofos, 2007). Clean display cases will give a positive impression about the establishment and possibly attract more customers. Some recommended good practices when cleaning is to keep a schedule and record of cleaning activities and clean all display cases on the same day to avoid cross-contamination (Hillphoenix, n.d.). Employees should consult the company’s policies to determine if any of the suggested procedures should be performed in a different manner. The training topics include personal protective elements required for the activity and safety precautions when performing the task. The activities needed to prepare the display case and surroundings for the cleaning procedure and some aspects to check as a precaution are also
described. Disassembling and removing parts that should be cleaned separately and transporting them to the cleaning area for this purpose should be done carefully and using a cart to avoid contact with non-food contact surfaces.

Cleaning the sink where the trays and other removable parts will be washed prior to washing the parts is critical to avoid cross-contamination. This cleaning procedure should include cleaning the transportation cart as it has come into contact with the dirty trays and parts. If the cart will not be washed, a different, clean cart should be used to transport the trays and removable parts back to the display case when reinstallation is required.

The training material describes the steps in cleaning all parts of the display case being careful to use low pressure water to prevent damage to the electrical components, tubing, and avoid the formation and spread of aerosols that could contain microorganisms, contaminating the surrounding area and recontaminating the display case. After enough time has been allowed for the case to dry off, reconnect the electrical components, reinstall removed parts, and remove any safety lock-out. Make sure to follow the company’s safety protocols for personnel safety. Once all verifications have been done, restart the case. Check that the display case is working properly. Once the temperature is below 41 °F or 5 °C, restock the case with the products. The detailed procedure is described in the training material, included in Annex B of this document.

**Proposed Implementation**

Food handlers in the US have very diverse ethnicities, educational level, and have different cultural backgrounds, increasing training challenges (Howton et al., 2016; Reynolds & Dolasinski, 2019). Age and generational differences can also affect the motivational drivers to safe food handling behavior (Ellis, Arendt, Strohbehn, Meyer, & Paez, 2010). Howton et al. studied the effectiveness of food safety programs and they observed that basic language (middle
school reading level) and easy to follow instructions were the preferred training methods (Howton et al., 2016). Adult trainees prefer shorter sessions and hand-on activities, with which they can relate (Merriam, 2001).

The demographics of retail food employees is that of low salary and low education, usually having a general equivalency diploma (GED) or a high school diploma (Data USA, 2019; Salary.com, 2019). In addition, retail employees have a high voluntary turnover in grocery stores (Lewis, 2019) and diverse cultural backgrounds (Howton et al., 2016; Reynolds & Dolasinski, 2019).

Food safety culture has been identified as a condition that once established in an organization, it will permeate into daily activities and be learned by newer employees (Arendt et al., 2015; Neal, 2014; Yiannas, 2009). A food safety culture starts with management commitment and participation (Neal, 2014). Employees want to see that principles are consistent in the organization and management is involved (Neal, 2014; Yiannas, 2009). Egan et al. report that in some cases, management is not properly trained to supervise and therefore lack the knowledge to assess risks (Egan et al., 2007). Research shows that work environments and safety culture are determinant in employee’s food handling practices (Abidin, Arendt, & Strohbehn, 2013). A company’s profitability depends on workers performance. A marked increase in worker and business performance can be observed when all employees in a company are aligned with the company’s business goals and objectives. Employees should know what is expected of them and how they align with the companies goals (SAP, 2019).

These training materials were developed based on the observations collected from various retail stores. The topics developed are only a reinforcement to the actual employee training. The varied formats to communicate food safety topics attempts to catch the attention of
employees. Basic language, relatable content, and short texts were used to make the training materials suitable for the diverse employee population. In order to quantify the effectiveness of the intervention, initial observations of the actual employee behavior would have to be done. Some studies on training effectiveness have focused on self-reported behavior but with this method, safe handling behavior is usually over-reported compared to observations of the activities (Redmond & Griffith, 2003).

Posters have the drawing (cartoon) that in itself tells the story on common situations occurring in the retail environment. The text refers to very specific situations that employees can relate to when handling produce. The posters cover situations and best practices that stocking employees routinely encounter and easily identify with. Adults like to acquire knowledge that is applicable to situations they can relate to (Merriam, 2001). Poster rotation would also be a good strategy that has been used in successful interventions (Abdelmassih et al., 2016) and this could increase its visibility among employees. Flipcharts also cover easily relatable topics. For these and other topics of training, integrating the trainings at the beginning of meetings would be highly recommended, as a Food Safety Toolbox Talk, which is a 2-5 minute refreshing food safety concepts session. This activity can also include the voluntary reflection from an employee or any pertinent discussion on food safety to keep the team motivated. This will maintain the idea of having proper food safety behavior present and gives food safety the leading importance that it should have in any food environment. This is a common strategy implemented to build personnel safety culture in high risk companies with excellent results (Jin & Chen, 2013). The training video should be provided to all personnel involved in the actual cleaning display case task. A small printout in booklet form can be provided in plastic covered (impermeable) format to follow along as the task is being done, in case any question may arise and to make sure critical
steps are not forgotten. Smaller versions of the flipcharts and a booklet style version of the video can be made available in breakrooms or sitting areas for employees. This can allow for curious employees to voluntarily go over the material on their own. Training information can also be uploaded to Apps or shared folders, easily accessible by employees for their voluntary reference. The use of various formats in food safety training has been shown to be successful (Geith, Vignare, Thiagarajan, & Bourquin, 2010; Howton et al., 2016).

These training materials aim to correct deviant food safety behavior in the retail establishment. Studies have shown that a food safety culture improves safe food handling behavior and permeates into the new employees (Yiannas, 2009). This implementation strategy aims at reinforcing proper food safety behavior and culture. To evaluate the effectiveness of the intervention, casual observations will be done and classified, similar to this study by a designated observer. Observations will be classified in the same categories or additional ones, if considered pertinent, and they can be positive or corrective. This will allow for future modification or reinforcement of the intervention. This is not a full training program, but knowledge assessments every 2 months can help measure the overall knowledge and gage the required frequency between food safety trainings and / or refreshers, as well as any improvements. To evaluate the effectiveness in correcting deviant behavior, this food safety reinforcement program should be implemented as a continuous observation program with periodic behavioral assessment. These measurements of knowledge and behavior will be graphed over time and discussed with employees, showing that the company and management are committed to the improvement of food safety. Sharing information within the company will reinforce a food safety culture (Powell, Jacob, & Chapman, 2011). The information collected will provide useful data on trends and opportunities. Discussion with employees can drive new interventions (Powell et al., 2011). If
the company has a Key Performance Indicators (KPI) system, these can be included for management follow-up. Periodic measurement of indicators can provide useful information for a continuous improvement program (Brown, 2009).

Conclusions

Food safety training can provide valuable knowledge for employees to perform their food handling jobs properly but this doesn’t necessarily mean that safe food handling practices will be applied to all situations. Employee observations can help show food safety practices that need to be corrected or conditions that need to be modified in the real work scenario. Reinforcement training materials developed from behavior observations can be a useful way to refresh concepts and translate the theoretical knowledge acquired through training to the real task in the work environment. Retail establishments need to improve their food safety culture and produce stockers should have proper safe food handling behavior. Continuous monitoring of employee knowledge can increase food safety behavior. Continuous monitoring of employee behavior and participation of managers in safety discussions can improve communication, enhance food safety practices, and increase food safety culture. The combination of food safety knowledge and behavior monitoring with corrective actions can provide a continuous improvement system that can reduce food safety risks.

References


CHAPTER 5. PFOA AND PFOS LEVELS IN MICROWAVE PAPER PACKAGING BETWEEN 2005 AND 2018

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Abstract

Per- and polyfluoroalkyl substances are synthetic environmental pollutants previously used for packaging applications as a grease, oil, and water-resistant coating. Exposure reported in previous studies highlighting potential concerns with public health. This study evaluated performance of coated paper packaging used for microwave popcorn, snacks, and sandwich bags for presence of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Current paper packaging materials: seven popcorn bags and three snack and sandwich bags were analyzed for PFOA and PFOS and compared to concentrations in microwave popcorn bags between 2005 and 2018. Only two microwave popcorn bags had average PFOA content above the limit of quantitation of 5.11 ng g⁻¹ paper. All other sample types had PFOA and PFOS values below the limit of detection (LOD) of 1.53 and 0.63 ng g⁻¹ paper, respectively. Results of this study follow trends from 2005 to 2018 suggesting a reduction in PFOS and PFOA concentrations in microwave packaging.
**Introduction**

Per- and polyfluoroalkyl substances (PFASs) are organic substances with a hydrocarbon backbone where fluorine has substituted all the hydrogens. These compounds are very stable and resistant to biological, chemical, and thermal degradation. PFASs are synthetic persistent organic pollutants (POPs) that bioaccumulate and have been detected in human blood serum (US median is equal to 4 ng/mL) (Steenland et al. 2010). Due to their stability, toxicity, bioaccumulation, and long half-lives in mammals, they have been classified as POPs (Lindstrom et al. 2011; Stahl et al. 2011; Surma et al. 2015; Schaidler et al. 2017). PFASs were first produced in the 1940s and 1950s to increase grease, oil, and stain resistance on surfaces. They were added to surfactants, cookware coatings, firefighting foams, and food contact paper packaging products, among other applications (EFSA 2008; Sun et al. 2017). Studies have shown they are widely spread throughout the environment and in certain concentrations are detrimental to human health (EFSA 2008; Lindstrom et al. 2011; Stahl et al. 2011; Martinez-Moral and Tena 2012; OECD/UNEP Global PFC Group 2013). PFAS compounds are all synthetic chemicals and include thousands of chemicals but some of the most common include perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) (Giesy and Kannan 2002). Some PFAS compounds degrade to form PFOA and PFOS resulting in many in depth research studies with these two compounds to better understand the spread in the environment and human exposure. Global research studies have attempted to understand potential human intake and health consequences, as well as their global abundance (Giesy and Kannan 2002; Lindstrom et al. 2011; Stahl et al. 2011). PFOA and PFOS have been detected throughout the environment: water, soil, plants, and animals. PFASs bioaccumulate and have been shown to pass on through the food chain. Higher concentrations of PFASs have been found in rural and industrialized areas, but they have been detected in remote areas such as the arctic, far away from any source (Giesy and Kannan 2002; Lindstrom et al.
2011; Newton et al. 2017; Sun et al. 2017). Significant differences in bioaccumulation and excretion have been reported between PFOA and PFOS for different mammalian species, including humans. Studies in primates and rodents have shown that PFOS and PFOA increase cancer risk, reduce childbirth weight and reduce gestational age, affect hormonal activity, metabolism, among many other health impacts that continue to be studied (Steenland et al. 2010; Geueke 2016). In humans, gender differences in PFOS and PFOA serum concentrations have been reported for Japan and the USA, being higher for males than females (Stahl et al. 2011). In addition, epidemiological studies in PFOS and PFOA on exposed workers are contradicting, but more recent studies show the relation between serum levels and liver, pancreatic, testicular, and breast cancer, tumor-promoting activities, immunosuppression, estrogenic and nonestrogenic hormonal disruptions, among other adverse effects to human health (Alexander et al. 2003; Lau et al. 2007; Steenland et al. 2010; Lindstrom et al. 2011; Barry et al. 2013; Vieira et al. 2013; Timmermann et al. 2017; Pierozan et al. 2018). Studies have shown increased triglycerides and other hormonal health effects, identifying PFOA as a thyroid hormone disruptor (Nelson et al. 2010; Lindstrom et al. 2011). The Scientific Panel on Contaminants in the Food Chain (CONTAM) has established that the Tolerable Daily Intake (TDI) for PFOS is 150 ng kg−1 body weight day−1 and 1500 ng kg−1 body weight day−1 for PFOA (EFSA 2008; Geueke 2016). The major pathways of PFOA and PFOS intake by humans have been identified as dietary intake from water, animals, plants, migration into foodstuff from packaging, and other environmental sources such as indoor dust (Trudel et al. 2008). Water has been identified as a major source of contamination of all food stuff (Trudel et al. 2008; Newton et al. 2017). The distribution and actual intake mechanisms are still ambiguous and continue to be studied (Picó et al. 2011; Domingo 2012; OECD/UNEP Global PFC Group 2013). Research has demonstrated the toxicity
of PFCs and regulatory bodies in some countries have tried to legislate against their production, but other countries have picked up production to fill in the need. After the year 2000, PFAS production in Japan, Western Europe, and the USA decreased, but China, India, Poland, and Russia have increased their production levels (Geueke 2016). PFOA and PFOS are listed in the Stockholm Convention as POPs. This is one of the most important international efforts to stop production and use of these compounds (Newton et al. 2017). The Environmental Protection Agency (EPA) collaborated with 3M and DuPont to voluntarily discontinue production of PFOS and related compounds. The EPA has continued to develop new legislation to eliminate long chain PFASs from emissions and products (Lindstrom et al. 2011). Manufacturers have started to replace the long chain PFAS with shorter chain PFAS or non-fluorinated compounds, but there is not enough information on the toxicity of these shorter chain PFASs (OECD/UNEP Global PFC Group 2013; Geueke 2016). Historically, PFCs were used in paper to provide water, oil, and grease resistance, as well as protection from external contaminants to the food. When food comes in contact with the package, these chemicals can migrate into food, becoming a food safety issue. (Begley et al. 2008; Zafeiraki et al. 2014).

Paper packaging has been analyzed for PFASs and the recovery rates have been reported. Some methods include liquid extraction with solvents, followed by liquid chromatography. Studies report using various types of ultrasound and high pressure-assisted techniques of extraction, as well as low, high and ultra-high pressure liquid chromatography (LC, HPLC, and UHPLC) coupled with different detection methods for quantitation. Some of these studies have identified PFOA and/or PFOS in their analysis; others were not able to detect either compound due to poor method sensitivity. It is an analytical challenge to quantify these compounds in food contact paper packaging. The development of liquid chromatography-tandem mass spectrometry
(LC-MS/MS), triple quadrupole tandem mass spectrometry coupled to liquid chromatography (LC- (QqQ)MS/MS) and liquid chromatography quadrupole time-of-flight tandem mass spectrometry (LC-QTOF) methods has proven to be able to detect PFASs at low levels (Jogsten et al. 2009; Moreta and Tena 2013, 2014; Zafeiraki et al. 2014; Surma et al. 2015; Schaider et al. 2017). The objective of this research study was to quantify the amount of PFOA and PFOS in popcorn bags and paper snack bags currently used in the market following the analytical method proposed and validated by Moreta and Tena (2014). Snack and sandwich paper packaging has been used by customers as an alternative to traditional microwave packaging for popping corn. The method used in this study was a focused ultrasonic liquid extraction using ethanol, with sample reconstituted in methanol and quantified using UHPLC-QTOF.

**Materials and Methods**

**Sample Preparation**

Seven unique unused, unfilled, single-gusseted microwave-printed popcorn bags were obtained from multiple international suppliers. Three lunch sacks were obtained from three unique retail grocery chains (Ames, IA). The lunch sacks are not printed. Sections with the adhesive were removed before sampling to avoid possible chemical interactions during extraction and chromatographic analysis. The susceptor was ground with the paper as it is in direct food contact. Eight bags of each packaging material were pulverized into uniform particle powder using an IKA A11 Analytical Mill (Wilmington, NC) with a fiber cutting blade attached. The mill and its components were cleaned completely between samples of different material to avoid cross-contamination. Calibration standards were prepared at 1, 5, 10, 25, 50, 75, and 100 ng ml⁻¹ of native, non-mass labelled, PFOA (Perfluoro-n-octanoic acid: PFOA), and PFOS (Sodium perfluoro-1-octanesulfonate:L-PFOS) standards obtained from Wellington Laboratories (Guelph, Ontario, Canada). An initial standard solution of 100 ng ml⁻¹ was prepared then diluted
separately to make 75, 50, 25, and 10 ng ml\(^{-1}\) standard solutions. Standards for 5 and 1 ng ml\(^{-1}\) were prepared from separate dilutions from the 10 ng ml\(^{-1}\) obtained standard. A 20 ng ml\(^{-1}\) spike solution was prepared using a combination of the native PFOA and PFOS standards obtained from Wellington Laboratories. Isotopically labelled Perfluoro-n-\([^{13}\text{C}_8]\) octanoic acid (M8PFOA) and isotopically labelled sodium perfluoro-1-\([^{13}\text{C}_8]\) octanesulfonate (M8PFOS) internal standard solutions, obtained from Wellington Laboratories (Guelph, Ontario, Canada), were prepared at 300 ng ml\(^{-1}\). Calibration standards, spike solution, and internal standard solutions were prepared in HPLC-grade methanol (Fisher Scientific; Hampton, NH) using glass volumetric flasks (20 ml, 100 ml, and 200 ml Pyrex® with glass stoppers, respectively), pipettes (Pyrex™, disposable, 10ml in 1/10), and micropipettes (Gilson pipetman, 20, 1000, and 5000 µl). Samples were stored protected from light via aluminum foil in the freezer of a conventional refrigerator (Frigidaire, FFTR1814TWO) at −16°C. Random samples from three different popcorn bags and one snack and sandwich bag were selected for spike recovery quantification for method validation. Pulverized paper samples were suspended in ethyl acetate (Fisher Scientific; Hampton, NH) and then spiked with a 20 ng ml\(^{-1}\) solution in a 1-L glass beaker (Pyrex®, 1000 ml) to give 20 ng spike per 1.5 g paper. This spike concentration was chosen because it was above the limit of quantification (LOQ) and close to the expected values in samples. The suspended and spiked samples were mixed thoroughly, then evaporated to dryness using a water bath set at 45°C, and ground again to ensure homogeneity. Spiked and nonspiked samples were stored in polyethylene bags wrapped in aluminum foil and refrigerated (Frigidaire, FFTR1814TWO) at 4°C.

**Focused Ultrasonic Liquid Extraction**

Extraction of PFOS and PFOA was performed using focused ultrasonic liquid extraction (FUSLE) procedure using a Misonix S-4000 Ultrasonic Sonicator (Farmingdale, 150 NY), with a
power of 600 W and an operating frequency of 20 kHz, equipped with a 3-mm titanium tip. Each packaging material was analyzed in three sampling repetitions. A known amount of processed paper (~1.5 g of homogenized sample) was placed into a 50-mL (34 mm x 100 mm) glass centrifuge tube and 24 mL of ethanol was added to each sample. The weight of sample used in each extraction was recorded and used to normalize the concentration of PFAS obtained per gram of paper. Before each extraction, 100 µL of 300 ng ml\(^{-1}\) mass labelled M8PFOA and M8PFOS internal standard solution was added. The probe was inserted in the mixture to a depth of 2 cm from the bottom of the test tube. Each individual tube was then secured in an ice bath and subsequently sonicated. Samples were exposed to 30% amplitude at 50% pulsed cycle for 10 s. Extracts were filtered through a 60 mL Pyrex® Buchner funnel with fritted disc and porosity 10–15 µm using a vacuum pump at 550 in Hg vacuum. The probe, glassware, and extracted samples were washed twice with 2.5 mL of ethanol each rinse. The total amount of filtered extract with rinses was transferred to a 55 mL Pyrex® culture tube without cap and immediately dried to completion in a nitrogen evaporator with water bath at 45°C. The dry residue was reconstituted with 1-ml HPLC-grade methanol and filtered into a 2-mL LC vial using a disposable polypropylene medical sterile syringe equipped with a 0.22-µm nylon filter.

**LC-MS Conditions**

Negative ion mode mass spectra were obtained using the Agilent (Santa Clara, CA) QTOF 6540 mass spectrometer equipped with the JetStream ESI ion source. The LC system consisted of the Agilent 1200 series binary pump and autosampler system. Sample mixtures were separated using an Agilent XDB C18, 4.6 × 150 mm, 1.8 um column at a flow rate of 700 µL min\(^{-1}\) at 30°C. Then, 1 µL of sample was injected. The mobile phases used were 0.1% Formic acid aqueous solution for Solvent A and 0.1% Formic acid acetonitrile solution for Solvent B. The phase composition was varied linearly from 28% to 50% Solvent B in 1.5 min, then
increased to 52% Solvent B in 1.2 min. Increased again to 72% Solvent B in 0.5 min and maintained at 72% Solvent B for 1.5 min more. Then increased again to 100% solvent B in 0.1 min and then maintained at 100% Solvent B for 10.2 min. The chromatographic separations took place in 15 min, with retention times between 8 and 12 min. After each run, 100% Solvent B for 3 min was used to clean the column prior to the next sample. The mass spectrometer was scanned from m/z 100 to 1000 and operated in the 4 GHz HRes mode. Accurate mass measurement was achieved by constantly infusing a calibrant (ions at m/z 121.0508 and 922.0098). Extracted Ion Chromatogram (EIC) peaks were displayed for native PFOA (m/z: 412.97), M8PFOA (m/z: 420.99), native PFOS (m/z: 498.92), and for M8PFOS (m/z: 506.957) standards. M8PFOA (m/z: 420.99) and M8PFOS (m/z: 506.957) were used as internal standards. Native PFOA (m/z: 412.97) and native PFOS (m/z: 498.92) peaks were observed and integrated at the same retention times of the corresponding mass labelled standards. Ratios of PFOA/M8PFOA and PFOS/M8PFOS versus concentration were plotted for accurate quantitation.

Calibration curves were run every 9 to 16 samples to check for column degradation. Blanks were run before and after calibration curves and in between samples of the same packaging material. Each paper packaging material was sampled three times, drawing paper sample from the combined ground matrix of eight bags. From each of these samples, repeated measurements from the same vial were run through the LC three nonconsecutive times. The three repeated measurements were averaged to obtain the ratio for each sample. The ratios were read from the developed calibration curve to obtain the concentration for the three samples for each paper packaging. These readings were normalized by the sample weights and then averaged to provide the concentration of PFAS in each paper packaging material.
Results

PFOA and PFOS were identified in the obtained chromatograms. Native and mass labelled PFASs were identified with the peak retention time for the mass labelled PFOA or PFOS, accordingly. Three non-consecutive injections for every sample were run to account for instrument variability. The ratio between peak areas associated with PFOA and M8PFOA internal standard peak areas for each injection was used as the response reading value. The same procedure was applied for PFOS and M8PFOS readings. Calibration curves were constructed plotting the ratio against the known concentrations.

LOD and LOQ

Calibration curves do not show a pattern overtime for PFOA and PFOS; therefore, no column degradation was evident, and the data were accepted. A weighted linear regression model (Ramsey and Schafer 2002) was fitted to the data from calibration curves using SAS statistical software (Statistical Analysis Systems Inc., Cary, NC, USA), as unequal variance was observed from the residuals plot. Significant values for slope and intercept were obtained. The estimated intercept and slope, with standard errors in parentheses, are 0.0267 (0.0013) and 0.03062 (0.00025) for PFOA and 0.0055 (0.00035) and 0.02737 (0.00016) for PFOS. The data obtained from the calibration curves (Figures 1 and 2) have unequal variance with respect to concentration so the Hubaux-Vos (Hubaux and Vos 1970) method for detection limits was applied to the data through an iterative process using SAS. The limit of detection (LOD) was found to be 1.53 ng PFOA g⁻¹ paper and 0.63 ng PFOS g⁻¹ paper. From the LOD, LOQ was calculated as 5.11 ng PFOA g⁻¹ paper and 2.11 ng PFOS g⁻¹ paper.
Figure 2 Calibration curve for PFOA

Figure 3 Calibration curve for PFOS
Measurement Results

With the linear regression model obtained from the calibration curves and the LOD, the concentrations for the samples were obtained as shown in Table 1. Results show two packaging samples have quantifiable amounts (Limit of quantitation, LOQ = 5.11 ng g⁻¹ paper) and one was detected but not quantified for PFOA (limit of detection, LOD = 1.53 ng g⁻¹ paper). The concentration of PFOS in all samples measured were below the limit of detection (LOD = 0.63 ng g⁻¹ paper, LOQ = 2.11 ng g⁻¹ paper). For PFOA measurements with a mean greater than 3 ng ml⁻¹, measurement uncertainty as pooled sd was 3.8 ng ml⁻¹, whereas for mean values less than 3 ng ml⁻¹ it was 0.63 ng ml⁻¹. For PFOS measurements with a mean value greater than 0.6 ng ml⁻¹ measurement uncertainty as pooled sd was 0.71 ng ml⁻¹ and for mean values less than 0.6 ng ml⁻¹ it was 0.06 ng ml⁻¹. Spike recoveries were obtained from three randomly selected different popcorn bags and one snack and sandwich paper bag. Spike recoveries for PFOA were between 71.8 and 96.4% and for PFOS were between 76.5 and 86.2%. The lowest spike recoveries were obtained in snack and sandwich bags for both PFOA and PFOS, as shown in Table 2. Previous studies of PFOA and PFOS on paper packaging report concentrations normalized to the surface area (Surma et al. 2015; Timmermann et al. 2017). In order to make these data comparable other data reported in the literature, concentrations were calculated per surface area in Table 3.
Table 6 PFOA and PFOS concentrations on samples.

<table>
<thead>
<tr>
<th>PACKAGING MATERIAL</th>
<th>SAMPLE</th>
<th>PFOA (ng g⁻¹)</th>
<th>PFOS (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popcorn 1</td>
<td>Sample 1</td>
<td>28.6 ± 0.3</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>27.0 ± 3.2</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>30.3 ± 7.2</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 2</td>
<td>Sample 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 3</td>
<td>Sample 1</td>
<td>19.8 ± 2.3</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>19.6 ± 2.1</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>15.3 ± 3.4</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 4</td>
<td>Sample 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 5</td>
<td>Sample 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 6</td>
<td>Sample 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 7</td>
<td>Sample 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 1</td>
<td>Sample 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 2</td>
<td>Sample 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 3</td>
<td>Sample 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td></td>
<td>Sample 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
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</tbody>
</table>
Table 7 PFOA and PFOS concentrations in paper packaging materials analyzed and spike recoveries.

<table>
<thead>
<tr>
<th>PACKAGING MATERIAL</th>
<th>PFOA (ng g⁻¹)</th>
<th>PFOS (ng g⁻¹)</th>
<th>SPIKE RECOVERY</th>
<th>PFOA (%)</th>
<th>PFOS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popcorn 1</td>
<td>28.6 ± 1.7</td>
<td>BELOW LOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popcorn 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popcorn 3</td>
<td>18.2 ± 2.5</td>
<td>BELOW LOD</td>
<td></td>
<td>96.4%</td>
<td>83.6%</td>
</tr>
<tr>
<td>Popcorn 4</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popcorn 5</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
<td></td>
<td>81.9%</td>
<td>86.2%</td>
</tr>
<tr>
<td>Popcorn 6</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popcorn 7</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
<td></td>
<td>80.4%</td>
<td>85.5%</td>
</tr>
<tr>
<td>Snack and Sandwich 1</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack and Sandwich 2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack and Sandwich 3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
<td></td>
<td>71.8%</td>
<td>76.5%</td>
</tr>
</tbody>
</table>

Table 8 PFOA and PFOS concentrations in paper packaging per surface area.

<table>
<thead>
<tr>
<th>PACKAGING MATERIAL</th>
<th>Paper Mass per surface area (g dm⁻²)</th>
<th>PFOA (ng dm⁻²)</th>
<th>PFOS (ng dm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popcorn 1</td>
<td>0.77</td>
<td>22.1</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 2</td>
<td>0.77</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 3</td>
<td>0.71</td>
<td>12.9</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 4</td>
<td>0.77</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 5</td>
<td>0.79</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
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<tr>
<td>Popcorn 6</td>
<td>0.82</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 7</td>
<td>0.81</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 1</td>
<td>0.55</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 2</td>
<td>0.56</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 3</td>
<td>0.45</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
</tbody>
</table>

Discussion

To fully interpret the data and possible human exposure to PFOA and PFOS in paper packaging, the data were also reported in concentration per bag, as shown in Table 4. This information provides a framework to understand the maximum potential ingestion by transfer from the packaging material to the food, assuming that all of the contaminant is transferred into
the food. The values for PFOA detected in popcorn bags 1 and 2 were 321.4 and 204.6 ng bag\(^{-1}\), respectively. Correcting for the methods with the lowest of 71.8\%, and assuming that all of the concentration could be transferred into the food, the maximum potential ingestion quantity would be 447.2 ng bag\(^{-1}\) and 285.0 ng bag\(^{-1}\), respectively. Compared to the TDI of 1.5 µg kg\(^{-1}\) body weight (equivalent to 1500 ng kg\(^{-1}\) body weight per day) for PFOA (EFSA 2008), the potential contribution of the paper packaging analyzed is below threshold. As an example, for a person that weighs 75 kg, the maximum TDI is 112,500 ng PFOA and the bag of popcorn with the highest concentration found could potentially contribute a maximum of 447.2 ng PFOA, or 0.4\%, of the maximum recommended daily intake. In a similar comparison, all concentrations for the bags analyzed were below the LOD for PFOS of 0.63 ng g\(^{-1}\) of paper packaging. One sample was above the LOD but below the LOQ. Taking a maximum content of PFOS equal to the LOQ, which is higher than the LOD, of 2.11 ng g\(^{-1}\) of paper packaging, the potential contribution of the paper packaging analyzed is also below threshold. Calculating the maximum potential ingestion, utilizing the lowest recovery rate observed at 76.5\% and the highest weight per bag of 12.3 g, a bag of popcorn can contribute 33.9 ng of PFOS, compared to the TDI of 150 ng PFOS kg\(^{-1}\) body weight. As an example, for a person that weighs 75 kg, the maximum TDI is 11,250 ng PFOS and a bag of popcorn with the concentration equal to the LOQ, above the observed concentrations could potentially contribute a maximum of 33.9 ng PFOS, or 0.3\% of the maximum recommended daily intake.
Table 9 PFOA and PFOS concentration in paper packaging per bag for samples analyzed.

<table>
<thead>
<tr>
<th>PACKAGING MATERIAL</th>
<th>Avg. Mass (g bag&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>PFOA (ng bag&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>PFOS (ng bag&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popcorn 1</td>
<td>11.2</td>
<td>321.4</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 2</td>
<td>11.9</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 3</td>
<td>11.2</td>
<td>204.6</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 4</td>
<td>7.9</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 5</td>
<td>11.7</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 6</td>
<td>12.3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Popcorn 7</td>
<td>12.2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 1</td>
<td>7.2</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 2</td>
<td>7.3</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
<tr>
<td>Snack and Sandwich 3</td>
<td>4.5</td>
<td>BELOW LOD</td>
<td>BELOW LOD</td>
</tr>
</tbody>
</table>

Choi et al. (2018) evaluated 312 samples of food contact materials from the Korean market for 16 different perfluorinated compounds, including PFOA and PFOS. The 11 samples of baking paper analyzed were negative for all 16 PFCs. Zafeiraki et al. (2014) also analyzed 42 different samples of various paper and paperboard food contact materials in the Greek market and did not find PFOA or PFOS in any sample. These results are consistent with the results obtained in this research. Table 5 shows compilation of data collected from different studies on PFOA and PFOS concentrations in microwave popcorn bags between 2005 and 2018, including this study. The data show a reduction overtime. Values reported in units per weight of packaging were transformed into ng dm<sup>-2</sup> by assuming 0.78 g of packaging per dm<sup>2</sup>, which was the average obtained in this study. This was done only to unify the units for comparative purposes and does not affect the trend overtime. Ongoing research is being conducted to standardize methods and threshold limits for PFOS and PFOA in food packaging materials in the USA. Recent efforts have utilized international standards such as the Danish Ministry of Environment and Food recommended limit value of 10 µg organic fluorine per square decimeter paper (Ministry of
Increased sensitivity of instruments, improved extraction methods, such as the one used in this research, is now capable of detecting POPs that are not intentionally added to packaging and serve no functional purpose.

Table 10 PFOA and PFOS concentration (ng dm\(^{-2}\)) in microwave popcorn bags from studies between 2005 and 2018, including this study (Zabaleta, et al. 2016).

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>PFOA (ng dm(^{-2}))</th>
<th>PFOS (ng dm(^{-2}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>U.S.</td>
<td>4.7*</td>
<td>NA</td>
<td>Begley et al. 2005</td>
</tr>
<tr>
<td></td>
<td>U.S.</td>
<td>226.2*</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>U.S.</td>
<td>470</td>
<td>NA</td>
<td>Sinclair et al. 2007</td>
</tr>
<tr>
<td>2011</td>
<td>Denmark</td>
<td>&lt;LOD</td>
<td>NA</td>
<td>Trier et al. 2011</td>
</tr>
<tr>
<td>2011</td>
<td>Australia</td>
<td>7.1*</td>
<td>&lt;LOD</td>
<td>Dolman and Pelzing 2011</td>
</tr>
<tr>
<td>2012</td>
<td>Spain</td>
<td>41.3*</td>
<td>&lt;LOQ</td>
<td>Martinez-Moral and Tena 2012</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
<td>154.4*</td>
<td>17.9*</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>Thailand</td>
<td>0.1</td>
<td>&lt;LOD</td>
<td>Poothong et al. 2012</td>
</tr>
<tr>
<td></td>
<td>Thailand</td>
<td>1.7</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>Spain</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>Moreta and Tena 2013</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
<td>10.9*</td>
<td>4.6*</td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>U.S.</td>
<td>22.1</td>
<td>&lt;LOD</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>U.S.</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td></td>
</tr>
</tbody>
</table>

*Values reported in ng g\(^{-1}\) were converted to ng dm\(^{-2}\) using 0.78 g dm\(^{-2}\) as the average grammage of microwave bags including the susceptor.
NA – Not analyzed
<LOD – Below the limit of detection
<LOQ – Below the limit of quantitation

Conclusions

Two of the ten samples analyzed for PFAS had average concentrations of PFOA above the LOD, whereas all PFOS data remained below LOD. Both PFOA containing samples were popcorn bags. The three snack and sandwich bags analyzed had average PFCs concentration below the LOD, although one of the samples of a snack and sandwich bag found detected concentrations above LOD and below LOQ. Calculating the maximum potential ingestion
quantities of PFOA and PFOS from the paper packaging samples analyzed, the amounts that each bag contributes are several orders of magnitude below the TDI amounts per day at 1500 ng kg\(^{-1}\) body weight per day for PFOA and 150 ng kg\(^{-1}\) body weight per day for PFOS. PFOA and PFOS are not currently being added to paper packaging, but rather appear as environmental contaminants from the materials used in manufacturing (water, fiber sources, etc.). Removing these compounds is very difficult due to the stability of the molecules and the persistent nature of the pollutant, and previously reported global environmental contamination. Increased attention and awareness have been given to materials used in food packaging to avoid unintentional presence of PFOA and PFOS in paper packaging. This increased awareness has resulted in low-level detection of PFOA and PFOS in packaging that is an unintentional POP that does serve any functional purpose in the packaging structure. This study provides useful information for the microwave popcorn industry, as well as the paper packaging industry, on current levels of PFOA and PFOS in packaging. While PFOA and PFOS were not found in some of the samples, further studies should include the identification and quantitation of other PFASs and their possible effects on human health. Consumers demand paper packaging that is water, oil, and grease resistant and paper offers a sustainable and convenient packaging material for some applications. More research is needed to develop other processes and/or chemicals that do not harm the environment and health, but can impart oil, grease, and water resistance to paper packaging materials.

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Citation


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CHAPTER 6. GENERAL CONCLUSIONS

Food safety is critical in providing safe food to people, while optimizing resources and avoiding food waste. This dissertation covers potential sources of food safety risks relevant to the food industry such as current refrigeration conditions of fruits and vegetables in display cases, food handling behavior of retail employees, and current levels of contaminants in food packaging that can migrate and be harmful to humans. Studies presented address the current situation for each of the topics, which are different from previous studies and therefore reveal a new updated perspective. The results obtained provide information that will help redirect research focus to new evolving risks.

Correct refrigeration temperatures, as emphasized by FDA, should be monitored and maintained below 41 °F or 5 °C for TCS foods such as fruits and vegetables. Current available retrofitted doored display cases have a positive impact in controlling temperatures and reducing the time that produce remains under temperature abuse (>5 °C). Doored display cases also reduce the variability of temperatures in display cases and improve relative humidity by increasing it to >90% RH. Differences in display case performance was observed in both open and closed display cases, depending on retailers. Retailers had a significant effect on case performance, evidencing the importance of self-checks and increased surveillance to avoid deviations and abuse conditions.

Produce stocker behavior was observed to be potentially unsafe in the following topics: employee health and hygiene, handwashing, cross-contamination, stocking and rotation, handling of fallen produce, refrigeration and temperature control, display case cleaning, and maintenance. Customers were also observed to be a source of potential food safety risks. Retail employees can impact produce food safety through their food handling practices. Knowledge is a
basic requirement that can improve correct food handling practices, but this doesn’t necessarily translate into correct food safety behavior. A strong food safety culture in the workspace can have a positive impact in safe food handling. Observations of employee behavior can help the retail industry focus on specific gaps of knowledge or behavior instead of spending large amounts of resources. Retraining in specific identified gaps can help maintain a food safety culture.

Food packaging can be a source of chemical food safety risks. Long-chain PFAS such as PFOA and PFOS used to be added to food packaging and this compound migrated into food products. Current food packaging such as popcorn bags and snack and sandwich bags show very low content or undetectable contents of PFOA and PFOS. An analysis between 2005-2018 indicates that the content of PFOA and PFOS in paper packaging has decreased, therefore reducing the risk of ingestion due to migration into food. Increased attention given to past reports on the presence of PFOA and PFOS in microwave popcorn paper packaging have a negative impact on the microwave popcorn industry and the food contact paper packaging industry. The data presented shows current levels and trends in popcorn paper packaging. The information provided redirects research focus from legacy PFAS such as PFOA and PFOS, to short-chain and GenX PFAS in paper packaging.

Food that can be acquired by consumers at the retail level has many potential food safety risks for both short time and long-time exposure. This dissertation has discussed the potential factors associated with refrigeration and display, food handling by retail employees, and contaminant migration from food packaging, as examples of commonly ignored risks. FDA is committed to providing safe foods and through FSMA has implemented a preventive approach to food safety risks. More preventive work must be done at all levels of the food industry and food
supply, to ensure that the correct and effective measures are implemented to avoid and prevent real food safety risks in our food supply. Focusing research on real current risks is necessary to address food safety risks in a timely manner. This dissertation serves as a current assessment of food safety in the retail environment.
APPENDIX A. RETAIL OBSERVATIONS

Produce retail display observations were divided into the following topics: display case cleaning, handwashing, stocking and rotation, employee health and hygiene, temperature control, fallen produce, display case maintenance, and customer behavior.

Employee Health and Hygiene

- Employee stocking herbs has soiled jeans.

Handwashing

- Workers don’t use gloves when stocking the produce section. The palms of their hands are soiled as they perform the stocking activity. No handwashing during the observation.
- No washing stations near produce section – bathrooms are public and far away.
- Employee is stocking cauliflower, unwrapping, uses gloves but touches the cart, the box, produce, opens new box, etc., without changing gloves.
- Employee stocking lettuce, has gloves, peels the plastic off, drops a leaf to the floor, picks up the leaf, puts it in a trash can he has for the task, takes the lettuce with the hand he picks up from the floor and puts it on display. Used dirty hand to stock and doesn’t change gloves.

Cross-contamination

- Shopping carts are brought in from parking lot and placed for customers to use directly. No cleaning procedure.
- Shopping carts have hairballs in wheels’ mechanism. A cart has a band-aid stuck to a wheel. Customer using this cart is inside the store.
- Service dog inside the store.
- Person recycling cans and bottles goes shopping without washing hands after handling garbage. There is a handwashing station with soap, clean running water, and paper towels. There are no signs to encourage handwashing. (Cross-contamination / Customer Behavior)
- Employee using handheld device to check price and then handles produce.
- Stocking produce from boxes, places box on top of other produce displayed. (Cross-contamination / Stocking and Rotation)
- Spit in sidewalk where people walk into the store. People step on it.

Stocking and Rotation
- When organizing racks, produce is set in shopping carts or on top of other products in the cases, while they organize
- Sales representatives stock their displays (juices) unsupervised – not an employee of the store so no control of their behavior
- Tomatoes, mushrooms and citric fruits rotten in display
- Boxes of produce have an opening on the top and bottom panels. Employees place these boxes directly on the floor and the opening lets produce come in contact with the floor. This area is a high transit area and therefore very dirty because it’s where people stand to choose their produce. This floor has grapes and strawberries stepped on. Very dirty floor.
- Very few containers of strawberries are left and have incomplete amount, evidencing some type of improper handling. Customer is combining into one box and purchased it. (Customer behavior / Stocking and Rotation)
- Black and white mold on strawberries. Juice leaking onto other surfaces and other boxes. Old spoiled boxes with good ones.
- Damaged peaches on display – bruised and mold.
- Very packed shelves of bagged leafy greens and other not so packed. This can disrupt the airflow in the display cases, causing warmer spots in the case. (Refrigeration and Temperature Control / Stocking and Rotation)

- Stocking produce from boxes, places box on top of other produce displayed. (Cross-contamination / Stocking and Rotation)

- Bagged salad mixes have longer best-by dates in the front than in the back. Lack of FIFO or intentionally stocked using LIFO?

- Ripe and non-ripe produce in the same display – mixed.

**Handling of Fallen Produce**

- Apples fall to the floor and are placed back in display by customers (Fallen produce / Customer behavior)

- Turnip is on the store’s floor. After 3+ hours of working on display cases, the store employee decides to put it back on display.

**Refrigeration and Temperature Control**

- Produce that customers don’t buy is taken back to the shelves after being in the cart for some time (temperature abuse?).

- Very packed shelves of bagged leafy greens and other not so packed. This can disrupt the airflow in the display cases, causing warmer spots in the case. (Refrigeration and Temperature Control / Stocking and Rotation)

- Small shelves very packed, then bigger shelves not so packed – disruption of airflow and improper refrigeration in packed areas.

- Big boxes of salad or fruit cover the vent for air curtain – bottom of display case.

- Guacamole container misplaced in the canned beans section.
Display Case Cleaning

- Black mesh or plastic cover under bottom rack is very dirty. When lifted, mold under this material
- On cut fruit display, juice is spilled on shelves
- In section where produce is misted, honeycomb near the spraying nozzles has mold.
- Under the bottom rack and under the air curtain rack, cases are very dirty. Slime, pieces of rotten fruit and vegetables in juices. Obstructed drains retain water and fallen produce residues. Hair and soil in air curtain vent. This is where the fans are located, and air is distributed to the rest of the display case. Potential source of cross contamination.
- Employee comments that they wash with a high pressure hose the shelves once every week. This can generate aerosols and contaminate the produce and other surfaces nearby.
- Employees use rags to clean spills on shelves. No cleaners or sanitizers used.

Maintenance

- Spraying system is set so that each shelf can be sprayed but only the top shelf sprays. Dead points? Don’t see drains for those sections out of service.
- Cleaning hoses used to wash the display cases are kept over the air curtain rack and are installed connected to the display case (non-removable). There is no air flow, but the case is an open front display case with no doors.

Customer Behavior

- Children touch and let produce fall to the floor. Parents pick up and put in display again – nectarines and peaches
- Very few containers of strawberries are left and have incomplete amount, evidencing some type of improper handling. Customer is combining into one box and purchased it. (Customer behavior / Stocking and Rotation)
- Containers of cherries fall to the floor, letting cherries fall out of the container. A customer picks them up and puts them directly onto the shelf.
- Customers buy fruits in containers (strawberries) and eat them directly as they shop, no washing.
- Apples fall to the floor and are placed back in display by customers (Fallen produce / Customer behavior)
- Person recycling cans and bottles goes shopping without washing hands after handling garbage. There is a handwashing station with soap, clean running water, and paper towels. There are no signs to encourage handwashing. (Cross-contamination / Customer Behavior)
APPENDIX B. TRAINING MATERIALS

Food Safety in the Supermarket

Wash display cases frequently with soap and water to remove accumulated filth. Wash all interior and exterior surfaces to remove harmful bacteria.

Frequent cleaning of shopping carts removes harmful bacteria and dirt that can contaminate the store and products.

Do not pick up and put back in display dropped fruits and vegetables. They can get contaminated with harmful bacteria and make customers very sick.

All personnel should wash their hands frequently and after going to the bathroom. Sick employees should not work with produce.

Temperature inside the display case should be monitored and kept below 41 °F (5 °C).

Do not place boxes of produce directly on the floor surface to avoid contamination. Use carts, crates, or other boxes to elevate the box.

Figure 4 Poster of food safety in retail stores covering topics related to display case cleaning, refrigeration, stocking, handwashing, cross-contamination, and handling of fallen produce.

Display Cases

Air Flow
- Do not interrupt air flow
- Do not cover the vent
- Clean honeycomb and vents frequently
- Air curtains help maintain temperature and improve energy efficiency

Cleaning
- Cleaning with soap and water removes harmful bacteria and viruses
- Clean drains, shelves, inner walls, racks, air vents, honeycomb, and under the bottom rack

Maintenance
- Have a periodic preventive maintenance program and keep records
- Remove product before starting maintenance work
- Clean the display case after maintenance
- Monitor temperature in display cases to avoid temperature abuse

Temperature Control
- Keep product below 41 °F (5 °C)
- Avoid overstocking

Stocking / FIFO
- Remove bruised or over-ripe product
- Do not overstock the case to avoid temperature variations
- Only stock refrigerated products
- Product with shorter shelf-life (closer best-by date) should be placed to the front, following FIFO - First In First Out

Figure 5 Poster of display cases good practices covering topics related to refrigeration, display case cleaning, stocking, and maintenance.
Figure 6 Handwashing flipchart explains the ways in which employees can get cross-contaminated and the importance of handwashing, following CDC’s recommended steps of proper handwashing technique.

- In this training we will be covering the basic concepts of cross-contamination and handwashing.
- Concepts covered in this training have been extracted from the CDC Handwashing recommendations and FDA’s Employee Health and Personal Hygiene Handbook.
- By the end of this module, you will be able to understand
  - Why handwashing is important in reducing food safety risks.
  - You will identify major routes of contamination,
  - and learn the proper handwashing technique.
Why Is Handwashing Important?

Do you think washing our hands is important?

Why is it important to wash our hands before touching food, even if it is packaged?

Activity (do not read):
Allow for the trainee to think and give their opinion on these questions.
Every day we touch surfaces that are covered with microorganisms and we cannot see. Healthy people can also spread pathogens (ServSafe).

Washing hands prevents illnesses and spread of infections to others (CDC).

Microorganisms are tiny organisms that can be found in most surfaces but cannot be seen directly with our eyes. They can be harmful for humans and animals, causing sickness and even death. Anyone can spread microorganisms.

ServSafe teaches us that every day we touch surfaces that are covered with microorganisms and we cannot see. Healthy people can also spread pathogens (ServSafe)

The Center for Disease Control recommends hand washing to prevent illnesses and spread of infections.
Your hands can have microorganisms, chemical, and/or physical contaminants that can cause sickness to you or others.

We can spread these contaminants by touching contaminated surfaces and then touching other surfaces or food without washing our hands or changing our gloves.

Handwashing is an effective way of avoiding spreading the contaminants in our hands.

Only a few cells of some microorganisms can make people sick and harmful microorganisms are not visible to the human eye.

Surfaces that appear to be clean may actually have harmful microorganisms.
Major routes of contamination

- Some ways in which our hands can be contaminated are
  - Contact with soil on shoes and clothes
  - Contact with toxic substances
  - Handling animals
  - Contact with contaminated surfaces
  - Using the restroom
  - Infected wounds and boils
  - Handling money
  - Having a foodborne illness or contact with an ill person
Major routes of contamination

<table>
<thead>
<tr>
<th>Sneeze or cough</th>
<th>Scratch or touch body</th>
<th>Contact with body fluids</th>
<th>Handling electronics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-contamination</td>
<td>Use of cleaning towels</td>
<td>Contact with non-food contact surfaces</td>
<td></td>
</tr>
</tbody>
</table>

- As well as:
  - Sneezing or coughing
  - Scratching or touching our body
  - Coming in contact with body fluids
  - Cross-contamination
  - Use of cleaning towels – improper handling
  - Contact with non-food contact surfaces: Handling objects in direct contact with floor – dropped produce
Many more...

Can you think of other sources of contamination?

There are many other sources of harmful microorganisms and ways in which they can be transferred.

Activity (do not read):
Allow for the trainee to reflect on other sources of contamination and transfer. Have the employee explain his/her thoughts on the topic.
How to Wash Hands

The complete handwashing process should take at least 20 seconds.

- We will now see the correct way to wash our hands step by step.
- Handwashing should be done in the areas designated for this purpose, not in any sink.
- You will need soap, clean running water, and paper towels or an air-drying system.
- Remember that the complete procedure will take at least 20 seconds and handwashing should be done frequently.
• **Wet** your hands and arms with clean, running water. The water used can be warm or cold.
• **Apply soap,** enough to cover your hands and arms.
• **Rub** your hands together with the soap and spread over hands and arms. Rub the backs of your hands, between fingers, and under your fingernails.
• **Scrub** your hands for at least 20 seconds. If you need to help with the correct amount of time for this step, humming the complete “Happy Birthday” song twice will help you track the time.
• **Rinse** your hands well under clean, running water.
• **Dry** your hands with a clean towel or air dry them.
• **Using a paper towel,** turn off the water and open the door to exit. This will avoid re-contaminating your hands.
Hand sanitizers

Hand sanitizers do not replace hand washing

Only use hand sanitizers after having washed your hands with soap and clean water.

Hand sanitizers

- Only use hand sanitizers after having washed your hands with soap and clean water.
- Hand sanitizers are not effective if used without previously washing hands.
- Hand sanitizers do not replace hand washing.
- Wait for the sanitizer to dry off before touching any surface.
Putting into practice

Identify the handwashing areas

Verify:

- Liquid hand soap
- Clean running water
- Paper towels or air-drying system
- Surfaces are clean

1. Wet hands
2. Soap
3. Wash for 20 seconds
4. Rinse
5. Dry
6. Turn off water with paper towel

- Identify the available hand washing stations
- Go to the hand washing stations and verify:
  - Liquid hand soap
  - Clean running water
  - Paper towels or air-drying system
  - Surfaces are clean

Take the hand washing diagram and follow the instructions. Track your time. Remember to follow this procedure at all times, even at home.
  - Complete process should take at least 20 seconds.

Activity (do not read):
Go to the designated handwashing stations available with the trainee and follow the handwashing procedure until the trainee feels comfortable washing his/her hands.
• There may be situations in which handwashing alone may not remove all pathogens from heavily contaminated hands.
• Infected food employees may not always be identified and removed from food contact activities.
• As of 2005 FDA recommends “No Bare Hand Contact” to prevent contamination of food that will not require further cooking from potential pathogens in employee’s hands.
• It is recommended to have employees use gloves when handling produce.
• The purpose of gloves is to protect the food from contamination caused by employees and should therefore be changed regularly.
• Congratulations! Now you have learned that:
  • Frequent handwashing is important in reducing food safety risks.
  • You have identified some of the major routes of contamination,
    • And practiced the proper handwashing technique.
• Do you have any questions or concerns?

*Activity (do not read):*
Listen to the trainee’s questions and concerns and go over any concepts that are not clear. Correct any abnormal situations that may be happening and may be an obstacle to implementing proper handwashing behavior and/or technique.
Figure 7 Stocking and Rotation flipchart that addresses recommended best practices and correct food handling procedures related to stocking of fruits and vegetables in retail stores.

- This training material covers some, but not all, recommended general best practices and correct food safety procedures. Verify additional recommendations with the store’s manager or PIC.
- Fresh fruits and vegetables are healthy and should be consumed regularly and abundantly. Customers are demanding more fruits and vegetables, with good quality, and at a reasonable cost.
- Customers prefer clean and organized displays. Color segregation and variety of products are also attractive to customers.
• Establish First-In-First-Out (FIFO) Procedures for your fruits and vegetables.

• Product rotation is important for both quality and safety reasons. First-In-First-Out (FIFO) means that the older produce is placed in a more accessible location for customers to take first. FIFO stocking procedures limit potential pathogen and spoilage microorganism growth. Product rotation will allow for fresher product on display.

• There may be times in which stocking produce may require Last-In-Fist-Out (LIFO) stocking procedures. Consult with your manager when this can be required.
• Check for bad product and remove them from display as necessary
• Pull products that are no longer appealing to customers
• Fallen produce can get contaminated with harmful bacteria when in contact with the floor. Fallen produce can also get bruised, causing it to spoil faster. Dispose of fallen produce because they can be a food safety risk for customers.
• Damaged or bruised product can favor harmful bacteria and spoilage microorganisms.
• One damaged apple in a package can affect others and make the other pieces spoil faster or become unsafe. It is important to check bags of produce and remove them as necessary.
There are many ways in which excess products or fruits and vegetables with minimally reduced quality can be used before they are wasted:

- Minimally damaged produce can be used in prepared foods such as smoothies, salad bars, soups, etc., by retailers that have food service bars in their establishments.
- Some stores will donate food products according to the rotation date, and others may sell it at a discount price before there is a loss of quality.

Activity (do not read):
- Explain in detail to the employee your store’s policies and procedures to follow with respect to excess or minimally reduced quality products.
- Make sure to explain how they should identify the products classified for this, how to label them or segregate them, and any other relevant information.
- Allow time for the employee to understand and make any necessary comments or questions.
Use carts or other elevated aids to avoid contact with the floor. Boxes of produce may have holes that allow for cross-contamination of the contained products.

When stocking display cases, make sure that the display case is clean to avoid cross-contamination.

Clean and organized display cases are attractive to customers.

Frequent restocking and removal of minimally damaged product will help maintain quality of other products and reduce food safety risks.

Use gloves when handling Ready to Eat food such as fresh fruits and vegetables.
If you are sick or have been exposed to foodborne illnesses, consult with the PIC or manager for authorization before handling produce.

Temperature Controlled for Safety (TCS) foods include many fruits and vegetables that should be stored and displayed below 41 °F or 5 °C. Verify the storage conditions of fruits and vegetables before stocking.

When stocking display cases, make sure to consider the design of the display case:

- Do not cover the air return grill by overstocking or with large containers
- Overstocking the case can interrupt air flow and affect temperature control of products
- Signs can block air flow
- Proximity to lights can impact product temperature
- And other considerations
These are some recommended general best practices and correct food safety procedures that apply to stocking of fruits and vegetables.
Do you have any questions or comments?

Activity (do not read):
Allow for the employee to ask or comment. Answer the questions and take into account the trainee's comments to improve situations or conditions in your store.
Figure 8 Employee health and personal hygiene flipchart for employees based on FDA’s handbook, covering information on symptoms related to foodborne illness

This training will cover the employee’s responsibilities when having symptoms of foodborne illnesses. The content is an adaptation of the Food and Drug Administration’s Employee Health and Personal Hygiene Handbook.

- Foodborne illness is commonly known as food poisoning
- Many microorganisms can occur in food and make people sick.
- When two or more people have a common food and become sick with similar illness, this is known as an outbreak.
Employees will be excluded from work if they have the following symptoms:
• Vomiting
• Diarrhea
• Jaundice (yellow skin or eyes) ← From Hepatitis A
• Sore throat with fever
• Infected cuts and burns with pus on hands and wrists
Vomiting and Diarrhea

➢ Stop working immediately AND Contact supervisor
➢ Stay home for at least 24 hours after vomiting and diarrhea symptoms have ended

• When having symptoms of vomiting and/or diarrhea, and you haven’t arrived to work:
  • stay home
  • inform the manager by phone
  • and don’t report to work until at least 24 hours after vomiting and diarrhea symptoms have ended.

• When having symptoms of vomiting and/or diarrhea at work:
  • stop working immediately
  • report to the manager
  • Go home and return after at least 24 hours have passed since the vomiting and diarrhea symptoms have ended.
Jaundice

- Report symptoms
- Seek immediate medical attention
- Return to work after cleared from doctor

- When having symptoms of jaundice, that is the yellowing of the white portion of the eyes, skin, and mucous membranes:
  - Report the symptoms to the manager or an employee from the health department
  - Immediately seek medical attention
  - Return to work after receiving clearance from a health practitioner
  - If jaundiced for more than 7 days, you will require clearance from the local health department
Cuts and Boils on Hands

- Report the wound
- Need to cover the wound
- Wear a single use glove over the bandage before going back to work

• When having an infected cut or burn:
  • Report the wound to the manager
  • Cover the wound with a clean and impermeable bandage
  • Wear a single use glove over the bandage before going back to work.
    This will prevent the bandage from falling into the food and becoming a choking hazard for consumers

• If you have a cut or wound in your hands or wrists, you may continue working if the wound has been covered with a tight-fitting bandage and protected with a single use glove at all times.
Sore throat and fever

- Sore Throat → Okay
- Fever → Okay
- Sore Throat and Fever → Stay Home
- Report symptoms
- Return to work after medical clearance

• When having symptoms of sore throat and fever:
  • Report the symptoms to the manager
  • Stop working and go home
  • Return to work after obtaining clearance from a health practitioner and presenting it to the manager
• With this, we have covered what employees should do in case of having symptoms of foodborne illnesses.

Activity (do not read):
Ask the trainee if he or she has any questions about the topic. Discuss their questions.
Employee Health and Hygiene for Managers – Extension Publication

Managers’ responsibilities on employee reporting foodborne illness and symptoms

Foodborne illness is commonly known as food poisoning. There are more than 200 types of microorganisms that can occur in food and cause foodborne illness. Common symptoms associated with foodborne illness include:

- Stomach pain
- Vomiting
- Diarrhea
- Fever
- Cold or flu-like symptoms

Infected wounds should be covered with a tight, impermeable barrier and a single-use glove to avoid food contamination and sharing bacteria. All open or bleeding wounds shall be reported to the person in charge, including fresh bites, as they may contaminate food. All equipment, cutting boards, and utensils shall be sanitized after use. Hands and arms, except for a clean working hand, shall be washed after use.

The manager or person in charge of the food establishment is authorized to exclude sick or otherwise unwell employees from their work. If there is a food safety risk, an exception is when the employee is the only person available to do the work. In such cases, the employee shall be instructed to report the situation to the manager, who shall then make the final decision. However, if the employee’s condition is when the activities performed by the employee are likely to cause the risk of transmitting a foodborne illness to others, the employee will be excluded from their work. If the employee has symptoms of vomiting and/or diarrhea, has a sore throat and fever (where the symptoms are from a communicable condition).

Refer to the Food Code 2017 subparagraph 2.1.1.1.2 Removal, Adjustment, or Retention of Employees and Facilitators before removing the employee and allowing the employee to return to work. If the employee is excluded, the manager may give the employee the necessary, regulatory transportation to the exclusion site and the employee is not allowed to return to work. The employee is not allowed to return to work until the necessary, regulatory transportation is given. The manager shall then give the employee the necessary, regulatory transportation to the exclusion site and the employee is not allowed to return to work. The employee is not allowed to return to work until the necessary, regulatory transportation is given.

Figure 9 Employee health and personal hygiene extension publication for managers based on FDA’s handbook, covering information on the decisions related to symptoms of foodborne illness

References:
Figure 10 Display case cleaning video that shows the general best practices to clean refrigerated display cases.
Recommendations

Cleaning the display cases:
- Reduces energy and maintenance costs
- Decreases the risk of food spoilage and contamination

Recommended good practices:
- Schedule and keep record of cleanings
- All display cases should be cleaned on the same day

What you need to clean display cases
- Rubber gloves
- Rubber apron or waterproof clothing
- Rubber boots
- Safety glasses
- Access to cold and warm to hot water
- Low pressure hose
- Bucket or container with warm to hot water
- Vacuum
- Mild detergent or cleaning solution (as recommended by manufacturer)
- Glass cleaning solution
- Sanitizing solution (as recommended by manufacturer)
- Foam cannon or soap container
- Spray bottle
- Bobble brush or sponge
- Paper towels or reusable rags
**IMPORTANT**

- Do not use cleaners containing corrosives, abrasive material, or ammonia
- Some of the materials listed can change according to manufacturer’s recommendations
- Avoid spraying cleaning solutions or water directly on fans or electrical connections

**Safety Uniform**

- Gloves
- Water-resistant apron
- Rubber boots
Preparing the display case for cleaning

Remove all products from the display case
Store food in the cooler at temperature of 
< 41°F or < 5°C

Verify the temperature of the cooler
Use signs to avoid injury as the area may be wet and slippery

- Close the refrigeration valve in the display case
- Identify location in manufacturer's manual
• Turn off the power supply
• Follow procedures of “lock out/ tag out” to avoid safety incidents and accidents

Cleaning the bottom tank of the display case
Remove the trays from the display case

Take out other removable parts from the display case
Place trays and all removed parts on a cart or clean surface

Do not place on floor

Wash the cleaning sink before use to avoid cross-contamination
• Wash the trays with warm to hot water and cleaning solution
• Use brush and sponge as required
• Rinse thoroughly with warm water

Disconnect fans to avoid possible injuries
Remove any pieces of ice, food, or any other foreign objects from the bottom and drain line of the display case.

Using a hose or a bucket with warm water, put water through the drain to check that the drain line is not plugged.
• If the drain line is plugged, remove everything from it to unplug it.

• Use a filter to prevent large objects from plugging the drain line again.

• Using a low-pressure hose with warm to hot water, wet the bottom of the display case and melt any residual ice.

• Do not hose directly the tubes and fans as this can damage the case.
• If refrigeration tubes are frozen, you can remove the ice using a container with warm water until it melts off.
• Do not force the ice off the tubes.

• Spread cleaning solution using a sponge or foam cannon
• Scrub all surfaces with the sponge or brush, as needed.
Allow time for the cleaning solution to remove filth

Rinse thoroughly using warm water to remove the cleaning solution
• Use a cleaning solution and sponge, cleaning towels or rag to clean the structure around the fans

• Make sure not to wet the electrical components

Rinse immediately with a bucket or low-pressure hose with warm water to remove soap and filth
• Allow the case to air dry completely
• Sanitize all surfaces, including the tank, if recommended by the manufacturer, and let air dry

Reconnect fans
Honeycomb

- Uninstall the honeycomb and carefully remove it
- Clean using a vacuum or low-pressure hose
- If the honeycomb is damaged, consider replacing it for a new one
Cleaning the rear baffles

Use cleaning solution and paper towels or rags.
Wipe off completely the cleaning solution.
Cleaning the shelves
Wash the shelves or clean with solution and paper towels

Remove any residue from the surfaces
You may need cleaning solution, sponge, and brush to remove all residue
Cleaning glass surfaces

Spray glass cleaner solution and clean with reusable rags
Cleaning the exterior
Clean the exterior with a cleaning solution and paper towels or reusable rags.

Clean the sliding doors
Clean all exterior surfaces with cleaning solution and wipe off using paper towels or cleanable rags

Sanitizing
Spread sanitizing solution and let air-dry

Reinstall and Restart
- Verify that the display case is dry
  Once it has dried completely, open the refrigeration valve and plug in the fans

Reinstall the trays and all removed parts
Verify that all work in the display case has been finished and that the case is ready to start working again.

Remove the lock out – tag out

Start the display case. Once the temperature is <41°F or 5°C, you may stock the display case again with food.
APPENDIX C. EXTENDED METHOD FOR PFAS EXTRACTION

Previously ground samples

Samples previously ground using an IKA-A11 mill are ground to a voluminous consistency with no evident intact pieces of the original paper; the sample has been completely separated into fibers, as seen in the following picture.

![Ground samples of paper packaging](image)

Figure 11 Ground samples of paper packaging

Internal Standard solution (IS)

Prepare a 300-ppb solution of mass labeled (13C8) PFOA and PFOS in MS grade methanol. Each sample will require 100 µL of IS so make sure to estimate the required amount for the testing. It is recommended to use the same batch of IS for each batch of samples.

Spike solution

Prepare a 20-ppb solution of native (non-mass labeled) PFOA and PFOS in MS grade methanol. Each sample of 1.50 grams will require 1 µL of spike solution. It is recommended to use the same batch of spike solution for each batch of samples.

Thoroughly mix the ground sample in its bag. If a spike recovery will be evaluated, divide the sample into two and place in two different bags for further processing.
Use appropriate bags to avoid possible contamination of the sample with PFASs from other sources.

**Spiked sample preparation**

In a large beaker, add enough ethyl acetate to fully cover and soak the sample that will be spiked as shown in the picture.

![Figure 12 Packaging to be spiked and enough ethyl acetate to fully cover the sample](image)

Add 1 µL of spike solution for every 1.50 g of sample. Mix well the spike solution into the ethyl acetate. Add the total amount of the sample to be spiked and mix thoroughly.

Place in a water bath at 45 °C to evaporate the ethyl acetate, mixing every hour to ensure that the sample dries evenly. Rinse the walls of the beaker to reduce the amount of spike lost on the sides of the beaker. Continue until the sample is completely dry.

Once the sample is completely dry, regrind the sample using the IKA mill and mix well in the storage bag. Spiked and non-spiked samples are stored in polyethylene bags wrapped in aluminum foil and aged for 2 weeks under refrigeration conditions (approximately 4 °C).
Sample extraction

In previously washed and dry 50 mL test tubes, weigh 1.50 grams of ground sample and label the test tube. Record the exact weight, as this will be used in the final calculation. Each sample is analyzed by triplicate (3 test tubes, each with 1.50 g for each sample).

![Weighed packaging sample in test tube](image)

Add 100 µL of IS to 24 mL of HPLC or higher purity grade ethanol.

Add the ethanol + IS mixture to the test tube with sample. Rinse the beaker where the solvent and IS were combined with approximately 1.5 – 2 mL, and transfer to the test tube. Rinse twice.

Place the test tube in an ice bath.

Insert the 3 mm titanium micro tip in the sample mixture to a depth of 2 cm from the bottom of the test tube.

Sonicate using Focused Ultrasonic Liquid Extraction (FUSLE) - Misonix S-4000 Ultrasonic Sonicator. Sonicate for 10 seconds, 30% amplitude, 50% pulsed cycle.

Remove the tip from the test tube and rinse the tip with approximately 1.5 – 2 mL of ethanol into the test tube. Rinse twice.
Filter the mixture through a 60 mL Pyrex® Buchner funnel with fritted disc and porosity 10-15 µm (medium porosity), into a 250 mL glass Erlenmeyer connected to a vacuum pump at 500-600 in Hg vacuum. Rinse the test tube with approximately 1.5 – 2 mL of ethanol and transfer to the fritted funnel. Rinse twice.

Rinse the sample on the fritted funnel with approximately 1.5 – 2 mL of ethanol. Rinse twice.

Once the sample is filtered, remove the vacuum and the funnel. Transfer the extract to a 40 mL vial. Rinse the Erlenmeyer with 1.5 – 2 mL of ethanol and transfer to the vial. Rinse twice.
Sample concentration and reconstitution

Evaporate in a nitrogen evaporator with water bath at 45 °C until the sample is completely dry.

Add one 1.0 mL of MS grade methanol to reconstitute the sample. Vortex the sample for at least one minute – vortex for a longer time if there is residue on the vial.

Using a 3 mL disposable polypropylene medical sterile syringe transfer the reconstituted sample and filter through a 0.22 μm nylon syringe filter into a 2 mL LC vial.
APPENDIX D.  LOD AND LOQ CALCULATION – EXTENDED EXPLANATION

The data obtained for the calibration curve have higher variability towards the higher concentration than the lower concentrations. The generally accepted method for determining the LOD for these unequal variance cases is the Hubaux-Vos method. The LOQ can be estimated from the LOD by multiplying by 10/3 because both estimates are proportional to sigma, so the multiplication is appropriate.

The standard deviation of the data changes with concentration due to the unequal variance so the Curie method (LOD = 3 σ / slope) of calculation is not suitable to the data set. The following SAS code, used to calculate the LOD, was provided by Dr. Philip Dixon from the Department of Statistics at Iowa State University. The algorithm initially calculates an approximate Hubaux – Vos LOD by calculating the upper prediction bound (LC) at a concentration equal to zero. The LOD is the smallest concentration with a lower prediction bound above the LC. Once the lower bound > LC is established using coarse increments, fines increments between the established lower bound and the LC are calculated to narrow down the estimate of the LOD. The method used was an iteration method determined by comparing the LC to the “lowerpred” value in the output. The code is the same for PFOA and PFOS but must be modified for the fine lower bound values in the iteration.

**SAS Code**

```sas
proc import datafile='PFC.csv' out=pfc replace;

run;

/* for Hubaux-Vos, need prediction interval across a range of concentrations */
```
data predconc;
/* preliminary runs to provide upper bound on LOD */
* do conc = 0 to 100;
/* fine grid of values around potential LODs, for equal and unequal variance */
/* probably need to change the limits when run pfos */
/* need a prediction at 0 as well */
do conc = 0, 1.5 to 3.0 by 0.05, 4 to 10, 11 to 13 by 0.1;
pfoa = .;
   pfos = .;
   output;
end;
run;
data plus;
set pfc predconc;
run;

proc reg data=plus outest = ests;
   model pfoa = conc;
   output out=preds p=pred lcl=lowerpred ucl=upperpred;
   title 'Regression of PFOA on concentration, constant error variance';
   run;
quit;
/* this data step computes the Currie lod and loq from the regression estimates */

/* Currie method: 3 sigma / slope and 10 sigma / slope */

data lod;
set ests;

call symput('slope', conc);
/* save the slope for use in unequal variance method */

/* Currie estimates of LOD and LOQ */
sigma = _rmse_;
slope = conc;
lod = 3*sigma/slope;
loq = 10*sigma/slope;
keep _depvar_ intercept slope sigma lod loq;
run;

proc print;
var _depvar_ intercept slope sigma lod loq;
title2 'Currie method LOD and LOQ';
run;
/* compute LOD using Hubaux-Vos method */

/* assumes prediction points are sorted in increasing X=conc */

data hvlod;
  retain lc lod;

set preds;
  where pfoa = .; /* only consider prediction points */

if conc = 0 then lc = upperpred;
  /* retain upper 95% prediction bound at conc = 0 */
  /* that is the value that must be 'beat' by lower pred bound at lod */
if (lc ne .) and (lod = .) and (lowerpred > lc) then do;
  lod = conc;
  output;
  end;

/* lod is smallest X (i.e. first time) lower pred bound > lc */
run;

proc print data=hvlod;
  var lc lowerpred lod;
title2 'Hubaux-Vos lod';
run;

/* first look at how variance increases with mean or with conc */
proc sort data=pfc out=pfcsort;
   by conc;
run;

proc means noprint data=pfcsort;
   by conc;
   var pfoa;
   output out=means mean=mean var=var;
run;

data means2;
   retain var0;
   set means;
   drop _type_ _freq_;

if conc = 0 then do;
   var0 = var;
   call symput('var0', var0);
   end;
/* save variance for conc0, both as a variable and a macro constant */

logmean = log(mean);
logconc = log(conc);
logvar = log(var);
logvarc = log(var-var0); /* variance increase from 0 conc */
run;

proc reg data=means2;
model logvar=logmean;
model logvarc=logconc;
title 'log-log regression of variance on mean and conc';
run;

/* I decide to go with var Y = c0 + slope^2*conc */
/* use sample variance at concentration = 0 (saved earlier) as c0 */
/* use the regression slope (saved earlier) to convert conc to Var y */

data plus2;
set plus;

wt = 1/(&var0 + &slope*&slope*conc);
if conc = 0 then call symput('wt', wt);

/* save weight for use in Hubaux-Vos */
run;

proc reg data=plus2 outest=estswt;
  model pfoa = conc;
  weight wt;
  output out=predswt pred=pred ucl=upperpred lcl=lowerpred;
  title 'Regression of PFOA on concentration, unequal variance';
run;
quit;

data lodwt;
  set estswt;

  /* Currie estimates of LOD and LOQ */
  sigma = _rmse_/sqrt(&wt);
  /* adjust rmse for 1/sqrt(wt) at conc = 0 */
  slope = conc;
  lod = 3*sigma/slope;
  loq = 10*sigma/slope;
  keep _depvar_ intercept slope sigma lod loq;
run;
proc print;
   var _depvar_ intercept slope sigma lod loq;
title2 'Currie method LOD and LOQ';
run;

/* compute LOD using Hubaux-Vos method */
/* assumes prediction points are sorted in increasing X=conc */

data hvlodwt;
   retain lc lod;
   set predswt;
   where pfoa = .; /* only consider prediction points */
   if conc = 0 then lc = upperpred;
      /* retain upper 95% prediction bound at conc = 0 */
   if (lc ne .) and (lod = .) and (lowerpred > lc) then do;
      lod = conc;
      output;
      end;
/* lod is smallest X (i.e. first time) lower pred bound > lc */
run;
proc print data=hvlodwt;
var lc lowerpred lod;
title2 'Hubaux-Vos lod';
run;
APPENDIX E. NO IRB REQUIRED STATEMENT

Does the research involve obtaining information about living individuals (45 CFR 46.102(f)) Answer Yes

Does the research involve intervention or interaction with the individuals (45 FR 46.102(f)(1), (2)): Answer No

Is the information individually identifiable (i.e. the identity of the subject is or may readily be ascertained by the investigator or associated with the information) (45 CFR 46.102(f)(2))? Answer No

Conclusion: The research is not research involving human subjects and 45 CFR Part 46 does not apply.
To: Angela Shaw  
Subject: Food Safety Outreach for Retailers  

Your responses on the Human Subjects Research Assessment form (Does My Study Require IRB Oversight) indicate that your research project does not involve human subjects per the federal regulations (45CFR46.102 and 21CFR56). Accordingly, IRB oversight is not necessary.

Please be aware that this assessment is based on the responses you provided. No individuals from the IRB Office or Committee have reviewed this form or your project plans. The Human Subjects Research Assessment form does not replace an IRB application and this determination was made solely on the information provided within the form. If there is information that was not accounted for when responding to the questions in this form, it could change the determination. We recommend completing a new Human Subjects Research Assessment Form if there are any changes to your project plans.

In addition to this notification, you may click "View as PDF" at the bottom of the Human Subjects Research Assessment form to save a copy of the information you submitted for your records.

Ana-Lorena Monge-Brenes <almonge@iastate.edu>

RE: Question on No IRB Requirement...Your Thesis: Minor changes required
2 messages

IRB Committee [ORR] <irb@iastate.edu>  
To: "Shaw, Angela M [FS HN]" <angelaml@iastate.edu>  
Cc: "Monge-Brenes, Ana-Lorena [FS HN]" <almonge@iastate.edu>

Good morning Angela,

The email you attached to your message below ("Human Subjects Research Assessment Results...") is documentation that IRB oversight is not necessary, based on the information entered into our online form.

Only studies that meet regulatory definitions of human subjects research require IRB oversight, and federal regulations establish several categories under which protocols can be determined exempt from most requirements of the human subject protections regulations. Because this study, based
on the information entered into our online form, does not involve human subjects, there is no exemption determination memo for this study.

Looking at your message below, the Human Subjects Research Assessment Results... email was included in the Appendix. If so, I encourage you to follow up with the reviewer to see why the email is not acceptable. Let me know if I can help address any of their concerns.

Don’t hesitate to be in touch,

Deirdre

**Deirdre Rosenfeld**  *IRB Administrator*
  *she/her/hers*

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