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A Multidisciplinary Approach to Food Safety Evaluation: Hummus Spoilage and Microbial Analysis of Kitchen Surfaces in Residential Child Care Institutions (rcci) in Massachusetts, U.S.A.

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A MULTIDISCIPLINARY APPROACH TO FOOD SAFETY EVALUATION: HUMMUS SPOILAGE AND MICROBIAL ANALYSIS OF KITCHEN SURFACES IN RESIDENTIAL CHILD CARE INSTITUTIONS (RCCI) IN MASSACHUSETTS, U.S.A.

A Thesis Presented

by

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DEDICATION

This work is dedicated to The Almighty God, my dad and mom; Prof J.E. Hagan and Ms Angela S. Ahlijah and to all my siblings; Fransiwaa and Nana Leo (Paa Takyi) Vande-Pallen and Kojo Hagan.

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ABSTRACT

A MULTIDISCIPLINARY APPROACH TO FOOD SAFETY EVALUATION: HUMMUS SPOILAGE AND MICROBIAL ANALYSIS OF KITCHEN SURFACES IN RESIDENTIAL CHILD CARE INSTITUTIONS (RCCI) IN MASSACHUSETTS, U.S.A.

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Food borne illnesses continues to be a public health challenge in the United States (U.S.); an estimated 9.4 million incident cases occurred in 2011. In view of this challenge we conducted two food safety studies; 1) related to product formulation (hummus spoilage challenge study) and 2) evaluating the microbial safety of domestic kitchen surfaces in Residential Child Care Institutions (RCCI pilot study).

Hummus is of Mediterranean origin but is currently eaten globally. This challenge study evaluates a variety of industrial hummus formulations (four in total, differing in pH and/or addition of a preservative (natamycin). Two batches were setup: batch 1; aseptically inoculated hummus with 100 CFU/g fungal isolates and batch 2; uninoculated hummus. Samples of both hummus batches were stored at both 20^{o}C (10 days accelerated testing) and 4^{o}C (84 days recommended temperature testing). Inoculated samples were analyzed for fungus, whiles both fungi and bacteria (standard plate count (SPC) and *Lactococci*) counts were done for uninoculated samples. Results indicate that accelerated

testing inaccurately predicts fungal growth at $4^{o}C$ in hummus, also fungal growth inhibition requires a pH $\leq 4.0 \pm 0.2$ and refrigeration.

Limited studies have specifically evaluated the prevalence of pathogenic bacteria in domestic kitchens in the U.S, for this reason we assessed the microbial safety of 6 RCCI locations in MA. Fifteen key food contact surfaces and dish washing sponges, if available at each RCCI facility were assessed for SPC, yeast and molds, total coliform and *E. coli, Listeria sp* and *Salmonella sp*. Microbiological assessments were conducted preceding and after a hazard analysis and critical control point (HACCP) food safety training and implementation at each location. Microbial growth varied by surface for each type of microorganism, wet surfaces had higher most probable number (MPN) counts. Compared to dry surfaces, wet surfaces had significantly higher mean total coliform counts. For both *E. coli* and total coliform, microbial load differed significantly by surfaces sampled (P = 0.0323 and 0.014) respectively. The surface and training interaction effect was highly significant for only *E. coli* (P = 0.0089). Training overall had no significant effect on reducing the microbial load on kitchen surfaces.

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CHAPTER 1

INTRODUCTION

The annual US incidence of food borne illness, caused by the major identified pathogens is estimated at 9.4 million; with 55,961 resulting in hospitalized morbidities and an additional 1,351 in mortalities (17). These prevailing high incidence rates continually challenges all stakeholders in the food industry, such as food retail facilities and legislators to work towards the prevention of food borne diseases. An additional consequence of these high food borne disease incidences is the increased demand for food safety guarantees, and trustworthiness of food products purchased from industries by consumers (3). For this reason food industries and stakeholders are continually challenged with monitoring, innovation and renovation of their food products to meet quality assurance standards and the demands of consumers.

Refrigeration technology allows the possibility of preserving these highly perishable commercially produced traditional foods for much longer than the home made ones. Refrigerated foods which gives the perception of ‘freshness’ to the consumer have fast become a multimillion dollar industry for the preservation of minimally processed, very often ready to eat foods for a relatively short time (16). A big challenge food industries face is achieving this perception of ‘freshness’ whiles, still delivering foods that are preservative free and thus perceived as ‘all natural’ (without added chemical preservatives), by the consumer (16). This challenge has driven the recent growth in innovation of refrigerated foods, particularly the minimally processed food for which heat processing cannot be adequately applied to achieve commercial sterility (16).

This study is a two part food safety study: the first focus, addresses food safety issues related to product formulation whiles the second focus is a study targeted at evaluating the impact of consumer knowledge and food handling practices in food safety.

The first part of this study was a challenge study to evaluate the shelf life of four industrially produced refrigerated hummus formulations. The introduction of new products and the expansion of existing product lines, may lead to unforeseen food quality and safety challenges for food industries, especially in recent times where foods that were once indigenous to a particular society, is now being eaten by a wide range of people. This new trend is as a result of increased global migration, leading to increased food diversity in communities, which are becoming more cosmopolitan. This shift in the diversity of populations is constantly impacting and driving continually changing trends in the food industry. As consumers continue to demand ready to eat, fresh and safe traditional foods that can be purchased in supermarkets, it has become necessary to prepare foods that were once made traditionally on a small scale, industrially for commercial and retail purposes. Food industries meet this demand, both on a small and large scale because of improvements in the processing, preservation and packaging of many traditional products that have been achieved, despite the rudimentary processing of traditional foods due to the use of simple equipments, lower energy input, and the availability of resources (24).

In addition the second part of this two part food safety evaluation study, was to evaluate the impact of consumer knowledge and food handling practices on food safety outcomes. The second part of this study assessed the microbial levels of kitchen surfaces

before and after a HACCP based Food Safety training and plan implementation in Residential Child Care Institutions (RCCIs) in Massachusetts.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction: hummus (hoummos)

Traditionally hummus was cooked and consumed domestically as an appetizer together with Arabic bread, but in recent times it is also being produced and packaged in 100 g to 300 g "press-to-seal" plastic packages for sale commercially (25). Hummus traditionally a widely eaten Middle Eastern delicacy, served as a relatively cheap source of protein in the diet, but in recent times though, hummus is being eaten globally (25).

2.2 Preparation and serving of hummus

Hummus is usually made using these ingredients: chickpeas (*Cicer arietinum L*), tehineh (an oily viscous liquid derived from milled dehulled roasted white sesame seeds), garlic, lemon juice or citric acid and salt (25). Traditionally the chickpeas were steeped overnight, and then softened by boiling with sodium bicarbonate (25). The soft cooked chickpeas are then cooled and then mixed with tehineh (tahini) and other ingredients (garlic, lemon juice or citric acid and salt) to obtain the basic smooth hummus mix (24, 25). Hummus traditionally is normally served off plates or dishes but in recent times commercially produced hummus may be served straight out of the packaging or tub. Often hummus is served with a topping of a special dressing made of lemon juice, ground pungent green capsicum and garlic, as well as olive oil and, occasionally, chopped parsley (25). The average nutrient content of a 100 g edible serving of hummus consists of 49.5, 9.6 and 19.7 g of water, protein and fat, respectively and 300 Kcal energy (25).

2.3 Food Safety Concerns; hummus spoilage

2.3.1 General Microbial Spoilage

A consumer's perception of the occurrence of visible food spoilage which makes foods unacceptable, according to Day, 1999, is when the visible characteristics of the foods such as the appearance, flavor, smell and texture changes (5). The most widely used and effective preservation techniques, currently used to prevent or delay food spoilage include temperature, pH, and water activity (a_w) reduction, as well as heat application (7). Food preservation is highly improved when techniques are used to alter these factors to produce a synergistic effect. Microbial spoilage of chilled foods is very diverse and may be as a result of the type of microorganism present, the nature of the food substrate and the effect of temperature on the food, subsequently different microorganisms may adapt to changes in condition and nutrient levels in order to survive in the foods (5).

2.3.2 Spoilage by Yeast and Molds

The survival, growth and metabolism of yeast and molds in ecosystems such as food, are regulated by interconnected strain and species interactions, which may involve interactions with bacteria cells and other fungi (6). Fungal infestations are of major concern in the food and agricultural industry globally and may start right in the field, particularly in the tropics where humidity is high (generally > 80%) and hence mold growth is favored (23). This occurrence may lead to very huge economic losses, because most food products either processed or fresh e.g. fresh fruits, berries, marmalades, juices,

cereals and grains, are susceptible to yeast and mold contamination and growth after harvesting (22, 23). With the recent surge of product development in the food industry coupled with food safety concerns, associated with opportunistic infections involving yeast and molds, as well as other adverse effects of yeast infection in humans, interest in understanding the survival and growth of yeast in foods has been heightened (6).

2.3.3 Effect of Temperature on Food Spoilage

Food spoilage is influenced by temperature because most biochemical activities are either slowed down at reduced temperatures or speeded up at increased temperatures (21). Elevated temperatures enhance food spoilage, by altering the biological mechanisms in the food, which may lead to enzyme or protein denaturation and a subsequent increase in solute concentration, which may subsequently cause changes in pH and ionic strength of the medium (food) (21). Subsequently the application of reduced temperatures (refrigeration) during food storage has become a widely accepted method of storing minimally processed foods as a means of controlling and decreasing the progression of biochemical and microbial degradation in the food.

Low temperature is effective in preserving chilled foods because it either totally inhibits the growth of microorganisms in the foods and or reduces subsequent growth of these microbes by prolonging the lag phase (5). Day, 1999, observed that at reduced temperatures, approaching the least possible growth temperature for a microorganism, the vulnerability of the microorganism to the effects of the preservative attributes of the food like acidity (pH) and water activity (a_w) is enhanced (5). Food safety in industrial production takes precedence over other food quality issues in the production of chilled

foods and foods in general, this is important because although chilled foods may appear wholesome it may still contain large numbers of pathogens and toxins (5).

2.3.4 Effect of pH on Food Spoilage (Low pH and Weak Acid Synergy)

The pH of the food influences the microbial, as well as enzymatic activity of the food and subsequently influences the rate and type of food spoilage observed for a particular food (7). An extensively used combination preservation technique is to enhance the effect of an antimicrobial acid within the food by lowering the pH of the food (7). Many useful food preservatives fall into this category and thus provide the synergistic effect that produces a low pH, mild acid environment (food), capable of inhibiting some microbial growth in the food (7).

There are two modes of action for the functionality of these antimicrobial acids which include inorganic preservatives, sulphite, nitrite and the weak organic acids. As the lipophilicity of organic acids increase, its effectiveness as a preservative is enhanced; e.g. an increasing order of lipophilicity and subsequently effectiveness is: acetic, propionic, sorbic, benzoic (7). The second important aspect of the mode of operation of these acids, are their dissociation constants, their undissociated forms are the most lipophilic and are the ones that easily diffuse through the membrane of the microbe, this is influenced by the pH value and the dissociation constant (pK) and together these determine the amount of the undissociated acid remaining (7). The scope of pK values of the usual weak organic acid preservatives span 4.2 for benzoic to 4.87 for propionic acid, hence at higher pH values their activity is greatly diminished (7). In the microbial cell cytoplasm these undissociated acids dissociate, producing hydrogen ions and their accompanying anions

because most microbes in foods maintain an internal pH higher than that of their environment (7). Additional energy is required by the cell to export the additional hydrogen ions produced through the above mechanism (7). Hence in an attempt to maintain an elevated internal pH, cell growth is limited, till the required additional energy is obtained, to enable the pH of the cytoplasm to finally decline to unfavorably low levels limiting progressive cell growth (7). Gould *et al*., 1996, thus concluded that the simultaneous decrease of pH plus the availability of weak acid preservatives in a food, will lead to higher energy requirements by the microbial cells in the food and subsequently limit the effective generation of ATP by these cells, resulting in their growth retardation and a subsequent decline in microbial food spoilage (7).

2.4 Refrigeration

2.4.1 Shelf Life Extension via Refrigeration (Low Temperature Storage)

Reactions that lead to spoilage of foods are of primary concern in evaluating shelf life extension possibilities in foods, especially in minimally processed foods such as hummus. Some preservation techniques are targeted at regulating several forms of spoilage that may occur; these may be physical, chemical, enzymatic or microbiological (7). Essentially, though the most important or prime focus of shelf life experiments in all cases is to control and reduce the growth of microorganisms (7). Numerous new trends in food preservation and processing emerged in the past decade, but “Freshness”, was identified as one of the most important trends in food preservation in the food industry to have occurred in the past decade (19).

2.4.2 Preservation by Mild Thermal Processing and Cold Storage

Mild thermal processing in addition to vacuum packaging of foods, held at well regulated low temperatures lead to the deactivation of less heat labile vegetative microflora and spores of psychrotrophic bacteria that could thrive at reduced temperatures (7). This mild thermal processing destruct the cold-growing fraction of possible spoilage microflora, this fractional destruction together with the low oxygen tension conditions created via vacuum packaging guarantees premium food quality (7). This process can lead to extended product shelf life (more than 3 weeks), when products are stored at temperatures under 3^{o} C, although gradually slow growth of psychrotrophic bacteria such as strains of *Bacillus* and *Clostridium* may result in spoilage with time (7). To achieve food safety, thermal processing at 90^{o} C for 10 minutes is necessary to guarantee the deactivation of spores of the coldest-growing pathogenic spore formers such as psychrotrophic strains of *Clostridium botulinum* (7).

2.4.3 Recommended Steps to Achieve Microbial Safety in Foods

Day (1999), recommended these general principles to be applied in achieving microbial safety in chilled foods: primarily food safety may be achieved if only high quality raw materials are used, and this is made possible if the microbial status of all raw materials is known (5). There is also the need for proper documentation (clearly defined procedures), monitoring and control of all processing stages coupled with the documentation and monitoring of the temperature and time of chilled storage, transport and display of products in retail is key (5). Food safety may also be achieved if these

temperatures are controlled throughout, especially, that of raw material handling and if possible extending the temperature control to home refrigeration by consumers (5).

Day, 1999 also cite the fact that hygienic practices carried out throughout the entire food process may also ensure the minimization of microbial growth (5). These recommendations may be achieved via the implementation of good manufacturing practices such as Hazard Analysis and Critical Control Points (HACCP) as well as strict adherence to legislative regulations on food safety.

2.5 Natamycin a Natural Antimicrobial as a Food Preservative

2.5.1 Background of Natamycin

Natamax® is the trade name for Danisco's formulation of Natamycin, a very common, very potent, widely accepted, safe, antifungal, natamycin initially was isolated in 1955, from a culture of *Streptomyces natalensis* a microorganism originally found in a soil sample in South Africa, natamycin is now produced industrially by fermentation using this microorganism (22). Other trade names for industrially available formulations of natamycin include Delvocid®, Natacyn® and Pimaricin®.

Natamycin is a creamy-white colored polyene macrolide antimycotic with an empirical formular of C_{33} $H_{47}NO_{13}$ and a molecular weight of 665.75, widely used today in food industries as a preservative especially for the surface treatment of yeast and mold growth (22). Medicinally it may be utilized as an antifungal for humans and animals, when applied externally, to treat fungal infections and candidosis (22). This wide range of applications is partly because natamycin has broad spectrum activity and secondly partly due to the fact that development of resistance to natamycin is rare (22). However

the use of natamycin as a natural antimicrobial or preservative in foods is limited by the fact that it has no inhibitory effect on bacterial growth, this property though, makes it well suited for use in food manufacturing processes in which bacterial growth and survival is beneficial, such as in the manufacture of cheese and sausages which involves a bacterial ripening processes in the absence of yeast and mold growth (22). Though very chemically stable and hence can be stored for long periods without loss of activity, key factors of concern in the food industry such as extreme pH values, light, oxidants, chlorine and heavy metals affect the stability of natamycin (22).

2.5.2 Physical and Chemical Properties of Natamycin

The solubility of natamycin is poor in neutral aqueous systems and in organic solvents (range; 30 to 100 ppm), however it is improved in strong acids or alkaline milieu (22). Stark (1999), reported that dissolved natamycin is less stable and more susceptible to chemical degradation in comparison to the usual crystalline (dry) state (22). Natamycin exhibits antimicrobial activity when the mycosamine moiety in its structure is split off in low pH environments (pH lower than 3), however in high pH environments (pH higher than 9), the lactone component of the natamycin compound is saponified leading to the formation of a natamycoic acid which no longer exhibits antimicrobial activity (22). Natamycin suspensions are thermally stable under thermal conditions of 50^{o} C for several days and it remains chemically active without a major loss of activity, it also remains stable under sterilization conditions of 30 min at 116^{o} C (22).

When using natamycin as a preservative in food industries, to inhibit the growth of yeast and molds in foods , particular attention must be paid to processes that expose the food suspension to light, since irradiation by ultraviolet light is known to decompose natamycin, as such natamycin is best stored in the dark (22). Natamycin is also readily decomposed by low concentrations of peroxides, oxidants and chlorines, hence possible contact of pure natamycin or natamycin in foods with these compounds commonly found in cleaning agents in food production must be regulated and avoided to prevent the inactivation of natamycin, which may result in mold problems (22).

In aqueous systems such as most food systems natamycin is readily converted to the more stable and soluble trihydrate form, which enhances its antifungal activity (22). Food industry specific properties of natamycin that make it an effective antifungal agent include these: key among its food safety application benefits is its specificity; its broad spectrum inhibitory activity against growth of yeast and molds, is beneficial in shelf life extension and maintenance as well as in the prevention of the production of mycotoxins such as aflatoxins in foods (22). Because natamycin is also ineffective on bacterial cells, it is very applicable in fermented products, as a specific antimicrobial (22). It is also safe for use because there is no reported allergic, or known fungal resistance to natamycin, also it has no negative effect on the sensory attributes such as taste, flavor or color of the food product (22). Natamycin is also very easy to apply in foods, chemically stable and known to remain on the surface with no migration into the food when applied onto the surface, hence making it very safe for consumers and limiting its concentration in the food product whiles making it a very effective treatment against the growth of molds which usually occur on food surface (22). In cheese production where natamycin has

been applied for surface treatment the penetration depth of natamycin has been determined to be approximately 1-4 mm into the food (22). Due to its natural source, chemical stability, prolonged activity period, and broad range of activity at low, neutral and high pH as well as its high efficacy at low concentrations, it a very cost effective natural preservative in industry (22). This diversity in its range of pH activity also permits easy applications in new formulations of food products.

2.5.3 Spectrum of Activity of Natamycin

Jacques (1999), report the sensitivity of most molds to natamycin as being lower than 10 ppm (generally $\leq$ 5 ppm), with that of yeast species being even lower making them more sensitive to natamycin (22). This enhanced sensitivity of yeast and molds to natamycin is important because only dissolved natamycin exhibits antifungal activity (22). The solubility of natamycin which Jacques (1999), state as 40 ppm, implies that this heightened sensitivity to natamycin is desirable because in most cases there would be sufficient quantities of the dissolved active form of natamycin present in a product to inhibit fungal yeast and mold growth (22).

The minimum inhibitory concentration of natamycin to some molds: *Aspergillus and Penicillium species* including *A. niger*, *A. flavus, P. expansum* and *P. camemberti* amongst others as well as *Cladosporium cladosporioides, Mucor racemosus,* and *Wallemia sebii* was reported by Jacques (1999), as $\leq$ 5 $\mu g ml^{-1}$, some key yeast species cited including *Aspergillus oryzae*, *Aspergillus versicolor*, *Fusarium spp*., *Penicillium roqueforti, Rhizopus oryzae*, and *Scopulariopsis asperula* were reported to have a

minimum inhibitory concentration of $\leq 10\ \mu g ml^{-1}$, and that of *Penicillium discolor* was given as $\leq 20\ \mu g ml^{-1}$ (22).

2.5.4 Mechanism of Action for Natamycin

Jacques (1999), give a possible 'action-interference' mode of action of natamycin, as the most important reason for the resistance free antifungal action of natamycin on fungal cells (22). This mechanism is due to natamycin's ability to bind to Ergosterol (a major compound in fungal cell membranes), resulting in cellular disintegration and subsequent leakage of cellular materials out of the fungal cell membrane (22). In vitro laboratory experiments where reduced levels of Ergosterol was induced in mutant strains of. *Aspergillus sp* and *Candida* species which cannot survive in nature, revealed a resistance to the antifungal action of natamycin (22).

An additional mode of action; a "single-hit" theory involves the indefinite existence of micelles of polyene antimycotics formed from natamycin in very dilute aqueous solution that enhances the chance of contact between these micelles and fungal cells in solution (22). It is assumed that the concentration of polyene around the cell is always higher and hence the antifungal property of natamycin is effected and the cells die, in the absence of this "polyene-fungal" contact, the fungal cells survive (22).

2.5.5 Regulatory Approval for Natamycin use in Foods

Like all other food additives and preservatives its application is regulated under different laws in different countries. In the United States, it is Generally Recognized as Safe (GRAS), by the Food and Drugs Administration (FDA). An Acceptable Daily

Intake (ADI) of 0.3 mg/kg body weight per day (22), was approved by a Joint Expert Committee on Food additives (JECFA) of the Food and Agricultural Organization and World Health Organization (FAO/WHO) after reviews in 1968, 1976 and 2002, making it a recognized, acceptable and safe food preservative in many countries (1). It is safe because of the impossibility of reaching the ADI, even when extreme quantities are ingested because of the low concentrations needed to effect antifungal action in foods (1). In Europe natamycin is generally considered safe; E-235 in the European Union (EU25) (1).

The first part of this project evaluated the shelf-life and microbial growth of freshly pasteurized commercial hummus of four formulations: T1, T2, T3 and T4: (pH 4.12, 4.27, 4.45 and pH 4.43 with Natamax® (Natamycin) respectively) at 20° C and 4° C. In addition, fungal strains isolated from post-shelf like packages of the hummus obtained from the manufacturer were added into the freshly prepared pasteurized hummus at low numbers to perform a challenge study at 20° C and 4° C.

The published literature review for the second part of this project focused on environmental food safety evaluations of kitchen surfaces is summarized below.

2.6 Microbial Assessment of Kitchen Surfaces

A substantial number of studies have being conducted to investigate the existence of pathogenic microbial contaminants in the home environment, Finch *et al* (1978), is accredited for the first of such studies to extensively evaluate bacterial contamination in domestic environments and homes (11). Finch *et al* 1978, revealed that coagulase negative, gram positive cocci and *Bacillus sp*, could thrive in both the wet and dry

environments in the home, with the wet areas such as the kitchen sink and drains harboring the most numbers of *Escherichia coli* and sometimes *Klebsiella pneumoniae, Citrobacter* and *Enterobacter sp,* on the contrary to, what would have been expected, the samples taken from the toilet environment had the least fecal contaminant counts (11).

Subsequently few studies have been conducted in the United States to specifically evaluate the prevalence of indicator bacteria such as coliforms and other pathogenic bacteria in domestic or residential kitchens, key among such studies was one done by Josephson et al (1997), which evaluated the effect of the use of either antibacterial disinfectant and regular disinfectants on the growth and survival of pathogenic and indicator bacteria in some domestic kitchens in the United States (12). Key findings of the study suggested the importance of the use of antimicrobial disinfectants or cleaners in cleaning the kitchen environment and utensils, in addition to the need for regulated cleaning regiments involving the proper application of these disinfectants on a regular basis in cleaning contaminated surfaces (12). Kitchens in which cleaning was being done without antimicrobial disinfectants were shown to be contaminated with pathogens including food borne pathogens such as *Escherichia coli*, (16.7% of all sink surfaces and 33.3% of all sponge samples taken) (12). In particular, samples taken out of 63% of sink and 67% of dish sponges sampled in these kitchens exhibited high concentrations of fecal coliform bacteria contamination (12). This study also demonstrated the need for consistent and regulated or targeted use of antimicrobial disinfectants in cleaning the kitchen environment, soon after contamination of the surface occurs, in order to obtain a desired reduction in the growth and survival of pathogenic microorganisms (12).

Although raw foods such as chicken are thought to be the main source of pathogenic contaminants in kitchens, both wet and dry kitchen surfaces such as dishcloths, dish sponges, cutting boards and refrigerator handles potentially harbor pathogenic bacteria (12, 20). A significant (> 99.9%), decrease in the microbial load of dish sponges or dish cloths was observed when soaked in hypochlorite (bleach) solutions for 5 minutes (10). However the efficacy of a detergent or bleach solution in reducing microbial load in used kitchen sponges, can be compromised or reduced by the presence of food particles or other decontaminating agents such as grease (13).

In the United States food borne diseases and infection continue to be a primary public health challenge, in 2011 the Centers for Disease Control and Prevention (CDC) reported an estimated 9.4 million foodborne illnesses; with 55,961 hospitalizations and morbidities and 1,351 deaths (17). Although there are numerous data on the incidence of food borne diseases, all these published literature are estimations or probable numbers, due to the underreporting of these illnesses, hence the numbers though may seem alarming as-is, may actually be way more (15). The reason for this underreporting has mainly been due to the fact that many of the food borne diseases, originating from domestic settings such as homes occur infrequently with no set patterns or trends in their occurrence (15). Another reason of this underreporting is also because they frequently go unnoticed by public health officials because they involve small groups of people at a time so they are given very little attention (15).

On the contrary, large food facilities such as cafeterias and restaurants are the primary public health focus and concern in the investigations of the origins of the food eaten in food borne disease outbreaks (15). However it may be of interest to pay closer

attention to foods consumed in private homes and domestic settings, as being a major contributor or source of food borne diseases in the United States especially in homes that have a lot of children who may be adversely affected by food related illnesses or may themselves be the agents for spreading the food borne pathogens due to unhygienic handling of foods prior to consumption, since published literature has shown that foods consumed in private homes are three times as likely to be the cause of food borne illnesses as compared to foods consumed in cafeterias (15). Majority of domestic food borne disease outbreaks in the past, have been caused be *Salmonella sp.* (15, 18).

The need to study the domestic environment as a potential source of food borne illness, especially in large domestic settings such as group homes, like the Residential Child Care Institutions we studied, is important because of various reasons, key amongst these reasons is the fact that the majority of the occupants in these homes are children who in some cases can be considered as immuno-compromised and as such very susceptible to food borne disease. The other reason according to Redmond and Griffith (2003), is the fact that in a home setting especially in large homes, a lot of pathogenic and nonpathogenic bacteria are continually brought into the home by the activities of its occupants, such as the humans and pets in addition to those of airborne origin (4, 15). This situation is even more pronounced when in some instances some domestic kitchens serve as laundry rooms, as well as the dining area and living spaces for pets (8). However studies have suggested that proper hygiene plans if efficiently and accurately implemented can eliminate the risk of food borne disease transmissions (12, 15).

CHAPTER 3

OBJECTIVES

The specific objectives of the hummus study are:

1) To determine the shelf life of un-inoculated hummus of four different formulations, manufactured at pH 4.12, 4.27, 4.45 and at pH 4.43 with the addition of Natamax®, stored at 4° C and 20° C.

2) To perform a challenge study of the four formulations of hummus (pH 4.12, 4.27, 4.45 and at pH 4.43 with the addition of Natamax®), inoculated with fungus at an initial concentration of 100 cfu/g, stored at 4° C and 20° C overtime.

3) To determine if accelerated shelf life testing at 20° C accurately predicts the trend observed over 12 weeks shelf life at 4° C storage.

Whereas the primary aim of the RCCI pilot study was:

4) To collect microbiological evidence in support of the need for food safety training, to assist Residential Child Care Institution (RCCI) personnel, especially those manning smaller RCCIs with less than 20 residents, with limited resources, to develop and implement a HACCP-based food safety plan as required by Section 111 of the Child Nutrition and WIC Reauthorization Act of 2004 (Public Law 108-265).

CHAPTER 4

HUMMUS SPOILAGE STUDY

4.1 Introduction: Hummus Spoilage Study

Most traditional foods like hummus were initially developed without specific recipes through trial and error by the indigenes; that lacked scientific food processing knowledge. These foods once prepared, were intended for immediate consumption or consumption within a relatively short time. The lack of detailed documented scientific knowledge of the processing or documented handling of these traditional foods by the indigenes, complicates industrial attempts to successfully produce these foods. Hence industrial production of these foods, rely heavily on continual research and development to improve their product and make it comparable to the traditionally processed ones that consumers are used to and expect from the store bought products as well.

Hummus is one such traditional food native to the Eastern Mediterranean Region which has now become a very popular food of choice among consumers of diverse backgrounds all over the world. The United States is one such country in which commercially produced hummus is becoming a viable industry, but with this comes the challenges of shelf-life extension. Typically only a minimal heat treatment is applied in the preparation of hummus (except for the boiling of the chickpeas, no other heat treatment is applied) (25). Hence most commercially produced hummus may only be considered as pseudo pasteurized products, because it is impossible to attain commercial sterility through this minimum thermal processing (14). As such there is the possibility of

different types of microorganisms thriving in hummus (a high water activity food), despite its low pH (mean pH of about 5.1) (24).

To achieve very low microbial loads in the final hummus product, industries endeavor to reduce the microbial load of each ingredient, in addition to implementing sanitary practices like Good Manufacturing Practices (GMP). For this reason industrially produced hummus which is not intended for immediate consumption is generally kept safe and the rate of microbial growth reduced via refrigeration, in addition to the addition of preservatives. However in the event that the product (hummus) experiences temperature fluctuations, resulting in higher temperatures than the recommended refrigeration temperatures in the supply and distribution chain, microbial growth may be intensified and hence spoilage may occur rapidly prior to the estimated expiry date of the product. The high probability of this food safety issue occurring, coupled with the demands of consumers for minimally processed ready to eat foods that are "all natural"; without added chemical additives and most importantly the need for food industries to comply with regulatory agency specifications, makes shelf life studies a very important process in food formulations and production.

Because of this relatively high susceptibility of minimally processed foods such as hummus to microbial spoilage shortly after production, food industries producing these ready to eat, traditional foods on commercial basis, have designed and implemented preservation techniques to maintain freshness and quality beginning from the point of raw material sourcing, through storage, processing, packaging and distribution. However the effectiveness of any food safety method or set of techniques such as a Hazard Analysis and Critical Control Point (HACCP) plan to control spoilage of a particular food

type would depend largely on knowledge of the mechanisms and types of spoilage involved; be it spoilage caused by bacteria or yeast and molds and their association with the food medium (9). Thus the understanding of the interaction of key factors in foods such as pH and acidity, functional ingredients such as; additives and preservatives, as well as external factors like temperature, on the microbial load of the food throughout its shelf life is essential in order to innovate, formulate, adapt and implement more effective strategies for controlling microbial deterioration of minimally processed foods such as hummus over their shelf life.

The economic effect of yeast and mold infestations in the food industry cannot be overlooked, because despite enormous that efforts many modern industries put into place to control the growth of these fungi, such as GMP's and HACCP to ensure product sterility, food spoilage still may occur. Hence the most effective way of controlling food spoilage may be by the addition of preservatives to the foods and natamycin (Natamax®) is one such readily available, natural, generally recognized as safe (GRAS) antifungal, which may be applied to foods to prevent yeast and mold growth.

The main objective of this hummus spoilage study was to conduct a challenge study of a variety of formulations of hummus, produced at a higher pH than is currently manufactured, along with the addition of a natural preservative and antifungal (Natamax®).

4.2 Materials and Methods: Hummus Spoilage Study

4.2.1 Expired Hummus Micro flora Sampling

The spoilage cultures for this study were isolated from industrially produced expired hummus of a brand commercially available in supermarkets in the United States of America was obtained from the factory. These samples had been stored under recommended temperatures for a period of three weeks past their use by dates. Each of the five expired product tubs was transported refrigerated to the laboratory. Each tub was visually evaluated to characterize the spoilage (degree, type and extent) and images were taken with a Kodak EDAS 290 (Eastman Kodak Company, Rochester, NY).

The product seal integrity was also examined and bulging due to gas production was also visually evaluated, pH readings were also taken per sample type. From each expired hummus tub, samples of visible fungal spoilage that were selected based upon differing shape, size and/or color were taken for isolation of yeast and molds and were also evaluated for bacteria. Other parts of the product that showed no visible spoilage were also sampled for microbial isolation.

Isolation was done by inoculating (streaking) individual microbial colony samples onto each of these three agar media plates in triplicates: Trypticase Soy Agar (TSA) (Difco, Detroit, MI) for cultivation of general heterotrophic microbes; Tryptone Glucose Yeast Extract Chloramphenicol (TGYC) agar or Plate Count Agar supplemented with 100 mg/L chloramphenicol (PCAC) for growth of yeast and molds, and *Lactobacillus* Selective Agar (LBSA) (BBL, Cockeysville, MD), for the cultivation of Lactobacilli. TSA and LBSA plates were incubated for 24 h at 32° C and 35° C respectively, prior to observation and PCAC plates were incubated for 4 days at 20° C.

Single colony isolates of the different types of microorganisms initially isolated from the expired products were streaked onto the same type of slanted agar media plates made from the three different types of media used previously, and incubated as before, to be used as pure inoculum specimens for the actual challenge study.

4.2.2 Preparation of Inoculated Hummus - Experimental Design

Freshly prepared packages of hummus of varying compositions (a total of four differing in pH and/or addition of a preservative) were transported cold to the laboratory from the factory. Each formulation was designated as T1, T2, T3 and T4 (Table 1). Two batches were setup for testing. Batch 1 consisted of tubs of each formulation that was aseptically inoculated with a fungal mixture to 100 CFU/g. Batch 2: consisted of intact hummus samples as received. The two batches were further divided into storage temperatures: 20^{o} C (accelerated testing) and 4^{o} C (recommended storage temperature). The key variations in the four different hummus formulations are presented in Table 1, below.

Table 1. Hummus pH and presence of Natamax

Formulation Code	Presence of Natamax	Day 0 Manufacturer pH readings	Day 0 Laboratory pH readings
T1	No	4.12	4.26
T2	No	4.27	4.41
T3	No	4.45	4.57
T4	Yes	4.43	4.67

4.2.3 Preparation of Fungal Inoculums

A total of 4 fungal isolates were isolated from the expired sampled hummus. These cultures were grown for 3 days at 20^{o} C and maintained on individual PCAC slants stored at 4^{o} C. To prepare the inoculums, 1.5 mL of 0.1% buffered peptone water (BPW), was aseptically added onto each of the four slants of unique colonies of fungi and with a sterile loop, the mycelia was gently scraped off the surface of the slant media into 0.1% BPW diluents. To prepare the inoculum solution, each fungi solution was added to a test tube and mixed using a vortex, speed 5 for approximately 30 seconds. The cell number of the inoculums solution was determined using a direct microscopic count in a hemacytometer (Buffalo, N.Y. Hausser Scientific, Horsham PA).

To introduce the fungal mixture (inoculum) into the hummus, the freshly prepared intact hummus containers were placed into a biological safety hood, and the plastic liner or seal was carefully sliced along one edge and 0.5 mL volume of the 10^{-6} dilution of inoculums stock was added to obtain the target inoculation concentration level of 100 CFU/g of hummus. The product was then stirred with a sterile spatula and the plastic lid was replaced tightly onto the package. A total of 288 packages were inoculated, 216 were incubated at 4° C and 72 were incubated at 20° C.

To confirm the concentration or cell numbers inoculated into the hummus, based on the direct microscopic count results, serial dilutions from each homogenized stock sample obtained was made with 0.1% BPW and dilutions were spiral plated (23), using both the 50 µl and 100 µl exponential plating mode per sample on an Autoplate 4000 spiral plater (Spiral Biotech Inc. Bethesda, MD) onto PCA + 0.1 gL^{-1} chloramphenicol (PCAC) agar media plate and incubated for 4 days at 20° C (23).

4.2.4 Microbial Evaluation of Hummus Samples

Analysis to determine mold counts was performed on the intact hummus and inoculated hummus on day 0 of the study to validate the level of inoculation, in our inoculated samples. For inoculated samples, only fungal counts were performed at both incubation temperatures (4° C and 20° C) with testing at 0, 2, 4, 6 and 10 days for the 20° C samples and 0, 2, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 and 84 days for the 4° C samples. For uninoculated samples, levels of fungi and bacteria (SPC and *Lactococci* count) was performed at 0, 2, 4, 6 and 10 days for the 20° C samples and 0, 2, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 and 84 days for the 4° C samples.

On each test day, 3 tubs of each formulation (T1, T2, T3 and T4) of each treatment (inoculated or uninoculated) were taken for analysis. From each storage temperature, 12 inoculated (I) samples (original plastic seal broken) and 12 uninoculated (U) samples (sealed intact samples) were analyzed for each temperatures. Prior to sampling contents of each tub was thoroughly mixed and 5 g of each sample weighed into sterile polypropylene conical tubes (Falcon®, Becton Dickinson Labware, and N.J.). A volume of 45 mL of sterile water was added to the 5 g sample to attain a 10^{-1} dilution. Overtime, when needed additional serial dilutions were performed in sterile water for the plating. Contents of each tube were vortexed for 30 s at speed 5 to homogenize the samples before plating. Duplicate samples were plated from each tube, per media type used.

To prevent carryover of contaminants in the spiral plater, all uninoculated samples were plated before the inoculated samples and the spiral plater was disinfected with 70% alcohol and 10% bleach solution within rinses during plating. Also as a precaution,

before plating samples the sterile water used as diluents (water control) was plated on a TSA media to validate its sterility.

4.2.4.1 Fungal Plate Count

Homogenized suspensions of the hummus samples obtained via serial dilutions as described above were plated. When necessary serial dilutions were made with 0.1% BPW prior to spiral plating, using both the 50 µl and 100 µl exponential plating mode per sample on an Autoplate 4000 spiral plater (Spiral Biotech Inc. Bethesda, MD) onto PCA + 0.1 gL^{-1} chloramphenicol (PCAC) agar media plate and incubated for 4 days at 20°C (23). Colony enumeration was done using a QCOUNT™ Automated Colony Counting System (Spiral Biotech Inc. Bethesda, MD) (23). Cell numbers were reported as colony forming units per gram (cfu/g) of hummus.

4.2.4.2 Standard Plate Count and *Lactobacilli sp*. Enumeration

This was both achieved by plating the above dilutions onto TSA and LBSA media and incubating the plates for 24 h at 32° C and 35° C, respectively prior to enumeration with the Q-count.

4.2.4.3 Accelerated Shelf Life Studies (Samples Stored at 20^{o} C)

Samples of each treatment from each batch stored at 20^{o} C were analyzed after 2, 4, 6 and 10 days, of incubation to evaluate changes in bacterial, and yeast and mold counts during storage.

4.2.4.4 Recommended Storage Shelf Life Studies (Samples Stored at 4^{o} C)

Samples of each treatment from each batch stored at 4^{o} C was analyzed after 2 and 7 days and on a weekly basis thereafter: week 2 or 14 days, week 3 or 21 days, week 4 or 28 days week 5 or 35 days, week 6 or 42 days, week 7 or 49 days, week 8 or 56 days, week 9 or 63 days, week 10 or 70 days and week 12 or 84 days of incubation to evaluate changes in bacterial, and yeast and mold counts during storage. Analysis for week 11 or 77 days was not performed.

4.2.5 pH Reading (After Plating)

The pH of each sample type was taken from the first 1:10 dilution (10^{-1}) sample after it was utilized in the spiral plating procedure to avoid contamination from the pH reading procedure. Standard protocols for pH determination via immersion pH (Accumet® Basic AB15 pH meter, Fischer Scientific) were followed and all products to be analyzed we allowed to equilibrate to approximately room temperature prior to sampling. Contents of each dilution test tube were thoroughly mixed again prior to pH assessment. pH readings were done in the order of treatment 1 to 4 (lowest to highest initial pH), for both uninoculated and inoculated samples and the pH reading apparatus was thoroughly disinfected with ethanol and rinsed with sterile double distilled water and recalibrated in between readings.

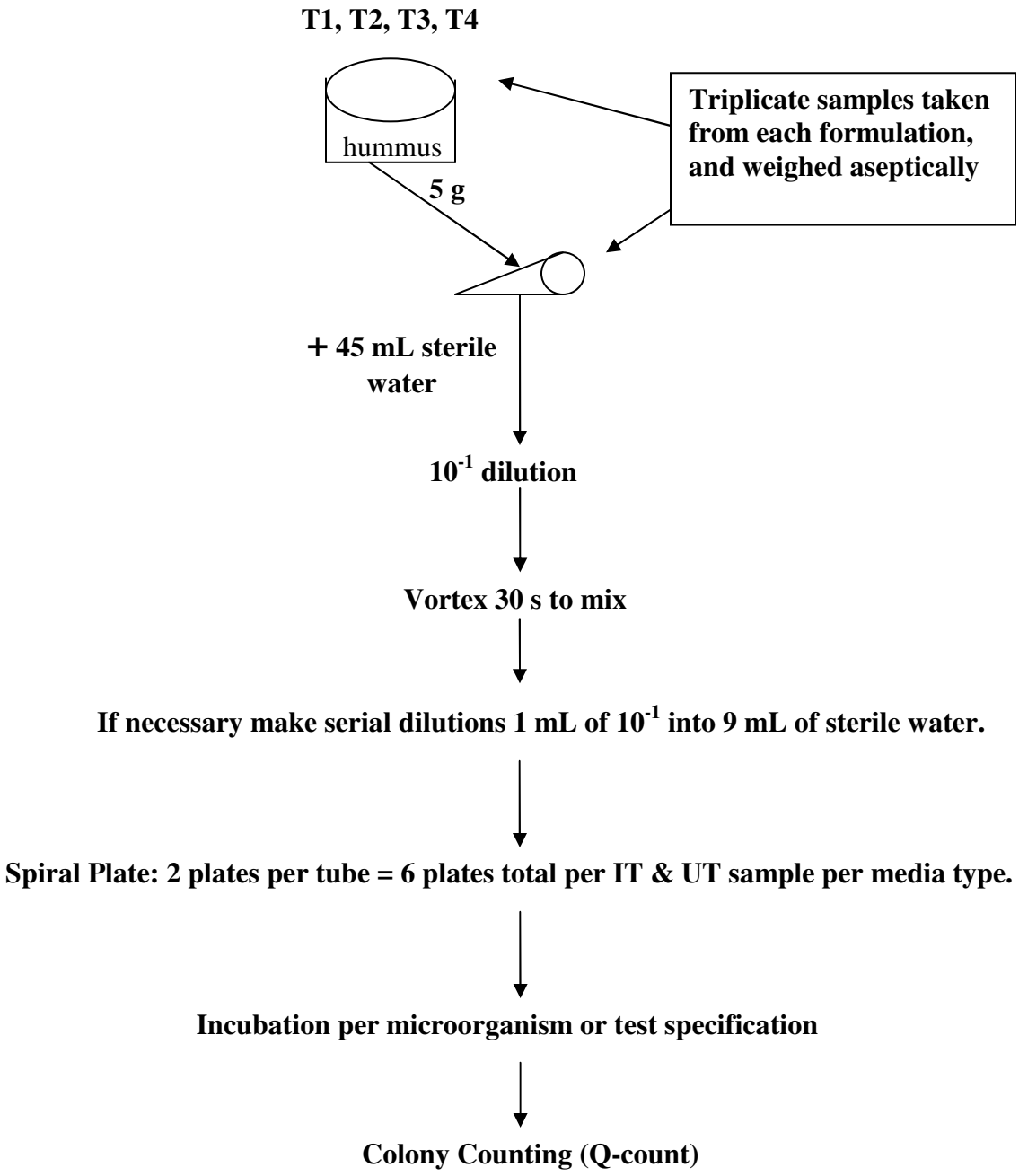

Figure 1. A flow chart illustrating the hummus sampling procedure.

4.3 Results and Discussion: Hummus Spoilage Study

4.3.1 Sampling of Expired Hummus for Spiking Inoculated Samples

Four different mold species were identified. Microscopic identification of the yeast and mold was attempted and mold samples were identified as probable strains of *Penicillium sp.* Slants were stored at 4° C for use later in the actual challenge study.

4.3.2 Inoculation of Hummus

The direct microscopic count (DMC), counting both spores and mycelium pieces in the inoculum using gave the concentration of the inoculum as $4.4X10^4$ DMC/ml. Therefore 0.5 ml of the 10^{-6} dilution of the stock inoculums was added to 8oz (or approximately 226.79 g of hummus), to obtain approximately 97 cfu/g of fungi in each inoculated tub inoculated. From our plate counts, we found our initial inoculation level to be 9.9×10^1 cfu/g which corresponded to the DMC, hence we were able to inoculate our sample at the targeted levels of 100 CFU/g.

4.3.3 pH Readings at the Time of Sampling

During manufacturing of the hummus, the pH of each batch was measured in the factory. Upon receipt of the product, we then measured the pH at Day 0 in the laboratory. In general, the pH readings in the laboratory were higher than measured during manufacturing. This variation in pH measures could potentially be due random measurement errors related to the instruments used for at each location.

The pH of the inoculated samples which were stored at 20° C holding temperature remained fairly stable, within 0.01-0.08 below of the pH reading taken in the laboratory

on Day 0 (Table 2). However, the pH of two of the uninoculated samples increased over the day storage at 20° C (Table 3), with the greatest increase in T4 (increase from pH 4.67 to pH 5.21), and a 0.1 unit increase in the pH of formulation T1 (from pH 4.26 to 4.36) and Formulations T2 and T3 (decrease of 0.05 and 0.07, respectively) over the 10 day incubation period (Table 2 and 3).

For samples stored at 4° C, in general, the pH levels in the inoculated and uninoculated samples were very similar (Tables 4 and 5). No notable change in pH was observed in formulations T2 and T3; the pH change after 84 days of storage was within hundredths (0.03-0.08) of the initial pH reading. For sample T1, there was little observed pH change in the uninoculated samples at 4° C (0.03 decrease in pH after 84 days), but a greater decrease in pH was observed in the inoculated samples (decrease of 0.12, from pH 4.26 to 4.14). The greatest change in pH was observed at 4° C for the T4 formulation; with both the inoculated and uninoculated samples the pH had increased by 0.3-0.4 pH units by day 84 (from pH 4.67 to 5.15 in the uninoculated samples and from 4.67 to 5.03 in the inoculated samples). The observed differences in pH changes between the uninoculated and inoculated samples, may have possibly been as a result of biochemical changes in the product due to the presence or absence of substantial microbial activity.

Table 2. pH readings of inoculated samples stored at 20^{o} C

Formulation Code	Factory pH readings DAY 0	Laboratory measured pH readings DAY 0	DAY 2	DAY 4	DAY 6	DAY 10
T1	4.12	4.26	4.21	4.18	4.21	4.23
T2	4.27	4.41	4.37	4.37	4.33	4.37
T3	4.45	4.57	4.54	4.54	4.51	4.53
T4 + Natamax	4.43	4.67	4.61	4.58	4.57	4.66

Table 3. pH readings of uninoculated samples stored at 20^{o} C

	Factory pH readings	Laboratory pH readings				
Formulation Code	DAY 0	DAY 0	DAY 2	DAY 4	DAY 6	DAY 10
T1	4.12	4.26	4.3	4.20	4.27	4.36
T2	4.27	4.41	4.43	4.33	4.4	4.36
T3	4.45	4.57	4.62	4.53	4.6	4.64
T4 + Natamax	4.43	4.67	4.67	4.53	4.63	5.21

Table 4. pH readings of inoculated samples held at 4^{o} C

	Formulation			
Day	T1	T2	T3	T4
	pH measured in factory after manufacturing			
0	4.12	4.27	4.45	4.43
	pH measured in laboratory			
0	4.26	4.41	4.57	4.67
2	4.25	4.41	4.56	4.64
7	4.18	4.31	4.55	4.57
14	3.98	4.1	4.25	4.35
21	4.18	4.31	4.53	4.56
28	4.12	4.3	4.48	4.47
35	4.16	4.28	4.5	4.59
42	4.07	4.25	4.43	4.47
49	4.35	4.39	4.48	4.69
56	4.1	4.27	4.52	5.16
63	4.2	4.28	4.61	5.1
70	4.12	4.21	4.58	5.21
84	4.14	4.25	4.6	5.15

Table 5. pH readings of uninoculated samples held at 4^{o} C

	Formulation			
Day	T1	T2	T3	T4
	pH measured in factory after manufacturing			
0	4.12	4.27	4.45	4.43
	pH measured in laboratory			
0	4.26	4.41	4.57	4.67
2	4.25	4.38	4.57	4.63
7	4.2	4.34	4.56	4.53
14	3.87	4.03	4.25	4.35
21	4.31	4.41	4.59	4.66
28	4.3	4.33	4.51	4.55
35	4.37	4.36	4.62	4.63
42	4.33	4.4	4.58	4.6
49	4.11	4.35	4.51	4.56
56	4.29	4.35	4.57	5.03
63	4.23	4.36	4.48	5.0
70	4.21	4.32	4.51	5.1
84	4.23	4.34	4.49	5.03

4.3.4 Inoculated Hummus stored at 20°C

Only fungi counts were performed on inoculated samples, the results are presented in Fig. 2. For inoculated samples at 20° C, formulation T1 with the lowest pH appeared to have the slowest rate of growth. Formulations T3 and T4 were identical, except for the addition of Natamax an fungal inhibitor in T4, however, the growth in these two formulations at 20° C were similar. This may have been caused by a number of factors. It is most likely that the high level of inoculums (100 CFU/g) may have been too great to achieve effective fungal inhibition, although it is possible that Natamax is chemically interacting with components of the hummus rendering it less effective, or the antimicrobial action of Natamax may be less effective at elevated incubation temperatures. The latter explanation is unlikely, since a similar effect was observed with inoculated samples at 4° C (Fig 5).

4.3.5 Uninoculated Hummus stored at 20° C

In uninoculated Hummus stored at 20° C, Standard Plate counts (SPC), yeast and mold counts and *Lactobacillus* counts were performed and can be seen in Fig 3 and 4. Even at 20° C for 10 days, very little fungi were detected, and only a slight increase was observed by day 10 (Fig 3). At 20° C, the numbers of bacteria increased to 10^6 CFU/g by day 10 (Fig 4). Bacterial growth was seen at day 4 in sample T4 and at day 6 in sample T3 (higher pH samples), with samples T2 and T1 showing the lowest SPC growth rate, most likely due to the lower pH of these formulations. These results show that Natamax® is not efficient against the inhibition of bacterial which was observed by Stark, (1999). The variation in microbial growth could also be an indication of random variations in

bacteria growth rate, within the stored samples due to the influence of factors such as air flow limitations due to stacking during storage. No *Lactobacillus* were detected over the 10 day course of the study, indicating that numbers were less than 9.9×10^1 cfu/g (results not shown).

4.3.6 Inoculated Hummus stored at 4° C

Only fungi counts were performed on inoculated samples at 4° C and the results can be seen in Fig. 5. Initially growth in formulation T4 (with Natamax) was slightly delayed compared with formula T3, with increasing cell numbers not detected until day 20, but by day 40 both T3 and T4 had fungi counts. There is a possibility of the effectiveness of Natamax being decreased due to the fact that the 4^o C samples were stored in a lighted refrigerator (20° C samples were stored in a dark refrigerator), however this would be similar to a lighted retail setting. At the end of the study (day 84), fungal counts in T1 and T2 were ($3.63X10^4 \pm 8.14X10^3$ standard deviations and $2.67X10^4 \pm 1.43X10^4$ standard deviations respectively) lower than those found in T3 and T4 ($8.17X10^5 \pm 6.16X10^5$ standard deviations and $1.04X10^6 \pm 1.45X10^6$ standard deviations respectively).

4.3.7 Uninoculated Hummus stored at 4° C

In uninoculated Hummus stored at 4° C, Standard Plate counts, yeast and mold counts, and *Lactobacillus* counts were performed and can be seen in Fig 6 and 7. No *Lactobacillus* was detected over the 84 day course of the study, indicating that numbers were less than 9.9×10^1 CFU/g (results not shown). Formulations T2, T3 and T4 showed

similar effectiveness in inhibiting fungal growth over the first 42 days of the study (Fig 6). Formula T1 had high fungal counts over days 14-54, but later decreased (Fig 6), this erratic growth pattern may be a reflection of a random contamination levels in the packages during manufacture rather than actual growth/death of organisms during storage or possibly due to higher bacterial contamination levels in formula T1 compared to the other formulations. As was observed at 20° C, once again bacterial counts were higher in formulation T4 containing Natamax. Acidic formulations T1 and T2 were most effective in inhibiting bacterial growth (Fig 7). Yamani (1994) cited the suitability of hummus as a very good medium for microbial growth due to its high content of sugars (available carbon source) and other nutrients that favor the growth of microorganisms (25).

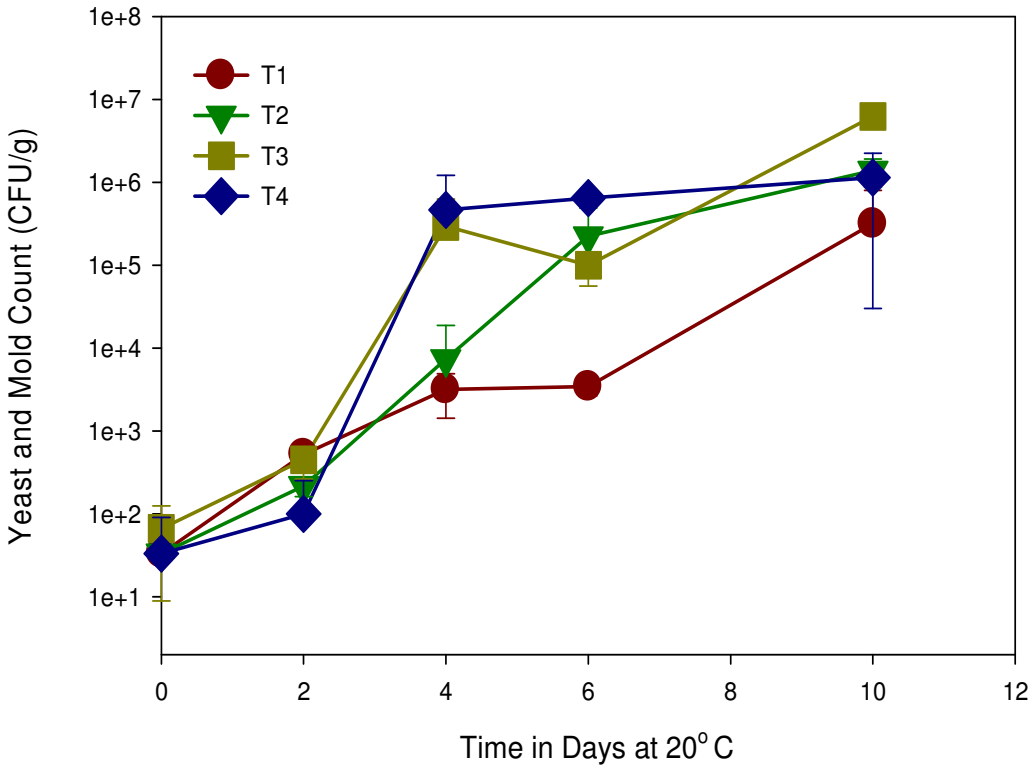

Figure 2. Growth of Yeast and Molds in inoculated hummus stored at 20° C over a 10 day period. Initial levels in hummus were approximately 100 cfu/g. T1, T2, T3 and T4 represents the four different hummus formulations.

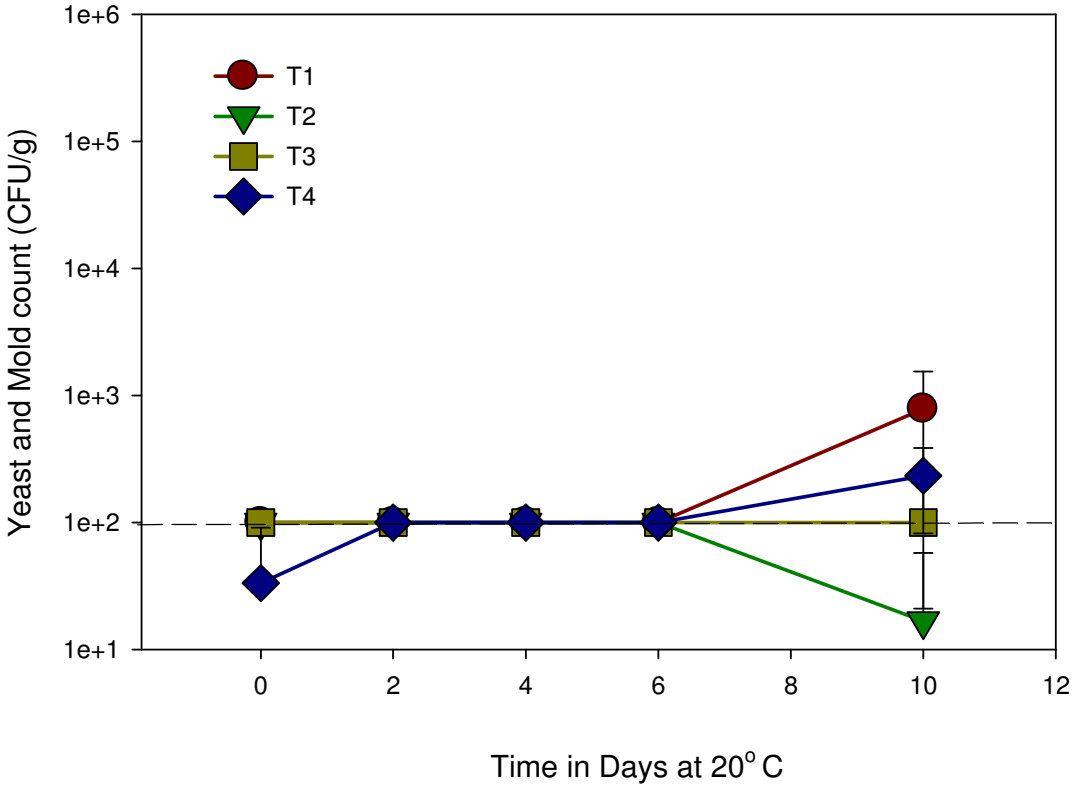

Figure 3. Growth of Yeast and Mold in uninoculated hummus, stored at 20° C over a 10 day period. T1, T2, T3 and T4, represents the four different hummus formulations.

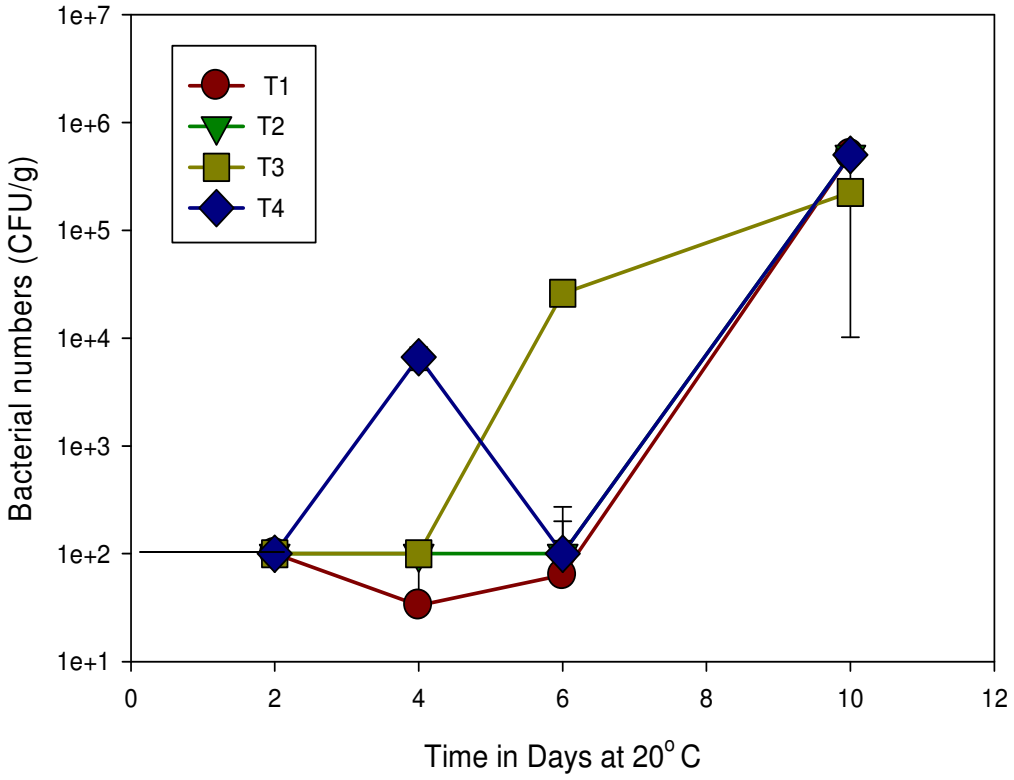

Figure 4. Bacteria growth in uninoculated hummus stored at 20° C over a 10 day period. T1, T2, T3 and T4, represents the four different hummus formulations.

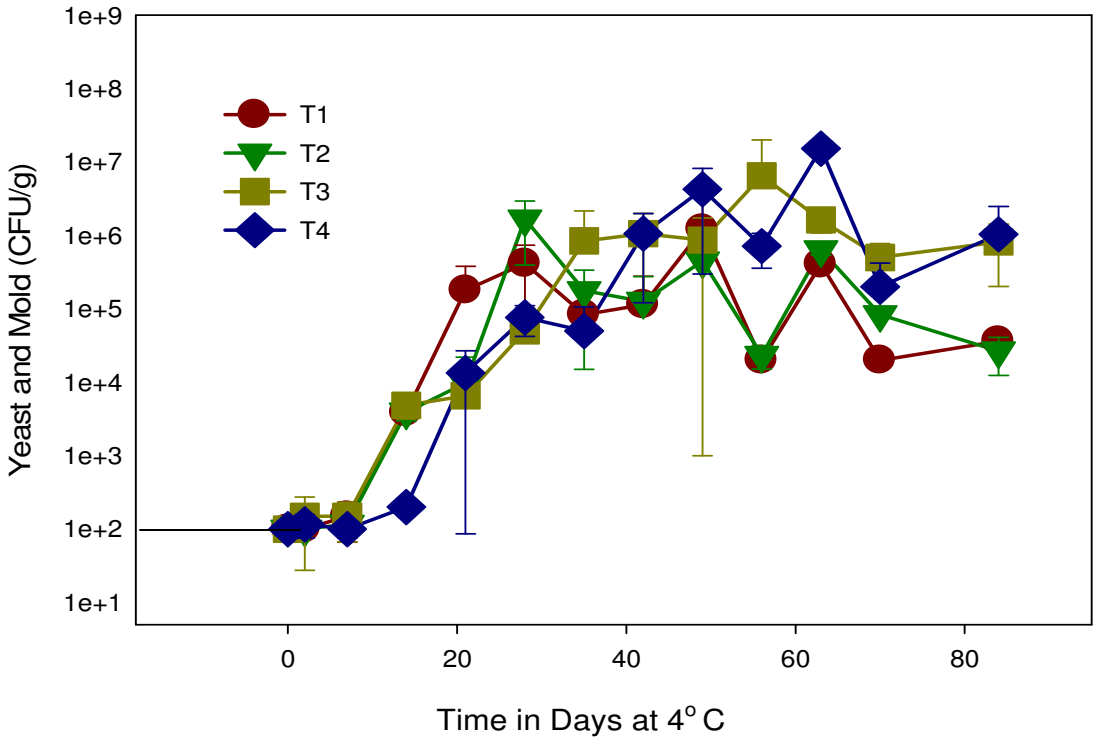

Figure 5. Growth of Yeast and Molds in inoculated hummus stored at 4° C over an 84 day (12 weeks) period. T1, T2, T3 and T4, represents the four different hummus formulations.

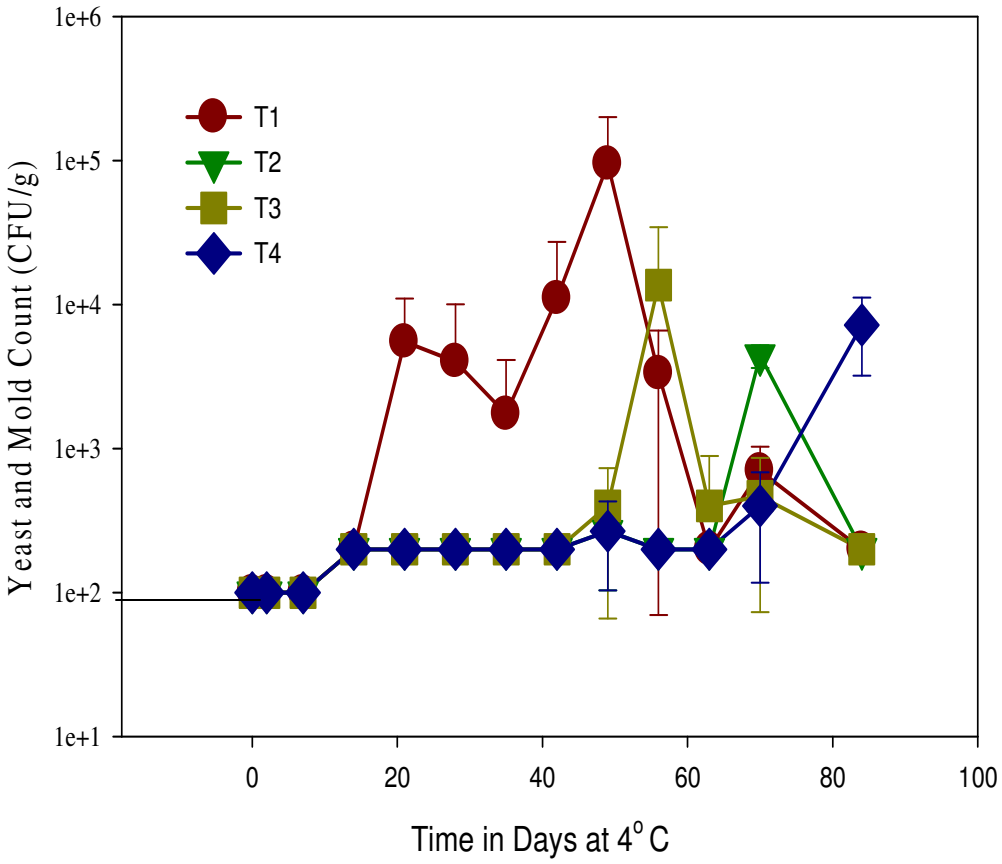

Figure 6. Growth of Yeast and Molds in uninoculated hummus stored at 4° C over an 84 day (12 weeks) period. T1, T2, T3 and T4, represents the four different hummus formulations.

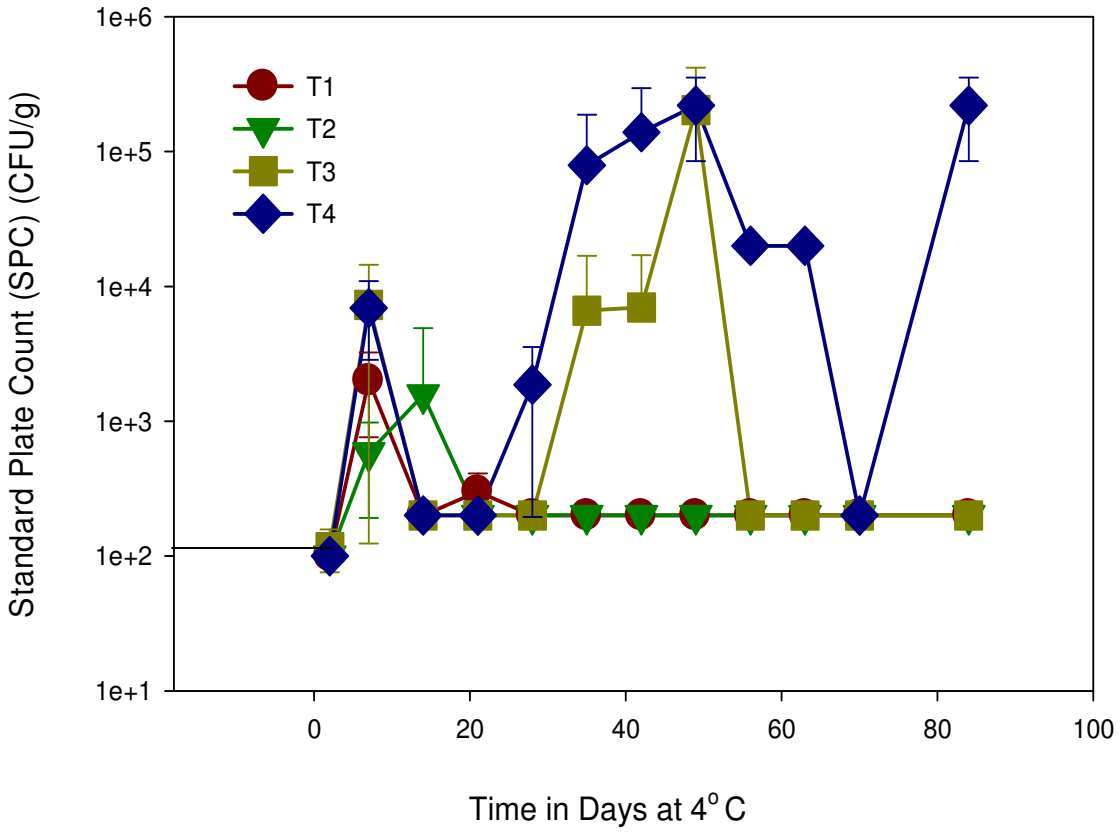

Figure 7. Growth of bacteria in uninoculated hummus stored at 4° C over an 84 day (12 weeks) period. T1, T2, T3 and T4, represents the four different hummus formulations.

4.4 Conclusion: Hummus Spoilage Study

The slight deviations noted in the trends in the hummus graphs may be attributed to variations in product runs, as samples were taken from different products (3 packages) and composited for each analysis. The drum drying step used in processing this hummus may also have contributed to the variations in the microbial content of the different production batches and hence that may also have accounted for the slight deviations seen in our observed trends of microbial growth. These deviations could also have been due to the possibility of the initial microbial load of the raw materials used being high or varying, which could have limited the efficiency of the pasteurization process, to efficiently inhibit microbial growth in all batches of samples analyzed in a similar manner. However to avoid this issues the industry producing this hummus pasteurizes their hummus using the drum heater technique and it has a strict HACCP plan in place to ensure very high and consistent raw material quality is for all production batches. Therefore their fungal issues were likely due to contamination between pasteurization and packaging of the product.

It is also important to note that for a challenge study such as this, the accelerated storage approach where the samples were stored at 20^{o} C instead of the recommended 4^{o} C holding temperature does not give a true representation of changes that may develop in the product when held at recommended temperatures, hence future product development attempts should be done over the right time frame, simulating all recommended product handling specifications or mimicking consumer handling of the product to gain a proper understanding of factors influencing the potential threat of fungal spoilage.

Irrespective of storage temperature, similar microbial growth trends were observed for the T3 and T4 samples (which were very similar in pH and only differed based on the addition of Natamax to T4), hence, we were unable to conclude that Natamax® (natamycin) was an effective inhibitor against the growth of yeast and mold, the main spoilage micro flora in hummus. For this reason its addition to hummus as a preservative to ensure the food safety of hummus over this extended shelf life of 12 weeks is unnecessary, because similar microbial growth inhibition can be achieved via pasteurization coupled with a decrease in product pH. Proper product refrigeration temperature is also certainly essential for the inhibition of the growth of yeast and molds in industrially produced hummus with extended shelf lives, whether or not preservatives or additives are added to inhibit the growth of these microorganisms. Also important to note is that at high holding temperature such as was demonstrated, using products stored at 20^{o} C, bacterial growth in the hummus samples escalates dramatically over a relatively short period of time e.g. 10 days, which also emphasizes the need to refrigerate this product whether opened or unopened. However the low microbial loads in our samples initially indicated that these hummus samples were produced under very hygienic conditions with high quality ingredients and it also illustrates that the HACCP plan being used by the manufacturer is effective.

Our results also indicate that bacterial growth increased with increasing hummus pH, hence it is recommended that the pasteurized hummus product be formulated at the pH of the T1 sample ($pH \leq 4.0 \pm 0.2$) or slightly lower if possible, this would ensure effective fungal and bacterial growth inhibition in industrially produced extended shelf-life hummus. However, this reduction in pH will have to be done with caution, as the

manufacturer reports that very slight changes in pH significantly alters sensory properties of hummus such as taste and texture. Additionally hummus needs to be properly refrigerated throughout the production and delivery system, to ensure its wholesomeness and reduce spoilage due to growth of yeast and molds and bacterial, and subsequently alleviate any potential harm these microorganisms, if allowed to grow may cause to humans when ingested. Thus alleviating any food safety concerns associated with the shelf life extension of hummus; a minimally processed food.

CHAPTER 5

RESIDENTIAL CHILD CARE INSTITUTIONS (RCCI) PROJECT

5.1 Introduction: RCCI project

A common cleaning practice for home kitchen surfaces, involves wiping these surface with a sponge or dish cloth soaked with some aqueous disinfecting solution. Sponges commonly used in these practices are often the same sponges used in dish washing, and hence, this practice, if left unchecked, has the potential of making the dish sponges a good habitat for food borne pathogens, thus making them a source of or a vehicle for microbial cross-contamination. This situation becomes inevitable, especially since the efficacy of disinfecting solutions and antimicrobials could potentially be compromised by the presence of the food residues in these dish sponges.

The efficacy of the cleaning procedures can be further compromised by the improper application of detergents and antimicrobials in the cleaning procedures. Improper application may result from the order or sequence in which, either a disinfectant or detergent is applied in cleaning, or possible from bad timing in its application (inadequate dwelling time allowed for the disinfectant to work on the surface being cleaned), or most often than not simply from the quantity, strength or concentration applied.

There are different types of facilities that offer child care that may be classified as a Residential Child Care facilities, basically, these facilities primarily provide care to children of all ages or age specific care to children, who have nowhere else to live and as such these facilities may be likened to orphanages or foster homes, with living situations

often comparable to that of a large family. Very often RCCIs are community based and locally funded and are called Group homes, because the facility serves as a shelter, crisis center or a home for children who for some reason or the other (may be delinquency, slight mental issues or abuse in the family), cannot live with their own parents and hence reside in these RCCIs, where they receive care, education and in some cases therapy.

Most Residential Child Care Institutions (RCCIs) are homes with more children than will normally be found in a regular domestic family home, yet most have kitchen settings very similar to that of family homes. Therefore there could potentially be heightened food safety concerns in these facilities, especially in those, in which large quantities of food preparation and cleaning is performed by untrained personnel; who depending on the setting in the home may include the children themselves or simply untrained guardians. Due to the relatively large volumes of people fed and the potential of greater seriousness of food borne illness in children, there has been heightened interest in the need for public health food safety education for Residential Child Care Institution facility operators.

In this study, two rounds of microbiological assessments were conducted at 6 RCCI locations in MA, one initially preceding the training and implementation of food safety training and a HACCP based food safety plan at each location. Follow-up microbiological assessments (post microbiological assessment), then occurred after the training, each participating RCCI was allowed enough time to implement strategies acquired from the food safety training. Fifteen surfaces at each RCCI facility was inspected and sampled, and the dish washing sponges, if available were collected from each RCCI location for analysis. The cleanliness of the surfaces sampled was visually

assessed for the presence or absence of food residues and validated using BioControl System's FLASH protein Rapid Cleaning Validation analysis. The surfaces assessed differed by location, but some major surfaces common to all RCCIs, such as the sink drains, and cutting boards were the top priority and considered primary sites to be sampled. In the absence of some primary sites at a particular RCCI location, other secondary sites present were chosen at the discretion of the analyst based on visual assessment of the relevance of the surfaces present for food preparation.

5.2 Materials and Methods: RCCI project

5.2.1 Overview of RCCI locations and kitchen surfaces selection and sampling methods

The six RCCI locations that participated in this pilot study were selected after a needs assessment survey was conducted asking one hundred and eighty six RCCIs facilities, in Massachusetts, U.S.A., to volunteer to participate in this pilot study. Some surfaces in the kitchen's and dining areas of six Residential Child Care Institutions (RCCI) were then chosen for this pilot study. The surfaces chosen were either in direct food contact with food during preparation for example the cutting boards, or indirect food contact surfaces such as the sink faucet. Out of the six homes sampled, only four homes were evaluated at both the pre and post training stages, whiles out of the remaining two, one was only evaluated at the pre training stage and the other at the post training stage. At each facility fifteen (15), "potential" key surfaces in the kitchens of these homes, on which microorganisms could grow and survive and hence serve as vehicles for cross-contamination of microorganisms to foods prepared, or stored in these kitchens were chosen. These surfaces included the refrigerator and freezer shelves,

drawers and handle, cutting boards, sink drain and sink faucets, preparation or work area (space) bench tops or table tops, dish sponges or cloths (if available) and microwave keypads were sampled based on availability. As such in the results presented and subsequent analysis, the surfaces assessed differed from RCCI to RCCI, based on the availability or absence of a particular surface at each RCCI.

Prior to each scheduled visit, kitchen staff were advised not to do any special cleaning, hence to ensure compliance to this, although staff were notified of the date of visit for the microbiological analysis, they had no definite information on the time of visit, nor the specific surfaces to be sampled. The visual appearance of the surfaces sampled were noted and documented as a visual measure of cleanliness, and the presence or absence of visible food particles noted as well. As a validation of the cleaning effectiveness, to get rid of food residue and substrates, for each area, the effectiveness of the cleaning of that area sampled, was analyzed using BioControl System's Flash Rapid Cleaning Validation kit (BioControl Systems, Incorporated, Bellevue, WA. USA), which detects the presence of protein residues on surfaces.

Each surface was swabbed or sampled in triplicates and the microbiological analysis was performed using a variety of commercially available rapid test microbiological test kits. For each sample collected, standard plate counts, yeast and molds counts and total coliform and *E. coli* counts were performed using BioControl Systems', Simplate Total Plate count MPN color indicator test, Simplate Yeast and Mold Color Indicator test and Simplate Total Coliform and *E. coli* color indicator test (BioControl Systems Incorporated, Bellevue, WA. USA). In addition enrichments for detection of *Listeria sp* and *Salmonella sp* were also preformed alongside using Strategic

Diagnostics Inc. (SDI)'s RapidChek® Lateral Flow Tests, (Strategic Diagnostics Incorporated (SDI), Newark, DE, U.S.A)

5.2.2 Sample collection at RCCI locations and kitchen surfaces

At each location, 15 food preparation surfaces were selected and sampled in triplicates, using appropriately labeled sponges pre-moistened with 10 ml of Difco™ DE Neutralizing Broth (BD Diagnostics, Sparks, MD) and sterile gloves. These samples were to be used for the Simplate testing (plate count MPN, yeast and mold MPN and coliform and *E. coli* MPN), as well as for the *Listeria sp* and *Salmonella sp* RapidChek® Lateral Flow Tests. A four inch or 10cm squared surface per sampling area was swabbed, by vigorously rubbing the moist sterile cellulose sponge, in a backward and forward motion for 30 s as recommended by both the SDI protocol and BioControl's environmental sampling protocols.

Sponges were then aseptically placed in their sterile Whirl-Pak* bags, resealed and transported to the laboratory on ice. Aseptic sampling and prevention of external contamination of sponges was achieved by using sterile gloves to handle the sponges during this sampling procedure.

From the triplicate sample sponges collected per surface sampled, a set of 15 sample sponges (one for each surface sampled), was used in performing all four Simplate analysis, with the second and third set of 15 sample sponges each being used in preparing the enrichment for *Listeria sp* and the *Salmonella sp*, testing respectively.

5.2.3 RCCI laboratory analysis

Immediately, upon return to the laboratory, approximately 40 mL of 0.1% Buffered Peptone Water (BPW) was added to each sample sponge for the Simplate Analysis which was then stomached for 30 s at normal speed to dislodge cells from the sponge into surrounding media. This resulted in a total volume of 50ml for each 100 cm^2 sampling area/sponge or a concentration of 2 cm^2/ml concentration. A single sponge was used for all four Simplates analysis tests that were performed according to the manufacturer's instructions. The *Listeria sp* and the *Salmonella sp*, enrichments was also done in the laboratory after sampling of the sponges, by adding 80 ml RapidChek® *Listeria* media (SDI, Newark DE) or 80 ml Lactose broth (BD Diagnostics, Sparks, MD) to each set of sample sponges for the *Listeria sp* and the *Salmonella sp*, testing respectively .

5.2.3.1 Simplate for Yeast and Mold (AOAC® approved Official Method 2002.11)

The dehydrated Y&M-CI medium supplied in the kit was rehydrated with aqueous supplement A solution, according to the manufacturer's instructions and 1ml of the sponge sample was added to 9 ml rehydrated Y&M-CI medium. In the case of heavily soiled sites such as the sink drain, 0.1 ml was added to 9.9 ml Y&M-CI medium with supplement A to achieve a 1:10 dilution. Each sample/medium mixture was thoroughly mixed, and then decanted onto the center of a Simplate®, the lid was replaced and the plate was swirled to distribute the sample/medium mixture evenly into all the wells. The Simplates were then incubated upright in the dark at room temperature (22-25^o C) for 72 h and the number of wells showing a color change was then counted and used for colony

calculations, using the SimPlate Normal Counting Range (NCR) conversion table to determine the total number of fungi per plate. The total number of fungi per cm^2, was then determined by multiplying the NCR count obtained by the appropriate dilution factor, where necessary. The total area sampled was $100cm^2$ ($10cm^2$ squared), and the initial volume of sample was 50 mL (10 mL NB + 40 mL BPW rinse solution). This resulted in an initial sampling test sensitivity of 2 cm^2 mL^{-1} equivalent to $100cm^2$ / 50mL. This implied that the original area sampled per mL or per plate was 2 cm^2 and hence the Most Probable Number (MPN) determination of bacterial count per cm^2 was made using this formula: (NCR count) / 2 cm^2 = # MPN cm^{-2}.

5.2.3.2 Simplate for Total Coliform and *E. coli* (AOAC® Official Method 2005.03)

The kit supplied dehydrated CEc-CI medium, which was rehydrated according to manufacturer's instructions and 1ml of the sponge sample was added to 9mL rehydrated CEc-CI medium. In the case of heavily soiled sites such as the skin drain 0.1 ml was added to 9.9 ml CEc-CI medium. Each sample/medium mixture was then decanted onto the center of a Simplate®, and was incubated upright in the dark at 37° C for 28 h. The number of wells showing a color change from the original background color was then counted and noted as positive wells for the Total coliform count. The *E. coli* presence and count was obtained by the number of wells showing a color change from the original background color, which in addition to the color change fluoresced green under a 366nm long range UV light held 15-30 cm above the SimPlate device.

The Simplate® Normal Counting Range (NCR) conversion table, was used to determine the total number of bacteria colony forming units (cfu) per plate,

corresponding to the observed number of positive wells for both Total Coliform and *E. coli* and the total number of bacteria colony forming units (cfu) per cm^2, was determined by multiplying the NCR count with appropriate dilution factor (if needed) and dividing by 2 cm^2: (NCR count) / 2 cm^2 = # MPN cm^{-2}.

5.2.3.3 Simplate Total Plate Count – (AOAC® Official Method 2002.07)

The kit supplied dehydrated TPC-CI medium was rehydrated according to manufacturer's instructions and 1ml of the sponge sample was added 9 ml rehydrated TPC-CI medium. In the case of heavily soiled sites such as the sink drain, 0.1 ml was added to 9.9 ml TPC-CI. Each sample/medium mixture was then decanted onto the center of a Simplate®, the lid was replaced and the plate was swirled to evenly distribute the sample/medium mixture into all the wells. The plate was tapped slightly to remove bubbles and any excess medium was discarded, by titling the plate and pouring over the sponge cavity. Prior to incubation the initial background color of all the wells, was noted. The Simplates® were incubated upright in the dark at 30° C for 28 h. The number of wells showing a color change from the original background color was then counted and noted as positive wells for the Total Plate Count.

The Simplate® Normal Counting Range (NCR) conversion table, was used to determine the total number of bacteria colony forming units (cfu) per plate corresponding to the observed number of positive wells for both Total Coliform and *E. coli* and the total number of bacteria colony forming units (cfu) per cm^2, was determined by multiplying the NCR count with appropriate dilution factor, (if needed) and dividing by 2 cm^2 (NCR count) / 2 cm^2 = #MPN cm^{-2}.

5.2.3.4 SDI RapidChek® *Listeria* species testing

Detection of the presence of *Listeria sp.* was performed with the SDI RapidChek® *Listeria sp* test kit, according to manufacturer's instructions (SDI, Newark, DE). Briefly, it was performed as follows. After adding 80 mL of the prepared RapidChek® *Listeria sp* media at room temperature to the specimen sponge in a stomacher bag, the bags were stomached at normal speed for 30 seconds and closed loosely for incubation at 30°C for 40 to 48 hours. After incubation, 400 µl of sample enrichment per sponge was transferred from each bag, into test tubes supplied in the kit and boiled in a water bath (100° C) for 10 minutes. Sample tubes were then cooled to room temperature and lateral immunoassay test strips were placed face down into each test tube, and allowed to stand uninterrupted for 10 mins. A test strip with two red lines was indicative of positive results, while those with a single red band (control line) indicated negative test results for the presence of *Listeria sp.*

5.2.3.5 SDI RapidChek® *Salmonella* species testing

Detection of the presence of *Salmonella sp.,* was performed with the SDI RapidChek® *Salmonella sp* kit, according to the manufacturer's instructions (SDI, Newark, DE). Briefly, it was performed as follows. After adding 80 mL of the prepared Lactose Broth (LB) media at room temperature to each specimen sponge in each stomacher bag, the bags were stomached at normal speed for 30 seconds and closed loosely for incubation at 35° C for 24 hours. After incubation the contents of each bag was gently mixed using gentle swirling motions and 1.0 mL of each enrichment was transferred into 10 mL aliquots of, freshly prepared, Tetrothionate (TT) original

formulation broth, containing iodine solution. These secondary enrichment aliquots were then incubated for 24 hours at 42° C ± 0.5° C. After 24 h of incubating the secondary enrichment, 500 µl of each sample was transferred into tubes provided in the kit and *Salmonella* RapidChek® assay test strips were then placed arrow facing down into samples and allowed to develop for 10 minutes uninterrupted. The test was interpreted as negative (absence of *Salmonella sp*), if only one red line; the control line, developed on the test strip and as positive (an indication of the presence of *Salmonella sp*), if two red lines both the test line and the control line developed, and as invalid if no line developed. In all cases samples were saved so that invalid tests could be repeated if they occurred.

5.2.4 Collection and Testing of Dish Sponges

When the RCCI home had a dishwashing sponge, it was retrieved and brought to the laboratory for *Salmonella sp* and *Listeria sp* detection. On site, excess liquid was removed from the dishwashing sponge by squeezing, prior to it being put into a sterile Whirl-Pak® homogenizing bag, for upright transportation on ice to the laboratory. In the laboratory each sponge was aseptically cut into approximately 5 X 5 cm size with sterile scissors and a piece each analyzed for *Salmonella sp* or *Listeria sp* respectively using the Strategic Diagnostics, Incorporated's (SDI) RapidChek® *Salmonella* Lateral Flow Test Kit and the SDI's RapidChek® *Listeria* Lateral Flow test kits as previously described.

5.3 Results and Discussion: RCCI project

5.3.1 Microbial counts (MPN) for each RCCI location and kitchen surface

A total of six RCCI facilities participated in the RCCI pilot study. However only four RCCI facilities; site 1JP, 3W, 4B and 5LT, participated in both the pre-training and post-training phase of the study. The remaining two RCCI facilities; site 2P and 6JC participated only in pre-training phase. Observations from the microbial analysis conducted at theses locations are presented below.

Results of the most probable number (MPN) calculations for each of the four microorganisms tested, as well as the rapid validation cleaning test are summarized in Table 6 to 15. Specifically, Table 6 and 7 shows the pre and post training microbial analysis, MPN counts for sites or surfaces sampled in "Site 1 JP" and that for "Site 3 W" is shown in Table 9 and 10 respectively. In addition the pre and post microbial analysis MPN results for "Site 4B" are summarized in Tables 11 and 12 respectively, and that of "Site 5LT" in Tables 13 and 14 respectively. Pre training microbial analysis data obtained for the two Sites (Site 2P and 6JC) are shown in Tables 8 and 15 respectively.

Microbial growth varied by surface for each of the individual microorganisms analyzed, the MPN counts were much higher on wet surfaces such as the sink drain and cutting boards that had cracks or crevices and were prone to being heavily soiled with food. This trend was more noticeable for *E. coli,* which was very low (below detection limit < 0.5 cfu/cm^2) on most of the surfaces sampled in all the RCCIs, but when present, the microorganism could be found growing on the wet surfaces such as the sink drain, sink handles and cutting boards.

For Tables 6 through 15, no specific microbial growth trends could be observed for the various surfaces sampled pre- and post- training, within or between the various RCCI locations, this is possibly because microbial growth rates differ depending on the prevailing conditions such as availability of moisture, initial microbial load or an organic source of food for the microbes. Hence even if sampling were done more frequently on these surfaces, it may still be difficult to observe a specific pattern in growth trends, another reason for this variation too is that, for each sampling done the microbial load on the surface sampled is reduced because some of the microbes are removed for the analyses hence a subsequent sampling following the initial sampling will not accurately depict the growth or survival pattern or rate of the microbes present on the surface overtime.

The rapid cleaning validation test done with BioControl's Flash Positive Control test was a good indication of the presence or absence of invisible food residues on the surface. However, the Flash tests on occasions indicated the presence of protein on a visually clean surface (See Appendix B). A positive Flash protein test implies cleaning was ineffective and the surface is still contaminated with food protein residues and as such should be considered dirty and a potential habitat for microbes. A negative Flash test on the other hand indicates adequate cleaning. Although on some few occasions a visually clean surface with food traces such as bread crumbs still tested negative.

All the *Salmonella sp* and *Listeria sp* detection tests were negative for all the surfaces sampled and dish sponges collected from all of the RCCI locations. Hence no results are shown in the tables for the RapidChek® analysis done, to detect these two microbes.

5.3.2 RCCI pilot study Statistical Analysis on MPN counts

Further statistical analyses were performed on the natural log transformed MPN data using, SAS version 9.2 software (SAS Institute, Inc., Cary, NC). A *P* value of = 0.05 was used to define statistical significance in all cases. Differences between the two training groups (pre and post training sampling), differences among the 10 surfaces common to the RCCI facilities and the interaction of surface and training group were assessed by Analysis of Variance Analysis (ANOVA). Differences among surfaces were assessed with Duncan's New Multiple Range test.

Overall, no statistically significant differences were noted for total plate counts among the individual surfaces tested at all 4 RCCI locations ($P = 0.3361$) (Table 16). The overall difference between pre-and post- training was non significant, and the interaction of training and surface was non significant.

For the total coliform test, however, overall a statistically significant difference was observed among the 10 individual surfaces tested at 4 RCCI locations ($P = 0.0140$) (Table 16). The mean total coliform counts for sink drain (SD) and sink drain dilution (SDdil) were higher than for microwave keypad (MK), prep/work area (PWA), refrigerator handle (RH), refrigerator shelf 1 (RS1), and stove top (ST). However, no statistical significant differences were observed between pre - and post – training, and the interaction of surface and training was non significant.

Overall, for the *E. coli* tests, a statistically significant difference was observed for the comparisons of overall differences in means among the 10 individual sites tested at 4 RCCI locations ($p = 0.0323$) (Table 16). *E. coli* counts for the sink drain (SD), were higher than that for microwave keypad (MK), prep/work area (PWA), refrigerator handle

(RH), refrigerator shelf 1 (RS1), and stove top (ST). The overall difference between training levels was non significant; however, a statistically significant interaction (P = 0.0089), was observed between surface and training. A comparison of the means for each surface pre and post training yielded statistically non significant differences for all the sampled surfaces except the sink drain and sink faucet. However the effects of training were different for both the sink drain and sink faucet; for the sink drain the training appears to have a positive effect on reducing the *E. coli mean* counts (pre training means: 0.84 MPN cm^{-1}, P = 0.0167 and post training means: 0.71 MPN cm^{-1}, $P <$ 0.0001), the inverse effect was observed for the sink faucet were the post training microbial load was higher than that observed prior to the training; (pre training means: 0.59 MPN cm^{-1}, $P < 0.0001$, and post training means: 0.84 MPN cm^{-1}, $P = 0.0167$) (Table 16). Although statistically significant, these difference are likely negligible, since these differences are within the error of the Simplate MPN counting range which is up to 738 MPN per plate (2).

For all the analysis of variance done on the natural log transformed yeast and mold MPN counts, statistically non significant differences were observed among surfaces, between training levels, and for the interaction (Table 16).

Overall our analysis may have been limited by the relatively small sample size (n = 10 surfaces). This could have led to inadequate power to detect statistically significant differences in most cases.

Table 6. MA RCCI pilot study Site 1 JP pre-HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**$6.0X10^0$**	< D.L.	< D.L.	**$3.0X10^0$**	Positive
Refrigerator Shelf 1	**$8.6X10^1$**	< D.L.	< D.L.	**$3.69X10^2$**	Positive
Refrigerator Shelf 2	**$2.35X10^2$**	< D.L.	< D.L.	**$3.69X10^2$**	Positive
Refrigerator Drawer 1	< D.L.	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Refrigerator Drawer 2	**$3.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Sink Handles	**$7.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Sink Faucet	**$9.0X10^0$**	**$1.0X10^0$**	< D.L.	**$8.6X10^1$**	Positive
Sink Drain	< D.L.	< D.L.	< D.L.	< D.L.	Positive
Sink Drain (1:10 dilution)	< D.L.	< D.L.	< D.L.	< D.L.	Positive
Cutting Board	**$1.1X10^1$**	< D.L.	< D.L.	< D.L.	Positive
Microwave Keypad	**$2.9X10^1$**	< D.L.	< D.L.	< D.L.	Negative
Stove Top	< D.L.	< D.L.	< D.L.	< D.L.	Negative
Prep / Work Area	**$2.0X10^0$**	< D.L.	< D.L.	< D.L.	Negative
Freezer Handle	**$5.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Freezer shelf 1	< D.L.	< D.L.	< D.L.	< D.L.	Positive
Freezer shelf 2	< D.L.	< D.L.	< D.L.	< D.L.	Positive

* Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 7. MA RCCI pilot study Site 1 JP post-HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**$5.0 X10^{0}$**	< D.L.	< D.L.	**$5.0X10^{0}$**	Positive
Refrigerator Shelf 1	**$1.5X10^{1}$**	< D.L.	< D.L.	**$3.69X10^{2}$**	Positive
Refrigerator Shelf 2	**$1.1X10^{1}$**	**$1.0X10^{0}$**	< D.L.	**$3.69X10^{2}$**	Negative
Refrigerator Drawer 1	< D.L.	**$1.0X10^{0}$**	< D.L.	**$2.0X10^{0}$**	Negative
Refrigerator Drawer 2	**$1.28 X10^{2}$**	**$1.6X10^{1}$**	< D.L.	**$2.0X10^{0}$**	Positive
Sink Handles	**$1.38X10^{2}$**	**$2.0X10^{0}$**	< D.L.	**$2.0X10^{1}$**	Positive
Sink Faucet	**$3.69X10^{2}$**	**$2.8X10^{1}$**	**$1.0X10^{0}$**	**$4.0X10^{0}$**	Negative
Sink Drain	**$2.0 X10^{0}$**	**$1.0X10^{0}$**	< D.L.	**$3.0X10^{0}$**	Positive
Sink Drain (1:10 dilution)	< D.L.	**$1.0X10^{0}$**	< D.L.	**$2.0X10^{0}$**	Positive
Cutting Board	**$1.5X10^{1}$**	< D.L.	< D.L.	< D.L.	Positive
Microwave Keypad	**$1.9X10^{1}$**	< D.L.	< D.L.	**$1.0X10^{0}$**	Positive
Stove Top	< D.L.	< D.L.	< D.L.	< D.L.	Positive
Prep / Work Area	**$3.0 X10^{0}$**	< D.L.	< D.L.	**$8.0X10^{0}$**	Negative
Freezer Handle	**$2.0 X10^{0}$**	**$1.0X10^{0}$**	< D.L.	**$2.0X10^{0}$**	Positive
Freezer shelf 1	**$1.0 X10^{0}$**	< D.L.	< D.L.	**$1.0X10^{0}$**	Positive
Freezer shelf 2	< D.L.	**$2.0X10^{0}$**	< D.L.	< D.L.	Negative

* Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 8. MA RCCI pilot study Site 2P pre HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**6.8 X10^{1}**	< D.L.	< D.L.	< D.L.	Positive
Refrigerator Shelf 1	**3.69X10^{2}**	< D.L.	< D.L.	**3.69X10^{2}**	Positive
Refrigerator Shelf 2	**4.3X10^{1}**	< D.L.	< D.L.	**3.69X10^{2}**	Positive
Stove knobs	**1.62X10^{2}**	< D.L.	< D.L.	**1.0X10^{0}**	Positive
Sink Handles	< D.L.	**3.69X10^{2}**	**1.0X10^{0}**	< D.L.	Negative
Sink Faucet	**3.12X10^{2}**	< D.L.	< D.L.	< D.L.	Negative
Oven handle bars	**7.5X10^{1}**	**6.2X10^{1}**	**1.0X10^{0}**	< D.L.	Positive
Sink Drain	**3.69X10^{2}**	**3.69X10^{2}**	**1.0X10^{0}**	< D.L.	Positive
Sink Drain (1:10 dilution)	**3.69X10^{2}**	**3.69X10^{2}**	**1.0X10^{0}**	< D.L.	Positive
Cutting Board (big white)	< D.L.	**5.2X10^{1}**	< D.L.	< D.L.	Positive
Microwave Keypad	**1.77X10^{2}**	< D.L.	< D.L.	< D.L.	Negative
Stove Top	**2.78X10^{2}**	**1.0X10^{0}**	< D.L.	< D.L.	Positive
Prep / Work Area	< D.L.	< D.L.	< D.L.	**3.0X10^{0}**	Positive
Cutting Board (small white)	< D.L.	**1.0X10^{0}**	< D.L.	< D.L.	Positive
Cold food serving area / holder	**1.49X102**	< D.L.	< D.L.	< D.L.	Positive
Hot food serving area / holder	**6.8X10^{1}**	< D.L.	< D.L.	**1.0X10^{0}**	Positive

Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^{2} (< 1.0 cfu/ 2cm^{2})

Table 9. MA RCCI pilot study Site 3W pre HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**$8.0X10^0$**	< D.L.	< D.L.	**$1.1X10^1$**	Negative
Stove Knobs	**$1.0X10^0$**	**$9.0X10^0$**	**$1.0X10^0$**	< D.L.	Negative
Refrigerator Shelf 1	**> $3.69X10^2$**	**$1.0X10^0$**	< D.L.	**> $3.69X10^2$**	Positive
Prep work Area 2	**$2.0X10^0$**	**$1.0X10^0$**	< D.L.	< D.L.	Negative
Staff hand sink handles	**$1.8X10^1$**	**$1.0X10^0$**	< D.L.	**$2.0X10^0$**	Negative
Sink Handles	< D.L.	< D.L.	< D.L.	**$2.0X10^0$**	Negative
Sink Faucet	**$5.0X10^0$**	**$1.0X10^0$**	< D.L.	**$3.0X10^0$**	Positive
Sink Drain	**> $3.69X10^2$**	**> $3.69X10^2$**	**$1.0X10^0$**	**$2.0X10^0$**	Negative
Sink Drain (1:10 dilution)	**> $3.69X10^2$**	**> $3.69X10^2$**	**$1.0X10^0$**	**$1.0X10^0$**	Negative
Cutting Board (prep foods)	**$6.8X10^1$**	**$7.3X10^1$**	**$1.0X10^0$**	< D.L.	Positive
Microwave Keypad	**$5.0X10^0$**	**$1.0X10^0$**	< D.L.	**$1.04X10^2$**	Negative
Stove Top (grilling top)	**$1.4X10^1$**	**$9.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Prep / Work Area 1	< D.L.	**$1.0X10^0$**	< D.L.	**$5.0X10^0$**	Positive
Residential kitchen Prep work area	**$5.0X10^0$**	**$2.0X10^0$**	< D.L.	**$8.0X10^0$**	Positive
Residential kitchen fridge shelf	**$5.2X10^1$**	**$1.0X10^0$**	< D.L.	**> $3.69X10^2$**	Positive
Residential kitchen fridge handle	**$6.0X10^0$**	**$2.0X10^0$**	< D.L.	**$1.8X10^1$**	Positive

Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 10. MA RCCI pilot study Site 3W post HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**$5.0 X10^0$**	**$1.0X10^0$**	< D.L.	**$3.69X10^2$**	Positive
Stove Knobs	**$2.0X10^0$**	**$1.0X10^0$**	< D.L.	**$4.0X10^0$**	Positive
Refrigerator Shelf 1	**$7.3X10^1$**	**$2.0X10^0$**	< D.L.	**$2.0X10^1$**	Positive
Prep work Area 2	< D.L.	**$2.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Staff hand sink handles	**$9.5X10^1$**	**$1.0X10^0$**	< D.L.	**$1.6X10^1$**	Positive
Sink Handles	**$1.5X10^1$**	< D.L.	< D.L.	< D.L.	Positive
Sink Faucet	**$2.0X10^0$**	**$1.0X10^0$**	< D.L.	**$7.0X10^0$**	Negative
Sink Drain	**$2.1X10^1$**	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Sink Drain (1:10 dilution)	**$1.0X10^0$**	**$1.0X10^0$**	< D.L.	**$2.0X10^0$**	Positive
Cutting Board (prep foods)	**$3.69X10^2$**	**$3.69X10^2$**	**$1.0X10^0$**	**$4.0X10^0$**	Positive
Microwave Keypad	**$2.4X10^1$**	**$2.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Stove Top (grilling top)	**$6.0X10^0$**	**$1.0X10^0$**	< D.L.	**$5.0 X10^0$**	Positive
Prep / Work Area 1	**$2.0X10^0$**	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Residential kitchen Prep work area	**$5.0 X10^0$**	**$1.0X10^0$**	< D.L.	**$6.0X10^0$**	Positive
Residential kitchen fridge shelf	**$3.69X10^2$**	**$1.9X10^1$**	< D.L.	**> $3.69X10^2$**	Positive
Residential kitchen fridge handle	**$9.0X10^0$**	**$1.0X10^0$**	< D.L.	**$4.0X10^0$**	Positive

* Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 11. MA RCCI pilot study Site 4B pre HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**$9.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Refrigerator Shelf 1	**$2.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Refrigerator Shelf 2	**$3.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Refrigerator Drawer 1	**$8.3X10^1$**	< D.L.	< D.L.	< D.L.	Positive
Refrigerator Drawer 2	**$4.0X10^0$**	< D.L.	< D.L.	**$1.0X10^0$**	Positive
Sink Handles	**$3.69X10^2$**	< D.L.	< D.L.	< D.L.	Negative
Sink Faucet	**$9.0X10^0$**	< D.L.	< D.L.	**$3.0X10^0$**	Negative
Sink Drain	**$3.69X10^2$**	**$3.69X10^2$**	**$1.0X10^0$**	**$6.0X10^0$**	Positive
Sink Drain (1:10 dilution)	**$3.69X10^2$**	**$3.69X10^2$**	**$1.0X10^0$**	**$6.0X10^0$**	Positive
Cutting Board	**$1.0X10^0$**	< D.L.	< D.L.	**$4.0X10^0$**	Positive
Microwave Keypad	**$1.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Stove Top	**$2.0X10^0$**	< D.L.	< D.L.	**$1.0X10^0$**	Positive
Prep / Work Area	**$4.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Freezer Handle	< D.L.	< D.L.	< D.L.	< D.L.	Positive
Freezer shelf 1	**$1.5X10^1$**	< D.L.	< D.L.	< D.L.	Positive
Freezer shelf 2	**$1.6X10^1$**	< D.L.	< D.L.	< D.L.	Positive

* Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 12. MA RCCI pilot study Site 4B post HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**$9.2 X10^1$**	< D.L	< D.L.	**$4.0X10^0$**	Positive
Refrigerator Shelf 1	**$5.0 X10^0$**	< D.L.	< D.L.	**$1.0X10^0$**	Negative
Refrigerator Shelf 2	**$5.0 X10^0$**	**$1.0X10^0$**	< D.L.	**$3.0X10^0$**	Positive
Refrigerator Drawer 1	**$3.69X10^2$**	**$1.1X10^1$**	< D.L.	**$1.0X10^0$**	Negative
Refrigerator Drawer 2	**$1.24X10^2$**	< D.L.	< D.L.	**$2.0X10^0$**	Negative
Sink Handles	**$3.69X10^2$**	**$1.4X10^1$**	< D.L.	**$3.69X10^2$**	Positive
Sink Faucet	**$3.69X10^2$**	**$2.8X10^1$**	**$1.0X10^0$**	**$5.0X10^0$**	Positive
Sink Drain	**$3.69X10^2$**	**$3.69X10^2$**	**$1.0X10^0$**	**$3.0X10^0$**	Positive
Sink Drain (1:10 dilution)	**$3.69X10^2$**	**$3.69X10^2$**	**$1.0X10^0$**	**$1.0X10^0$**	Positive
Cutting Board	**$4.7X10^1$**	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Microwave Keypad	**$2.5X10^1$**	< D.L.	< D.L.	< D.L.	Positive
Stove Top	**$5.4X10^1$**	< D.L.	< D.L.	**$2.0X10^0$**	Positive
Prep / Work Area	**$3.69X10^2$**	< D.L.	< D.L.	**$3.0X10^0$**	Positive
Freezer Handle	**$1.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Freezer shelf 1	**$3.8X10^1$**	< D.L.	< D.L.	< D.L.	Positive
Freezer shelf 2	**$7.5X10^1$**	< D.L.	< D.L.	**$4.0X10^0$**	Positive

* Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 13. MA RCCI pilot study Site 5LT pre HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**$5.0X10^0$**	**$3.0X10^0$**	< D.L.	**$1.2X10^1$**	Positive
Refrigerator Shelf 1	**$>3.69X10^2$**	< D.L.	< D.L.	**$1.0X10^0$**	Negative
Refrigerator Shelf 2	**$2.1X10^1$**	**$6.0X10^1$**	< D.L.	**$4.0X10^0$**	Positive
Cutting board 1 (cooked food)	**$4.0X10^0$**	< D.L.	< D.L.	< D.L.	Negative
Plate drying rack	**$2.54X10^2$**	**$1.0X10^0$**	< D.L.	**$>3.69X10^2$**	Positive
Sink Handles	**$>3.69X10^2$**	**$>3.69X10^2$**	**$1.0X10^0$**	**$>3.69X10^2$**	Negative
Sink Faucet	**$2.2X10^2$**	**$1.44X10^2$**	**$1.0X10^0$**	**$7.8X10^1$**	Negative
Sink Drain	**$>3.69X10^2$**	**$>3.69X10^2$**	**$1.0X10^0$**	**$2.10X10^2$**	Positive
Sink Drain (1:10 dilution)	**$>3.69X10^2$**	**$>3.69X10^2$**	**$1.0X10^0$**	**$1.0X10^0$**	Positive
Cutting Board 2 (Red: meat)	**$6.0X10^1$**	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Microwave Keypad	< D.L.	< D.L.	< D.L.	**$1.0X10^0$**	Positive
Stove Top	**$1.0X10^0$**	< D.L.	< D.L.	**$1.04X10^2$**	Positive
Prep/Work Area	**$1.0X10^0$**	< D.L.	< D.L.	**$1.0X10^0$**	Positive
Freezer Handle	**$3.0X10^0$**	< D.L.	< D.L.	**$1.0X10^0$**	Negative
Freezer 1 shelf 1	**$>3.69X10^2$**	**$5.8X10^1$**	**$1.0X10^0$**	**$3.0X10^0$**	Negative
Freezer 2 door shelf 2	**$3.69X10^2$**	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Dish sponge	-	-	-	-	-

* Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 14. MA RCCI pilot study Site 5LT post HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	*E. coli* Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle	**> $3.69X10^2$**	**$4.0X10^0$**	< D.L.	**$1.1X10^1$**	Positive
Refrigerator Shelf 1	**> $3.69X10^2$**	< D.L.	< D.L.	< D.L.	Positive
Refrigerator Shelf 2	**> $3.69X10^2$**	< D.L.	< D.L.	**$1.5X10^1$**	Positive
Cutting board 1 (cooked food)	< D.L.	< D.L.	< D.L.	< D.L.	Negative
Plate drying rack	**$1.62X10^2$**	**$6.0X10^0$**	< D.L.	**$5.0X10^0$**	Positive
Sink Handles	**> $3.69X10^2$**	**> $3.69X10^2$**	**$1.0X10^0$**	< D.L.	Negative
Sink Faucet	**> $3.69X10^2$**	**$4.0X10^1$**	**$1.0X10^0$**	< D.L.	Negative
Sink Drain	**> $3.69X10^2$**	**> $3.69X10^2$**	**$1.0X10^0$**	**$4.0X10^0$**	Positive
Sink Drain (1:10 dilution)	**> $3.69X10^2$**	**$6.4X10^1$**	< D.L.	< D.L.	Positive
Cutting Board 2 (Red: meat)	**$2.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Microwave Keypad	**$2.0X10^0$**	< D.L.	< D.L.	< D.L.	Negative
Stove Top	**$1.6X10^1$**	< D.L.	< D.L.	< D.L.	Positive
Prep/Work Area	**$1.0X10^0$**	< D.L.	< D.L.	< D.L.	Positive
Freezer Handle	**$3.7X10^1$**	< D.L.	< D.L.	**$2.4X10^1$**	Negative
Freezer 1 shelf 1	**$6.2X10^1$**	**$2.0X10^0$**	< D.L.	< D.L.	Positive
Freezer 2 door shelf 2	**> $3.69X10^2$**	**$3.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Dish sponge	-	-	-	-	-

* Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 15. MA RCCI pilot study Site 6 JC pre HACCP training microbial MPN count data.

Swab Locations	Total Plate Count Most Probable Number (MPN / cm^{-2})	Total Coliform Count Most Probable Number (MPN / cm^{-2})	E. Coli Count Most Probable Number (MPN / cm^{-2})	Yeast & Molds Count Most Probable Number (MPN / cm^{-2})	Rapid Cleaning Validation BioControl Flash Positive Control Test
Refrigerator Handle (Kitchen: all foods)	**$1.0X10^0$**	**$1.0X10^0$**	< D.L.	< D.L.	Positive
Refrigerator Shelf 1 (Kitchen: all foods)	**> $3.69X10^2$**	**$3.0X10^0$**	< D.L.	**$2.0X10^0$**	Positive
Meats Refrigerator metal base (Storage room)	**$5.0X10^0$**	**$1.0X10^0$**	< D.L.	**$4.0X10^0$**	Positive
Meats Refrigerator handle (Storage room)	< D.L.	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Negative
Cutting Board (Blue: salads)	**$1.0X10^0$**	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Sink Handles	**$1.24X10^2$**	**$2.0X10^0$**	< D.L.	**$1.0X10^0$**	Positive
Sink Faucet	**$1.0X10^0$**	**$1.0X10^0$**	< D.L.	< D.L.	Negative
Sink Drain	**> $3.69X10^2$**	**> $3.69X10^2$**	**$1.0X10^0$**	< D.L.	Positive
Sink Drain (1:10 dilution)	**> $3.69X10^2$**	**> $3.69X10^2$**	**$1.0X10^0$**	< D.L.	Positive
Cutting Board (Red: meats)	**> $3.69X10^2$**	**$9.0X10^0$**	< D.L.	**$7.0X10^0$**	Positive
Microwave Keypad	**$3.0X10^0$**	**$1.0X10^0$**	< D.L.	< D.L.	Positive
Stove Top	**$1.0X10^0$**	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Negative
1° Prep / Work Area (with main sink)	**$1.0X10^0$**	**$1.0X10^0$**	< D.L.	< D.L.	Positive
Milk Fridge Handle	< D.L.	**$1.0X10^0$**	< D.L.	**$1.0X10^0$**	Negative
Milk Fridge shelf 1	**> $3.69X10^2$**	**$1.0X10^0$**	< D.L.	**$4.7X10^1$**	Positive
Milk Fridge drawer	**> $3.69X10^2$**	**$1.0X10^0$**	< D.L.	**$4.0X10^0$**	Positive

* Simplate sponge counted as well. * < D.L. implies lower than detection limit = < 0.5 cfu/cm^2 (< 1.0 cfu/ $2cm^2$)

Table 16. Differences among various kitchen surfaces at MA Residential Child Care Institutions (RCCI). Numbers presented are the means of samples taken before and after training at four RCCIs (Site: 1JP, 3W, 4B and 5LT).

Swab locations (Surfaces)	Mean Total Plate Count (TPC) (MPN/cm^2)	Mean *E.* coli (MPN/cm^2)	Mean Total Coliform Count (TCC) (MPN/cm^2)	Mean Yeast Mold Count (YMC) (MPN/cm^2)
Cutting Board (CB)	11.85^{a}	0.59^{ab}	2.32^{ab}	0.92^{a}
Microwave Keypad (MK)	5.99^{a}	0.50^{b}	0.65^{b}	1.26^{a}
Prep/Work Area (PWA)	3.11^{a}	0.50^{b}	0.59^{b}	1.40^{a}
Refrigerator Handle (RH)	10.78^{a}	0.50^{b}	0.88^{b}	7.96^{a}
Refrigerator Shelf 1 (RS1)	38.40^{a}	0.50^{b}	0.65^{b}	11.22^{a}
Sink Drain (SD)	58.85^{a}	0.77^{a}	36.89^{a}	2.86^{a}
Sink Drain (1:10 dilution) (SDdil)	33.82^{a}	0.71^{ab}	29.64^{a}	1.25^{a}
Sink Faucet (SF)	41.60^{a}	0.71^{ab}	6.23^{ab}	5.05^{a}
Sink Handles (SH)	58.32^{a}	0.59^{ab}	4.70^{ab}	4.91^{a}
Stove Top (ST)	3.71^{a}	0.50^{b}	0.78^{b}	1.84^{a}
Significance (P > F)				
Surface	0.3361	0.0323	0.014	0.2737
Training	0.2204	0.7608	0.9528	0.6391
Surface X training	0.1878	0.0089	0.1522	0.6048

Mean separation within column by Duncan's New Multiple Range Test (P = 0.05).

5.4 Conclusion: RCCI project

Irrespective of the Residential Child Care Institution location, or the kitchen surfaces sample nor the type of microorganism tested, training had no significant effect in significantly reducing the microbial load on the surfaces analyzed. Despite this observation it would be erroneous to conclude that training is unnecessary for personnel of these RCCIs, mainly because many uncontrollable factors such as compliance and adherence issues, related to human behavior influences the effectiveness of a training program such as this in efficiently producing consistent repeatable reductions in microbial growth.

The microbial load differed significantly by surfaces sampled in both the *E. coli* and Total Coliform count analyzes (P = 0.0323 and 0.014) respectively. For *E. coli* and Total Coliform, as was expected the wet or moist surfaces with heavier food residue contamination such as the sink drain had significantly higher microbial counts cm^{-2} in comparison to the other surfaces sampled. The interaction between surface and training effect was highly significant for only *E. coli* (P = 0.0089).

Our results also show that the presence of food residues as confirmed by the BioControl Flash Positive Control tests does not necessarily predict the presence of high counts of microorganisms on all kitchen surfaces.

APPENDIX A

PICTURES OF EXPIRED HUMMUS SHOWING MICROBIAL FOOD SPOILAGE.

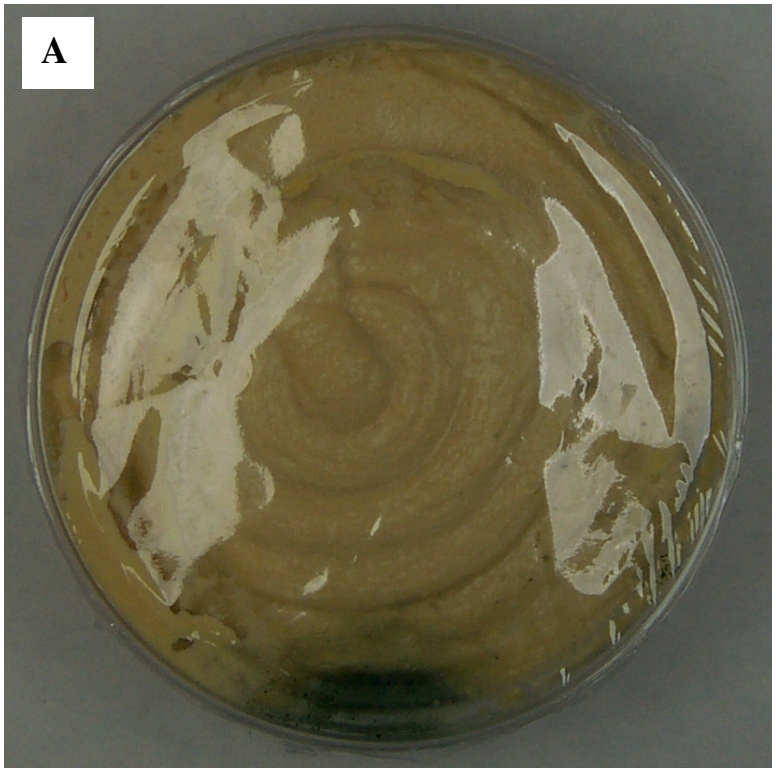

Figure 8. A photograph of an unopened expired hummus tab with an intact rubber seal and visible microbial growth.

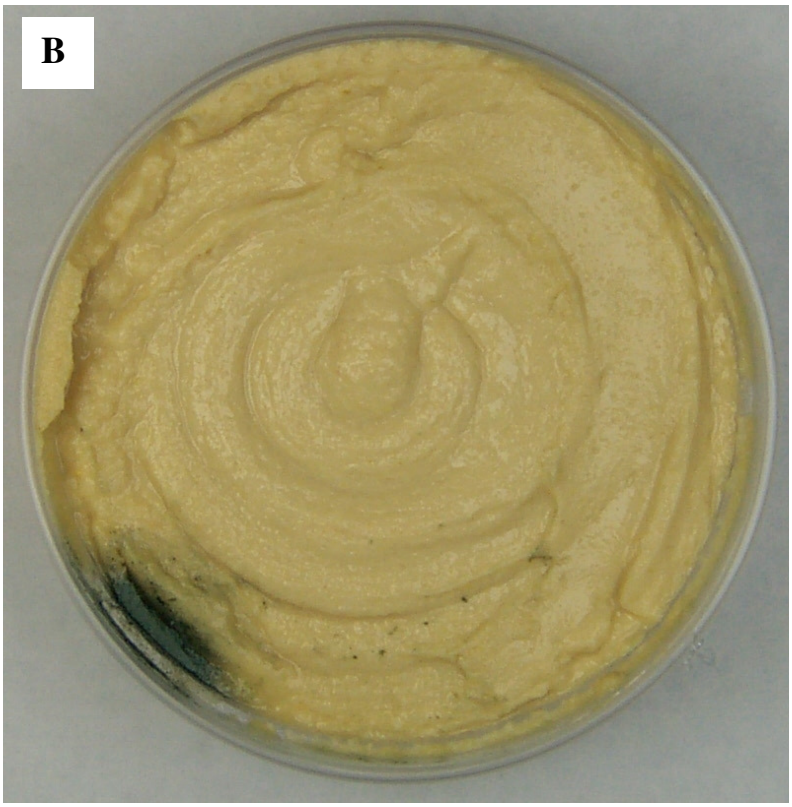

Figure 9. Visible microbial growth as seen from the top of an opened expired hummus tab without the rubber seal.

Figure 10. Visible microbial growth on the top sidewall of a sealed unopened expired hummus tab.

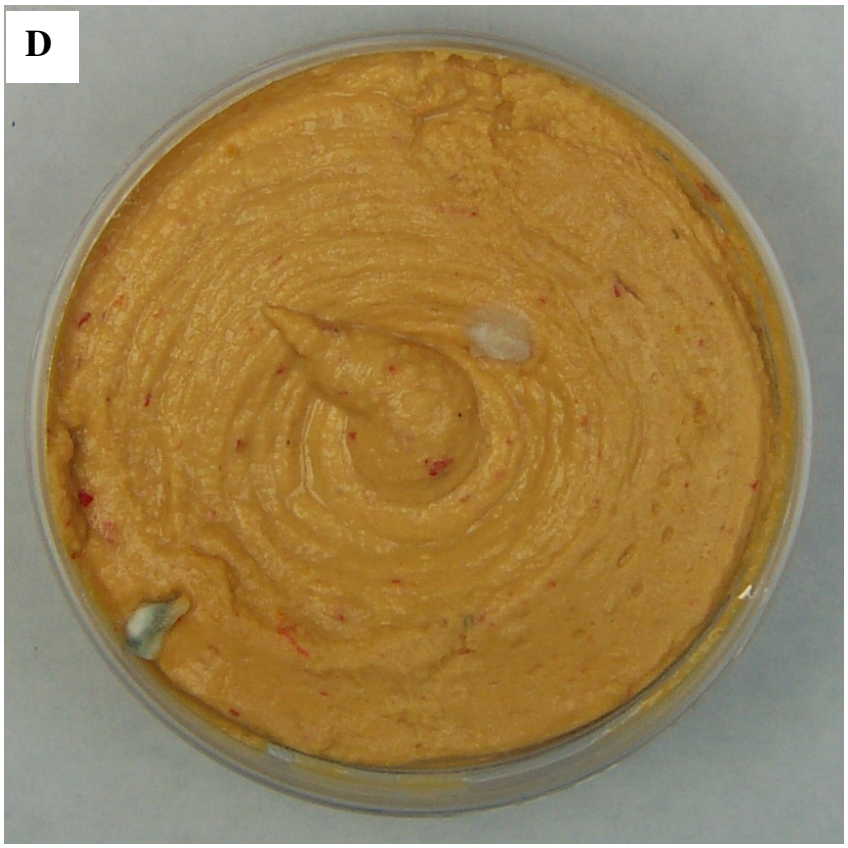

Figure 11. Visible yeast and mold growth on the top of an opened expired hummus tab. Visible red specks are pieces of ground pepper.

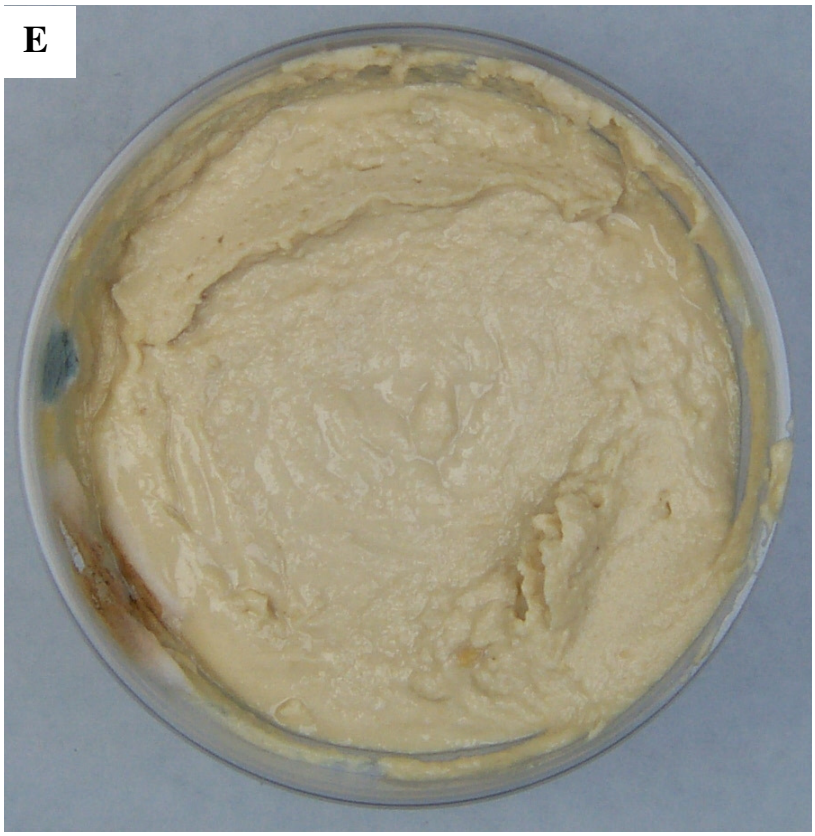

Figure 12. Visible yeast and mold growth on the hummus surface in an opened expired hummus tab.

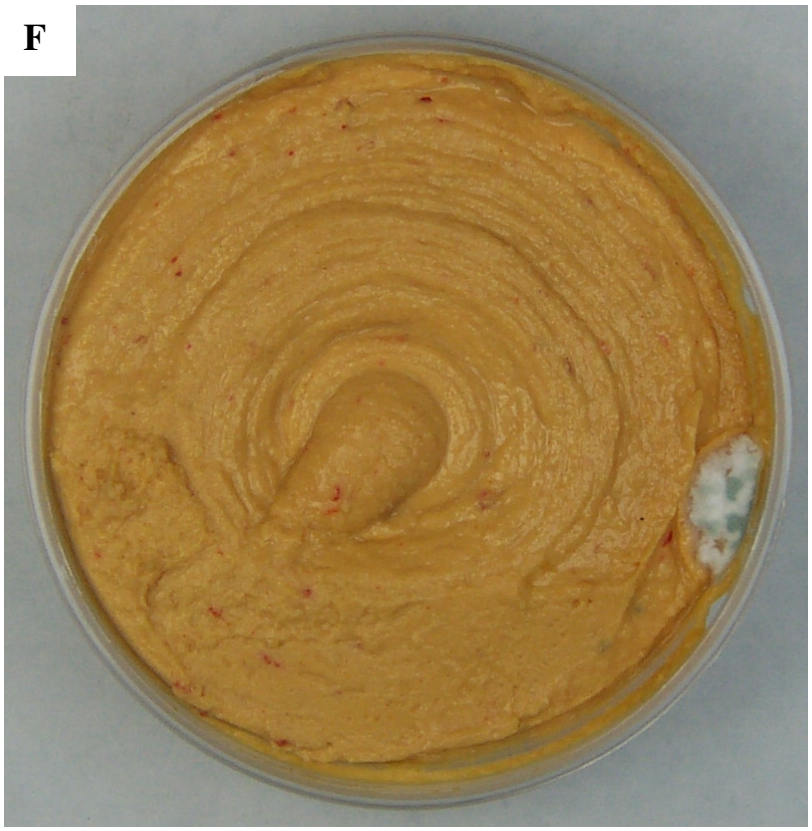

Figure 13. Top view of an opened expired hummus tab showing a cluster of visible yeast and mold colonies clustered on one side.

APPENDIX B

SUMMARY OF CLEANNESS OF TESTED RCCI KITCHEN SURFACES

The apparent cleanness of a kitchen surface, as indicated by the absence of visible food particles or grease may not necessarily imply the absence of food residues on that surface. Hence the need to validate a visual cleanness perception with a tool such as the Flash Positive Control test which is able to detect protein residues, which are generally more difficult to remove from surfaces but could serve as a nutrient source for some microorganisms. Table 17 to 22, shows a summary of the visual perception of cleanness with the corresponding Flash test result for each RCCI site, pre and post training when available.

Table 17. Visual description of cleanness of surfaces and Flash validation test, Site 3W MA RCCI study

	Pre-training sampling		Post-training sampling	
Swab Locations	Visual Description of area	Flash Test	Visual Description of area	Flash Test
Refrigerator Handle	Clean	-	Clean, but greasy	+
Stove Knobs	Fairly clean but greasy	-	Fairly clean but greasy	+
Refrigerator Shelf 1	Clean	+	Clean, with few food particles	+
Prep work Area 2	Clean	-	Clean	+
Staff hand sink handles	Clean	-	Clean	+
Sink Handles	Fairly clean, food particles traces	-	Clean	+
Sink Faucet	Clean, traces of food around base	+	Clean, traces of food around base	-
Sink Drain	Clean no traces of food	-	Clean no traces of food	+
Sink Drain (1:10 dilution)	Clean no traces of food	-	Clean no traces of food	+
Cutting Board (prep foods)	Clean	+	Clean	+
Microwave Keypad	Clean	-	Clean with traces of food	+
Stove Top (grilling top)	Greasy with traces of food particles	+	Greasy with traces of food particles, but clean	+
Prep / Work Area 1	Clean	+	Clean	+
Residential kitchen Prep work area	Clean	+	Clean	+
Residential kitchen fridge shelf	Fairly clean food particles on base shelf	+	Fairly clean food particles on base shelf	+
Residential kitchen fridge handle	Fairly clean greasy with finger prints and traces of food particles on base shelf	+	Fairly clean greasy with finger prints and traces of food particles	+

"+" is positive for food residue (implying dirty), "-" is negative for food residue (implying clean)

Table 18. Visual description of cleanness of surfaces and Flash validation test, Site 4B MA RCCI study

	Pre-training sampling		Post-training sampling	
Swab Locations	Description of area	Flash Test	Description of area	Flash Test
Refrigerator Handle	Fairly clean but greasy with finger prints	+	Fairly clean but greasy with finger prints	+
Refrigerator Shelf 1	Fairly clean, few food particles	+	Clean, no visible food particles	-
Refrigerator Shelf 2	Fairly clean, few food particles	+	Fairly clean, few food particles	+
Refrigerator Drawer 1	Fairly clean, few food particles	+	Clean, no visible food particles	-
Refrigerator Drawer 2	Fairly clean, few food particles	+	Clean, no visible food particles	-
Sink Handles	Fairly clean but greasy with finger prints	-	Fairly clean but greasy with finger prints	+
Sink Faucet	Greasy with finger prints and traces of food	-	Clean no traces of food	+
Sink Drain	Fairly clean, few food particles	+	Lots of food particles in drain net	+
Sink Drain (1:10 dilution)	Fairly clean, few food particles	+	Clean	+
Cutting Board	Clean	+	clean	+
Microwave Keypad	Clean	+	Few finger prints but clean	+
Stove Top	Clean	+	Clean, few traces of food	+
Prep/Work Area	Clean	+	Clean	+
Freezer Handle	Clean	+	Finger prints, food traces	+
Freezer shelf 1	Clean	+	Lots of food particles	+
Freezer shelf 2	Clean	+	Lots of food particles	+

"+" is positive for food residue (implying dirty), "-" is negative for food residue (implying clean)

Table 19. Visual description of cleanness of surfaces and Flash validation test, Site 1JP MA RCCI study

	Pre-training sampling		Post-training sampling	
Swab Locations	Description of area	Flash Test	Description of area	Flash Test
Refrigerator Handle	Clean with traces of food	+	Very Clean	+
Refrigerator Shelf 1	Walk in refrigerator with clean metal shelves with	+	Walk in refrigerator with clean metal wire shelves	+
Refrigerator Shelf 2	bars in them	+	with bars on them	–
Refrigerator Drawer 1	Very clean	+	Very clean	_
Refrigerator Drawer 2	Very clean	+	Clean, with traces of food	+
Sink Handles	Clean with food residues	+	Clean with food residues	+
Sink Faucet	Very clean	+	Very clean	_
Sink Drain	Fairly clean with food	+	Clean with no visible	+
Sink Drain (1:10 dilution)	residues, sink only used to drain liquids.	+	food, sink only used to drain liquids from food.	+
Cutting Board	Very clean color coded for produce and meat	+	Very clean color coded for produce and meat	+
Microwave Keypad	Very clean	-	Very clean	+
Stove Top	Very clean	-	Clean	+
Prep / Work Area	Very clean	-	Very clean	_
Freezer Handle	Very clean	+	Very clean	+
Freezer shelf 1	Clean with food traces	+	Clean with few food traces	+
Freezer shelf 2	Clean with food traces	+	Clean with food traces	_

"+" is positive for food residue (implying dirty), "-" is negative for food residue (implying clean)

Table 20. Visual description of cleanness of surfaces and Flash validation test, Site 2P MA RCCI study

Swab Locations	Post-training sampling	
	Description of area	Flash Test
Refrigerator Handle	Clean	+
Refrigerator Shelf 1	Walk in refrigerator with clean metal wire shelves with bars on them	+
Refrigerator Shelf 2		+
Stove knobs	Clean but greasy	+
Sink Handles	Clean, with traces of food	-
Sink Faucet	Clean	-
Oven handle bars	Very but slightly greasy	+
Sink Drain	Clean with few visible food, sink only used to drain liquids from food.	+
Sink Drain (1:10 dilution)		+
Cutting Board (big white)	Fairly clean with traces of food	+
Microwave Keypad	Very clean	-
Stove Top	Fairly Clean with traces of food	+
Prep / Work Area	Clean	+
Cutting Board (small white)	Fairly clean with traces of food	+
Cold food serving area / holder	Clean	+
Hot food serving area / holder	Clean	+

"+" is positive for food residue (implying dirty), "-" is negative for food residue (implying clean)

Table 21. Visual description of cleanness of surfaces and Flash validation test, Site 5LT MA RCCI study

Swab Locations	Pre-training sampling		Post-training sampling	
	Description of area	Flash Test	Description of area	Flash Test
Refrigerator Handle	Fairly clean	+	Fairly clean and grease evident	+
Refrigerator Shelf 1	Poor cleaning: food particles and grease evident	+	Clean, no visible food particles	-
Refrigerator Shelf 2		+	Fairly clean with food traces	+
Cutting board 1 (cooked food)	Fairly clean with traces of food	-	Clean	-
Plate drying rack	Fairly clean	+	Fairly clean with food particles	+
Sink Handles	Fairly clean	-	Fairly clean and grease evident	-
Sink Faucet	Clean	-	Clean	-
Sink Drain	Food particles in drain	+	Clean, no visible food particles	+
Sink Drain (1:10 dilution)		+		+
Cutting Board 2 (Red: meat)	Clean	+	Clean	+
Microwave Keypad	Clean	-	Clean	+
Stove Top	Fairly clean, greasy with traces of food	+	Clean with traces of food	+
Prep/Work Area	Fairly clean	+	Clean	+
Freezer Handle	Fairly clean, greasy with finger prints	-	Clean	-
Freezer 1 shelf 1	Food particles (cheese) and dirt	+	Clean	-
Freezer 2 door shelf 2	Very dirty and food soiled	+	Clean with few traces of food	+

"+" is positive for food residue (implying dirty), "-" is negative for food residue (implying clean)

Table 22. Visual description of cleanness of surfaces and Flash validation test, Site 6JC MA RCCI study.

Swab Locations	Post-training sampling Description of area	Flash Test
Refrigerator Handle (Kitchen: all foods)	Clean	+
Refrigerator Shelf 1 (Kitchen: all foods)	Clean	+
Meats Refrigerator metal base (Storage room)	Clean, few food traces	+
Meats Refrigerator handle (Storage room)	Clean	-
Cutting Board (Blue: salads)	Clean	+
Sink Handles	Clean	+
Sink Faucet	Clean	-
Sink Drain	Clean few traces of food	+
Sink Drain (1:10 dilution)		+
Cutting Board (Red: meats)	Clean	+
Microwave Keypad	Clean	+
Stove Top	Clean	-
1^{o} Prep / Work Area (with main sink)	Clean	+
Milk Fridge Handle	Fairly clean	-
Milk Fridge shelf 1	Fairly clean with traces of spilled milk	+
Milk Fridge drawer	Fairly clean, traces of food particles	+

"+" is positive for food residue (implying dirty), "-" is negative for food residue (implying clean)

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