

**MULTIPLE APPROACHES TO QUANTITATIVELY EVALUATING BACTERIAL
PATHOGEN TRANSFER BETWEEN FOOD PRODUCTS AND CONTACT SURFACES**

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A DISSERTATION

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

Biosystems Engineering - Doctor of Philosophy

2017

ABSTRACT

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Various bacterial pathogens have been identified as causes of foodborne disease outbreaks linked to produce and other ready-to-eat food products. Numerous studies have evaluated bacterial transfer via processing equipment. The overall goal of this dissertation was to improve the understanding of *Salmonella* transfer between contact surfaces and food products using three approaches: 1) experimental testing of *Salmonella* transfer between a model food product (potato) and stainless steel; (2) development and meta-analysis of a bacterial transfer database; and (3) formulation of a novel modeling approach using dimensional analysis. The three approaches were aimed at improving the understanding of generalized relationships between physical variables and bacterial transfer to/from food and food contact surfaces.

Experimental testing of bacterial transfer (*Salmonella* Typhimurium LT2) between a potato sample and stainless steel was achieved by bench-scale experiments via static and dynamic (sliding) contact. Physical variables of pressure, speed, potato surface moisture, and contact time were evaluated. Bacterial transfer increased ($p < 0.0001$) with contact time (from 5 to 40 s) during static contact. In bacterial transfer via 18 multiple static contacts, higher bacterial transfer occurred at the highest pressure ($p = 0.0226$). Additionally, the number of *Salmonella* remaining on a contaminated potato sample decreased ($p < 0.0001$) from ~6.5 Log CFU to ~5.5 Log CFU after 18 sequential static contacts with stainless steel. In dynamic contact tests, greater bacterial transfer ($p = 0.0098$) was found at 7.75 than at 3.75 mm/s when bacteria were

transferred from the potato to the plate, whereas no effect ($p = 0.4947$) was found when bacteria were transferred from the plate to multiple potatoes at 3.75, 5.00, and 7.75 mm/s. Overall, potato surfaces acquired more bacteria from the stainless steel than bacteria transferred from the potato to a clean stainless steel surface, with ~ 3 Log CFU difference between them, implying preferential transfer affinity to the potato, as compared to stainless steel.

Development of a new bacterial transfer database included 321 data sets from 71 published studies, with 25 studies included in a meta-analysis. Regression analysis of the aggregated data showed, via the Weibull model rate parameter, that bacterial transfer via dynamic contact decreased as fat and protein increased ($p < 0.05$). The same parameter increased as moisture content increased ($p < 0.05$). Only five studies measured surface roughness, and regression analysis conducted on the intercept parameter revealed that if material roughness increased, intercept decreased ($p < 0.05$).

A novel modeling approach also was used to describe the dependency of bacterial transfer on physical variables. Among the relevant variables for formulating a model for bacterial transfer were initial inoculation level, bacteria transferred, pressure, viscosity, friction force, and speed. Although insufficient data were available for complete evaluation and validation, the resulting models, demonstrated the conceptual feasibility of applying such an approach to predict bacterial transfer. It was possible to illustrate from experimental results that bacterial transfer from food to food contact surface increased with pressure and decreased as friction force increased. Overall, the three approaches in this dissertation supported each other. The result is a multidimensional demonstration of the importance for bacterial transfer studies to control and document physical variables. Only then will the growing body of work in bacterial transfer yield more generalizable knowledge and tools.

ACKNOWLEDGMENTS

My major professor Dr. Marks for his excellent guidance, his continuous motivation during my time at MSU, his commitment on giving me the tools to conduct my research at MSU and my future academic success.

My committee members: Dr. Ryser, Dr. Almenar, Dr. Dolan, and Dr. Beaudry thanks for the teamwork, especially at the beginning of my program, and their commitment for reading my work, giving good suggestions, and always being available to meet.

The sponsors from Costa Rica government that I had during my stage at MSU, the University of Costa Rica specially the Biosystems engineering department, and the Ministry of science technology and telecommunications (MICITT) of Costa Rica.

In addition, this work was supported by AFRI grant No. 2012-67017-3018 from the USDA National Institute of Food and Agriculture, the MSU office of international students and scholars: tuition award (spring and fall 2017), and the Department of Biosystems Engineering: fellowship (summer 2017).

My lab-mates, Emma who help me to collect and to organize the data for doing the meta-analysis, Ian and Quincy for their help on data analysis and writing codes, Nurul and Pichamon for their support in multiple activities such as plating and reading my writing.

The lab-managers Nicole and Mike for their assistance during the set-up and conduction of the experiments, and the edition process during the writing process of my dissertation.

Dr. Srivastava for helping on the dimensional analysis.

The writing consultants at the writing center specially to Jessica, Seven, and Rachel for their motivation during my writing and teaching me how to write for readers from other fields.

My parents, my brother, and Bet for their loyal support and their trust in me and my work from beginning to end, also to my friends in Costa Rica and at MSU for being present in many ways.

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KEY TO SYMBOLS

a	parameter of a general equation
AC	acrylic
AIC_c	Akaike's Information Criterion
b	parameter of a general equation
C	parameter of a general equation
C_{-1}	initial clean stainless steel contact area
C_0	inoculated contact area
C_1 to C_{17}	clean stainless steel contact area
CFU	colonies forming units
F	friction force
GGP	Glo Germ TM
HDPE	high density polyethylene
k	rate parameter on the Weibull model or slope parameter on the Log-linear model
K	number of parameters in a model
L	characteristic length of the potato
MTSA	modified trypticase soy agar
n	number of data points (contact or slice number)
n_c	critical value on the linear-Weibull model
N	bacteria transferred on the Weibull, Log-linear, and linear-Weibull model
N_0	initial number of bacteria on the Weibull, Log-linear, and linear-Weibull model
N_i	initial inoculation level

N_t	bacteria transferred
p	shape factor in the Weibull model
P	pushing force in the schematic of Figure 3.3
P	normal pressure used for the dimensional analysis
PP	polypropylene
R	reaction force to the weight
RMSE	root mean squared error
SS	stainless steel
SSE	squared residual errors
t	contact time
V	speed
W	weight
Y	response variable
x_1, x_2, x_3	independent variables of the experimental design
Π	Pi term
Π_{c1}	first Pi term for bacterial transfer via static contact
Π_{c2}	second Pi term for bacterial transfer via static contact
Π_{s1}	first Pi term for bacterial transfer via dynamic contact
Π_{s2}	second Pi term for bacterial transfer via dynamic contact
Π_{s3}	third Pi term for bacterial transfer via dynamic contact
Π_{s11}	Pi term for bacterial transfer via dynamic contact from the plate to the potato
Π_{s12}	first Pi term for bacterial transfer via dynamic contact from the potato to the plate
τ	general equation for bacterial transfer via dynamic and static contact

σ	surface tension
μ	roughness
ν	viscosity
$\beta_1, \beta_2, \beta_3$	model parameters in a factorial design and in a randomized complete block design

INTRODUCTION

1.1 Background

Foodborne illness due to the consumption of contaminated produce is a concern, especially for children, pregnant women, and those who are immunocompromised and/or might have an elevated risk of becoming sick or hospitalized. Fresh produce is important for good dietary health; however, there typically is no microbial kill step during processing, which affects the risk for pathogenic bacteria to be present. The spread of bacterial contaminants can occur by various means throughout the food processing chain.

From harvest to consumption, fresh produce can become contaminated with microorganisms. Food processing includes many different steps, such as shredding, passage on conveyer belts, washing in flume tanks, centrifugation, packaging, and handling during retail distribution or/and preparation for consumption (Buchholz, Davidson, Marks, Todd, & Ryser, 2012a, 2012b; Buchholz, 2012; Ren, 2014). There are six forms of bacterial transfer within these processes: surface (or water) to product, product to surface (or water), and product to product (or water) (Luo et al. 2012). The risk starts when one product previously contaminated in the field or in handling comes into contact with equipment surfaces or water, which can lead to contamination of other products. There are a plethora of contamination sources, such as workers' hands, gloves, water, equipment surfaces, biofilm development on equipment, and the processing environment.

Ultimately, cross-contamination through these means leads to increased risk for consumers. Microorganisms, such as *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7, have been linked to outbreaks involving fresh produce. For example, in 2006, an outbreak caused by *E.coli* O157:H7 on spinach infected 199 people in 26 states (CDC, 2006).

From 2011 to 2015, 26 outbreaks were linked to consumption of fresh produce. For example, in 2015, Dole Fresh Vegetables recalled 22 varieties of bagged salad that were distributed in 13 states, due to *Listeria* contamination (CDC, 2015). That same year, prepackaged caramel apples were contaminated with *Listeria* in 12 states (CDC, 2015), and cases of salmonellosis linked to the consumption of cucumbers were reported (CDC, 2015).

Many bacterial transfer and cross-contamination studies have been conducted at a microscale, focused on understanding bacterial physiology and mechanisms of attachment and adhesion to surfaces (see Chapter 2). There is a robust body of literature addressing the fundamentals of bacterial adhesion, attachment, and biofilm formation (Chapter 2). For example, Wagner & Hensel (2011) reported the adhesive mechanisms of *Salmonella enterica*, and Krishnan & Narayana (2011) presented a study that illustrated the common structural details between surface proteins and pili, which have attachment functions, of Gram-positive bacteria. A few studies also tested the effects of physical variables (e.g., contact pressure or surface roughness) on bacterial transfer (see Chapter 2); however, very few of these studies have reported the treatments in terms of fundamental physical units. Therefore, there is insufficient information on the relationships between general physical variables and bacterial transfer.

1.2 Cross-contamination

Cross-contamination processes can contribute to the scope and severity of foodborne illness outbreaks. Cross-contamination should be prevented, but, to do so, the mechanisms of cross-contamination must be better understood. Studies have been conducted considering different variables and different conditions that affect cross-contamination, such as initial inoculation level (Fravalo, Laisney, Gillard, Salvat, & Chemaly, 2009; Aarnisalo, Sheen, Raaska,

& Tamplin, 2007), direction of transfer, and the specific handling process (van Asselt, de Jong, de Jonge, & Nauta, 2008). Such studies generally have been conducted to understand bacterial behavior and the causes of bacterial transfer. In contrast, very few studies have analyzed bacterial transfer in terms of fundamental physical variables such as contact pressure, surface roughness, contact time, and surface hydrophobicity.

1.3 Bacterial transfer during food handling

Slicers/dicers, shredders, conveyer belts, flume tanks, and packing equipment are widely used in the food industry. Bacterial transfer occurring in multi-stage processes has been studied (Buchholz et al. 2012a, 2012b; Buchholz, 2012; Ren, 2014), as well as transfer occurring at the point of slicing (Aarnisalo et al. 2007; Chaitiemwong, Hazeleger, Beumer, & Zwietering, 2014; Keskinen, Todd, & Ryser, 2008a; Perez-Rodriguez et al. 2007; Scollon, 2014a; Sheen, 2008; Sheen, Costa, & Cooke, 2010; Sheen & Hwang, 2010; Shieh, Tortorello, Fleischman, Li, & Schaffner, 2014; Vorst, Todd, & Ryser, 2006a). Other studies have quantified bacterial transfer via different utensils, such as knives (Jensen, Friedrich, Harris, Danyluk, & Schaffner, 2013) and graters (Erickson, Liao, Cannon, & Ortega, 2015). Additionally, washing protocols have been studied, including the general process (Palma-Salgado, Pearlstein, Luo, Park, & Feng, 2014) and application of sanitizer treatments (Luo et al. 2012). Models based on probability analysis developed by, for example, Munther, Luo, Wu, Magpantay, & Srinivasan (2015), Perez-Rodriguez et al. (2010), and Perez-Rodriguez et al. (2011), yielded recommendations regarding how such modeling tools are useful and how the outputs provide insight into potential sources of cross-contamination. Benefits from these models include information regarding the risk and level

of bacteria that might come to the industry during processing, and the application of control measures to reduce cross-contamination (e.g. hygiene measures, chlorination, active packaging).

1.4 Problem statement

Numerous studies have addressed bacterial transfer, adhesion, attachment, and detachment (see Chapter 2). In fact, the number of published studies in this area (Figure 1.1), related to food, have increased significantly in recent decades (Benoit, Marks & Ryser, 2013). However, previous studies have revealed gaps in information and approaches to such work. For example, statistical analysis of treatment effects is common in the literature, but few studies reported development or critical analysis of transfer models. Some authors have developed and reported probability distributions based on transfer rates (Hoelzer et al. 2012; Moller, Nauta, Christensen, Dalgaard, & Hansen, 2012; Rodriguez et al. 2011). However, understanding the complex bacterial interaction with food products, in the absence of standard models, is difficult, particularly without consideration of the fundamental physical variables involved.

Prior studies that have focused on testing or developing a transfer model with the goal of understanding the process can be classified into three categories (Table 1.1): curve-fit, complex-system or “black box” approaches, or probabilistic models. From those previous studies, it is difficult to draw general conclusions regarding the effects of physical variables on bacterial transfer. Additionally, it is not possible to infer yet which model best describes bacterial transfer under any particular condition. From the aforementioned studies, very little has been done to aggregate data or cross validate results across multiple studies, in order to draw generalized conclusions relating fundamental physical factors to bacterial transfer outcomes.

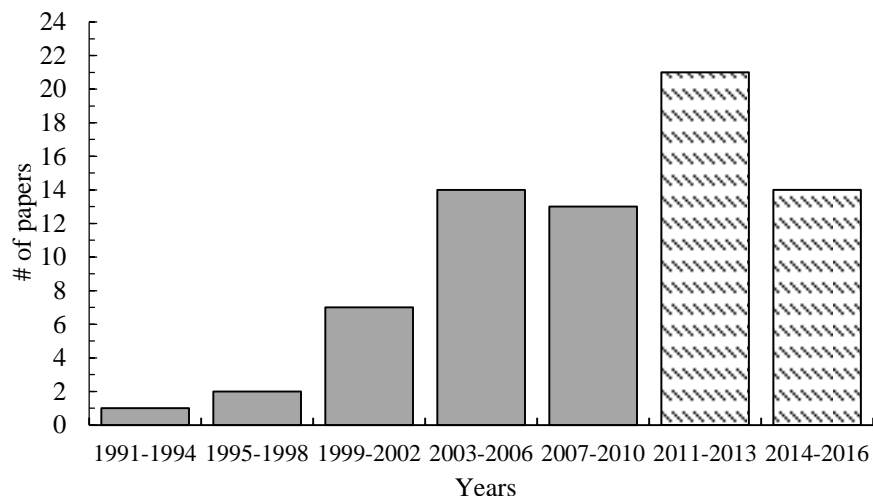


Figure 1.1 Number of peer-reviewed journal articles assessing surface attachment and transfer of foodborne pathogens, published from 1991 - 2016 (Benoit et al. 2013), studies published from 2011 to 2016 were updated.

Table 1.1 Classification of models available from the literature on bacterial transfer via surface and water.

Model type	Examples
Curve fit	Aarnisalo et al. 2007; Shieh et al. 2014; Sheen, 2008; Sheen et al. 2010; Sheen & Hwang, 2010; Wang, 2015.
Complex system	Buchholz, 2012; Buchholz et al. 2012a, 2012b; Flores & Tamplin, 2002; Ren, 2014.
Probabilistic	Hoelzer et al. 2012; Moller et al. 2012; Munther et al. 2015; Perez-Rodriguez et al. 2007; Perez-Rodriguez, Gonzalez-Garcia, Valero, Hernandez, & Rodriguez-Lazaro, 2014; Yang, Li, Griffis, & Waldroup, 2002.

1.5 Overall goal, hypotheses, and objectives

1.5.1 Overall goal

The overall goal of this dissertation was to improve the understanding of *Salmonella* transfer between contact surfaces and food products using three approaches: 1) Experimental testing of *Salmonella* transfer between a model food product (potato) and stainless steel; (2) Development and meta-analysis of a new *Salmonella* transfer database; and (3) Formulation of a novel modeling approach using dimensional analysis.

1.5.2 Hypotheses

Hypothesis 1: *Salmonella* transfer from food to a contact surface increases with moisture content, contact time, and pressure, and decreases with increasing speed.

Hypothesis 2: Across multiple studies, it can be shown that *Salmonella* transfer increases with contact surface roughness, pH, protein, fat, and water content.

Hypothesis 3: *Salmonella* transfer between food and contact surfaces can be modeled as a function of fundamental physical variables.

1.5.3 Objectives

1. To quantify the effects of fundamental physical variables (surface finish, pressure, sliding speed, and product moisture) on *Salmonella* transfer to and from stainless steel and a model produce tissue (potato) during sliding and multiple contacts (**Hypothesis 1**).
2. To conduct a quantitative meta-analysis of existing data on *Salmonella* transfer to and from food and food contact surfaces compiled in a standardized database format, to

identify generalizable trends between product contact variables and *Salmonella* transfer response (**Hypothesis 2**).

3. To propose a mathematical model for relationships between *Salmonella* transfer and fundamental physical variables, based on a dimensional analysis approach (**Hypothesis 1, 2, and 3**).

LITERATURE REVIEW

The purpose of this literature review was to synthesize studies published on bacterial transfer. Studies were found on the topics of fundamentals of food microbiology, bench-scale and pilot plant studies, best fit models, and data aggregation. The body of work on bacterial transfer to/from food products has grown significantly, but some gaps were found in standardization of methods for transfer studies. In addition, there remains a significant need/opportunity to quantitatively evaluate the data that have been published to date, to determine whether any generalizable relationship can be elucidated. The current work collected data from previous studies including various food products, microorganisms, and surface materials for further comparison with data collected from original experimental designs, with a primary focus on the effect of physical variables.

2.1 Foodborne illnesses and contamination

2.1.1 Foodborne illness caused by *Listeria*

Listeria monocytogenes is a Gram-positive bacterium that can cause meningitis, septicemia, and abortion (Montville & Matthews, 2008). It is a facultative anaerobic microorganism, psychrotrophic, and grows in human phagocytes. *L. monocytogenes* is capable of growing under a variety of environmental conditions, including temperatures from 0 to 45°C (Montville & Matthews, 2008). A higher production of biofilm was found at 30°C after 24 h (Stepanovic, Cirkovic, Mijac, & Svabic-Vlahovic, 2003). It can be killed at temperatures higher than 50°C. Growth is possible at a pH of 4.2, and survival (but not growth) can occur at a lower pH. It can grow at water activities above 0.92. *L. monocytogenes* exhibits tumbling motility at

ambient temperature due to peritrichous flagella, and can attach to materials such as stainless steel, glass, and rubber (Montville & Matthews., 2008).

The CDC has reported listeriosis outbreaks linked to the consumption of fresh produce. One listeriosis outbreak was found to be connected to consumption of sprouts (CDC, 2014). During this outbreak, a total of five people were hospitalized, and two deaths were reported. Another outbreak reported in 2011 was linked to whole cantaloupes and included 143 hospitalizations and 33 deaths in 28 states (CDC, 2011). Food products such as ackawi cheese, chives cheese, Mexican style cheese, blue-veined cheese, and cantaloupe also were reported as food vehicle leading to listeriosis (CDC, 2011).

2.1.2 Foodborne illness caused by *Salmonella*

Salmonella is a Gram-negative, rod-shaped facultative anaerobic bacterium belonging to the family *Enterobacteriaceae* (Montville & Matthews, 2008). It is resilient and capable of adapting to extreme environmental conditions. It can grow at pH ranging from 4.5 to 9.5 and in environments of high salinity (>2%). Some strains can adapt to a temperature of 54°C, or at 2 to 4°C can exhibit psychrophilic properties. Water activities less than 0.96 do not support the growth of *Salmonella*, and salt concentrations of 3 to 4% inhibit the microorganism (Montville & Matthews, 2008).

According to the CDC (2015), there are more than 2,500 different serotypes of *Salmonella*. It is a large genus that includes more than 2000 distinct strains. The most common in the United States are *Salmonella* Typhimurium and *Salmonella* Enteritidis. *Salmonella* causes an estimated one million illnesses in the United States, with 19,000 hospitalizations and 380 deaths, annually (CDC, 2012). In one salmonellosis outbreak linked to cucumbers (CDC, 2015), 732

cases were reported in 35 states, resulting in 4 deaths and 150 hospitalizations. Such large outbreaks likely indicate a problem with cross-contamination somewhere in the harvest, handling, packing, processing, and distribution systems.

2.1.3 Foodborne illness caused by *Escherichia coli*

Escherichia coli is a Gram-negative bacterium (Montville & Matthews, 2008). Most *E. coli* strains are harmless. However, some are pathogenic and can cause diarrheal disease. *E. coli* O157:H7 causes Hemolytic Uremic Syndrome (HUS) and TTP (Thrombotic Thrombocytopenic Purpura), the adult form of HUS. Fewer than 100 cells, and possibly as few as 10 cells, are enough to cause an illness (Montville & Matthews., 2008). It can grow at a minimum pH of 4.0 to 4.5. *E. coli* is less heat resistant than many other pathogens and is unable to grow well at temperatures higher than 44.5°C (Montville & Matthews., 2008).

According to the CDC (2014), *E. coli* is still an important cause of human illness in the United States. In one outbreak in 2014, 19 cases were reported in six different states, linked to the consumption of raw clover sprouts (CDC, 2014). Although no deaths were reported, 44% of the cases required hospitalization.

2.2 Fundamentals of bacterial adhesion

2.2.1 Factors affecting attachment

Ultimately, it is important to understand both the pathogens involved and their interactions with food products and contact surfaces, to improve understanding of bacterial transfer processes. Prior work in this area includes attachment, biofilm development, transfer, and modeling. A few studies have considered internalization (Burnett, Chen, & Beuchat, 2000),

gene expression (Salazar et al. 2013), and model development (Hoelzer et al (2012), Moller et al (2012), Yang et al (2002), and Zilelidou, Tsourou, Poimenidou, Loukou, & Skandamis (2015)).

Bacterial attachment is a complex process that depends on a significant number of factors and the interactions between them, such as environmental factors, surface characteristics, bacterial physiology, etc. These factors influence the rate and degree of attachment to the surface. Bazaka, Crawford, Nazarenko, & Ivanova (2011) reported that such factors also include the surface energy of the structure, the hydrophobicity of the bacterial cell, the presence of fimbriae and flagella, the extent of extracellular polysaccharide (EPSs) production, and the type of polymeric materials being produced by the cell.

Environmental factors such as the attachment surface make this process even more difficult to fully understand. Geng & Henry (2011) affirmed that bacterial attachment to artificial surfaces appears to occur via several types of dynamic processes that have often been confused: cell-surface association, surface link maturation, and adhesive substrate property alterations. The combination of factors makes identifying and understanding the mechanism difficult; furthermore, the limitations of such studies are affected by measurement capabilities and accuracy.

2.2.2 Definition of attachment

Tsang, Li, Brun, Ben Freund, & Tang (2006) stated that to fully understand the mechanisms of biofouling and biofilm formation, it is essential to comprehend the nature, biosynthesis, and properties of the adhesives that mediate the bacterial attachment to surfaces. There are two types of attachment, reversible and irreversible. Initially, bacteria are transferred from a previously inoculated/contaminated surface to a food product or vice versa. The first step

is bacterial transfer followed by attachment, and then possibly bacterial growth. Ong, Razatos, Georgiou, & Sharma (1999) defined bacterial adhesion to surfaces as the initial attraction of the cells to the surface followed by adsorption and attachment.

2.2.3 Characterization

The concept of adhesion or attachment is described in the literature as the interaction between microorganisms and surfaces, and the bacteria's physiology that leads to the cell's anchorage to a surface (Mafu, Roy, Goulet, & Magny, 1990; Silva, Teixeira, Oliveira, & Azeredo, 2008; Tuson & Weibel, 2013). The words adhesion and attachment are used interchangeably. In a literature review covering areas other than food applications, Tuson & Weibel (2013) explained that reversible attachment takes as little as 1 min. In addition, other authors studied the behavior of bacterial attachment during short time periods (15 min to 1 h). For example, Mafu, Roy, Goulet, & Magny (1990) evaluated short contact times between *Listeria monocytogenes* and different materials, such as stainless steel and polypropylene, which are frequently used in the food industry. From this, attachment was defined as the surface-material interaction over short time periods with weak bacteria-surface interactions.

Tuson & Weibel (2013) defined adhesion as a process where van der Waals forces prevail and electrostatic forces of attraction and repulsion are the primary forces influencing attachment. They further defined attachment as a series of hydrophobic interactions, during which interactions among curli, flagella, and pili prevail, and genetic regulatory networks start to change gene expression profiles. The interpretation from this definition is that adhesion involves changes in bacterial physiology and related effects that impact surface-bacteria interactions.

In another literature review, Goulter, Gentle, & Dykes (2009) identified three causes for differences observed among bacterial attachment studies: (1) a lack of standardization across methods, resulting in conflicting data, (2) low sensitivity of methods, and (3) the study of the bulk properties of many bacteria as opposed to individual cells. Considering the gaps and challenges encountered in different studies and the definitions found in the literature, significant gaps remain in the knowledge base relating the fundamentals of bacterial attachment to actual bacterial transfer outcomes between foods and contact surfaces.

2.2.4 Contributing forces

Bacterial attachment is a complicated process, Giaouris et al. (2014) listed different factors affecting bacterial attachment, such as food composition, texture (homogeneity and roughness), and physicochemical properties (hydrophobicity and surface electrical charge) of surfaces employed (abiotic or food surface), the blade speed-size-sharpness-material, cutting force, slicing speed, the microorganism characteristics (growth phase, strain, inoculum size, capability to adapt to different stresses, ability, and strength of adhesion to surfaces), and finally the environmental conditions (temperature and relative humidity). Although it is difficult to simply and accurately define bacterial attachment, it is important to consider all of the factors involved.

2.2.5 Van der Waals interactions

Van der Waals interactions play a fundamental role in the initial attachment of bacteria to a surface. Ong et al. (1999) described that the initial adhesion of bacteria to natural or artificial surfaces correspond to van der Waals interactions. The same criteria were used by Tuson &

Weibel (2013). Additionally, electrostatic, hydration, and hydrophobic interactions play an important role in the beginning phases of attachment.

In the model developed by Ong et al. (1999), polar (or hydrophobic) and steric interactions were added to the conventional van der Waals attraction and electrostatic components. They acquired data for tip deflection (nm) versus piezo position (nm). Plots of force (nN) versus distance of separation (nm) were presented as insets to plots of tip deflection versus relative distance of separation. A similar study was reported by Tsang et al. (2006), who also measured the deflection of a thin flexible pipette. There were differences between the studies, materials and methods, but the fundamental variables used to evaluate attachment force were the same, which makes the results comparable in terms of fundamental units of the mechanisms of attachment (i.e., force and distance).

2.2.6 Surface hydrophobicity

Ong et al. (1999) found that bacterial attachment is enhanced by surface hydrophobicity of the substrate. They found that the attractive force for a more hydrophobic strain (D21f2) of *E. coli* increased with the hydrophobicity of the substrate. The materials tested were listed according to increasing order of hydrophobicity: mica, glass, polystyrene, and Teflon.

Donlan (2002) notes that studies on this topic are often contradictory because no standardized methods exist for determining surface hydrophobicity. Hydrophobic interactions between the cell surface and the substratum are stronger than the repulsive forces and form irreversible bonds. Different authors, for example Wang, Feng, Liang, Luo, & Malyarchuk (2009) and Oliveira, Oliveira, Teixeira, Azeredo, & Oliveira (2007), measured surface hydrophobicity differently. Wang et al. (2009) measured surface hydrophobicity on produce and

metals using a goniometer through a microscope. Oliveira et al. (2007) applied the sessile drop method. They affirm that the mechanisms governing adhesion of *Salmonella* spp. to inert surfaces are not completely understood. Overall, hydrophobicity is a general concept that cannot be directly measured for individual bacterial cells, but only estimated by observing the bulk properties of numerous cells and interpreting these interactions as reflecting molecular interactions (Goulter et al. 2009).

2.2.7 Polysaccharides

A biofilm is mainly composed of exopolysaccharides (EPS) and microbial cells (Donlan, 2002). Exopolysaccharides are the primary matrix material of biofilms and are responsible for biofilm conformation, rigidity, deformation, and solubility or insolubility (Donlan, 2002). Oliveira et al. (2007) stated that the EPS should be studied further because, in addition to variation among strains, EPS may play a major role in adhesion.

Bazaka et al. (2011) explained that capsular polysaccharides and free EPS are present in the outermost layer of a cell. As a result, they form an additional barrier between the membrane of the bacterium and its environment. Distribution of these extracellular polymeric substances is also influenced to a great extent by the nature of the cells' ambient conditions, such as solution chemistry, abundance of nutrients, and the growth phase of the cells. Ong et al. (1999) found that force measurements on a variety of substrates show that the lipopolysaccharides (LPS) molecules coating the cell surface greatly influence bacterial adhesion.

Polysaccharides have three different important functions affecting the interaction of bacteria with surfaces (Bazaka et al. 2011). They facilitate adhesion, give protection, and provide nutrition. Adhesion is improved with surface roughness due to the associated increase in surface

area available for colonization (Bazaka et al. 2011). Polysaccharides also are mediators that can increase the function of fimbria and flagella. During the attachment process, polysaccharides shield bacteria from the effects of a changing environment. Any spatial or temporal variations in the location of bacteria may directly or indirectly select for certain capsular polysaccharides (Bazaka et al. 2011).

2.2.8 Other factors

Other factors have been identified that affect bacterial transfer, including starvation and electrolyte concentration. The effect of starvation on the attachment of *E. coli* O157:H7 to fresh produce has not yet been addressed (Van der Linden et al. 2014). Ong et al. (1999) tested adhesion forces between *E. coli* and mica, hydrophilic glass, hydrophobic glass, polystyrene, and Teflon and concluded that electrolyte concentration is an environmental factor affecting adhesion processes.

2.3 Bacterial physiology and attachment to food contact surfaces

Many studies have evaluated bacterial attachment to food contact surfaces present in industrial facilities (Abban, Jakobsen, & Jespersen, 2012; Kim & Silva, 2005; Mafu et al. 1990; Mafu et al. 1991; H. D. N. Nguyen, Yang, & Yuk, 2014; V. T. Nguyen, Turner, & Dykes, 2010; Oliveira et al. 2007). Physiological factors that contribute to bacterial attachment to surfaces have been studied for numerous organisms, including *Yersinia* (Leo & Skurnik, 2011), *Salmonella enterica* (Wagner & Hensel, 2011), *Borrelia burgdorferi* (Antonara, Ristow, & Coburn, 2011), *Bartonella* spp. (O'Rourke, Schmidgen, Kaiser, Linke, & Kempf, 2011), *Xanthomonadaceae* (Mhedbi-Hajri, Jacques & Koebnik, 2011), *Corynebacteria* (Rogers, Das, &

Ton-That, 2011), and Staphylococci (Heilmann., 2011). The authors reported the attachment strength of both single bacterial cells and colonies. They found that the adhesion profile changed as a function of shear stress and presence of proteins, as determined using varying flow conditions, and they include motility as an important factor affecting bacterial adhesion.

Tsang et al. (2006) developed a method for measuring the attachment force of one cell of *Caulobacter crescentus* in the microNewton range, and reported the largest adhesion force ($0.59 \pm 0.62 \mu\text{N}$) ever measured on this scale. Ong et al. (1999) looked at bacterial strain as one of the factors that affect adhesion and affirmed that *E. coli* D21 and *E. coli* D21f2 behave completely different on the same material, supporting the premise that strain is a key factor affecting bacterial attachment.

De Figueiredo, de Andrade, Ozela, & Morales (2009) stated that genotypic factors, including expression of the genes encoding for flagella, fimbria, pili and exopolysaccharides production, affect the adhesion process. However, they also noted that interactions between surfaces and bacteria are not well understood yet. Adhesion of 22 strains of *L. monocytogenes* were tested by Mafu et al. (1991), and the strain Scott A had a higher energy of attraction to polypropylene and rubber than glass and stainless steel. In addition, they affirmed that the presence of exopolymer may affect bacterial adhesion to food contact surfaces. The study of the effect of different surfaces on bacterial transfer might help to understand if roughness facilitates or impedes this transfer.

Mafu et al. (1990), Nguyen et al (2014), and Nguyen et al. (2010) performed complex transfer studies that involved many variables, which make the results difficult to analyze. Mafu et al. (1990) reported that rubber and polypropylene surfaces had lower surface energies than stainless steel and glass. However, *L. monocytogenes* could attach to porous and nonporous

surfaces. It can be inferred that bacteria have a different pattern of attachment that also depends on time and temperature. Nguyen et al. (2014) evaluated *Salmonella* Typhimurium attachment to two different surfaces and reported significant differences in the population attached after 24 h; however, 48 h later, there were no significant differences. Nguyen et al. (2010) demonstrated a lower probability of detachment for five of six strains at 25°C as opposed to 4°C. Knowledge of the relationships between fundamental variables is necessary to enable researchers to simplify the system, in terms of quantifying and modeling attachment and transfer outcomes.

Different results were reported by Kusumaningrum et al. (2003), who reported that cross-contamination from a sponge to a stainless steel surface was not dependent on the microorganism type ($p = 0.07$) or the initial inoculation levels ($p = 0.30$). These results suggest that differences in methodologies and strains have an effect on observed outcomes and effects related to bacterial transfer. Standardization of methods, the control, the measurement, and the analysis of individual variables would better allow researchers to generalize what key generalizable factors affect bacterial transfer.

2.4 Bacterial attachment to food products

Compared to attachment to food contact surfaces (e.g., stainless steel), bacterial attachment to different food product surfaces is even more complex, given so many variables involved, including surface material composition and biological interactions with the food matrix. One possible classification of the many variables is chemical or physical. Material characteristics of food contact surfaces can be characterized relatively easily, as there are few differences between units. There are significant differences between microorganisms and food products (e.g., individual pieces of fresh produce), as they vary with age, cultivar, and maturity.

Chua & Dykes (2013) assessed a study on the attachment of foodborne pathogens to banana leaves, using three microorganisms. They found significant differences between strains in attachment to leaves. An important contribution is that they characterized the wax content of the leaves and identified that the number of attached bacteria was similar for spinach and lettuce. However, data are not comparable with other studies, because different methods were used among them and physical roughness of the leaf surfaces was not characterized.

Ukuku & Fett (2002) investigated the theory that bacterial surface charge and hydrophobicity may affect bacterial attachment and complicate bacterial detachment from cantaloupe surfaces. Their results show different behavior in bacterial attachment based on the variables measured for each microorganism, which makes it difficult to draw general conclusions. In addition, the relationship between surface hydrophobicity and surface roughness was undetermined. Similarly, Wang et al. (2009) identified a lack of information on the effect of surface hydrophobicity of fruits and vegetables on bacterial adhesion. They found a linear relationship between surface roughness and surface hydrophobicity and reported an increase in bacterial adhesion as surface roughness increased. Few studies like this have tried to elucidate the behavior of bacterial transfer versus a fundamental physical variable.

In addition, Midelet & Carpentier (2004) demonstrated the influence of three factors on bacterial transfer: substrate material, bacterial species, and prior contact with a sanitizer. These factors are outside the scope of this study, but they contribute to the understanding of the fundamentals of bacterial transfer. The method consisted of transferring bacteria from a pre-treated biofilm to a model food. The study focused on the cell scale as they compared micro-colonies to single cells, and presented similarities with other studies.

Kusumaningrum et al. (2003) reported that bacterial behavior depends on surface attachment (food product versus food contact material). They investigated the effect of different conditions on bacterial transfer and survival using two sampling methods. They applied a pressure and studied four microorganisms. They recommended further studies on the effect of moisture content of the surface. Relative to moisture state, Schaffner & Schaffner (2007) found a significant difference between frozen and unfrozen food products. In terms of bacterial transfer they hypothesized that this difference could be due to the difference in liquid moisture present on the surface of unfrozen versus frozen products. Given the importance of water in fresh produce, the effect of water content on bacterial transfer during surface contact events could be a critical variable with generalizable trends discernable either by systematic experimental investigation or meta-analysis of multiple studies that reported this variable.

2.5 Process of biofilm formation

Biofilm formation occurs as cells grow and stick to each other and attach to a surface, thereby affecting potential bacterial transfer to/from surfaces. The process of biofilm formation consists of three stages that occur sequentially: attachment, maturation, and dispersal (Cappitelli, Polo, & Villa, 2014). Garrett, Bhakoo, & Zhang (2008) reported that various environmental variables can affect biofilm development including: pH, rheological and adhesive properties of biofilms, and temperature. Other factors influencing biofilm formation mentioned by Giaouris et al. (2014) are related to nutritional conditions, bacterial co-aggregation, metabolic requirements, exposure to antimicrobial agents, and other environmental factors.

2.5.1 Effect of temperature on biofilm formation

According to Cappitelli et al. (2014), few biofilm studies have focused on fluctuating temperature, despite the fact that food processing plants frequently experience varying environmental conditions. Nguyen et al. (2010) included four temperatures in a study in which they quantified bacterial detachment from a previously inoculated stainless steel coupon (plate) to an agar plate. They found that an increase in temperature increased the number of *C. jejuni* cells transferred to the agar. They reported $\sim 4 \log \text{ CFU/cm}^2$ transferred at 4°C and $\sim 5 \log \text{ CFU/cm}^2$ at 55°C . In another study, Nguyen et al. (2014) reported that temperature and pH could have an effect on the rate of bacterial attachment during the first 14 h. They defined trends on bacterial attachment during periods up to 240 h. Biofilm formation can lead to migration of bacteria to other surface materials, where bacteria can be transferred to food products.

2.5.2 Availability of nutrients

In order to grow and survive, bacteria need nutrients. Midelet, Kobilinsky, & Carpentier (2006) studied attachment strength and transfer of *L. monocytogenes* from pure or mixed biofilms after contact with a solid model food. Four different media were used that varied in composition, glucose, calcium, incubation temperature, and age. They found differences in detachment of bacteria as a function of the contact number. The strength of bacterial attachment depended on the number of sequential contacts. Midelet et al.'s study focused on the effect of chemical shock that is achieved with different compositions of the substrate. They showed that the layers of bacteria present different detachment behavior depending on the contact number, consistent with the reasoning of Kusumaningrum et al. (2003).

2.5.3 Gene expression effects on biofilm formation and bacterial adhesion

The previously discussed studies also reported that the type of microorganism and genetics affect bacterial attachment. Ukuku & Fett (2002) found that attachment of *Salmonella* strains to cantaloupe was the strongest, and the attachment of *E. coli* was more extensive than that of *L. monocytogenes*. Oliveira et al (2007) compared adhesion of four strains of *Salmonella* Enteritidis (EMB, MUSC, AL, and PC) on stainless steel 304. The strains they tested showed no significant differences in the values of hydrophobicity degree, for instance, and no significant differences were found among the level of adhesion.

More specifically, Bonsaglia et al. (2014), in a study on biofilm production of *L. monocytogenes*, found that the product of the *inlA* gene is responsible for facilitating the entry of the microorganism into epithelial cells that express the receptor E-cadherin, which also participates in surface attachment. In another study performed on gene expression by Salazar et al. (2013), they reported that the deletion of the gene *ycfR* in *Salmonella* Typhimurium significantly reduced bacterial chlorine resistance and attachment to plant surfaces after chlorinated water washes. Giaouris et al. (2014), in a review, concluded that significant changes in gene expression occur in bacterial cells from initial interaction with a substratum to the sessile growth.

2.6 Bench-scale transfer experiments

As noted above, previous studies have linked the cellular-level processes to bacterial interactions with surfaces. However, such studies are still well removed from the actual, complex processes that occur at the macroscopic level, when bacteria transfer between real food products

and food contact surfaces. Consequently, bench-top scale experiments are typically designed to evaluate bacterial transfer in these types of scenarios.

2.6.1 Objectives of bench-scale experiments

Bench-scale experiments allow examination of the variables involved in bacterial transfer phenomena. Most such studies isolate and analyze these variables independently. This approach allows researchers to conclude the effect of specific variables on the behavior of bacterial transfer. Most of the studies were developed independently. However, they do contain novel ideas, because they reported new inoculation methods, they test a variety of products, and the data collected allow for further analysis.

Most studies of this type are designed to quantify bacterial populations transferred. A variety of studies have been conducted on food products coming in direct contact with a surface (Ak et al. 1994a, 1994b; Kusumaningrum et al. 2003; Midelet & Carpentier, 2002; Midelet et al. 2006; Moore, Blair, & McDowell, 2007; Sharps, Kotwal, & Cannon, 2012), multiple contacts (Benoit, 2013; Kim & Silva, 2005), and slicing processes (Aarnisalo et al. 2007; Chaitiemwong et al. 2014; Keskinen et al. 2008a; Perez-Rodriguez et al. 2007; Scollon, 2014b; Sheen, 2008; Sheen et al. 2010; Sheen & Hwang, 2010; Shieh et al. 2014; Vorst et al. 2006a; Vorst, Todd, & Ryser, 2006b; Haiqiang Wang, 2015). These studies were developed independently, and there is an absence of a unifying approach to such studies, in order to determine which variables significantly affect bacterial transfer across multiple product and surface types.

Bench-scale experiments were also developed to simulate kitchen conditions (Ak et al. 1994a, 1994b; Erickson et al. 2015; Mafu et al. 1990). Ak et al. (1994a, 1994b) isolated the problem of bacterial cross-contamination on cutting boards in the kitchen. They listed and evaluated variables that might have an effect on bacterial transfer. That study identified that the

type of material in contact with the food product has a significant effect on bacterial transfer. No significant difference was found between the different types of wood and plastic evaluated. It is difficult to compare the results of this study to others because different variables and modes of transfer were evaluated. A similar gap was found in the study by Mafu et al. (1990).

Erickson et al. (2015), developed a study on cross-contamination during fresh produce slicing. They included common kitchen utensils and focused on storage, residue formation, and inoculation protocols. They focused on the ability of microorganisms to attach to different stainless steel utensils and reported differences among produce types (cantaloupe, carrot, cucumber, honeydew, strawberry, and tomatoes). This is one of the few studies conducted on a wide range of fresh produce. The authors drew important conclusions that contribute to the understanding of bacterial transfer behavior. For example, the residue remaining on the kitchen utensil did not affect contamination of the utensil when used to process contaminated produce items, and the risk of contaminating the utensil depends on the product type. A grater was used on carrots, whereas a knife was used for the remaining items, and the effect of residues was evaluated only on strawberries and carrots, which makes these conclusions non-generalizable.

All food contact surfaces along a processing line are potential sources of bacterial transfer. Montville & Schaffner (2003) measured transfer rates between different kitchen items and food products, such as chicken to cutting board, chicken to bare hand, bare hand to lettuce, bare hand to spigot, and gloved hand to lettuce. That study was designed to determine whether initial inoculum levels can significantly affect experimental results. Ultimately, they recommended including the total number of bacteria and high initial inoculation levels in future cross-contamination studies.

In all the studies mentioned above, differences between them had been identified which consist mainly on the order of magnitude of the experiments set-up, the scale of the experimental design, the food product type, the microorganism, the data generated, the material type, and the treatments applied to the food products. Such differences among experimental designs and methods make it very difficult to draw general conclusions about factors affecting bacterial transfer.

2.6.2 Physical variables studied in bench-scale experiments

This dissertation focuses on the effect of physical properties on bacterial transfer. Physical variables are defined as a way to observe and describe matter. Physical properties govern the process of preparing fresh produce for the market. Physical variables, such as contact speed, pressure, and surface roughness, are a combination of the fundamental variables (mass, length, and time) from an engineering point of view, and govern the behavior of bacterial transfer during food processing and preparation.

Many studies have focused on the effect of specific variables, either product or processing, on bacterial transfer. For example, Perni, Read, & Shama (2008) studied slicer blade rotational speed. Wang, Liang, Feng, & Luo (2007) studied the effect of varying the speed of water flow on the removal of bacteria from the surface of fresh produce during washing and found that the Weibull model best described the results. The goodness-of-fit was described by R^2 , mean square error (MSE), and accuracy factor (A_f). Goulter-Thorsen, Taran, Gentle, Gobius, & Dykes (2011) evaluated six *E. coli* strains and three different materials with varying surface roughness. Bacterial attachment was higher on stainless steel SS8 than other finishes (SS4 and SS2B). Kusumaningrum et al. (2003) evaluated the effect of force on bacterial transfer and

survival from a stainless steel coupon to a plate with agar by contact, and quantified that a single contact transferred 50-60% of the total population. In a study performed by Silva, Teixeira, Oliveira, & Azeredo (2008), a variety of materials were analyzed (stainless steel 304, marble, granite, glass, polypropylene, and silestone) as well as a variety of variables (chemical composition of the surface, characteristics of the liquid surrounding the microorganism and the surface, and gene expression). They affirmed that *L. monocytogenes* adhered more tightly to granite and marble, followed by stainless steel 304 and glass. However, only a limited number of variables are typically measured and included in any single study. Due to this, the information and data available from an individual study are generally insufficient to develop a mathematical model of bacterial transfer.

The effect of surface roughness on bacterial attachment also was studied by Wang et al. (2009) and Sheen (2008). Both studies identified gaps in the literature. On one side, the relationship between surface hydrophobicity and surface roughness is largely unknown, and on the other side there is no universal method of measurement and/or instruments for quantifying transfer responses.

In terms of consecutive contact events, and contact times, Aarnisalo et al. (2007), Verran, Packer, Kelly, & Whitehead (2010), Kim & Silva (2005), Keskinen et al. (2008a), and Smid, de Jonge, Havelaar, & Pielaat (2013) quantified bacterial transfer as a function of observation number; however, different observational units were evaluated as the independent variable. Other authors also have studied bacterial transfer as a function of time, such as Takhistov & George (2004), Demoz & Korsten (2006), Raya et al. (2010), Perni et al. (2008), Ukuku & Sapers (2007), Kroupitski, Pinto, Brandl, Belausov, & Sela (2009), Liao & Sapers (2000),

Harapas, Premier, Tomkins, Franz, & Ajlouni (2010). Moore et al. (2007) studied bacterial transfer as a function of time, but time intervals and units used to report data were different.

After a thorough review of the literature data on bacterial transfer via surface equipment, data gaps were found mainly in information available on the physical variables of roughness and firmness. A majority of the studies did not include firmness as a variable in their evaluations. In contrast, food composition generally was specified in detail. In order to draw general conclusion, it is important to characterize the food product and contact surfaces in terms of both chemical and physical properties.

2.6.3 Contribution of bench-scale experiments to future studies

The objective of this section is to show the gaps identified in data collected from bench-scale experiments. The gaps can be classified as follows: data gathered, experimental design, and modeling. Few studies reported the results using fundamental units; most of them developed different methods, and few of them fit models or included various data from other studies. The identification of these gaps contributed significantly to the proposed use of the three synergistic approaches of this study.

Overall, the prior bacterial transfer studies have contributed fundamental knowledge about bacterial behavior, and helped to identify challenges in the food industry. Mafu et al. (1990) reported images from scanning electron microscopy of bacteria attached to different materials. These results are considered qualitative and show differences in bacterial population as a function of the treatment applied. Ukuku & Fett (2002) affirmed that the ability of pathogenic bacteria to adhere to surfaces of fruits and vegetables continues to be a potential food safety problem of great concern in the produce industry. They reported strength of attachment as

the ratio between strongly and loosely attached bacteria. Unfortunately, the results were dimensionless, because they used a qualitative scale for defining the strength of bacterial attachment. Garrett et al. (2008) affirmed the usefulness of environmental scanning electron microscopy, optical microscopy, and confocal laser microscopy are powerful in investigating bacterial transfer, but these are observational tools that do not directly measure attachment of bacterial populations. Micromanipulation is the only technique that enables direct measurement of biofilm adhesion. Further research in this area targeted at unifying units and methods would be beneficial to understanding the problem and developing a solution.

Studies conducted on slicing processes have identified a variety of conditions that enhanced bacterial transfer. For example, Wang (2015), Scollon (2014), Shieh et al. (2014), and others conducted similar tests. They point out the fact that bacterial transfer behaves as a continuum. Erickson et al. (2015) also tested transfer with a diversity of produce; however, the results were reported as positive or negative for pathogen presence on each of the produce items. An important conclusion was that longer contact times and greater degrees of force during grating, led to greater bacterial transfer, however force was not quantified. Their experimental design was not focused on the measurement of the physico-chemical variables and quantification of bacterial population per slice.

2.7 Pilot-scale studies on bacterial transfer

2.7.1 Overall purpose, equipment, and gaps

Buchholz et al. (2012a, 2012b), Buchholz (2012), Perez-Rodriguez et al. (2011), Yang et al. (2002), and Ren (2014) have conducted complex pilot-scale experiments using mostly fresh produce (lettuce). They included different equipment units and process operations in an attempt

to understand bacteria and surface material interactions. They included process operations as experimental units, such as a slicer, flume, shredder, centrifuge, and workers' hands and gloves, but the fundamental physical variables were not included in the experimental design, nor measured or reported.

Yang et al. (2002) conducted a study on cross-contamination of poultry with *Campylobacter jejuni* and *Salmonella* Typhimurium during the chilling process. They affirmed that no prediction model had been reported for the prediction of possible outcomes of bacterial cross-contamination, thereby identifying a knowledge gap surrounding what is essentially a bacterial transfer risk point in poultry processing systems.

In a bacterial transfer study using a pipe system for dairy products, a decreasing trend was found in the ratio between the speed and the bacteria adhered to the equipment pieces (de Figueiredo et al., 2009). They included, in the experimental design and in the analysis, basic physical variables, such as contact area, speed, Reynolds number, and time in the experimental design; in addition, they reported temperature, sanitizer concentration, and time of the cleaning procedures, as well as the conduction of the experiments. Although this prior study was on transfer to/from liquids rather than solid surfaces, the study of the fundamental variables agrees with the objectives of the experimental design of the present study.

2.7.2 Pilot-scale research of fresh produce during processing

The processes of peeling, cutting, shredding, cleaning, washing, and drying are fundamental in the fresh-produce industry. These operations change according to the fresh-produce type. Several studies have reported on bacterial transfer via water during washing used in model food systems of leafy greens (Buchholz et al., 2012a, 2012b; Buchholz, 2012; Palma-

Salgado et al., 2014; Ren, 2014; Rodriguez et al., 2011; Haiqiang Wang, 2015). They evaluated different water treatment and sanitizers during washing (Palma-Salgado et al., 2014; Wang et al., 2007). For example, Palma-Salgado et al. (2014) evaluated the effect of washing a whole head of Iceberg lettuce (*Latuca sativa* L.) prior to cutting, on recovery of *E. coli* O157:H7. They found that prewashing the head diminishes post cutting recovery of bacteria. They also affirmed that the hydrodynamic flow conditions play an important role in the effectiveness of a sanitizer, but physical variables were not included in the treatments.

In another pilot-scale washing study, Luo et al. (2012) evaluated the effect of applying T128 to adjust the pH of the water in a wash tank with sanitizer to decrease *E. coli* O157:H7 attachment to leafy greens treated with chlorine. The results showed that longer contact times were necessary for inactivating *E. coli* O157:H7 at low chlorine concentration. The purpose of this research was to evaluate T128, which adjusts the pH of wash water. General conclusions regarding bacterial transfer and physical variables of washing processes were not obtained from the previous studies, because their purpose was to study probability distributions and sanitizer effectiveness.

2.8 State-of-the-art for analysis of bacterial transfer systems

2.8.1 Bacterial transfer modeling

Several studies have modeled bacterial transfer, including Moller et al (2012); Nauta, van der Fels-Klerx, & Havelaar (2005), Hoelzer et al (2012), Perez-Rodriguez et al (2007), and Munther et al (2015). A majority of the studies used probabilistic or best-fit models. Some of the studies collected data from different authors, but most focused on analyzing data only from their

own experiments. None of the studies included a general form of model based on fundamental physical variables or worked to aggregate and analyze data from multiple studies.

In a quantitative pilot-scale study on *Salmonella* distribution in lettuce, Perez-Rodriguez et al. (2014) reported that initial inoculation levels affected cross-contamination and that the cutting, mixing, and washing steps produced a homogenous distribution of contamination during processing. As a result, they obtained probability distributions. However, they included very few fundamental physical variables in their study.

Model fitting is an important step in the development of a general model. Several bacterial transfer studies have addressed this topic, such as Shieh et al. (2014), Aarnisalo et al. (2007), Keskinen et al. (2008a), Keskinen, Todd, & Ryser (2008b), Perez-Rodriguez et al. (2007), Scollon (2014), Vorst et al. (2006b), Vorst et al. (2006a), and Wang (2015). Shieh et al. (2014) obtained the best fit for a log-linear model and they also tested a Weibull-type model. The correlation coefficient (0.905) was the criteria for selecting the model that best fit the data. However the conclusions of that study are specific to one microorganism, one product, and one process, which limits utility in other applications.

Nauta et al. (2005) pointed out the need to develop a mechanistic model for bacterial transfer during poultry processing. In addition, they emphasized the importance of subdividing a model according to the transfer type and the sources of contamination, such as water, air, and surface. In another study, Moller et al. (2012) applied the model to pork, rather than poultry. The database later discussed in this dissertation, and the design of additional experiments, contribute to filling one of the gaps (contamination via surface) identified by these authors.

According to Giaouris et al. (2014), models for bacterial transfer via a slicing machine or via multiple contact, similar to the study developed by Nguyen et al. (2010), are empirical. These

models may provide a useful tool in developing risk assessments, since they may be applied to predict the number of slices that may be contaminated by a pathogen-contaminated slicer during slicing operation. However, these models are both microbial-load and contamination-route dependent, which might limit their applications to other specific conditions.

The modeling work of Hoelzer et al. (2012) and Munther et al. (2015) used data from other studies and similar methods already developed by Rodriguez et al. (2011). These publications include bacterial transfer via surface and via water. Munther et al. (2015) developed a model based on rates. The free chlorine concentration was one component of the model, as well as the chemical oxygen demand. They discussed how pilot-plant practices affect the model fitting. In addition, they pointed out that the difference in the scale among experiments shows a discrepancy in the inactivation rate of free chlorine in the water. The current study proposes to develop a similar analysis but focuses on bacteria transferred via food contact surfaces.

Models for bacterial transfer to/from food and food contact surfaces can be classified as: complex model systems, probabilistic, and best-fit models. Buchholz et al. (2012b, 2012a, 2012) developed complex model systems, whereas Sheen & Hwang (2010), Sheen et al. (2010), Sheen (2008) developed best fit models. Probabilistic models were developed by Zilelidou et al. (2015), Hoelzer et al. (2012), Moller et al. (2012), and Perez-Rodriguez et al. (2011).

Few studies have been published on modeling bacterial transfer to/from contact surfaces. Zilelidou et al. (2015) developed a semi-mechanistic model that considered bacterial transfer during the preparation of fresh-cut salads, particularly during cutting and shredding. Transfer scenarios in these studies were similar to those used by Erickson et al. (2015), Perez-Rodriguez et al. (2011), Buchholz et al. (2012b), Buchholz et al. (2012a), Buchholz (2012), Ren (2014), and Shieh et al. (2014). They evaluated post-contamination time the same way as Wang (2015).

There were differences in the sampling method of the surface material. However, they developed a system of three equations based on transfer rates.

Zilelidou et al. (2015) analyzed the frequency of the transfer rates at a logarithmic scale. They studied two transfer scenarios from a knife to lettuce and from lettuce to the knife. They compared their study with other research contributing to bacterial transfer, such as Perez-Rodriguez et al. (2011), Buchholz et al. (2012b), Buchholz et al. (2012a), Buchholz (2012), Hoelzer et al. (2012), and Kusumaningrum et al. (2003). They affirmed that comparison among studies is difficult due to differences in methodologies and difficulties in controlling all factors involved in bacterial transfer phenomena. They explained from their results how complex bacterial transfer phenomena are based on interactions between microorganisms and surfaces, and the availability of nutrients and lettuce moisture content.

Few studies have quantified actual contact areas between two materials during bacterial transfer processes. Benoit (2015) quantified the interaction between *Listeria* and different transfer materials during bacterial transfer via static contact. A fluorescent powder (*Glo GermTM*, GGP) was used to quantify transfer from donor to receiver. These results were used to mathematically compare them to the rate of *Listeria* transfer during static contact. Various materials were used as the donor and/or receiver (stainless steel, high density polyethylene, turkey, and ham). Calibration curves for powder concentration (ppm) vs. intensity of the ultraviolet light were obtained, and first-and second-order curves fit the data well. Transfer results using GGP were compared with results using *Listeria*, and they allowed to conclude that GGP could be used as an approximation for *Listeria* transfer. The author affirmed that the method to obtain and to analyze the image of GGP on the surfaces was critical because subsequent analysis can be affected by the quality of the image. This knowledge of physical

variables, such as true surface contact area, is critical to quantify the fundamentals of surface to surface bacterial transfer.

2.8.2 Previous analysis conducted on multiple studies on bacterial transfer via surfaces

Multiple studies have been conducted on bacterial cross-contamination via slicer machines, food contact surfaces, and other equipment. Furthermore, bacterial transfer studies have included different surface contact materials and food processes. Differences in experimental methods amongst the various studies may account for variation in bacterial transfer as a function of treatment variables. Hoelzer et al (2012) found that the fraction of transferred bacteria seemed to vary by several orders of magnitude depending on source, recipient, and individual study. Few studies have considered physical variables such as dimensions, mass, roughness, and coefficient of friction as an example, few studies have reported roughness (Goulter-Thorsen, Taran, Gentle, Gobius, & Dykes, 2011; Sheen, 2008; Wang, Feng, Liang, Luo, & Malyarchuk, 2009). The current study focuses specifically on the behavior of bacterial transfer as a function of mainly physical variables.

Hoelzer et al (2012) compiled data from studies that used sanitizers during washing and slicing. They measured transfer coefficients, and they compared eight different distributions. The limitation of their probabilistic approach is that it is difficult to elucidate the causes of cross-contamination, because they focused on the overall bacterial response, rather than fundamental relationships with fundamental physical variables.

Nauta, van der Fels-Klerx, & Havelaar (2005), presented a quantitative microbiological risk assessment model that included cross-contamination as a component. They analyzed five stages of poultry processing and three potential means of contamination, as well as the

distributions of the bacteria transferred from the carcass to the environment and vice versa. A stochastic model was developed that assumed normal distributions in some cases. Similarly, McKellar et al (2014) used data collected from the field by three different authors to fit three transfer models and used the fits to study the impact of different distributions.

In addition, Perez-Rodriguez et al (2011) performed pilot-scale studies where clean lettuce was contaminated from previously inoculated product. They tested different initial inoculation levels, calculated transfer coefficients, and fitted probability distributions. They tested different scenarios to study the probability of an outbreak. Perez-Rodriguez et al (2010) studied the slicing process of cooked meat and ham. They performed a thorough statistical analysis to detect the presence of microorganisms, and found a high prevalence for *Listeria*. Perez-Rodriguez et al (2007) suggested that the medium type used to inoculate the blade or the contaminated area should be investigated for potential effect on the transfer coefficient. Other studies reported similar findings, for example Sheen et al (2010), used agar in place of deli meat to reduce the variability effects of medium type and microbial death. Perez-Rodriguez et al (2007) and Vorst, Todd, & Ryser (2006) found that bacterial transfer decreased logarithmically during processing. Variables such as initial concentration of bacteria and detection methods were thoroughly studied which gave some insight in how bacterial transfer doing repeated events.

2.9 Summary of the literature review

Outbreaks have been associated with consumption of foods contaminated with *Salmonella*, *Listeria*, and *E. coli*. Bacterial transfer via contact surfaces was identified as a source of cross-contamination. Many prior studies focused on fundamental concepts of bacterial adhesion at a microscale. Basic concepts of bacteria and surface interactions have been

intensively studied and understood, such as hydrophobicity, surface energy, and van der Waals interactions. These investigations focused on the identification and study of factors responsible for bacterial attachment. Other studies evaluated polysaccharides and lipopolysaccharides, which are mediators in the adhesion processes. Also, contact time was identified as a physical variable that affects attachment mechanisms. However, it generally was affirmed that there remain some gaps in standardization of methods for transfer studies.

On the other hand, studies performed at a macroscale were classified as bench-scale and pilot-plant scale experiments, often designed to identify factors affecting bacterial transfer. Variables such as microorganism, initial inoculation level, surface contact material, product type, contact time, contact number, and process type have been shown to affect bacterial transfer. However, very few prior studies have reported bacterial transfer results in terms of fundamental physical variables. Although the body of work on bacterial transfer to/from food products has grown significantly, there remains a significant need/opportunity, to quantitatively evaluate the data that have been published to date, to determine whether any generalizable relationship can be elucidated.

Development of bacterial transfer models have contributed to the understanding of bacterial transfer via contact surfaces. However, most of models reported were probabilistic or best-fit. As a conclusion, a key limitation of the prior literature is that a majority of the results are specific to one product, one process, and one microorganism, which makes it difficult to draw general conclusions. Few meta-analysis have been conducted, with most focused on food composition and distribution of bacteria along the processing line. Therefore, there remains a need for studying the physics of bacterial transfer systems in terms of fundamental units of

physics, and evaluate other physical variables such as, friction force, roughness of materials, contact area, and process speed (i.e., shredder, dicer).

INFLUENCE OF PHYSICAL VARIABLES ON THE TRANSFER OF *SALMONELLA* TYPHIMIRIUM LT2 BETWEEN POTATO (*SOLANUM TUBEROSUM*) AND STAINLESS STEEL VIA STATIC AND DYNAMIC CONTACT

3.1 Overview

These analyses address the first objective of the dissertation which is to quantify the effects of fundamental physical variables (pressure, sliding speed, material moisture, contact time, and contact distance) on *Salmonella* transfer to and from stainless steel and a model produce tissue during dynamic (sliding) and static (multiple) contacts. Bacterial transfer data via static and dynamic contact were analyzed as a function of physical variables, which was the first step to elucidate which factors affected bacterial transfer. Given that few fundamental physical variables were included in previous studies, these results give a new approach to conduct future bacterial transfer studies.

3.2 Methods

3.2.1 Overall approach

Potato samples (1 x 3 x 3 cm samples of potato *Solanum tuberosum*), stainless steel plates, and *Salmonella enterica* Typhimurium LT2 were used in a model bacterial transfer system. Potatoes were chosen because they are relatively homogenous, easy to cut for consistent surface contact area, and the water they release is not as excessive as is observed in other fresh produce. Color change of the potato was prevented by controlling the time for conducting the experiment, such that the duration of the experiment was not enough for the potato exhibit any visible browning.

Inoculated potato samples were either pulled across a stainless steel plate for dynamic (sliding) experiments or lifted and placed onto pre-marked stainless steel sample areas for single

and multiple sequential static contacts. Surface-to-surface bacterial transfer was quantified. Treatment variables included moisture content, pressure, sliding speed, contact time, and contact distance. The purpose of the experiments was to evaluate bacterial transfer via dynamic contact and static contact, as influenced by the aforementioned physical variables. The general experimental design was conceptually analogous to a slicer or knife blade sliding along the cut surface of a product (dynamic) or a product contacting and being lifted from a conveyor belt, cutting plate, or table top (static).

3.2.2 Equipment

For dynamic experiments, a controlled speed-force machine, also known as a texture analyzer (TA HDi Texture Analyser, Stable MicroSystems, Surrey, United Kingdom) with a custom pulley system was used to pull potato samples across a stainless steel plate (304; ASTM A240 standard; fabrication consisted of cold worked and heat treated, the hardness is Rockwell B80 (medium), and softened temper rating) for a programmed distance at a controlled speed. The dimensions of the stainless steel plate were ~46 x 46 x 0.09 cm (18 x 18 x 0.036 in). A metal pulley 3.81 cm in diameter was attached to a stainless steel platform (brushed finish), which was attached to the texture analyzer (Figure 3.1a). An eye screw was inserted into the potato sample ~4 mm above the contact surface (Figure 3.1d and 3.2) and was connected to the texture analyzer by a nylon cord, which was then looped through the pulley (90° turn) and attached to the texture analyzer test head (Figure 3.1b and 3.1c). The texture analyzer was used to control sliding speed of the sample and the distance the piece was pulled across the stainless steel surface. Potato samples were collected at the end of the predetermined path.

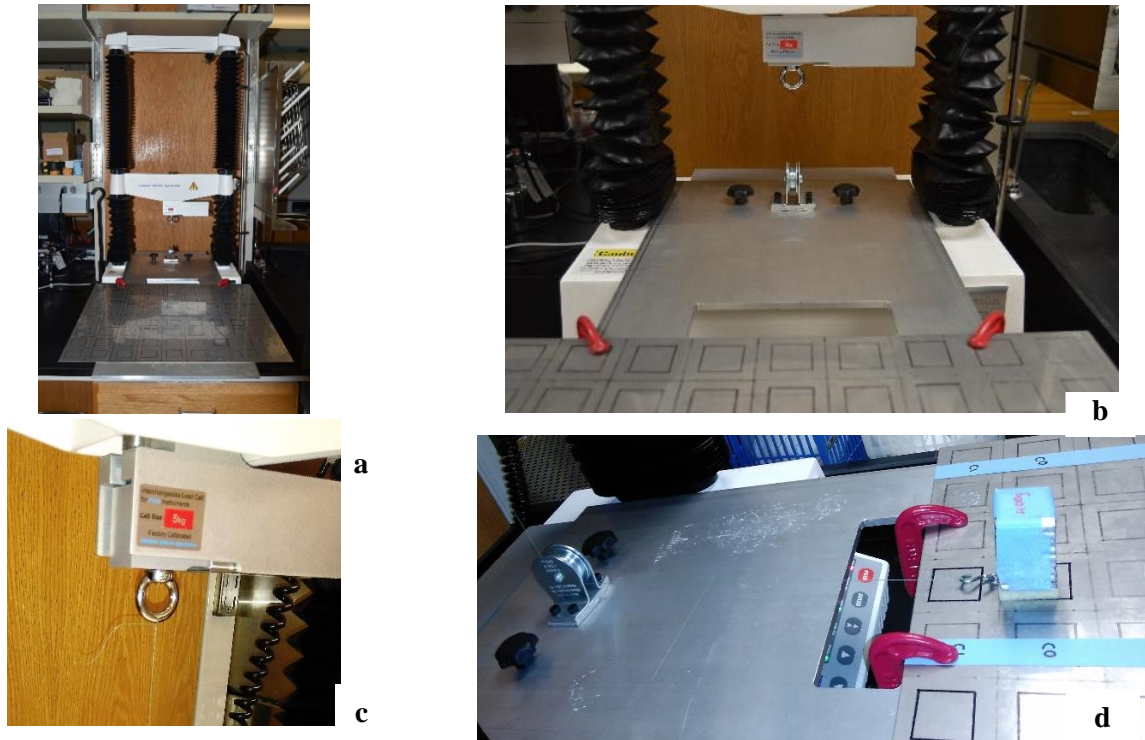


Figure 3.1 Experimental set-up: TA HDi Texture Analyser and platform used to pull a potato sample (3 x 3 x 1cm) across a previously inoculated stainless steel plate. (a) Texture analyzer with custom platform (b) close up of the platform with stainless steel plate attached, and the pulley (c) close-up of the hook attached to the texture analyzer (d) close up of the pulley and a potato sample with additional mass on top to control contact pressure.

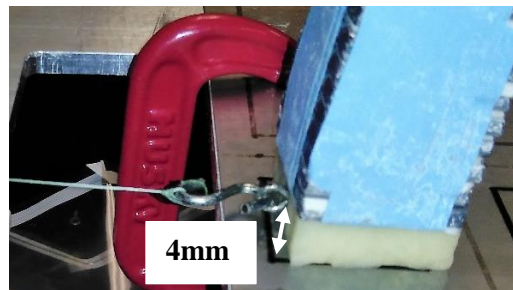


Figure 3.2 Screw eye attached to the potato ~4 mm above the stainless steel surface, which connects the potato with the texture analyzer.

The platform at the end of the path over which the potato sample was pulled had a hole with dimensions ~5.08 x 16.19 x 0.95 cm. The hole was designed to allow dynamic contact (sliding) from the beginning to the end of the path, where the sample slid off the end of the plate.

3.2.3 Inoculum preparation

Avirulent *Salmonella enterica* Typhimurium LT2 was used as the inoculum. This strain was previously obtained from Dr. Michelle Danyluk at the University of Florida (Gainesville, FL). Stock cultures were stored in tryptic soy broth (TSB; Difco, BD, Sparks, MD) containing 20% (vol/vol) glycerol at -80°C . A scraping of frozen stock culture was transferred to separate 9 mL tubes of TSB containing 0.6% (wt/vol) yeast extract (TSB-YE; Difco, Becton Dickinson, Sparks, Md.) and incubated for 24 h at 37°C . After 24 h, a loopful (10 μL) of each TSB-YE culture was transferred to new 9 mL tubes of TSB-YE and incubated ~24 h at 37°C before being used for sample inoculation, resulting in $\sim 9 \pm 0.3$ Log CFU/mL average from samples taken from the pure culture.

3.2.4 Sample preparation and inoculation

Commercially available red potatoes (*Solanum tuberosum*) were purchased at a local grocery store, stored at room temperature, and used within five days. The potatoes were grown in Michigan and were free of visible diseases, damage, or brush marks. Potatoes were cut manually into ~3 x 3 x 1 cm samples weighing ~11 g (Figure 3.4a). A potato sample was collected after purchase to determine if *Salmonella* was present on the potato tissues, and the results were negative. A 5.08 x 7.62 cm (2 x 3 in) aluminum miter box (Fit Tools; Figure 3.3a) was used to achieve straight cuts and 90° angles. After cutting, a 2,275 g (5 lb) weight, which corresponds to

22.32 N, was put on each potato sample for one minute. This pretreatment smoothed out the potato surface in contact with the stainless steel plate in order to increase the true contact area. The contact area achieved with this pretreatment was ~82% (analysis detailed in subsequent section). Potato pieces were put into plastic bags (532 mL) for a maximum of ~20 min (Figure 3.4b) before being used in the transfer experiments. This experimental set-up yielded the force diagram presented in Figure 3.3b, where the forces of friction (F), pulling (P), weight (W), and reaction to the weight (R) are interacting, which are the same forces interacting in slicer processes.

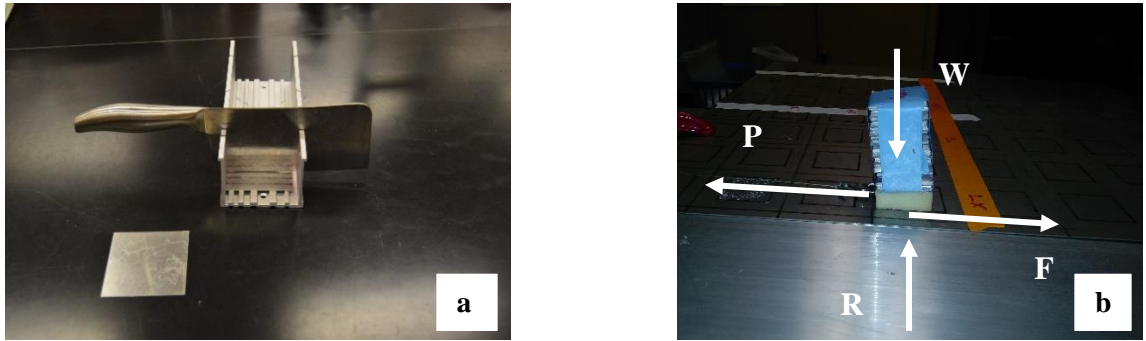


Figure 3.3 Frame, knife (a) used to cut potato samples (3 x 3 x 1 cm), and example of a potato piece (b).

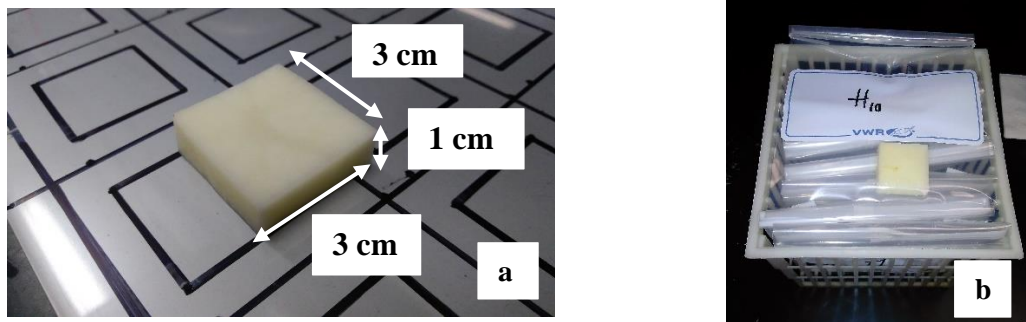


Figure 3.4 Potato (3 x 3 x 1 cm) after cutting (a), and in plastic bags before the experiment (b).

A stainless steel plate was inoculated (46 x 46 x 0.09 cm, Figure 3.5) similar to the method described by Kusumaningrum, van Putten, Rombouts, & Beumer (2002), Perez-Rodriguez, Valero, Carrasco, Garcia, & Zurera (2008), and Posada-Izquierdo, Perez-Rodriguez, & Zurera (2013). A single 3 x 3 cm square labeled as C_0 on the plate was inoculated with 0.1 mL inoculum, evenly distributed with the aid of a spreader (lazy-L, Fisher scientific). The inoculum was allowed to dry in the biosafety hood for 1 h, during which time the inoculum was spread 8 times every 5 min during the first ~35 min to enhance even distribution of the inoculum on the 9 cm² square, to avoid concentration of the inoculum on the center of the square, and to allow *Salmonella* to be attached to the stainless steel surface. The initial inoculation level on the plate ($\sim 6.23 \pm 0.32$ Log CFU/cm²) was determined by inoculating 12 – 3 x 3 cm squares on the stainless steel plate, assaying the squares using the 1-ply Kimwipe® method for swabbing the surface (Section 3.2.5), and calculating the average from the samples collected.

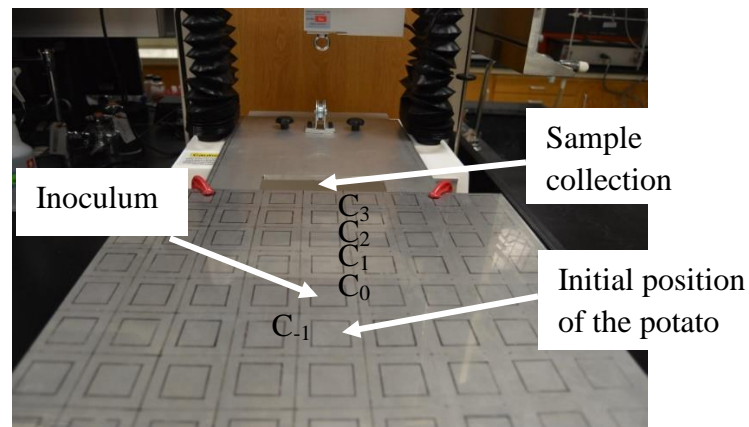


Figure 3.5 Inoculated plate with the initial position of the potato (C_{-1}), inoculated square (C_0), and sample collection, the example is for a dynamic sample with contact speed of 3.75 mm/s and a contact distance of 150 mm.

3.2.5 General methods bacterial enumeration

3.2.5.1 Method of bacterial recovery from the plate

The path of each potato sample in contact with the stainless steel plate was divided into 5 x 5 cm squares. In each 5 x 5 cm square, a 3 x 3 cm square was drawn in the center as a guide to collect the samples with bacteria from the plate, and to avoid cross-contaminating adjacent subsequent surface samples. A square labeled as C_{-1} corresponded to a sterile square where initially the potato sample was set before dynamic (sliding) experiments. Square C_0 corresponded to the inoculated square, and sterile contact squares were identified as C_1 to C_n , depending on the number of squares contacted in the experimental path (up to 18). Surface samples were taken using the Kimwipe® sampling method (Vorst, Todd, & Ryser, 2004). The same sampling protocol was followed for static and dynamic transfer experiments. Each 3 x 3 cm square was swabbed 10 times vertically and 10 times horizontally with a 1-ply Kimwipe® tissue, folded 6 times and moistened with 1 mL of sterile peptone water. After swabbing, the tissues were transferred to 9 mL of 0.1% of sterile peptone water.

The samples (Kimwipe tissues or 3x3x1 cm potato sample) were stomached for 3 min (Neutec Group Inc, model 1381/471, New York, United States). A 1 mL aliquot was serially diluted, and appropriate dilutions were plated in duplicate on modified trypticase soy agar (MTSA) and incubated at 37°C for 48 h before enumeration. The stainless steel plates were disinfected, cleaned with ethanol (75%), and autoclaved between tests.

3.2.5.2 Sample recovery for bacterial transfer via static contact

An inoculated potato sample was placed for a 5 s contact time on the inoculated square C_0 . The same potato sample then was lifted and placed on sterile square C_1 . For bacterial transfer

experiments via single contact, samples were only collected from C_0 and C_1 . For bacteria transferred via multiple contacts, the potato sample was lifted and placed sequentially on 8 or 18 sterile contact squares (C_1 to C_8 , or C_1 to C_{18}). At the end of each test, a sample from the inoculated square (C_0) and each subsequently contacted square (C_1 to C_8 , or C_1 to C_{18}) was collected. Samples from the plate were 3 x 3 cm squares, because that corresponded to the nominal contact area between the potato and the stainless steel plate.

3.2.5.3 Sample recovery for bacterial transfer via dynamic contact assays

Potato samples were pulled across a stainless steel plate. Total sliding distances of 10, 20, and 35 cm were used, allowing 2, 4, or 7 total squares to be sampled, respectively. Potato samples were pulled across the steel plate, starting in C_{-1} , until the target contact distance was achieved. The total number of surface samples included the sample collected from the inoculated square (C_0), which contained the bacteria remaining from the original inoculum after the sliding contact occurred across C_0 .

The same square size 3 x 3 cm sampling as the static contact was used for consistency. In addition, this sampling size avoids cross-contamination when sampling the squares. For C_1 to C_7 , an interpolation was performed in order to obtain the total bacteria transferred to 15 cm^2 , which was the actual total contact area between the potato sample and the stainless steel plate during sliding contact across a 5 x 5 cm square.

3.2.6 Experimental design and treatments

3.2.6.1 Bacterial transfer via static contact

Bacterial transfer experiments via static contact were performed to evaluate the effect of two contact times (5 and 40 s), multiple pressures, moisture content, and multiple sequential contacts. First, experiments on bacterial transfer via 8 multiple contacts (Figure 3.6) had two purposes: (1) to evaluate the effect of potato surface moisture content on bacterial transfer, and (2) to determine the shape of the curve obtained for bacterial transfer versus contact number. These results identified the need to increase the number of contacts to 18 (Figure 3.7) to subsequently be able to fit the Weibull model.

Metal coupons (~3 x 1.5 cm) and 21 g reference weights were added to the top of the potato sample to achieve total normal contact pressures of ~1,217, 2,307, 4,487, 5,247, 7,473, or 8,869 Pa. These values were selected based on preliminary experiments measuring the contact and quantifying reproducibility. The number of replicates was selected according to the variability of the measurements in preliminary trials.

After being in contact with the inoculated square for 5 s, each potato sample was lifted and then placed sequentially on subsequent 9 cm² sterile stainless steel squares. For experiments on bacterial transfer via single static contact, one 40 s contact was achieved, and for bacterial transfer experiments via multiple static contacts, eight 5 s contacts were achieved, accounting for 40 s of total contact time. The same contact time (40 s) achieved at different contact numbers allowed comparison of bacteria transferred after different static contact scenarios. An additional set of experiments was completed to increase the contact number to 18 (Figure 3.7).

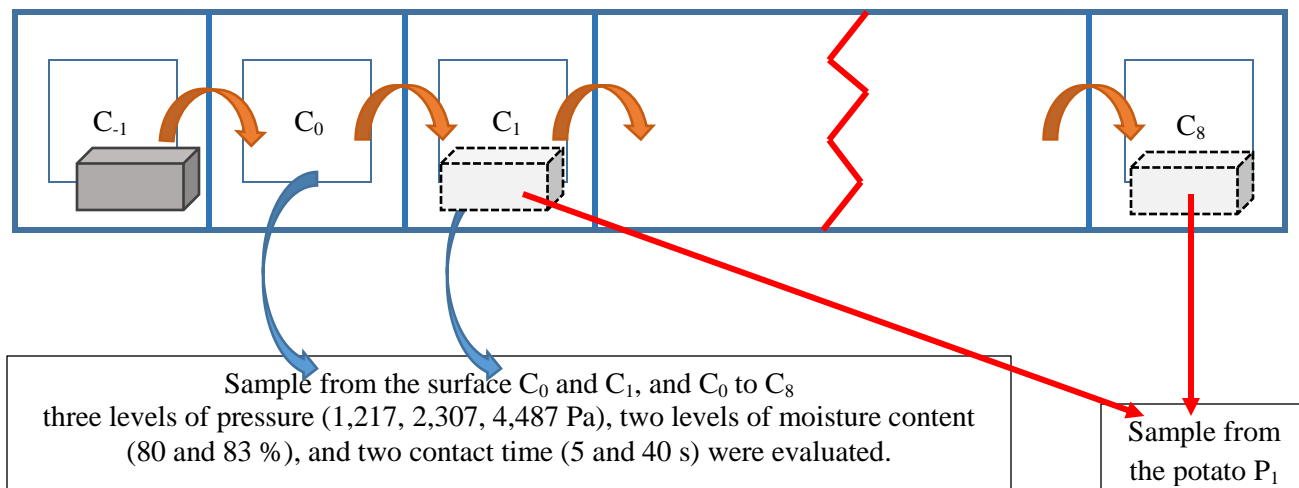


Figure 3.6 Bacterial transfer via multiple static contacts; potato sample was in single contact (C₁) or multiple sequential contacts (C₁ to C₈) with 3 x 3 cm squares of a stainless steel plate; samples were collected from the potato and the contact area.

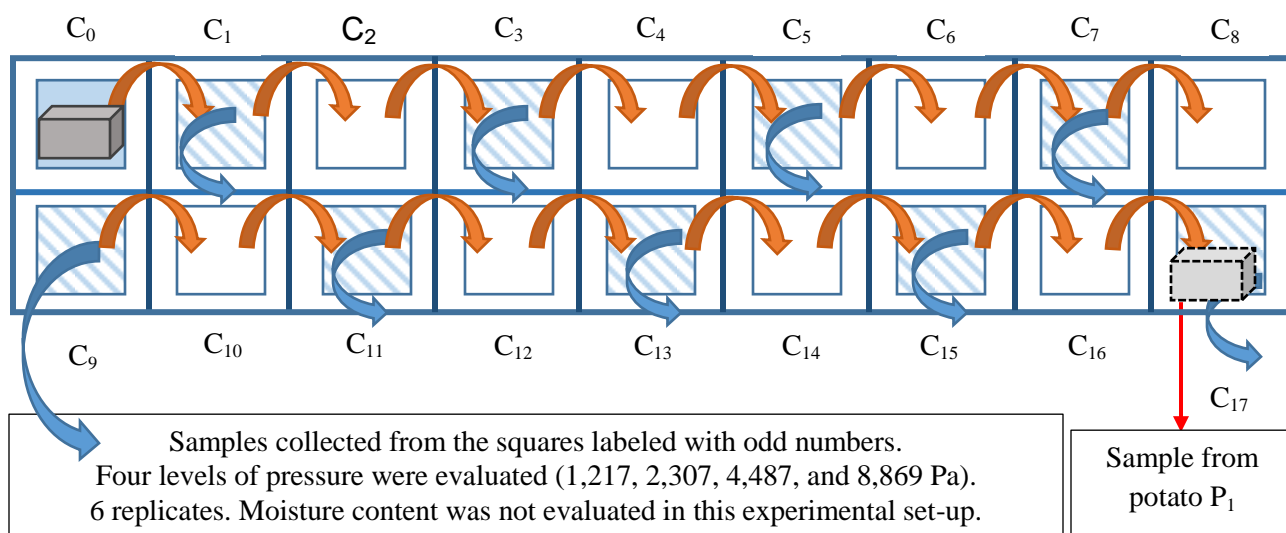


Figure 3.7 Bacterial transfer via static contact; potato sample was in contact with 18 sequential 3 x 3 cm squares (C₀ then C₁ to C₁₇) of a stainless steel plate; samples were collected from the potato and contact area; 12 replicates were used.

A factorial experimental design was used to evaluate bacterial transfer via single and multiple static contacts. Contact treatments consisted of a combination of three physical variables: pressure, potato surface moisture content, and/or contact time (Table 3.1). Normal pressure values included the weight of the potato sample. An uninoculated potato sample was in direct contact with an inoculated 9 cm² stainless steel square for 5 s. The potato sample was subsequently moved to the next 9 cm² clean stainless steel square until all contacts were achieved. Samples were collected from the potato and the plate for microbial analysis.

The purpose of every set of experiments (Table 3.1) was to evaluate the effect of: (1) surface moisture content on bacterial transfer via single contact, (2) surface moisture content on bacterial transfer via 8 multiple contacts, (3a) normal pressure on bacterial transfer via single contact, (3b) different levels of normal pressure on bacterial transfer via single contact (from these sets, it was determined to use 6 replicates on the remaining experiments) and (4) 4 levels of normal pressure on bacterial transfer via 18 multiple contacts. Normal pressure corresponded to the force per contact area due to the sum of the potato sample weight and the weight added on the potato sample.

Table 3.1 Experimental design for testing effects of contact time, pressure, contact number during static contact.

Set	Contact time (s)	Pressure (Pa)	Contact (#)	Potato water content on the surface (%)	Replicates
1	5	7,473	1 (C ₀)	80, 83	12
2	5	7,473	8 (C ₀ to C ₈)	80, 83	12
3a	40	7,473, 5,247	2 (C ₀ , C ₁)	83	12
3b	40	8,869, 4,487	2 (C ₀ , C ₁)	83	6
4	5	8,869, 4,487, 2,307, 1,217	18 (C ₀ to C ₁₈)	83	6

For bacterial transfer via multiple static contacts, a stainless steel plate (40 cm long) was divided into eight squares of 5 x 5 cm, and a 3 x 3 cm square was drawn in the center of each 5 x 5 square to identify the contact area (82%). A clean potato sample was placed on an inoculated square (C₀) and then sequentially transferred to sterile squares on the brushed finish stainless steel plate. The same cumulative net contact area and contact time were achieved as in the dynamic transfer scenario (next section). Samples were collected from the potato and each contact square. In the 18-contact experiments, samples were collected from the odd numbered squares. These experimental results were compared to bacterial transfer data via static and dynamic contact.

3.2.6.2 Bacterial transfer via dynamic contact

3.2.6.2.1 Physical forces during slicing and sliding

Experiments on bacterial transfer via dynamic contact (sliding) were designed to include the forces that were acting between a food product contact area and a cutting tool surface. In a slicing process, a dynamic contact interaction occurs after the tissues are cut. The forces in interaction during dynamic contact are: friction force (F), pulling force (P), and normal force due to the weight (W). The friction force ($F = \mu W$) is defined as the normal force multiplied by the coefficient of friction (μ). When the pulling force exceeds the friction force ($P > F$), for instance, the potato sample moves over a stainless steel surface. The slicing force corresponded to the force necessary to cut tissues of a food product (Figure 3.8a), and it is different from the sliding (pulling) force (Figure 3.8b), which corresponds to the sliding interaction between the side of a blade and the cut surfaces of the sample after blade edge moves through the tissue.

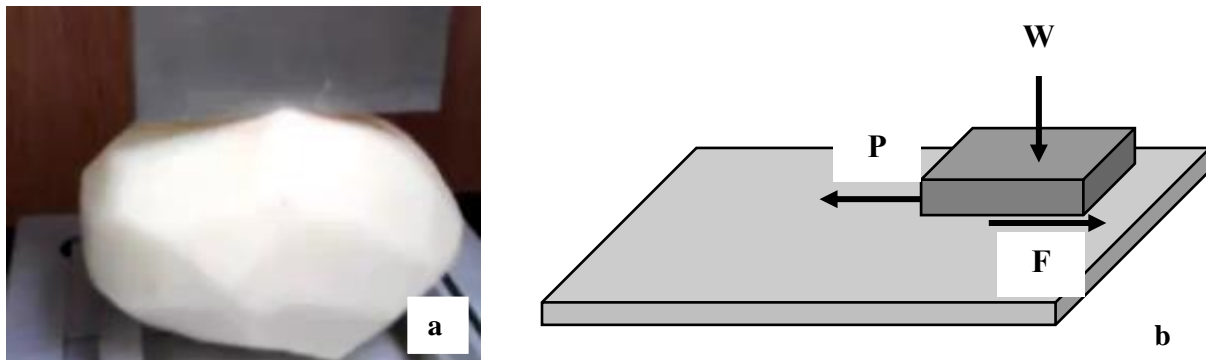


Figure 3.8 Example of a cutting force (a) and a sliding force (b) or dynamic contact.

3.2.6.2.2 Bacterial transfer via dynamic contact for 40 s at two speeds

The purpose of these experiments (Figure 3.9) was to test the effects of pressure and speed (3.75 and 7.75 mm/s) on bacterial transfer during sliding (dynamic) contact for a fixed

time (40 s). The same contact time was achieved at different contact distances of 15 or 30 cm, which corresponded to speeds of 3.75 mm/s and 7.75 mm/s, respectively.

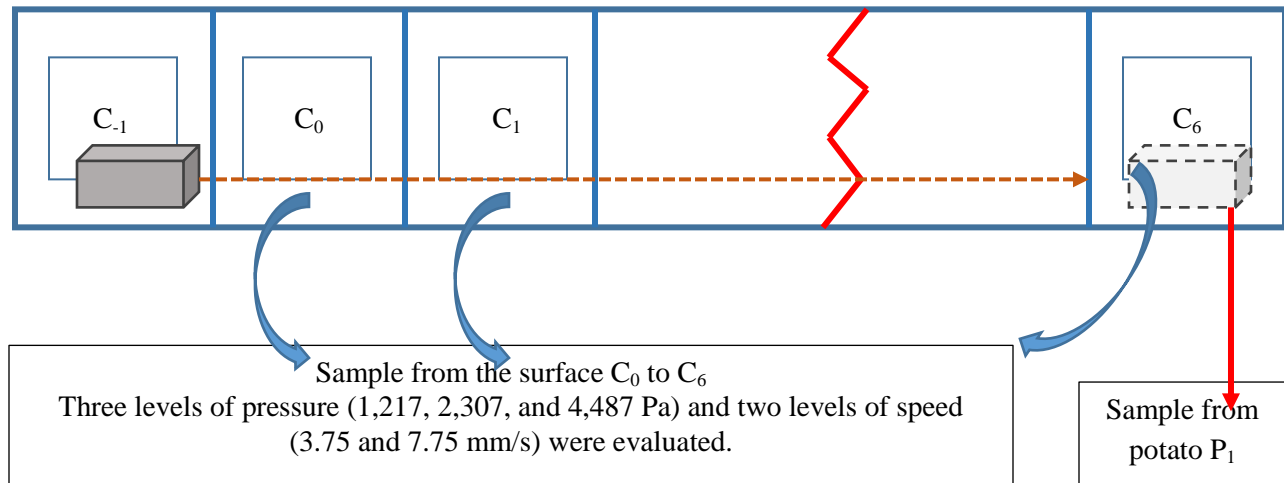


Figure 3.9 Bacterial transfer via dynamic contact, potato was in contact with the plate for a distance of 15 or 30 cm (C_{-1} to C_3 , or C_{-1} to C_6 , respectively).

In these experiments, the potato samples were pulled across the plate (C_0) after plate inoculation and 1 h of drying. The product cross-contaminated the plate (i.e., from C_0 to C_1 - C_3 or C_1 - C_6) during dynamic contact (sliding) (Figure 3.9). After sliding was completed, bacteria were assayed from every square along the sliding path. Potato samples were collected at the end of the path, then transferred to 20 mL of 0.1% of sterile peptone water in a 532 mL polyethylene bag for immediate microbial analysis (section 3.2.5).

A randomized complete block experimental design was used (Table 3.2 and Figure 3.9) to obtain 40 s of contact time in all treatments. Each sliding contact treatment consisted of a combination of four physical variables: pressure, sliding speed, contact distance, and contact time. Six replicates were evaluated per treatment.

Table 3.2 Experimental design for testing effects of speed and pressure on bacterial transfer over a fixed time during dynamic contact.

Speed (mm/s)	Pressure (Pa)	Distance (mm)	Contact time (s)	Replicates
3.75	1,217	150	40	6
	2,307			
	4,487			
7.75	1,217	300		
	2,307			
	4,487			

3.2.6.2.3 Bacterial transfer via dynamic contact at three speeds for 5 cm

The purpose of these experiments was to quantify initial transfer from the inoculated plate (C_0) to sequential potato samples (Figure 3.10).

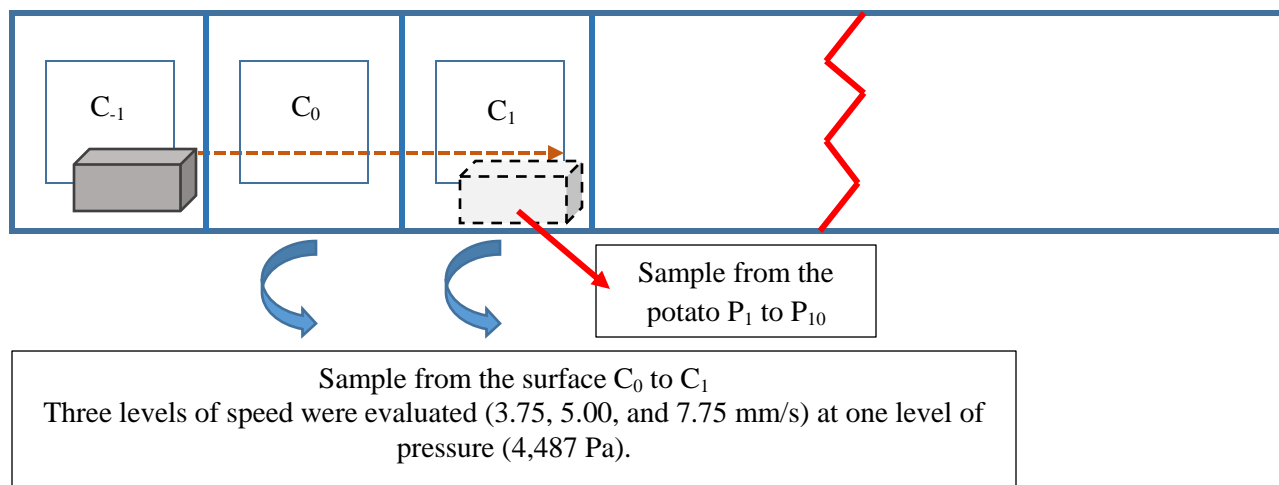


Figure 3.10 Bacterial transfer via dynamic contact, with 10 potato samples contacted at a fixed distance of 5 cm.

A randomized complete block experimental design was used to assess bacterial transfer via dynamic contact (sliding) at different speeds (3.75, 5.00, and 7.75 mm/s) and the same contact distance (5 cm) and pressure (4,487 Pa). Three replicates were used per treatment. The fixed variables were contact distance and pressure (Table 3.3). The process of bacterial transfer via dynamic contact was achieved using the same equipment described in section 3.2.2.

Table 3.3 Experimental design for testing the effect of speed on bacterial transfer from an inoculated square (C_0) to 10 consecutive potatoes.

Speed (mm/s)	Distance (mm)	Pressure (Pa)	Potato samples per run	Replicates
3.75	50	4,487	10	3
5.00				
7.75				

Bacterial transfer was completed over a 5 cm contact distance (Figure 3.10). The first sterile square (C_{-1}) corresponded to the start point before sliding. The second square (C_0) on the plate was inoculated as previously described. Ten consecutive potato sample were pulled from C_{-1} across C_0 and fully onto C_1 . The total contact distance was 5 cm, which was the length of one square drawn on the plate. After the sliding treatment, each of the 10 potato samples were immediately lifted vertically from the stainless steel surface, and transferred to 20 mL of 0.1% of sterile peptone water in a 532 mL polyethylene bag for immediate microbial analysis.

Bacteria remaining on C_0 and bacteria transferred to the sterile square (C_1) were collected after all 10 samples were slid across the plate. Surface samples were taken using the Kimwipe® sampling method (Vorst, Todd, & Ryser, 2004) described in Section 3.1.5.

This experiment also was conducted using a single potato sample to characterize bacterial transfer from the potato to the plate surface after a single contact. Samples were collected from the potato, the bacteria remaining on the plate (C_0), and the bacteria transferred from the potato to the sterile square of the plate (C_1). These measurements were done on one potato to assess the bacteria remaining on the plate after contact with ten potatoes.

This overall experiment was analogous to prior studies used to develop a meta-analysis for bacterial transfer (Chapter 4). It conceptually corresponded to the transfer of bacteria from a contaminated piece of equipment (e.g., a slicer blade) to multiple sequential uncontaminated product samples.

3.2.7 Determination of the true potato contact area on stainless steel

Very few prior studies on bacterial transfer in food systems report even nominal contact area (see Chapter 2), and almost none reported true contact area. Because the present study focused on fundamental physical variables, it was critically important to document the true contact area between the food material (i.e., potato) and control surface (i.e., stainless steel). In addition, preliminary evaluations revealed a high variability among replicates, which might be due to heterogeneity among potato samples. Specifically, true surface contact for the potato samples was potentially variable due to differences in the flatness of the cut surface. Therefore, improving consistency in the true contact area would contribute to decreased variability in bacterial transfer results. Although it was impractical to do that on a microscopic scale, even a macroscopic method is an improvement over using only the nominal area, because it better represents the actual contact area over which bacterial transfer can occur. In addition, this step increased the contact area between potato sample and stainless steel surface.

The method to do this utilized ink transfer and an image analysis tool. Preparation of the sample consisted of achieving a flat cut on the surface and adding an extra-weight on the potato. Potatoes were cut to measure 3 x 3 x 1 cm. The cutting method was previously established to achieve the best cut practically possible on the potato. As described in Section 3.2.4, a 2,275 g (5 lb) weight was placed on the cut potato sample for one minute. Subsequently, the contact side of the potato sample was placed in contact with an ink pad. The potato side covered with ink then was put in contact with the stainless steel plate, and an additional mass (Table 3.4) was added to the potato during 5 s of contact with the plate. A picture was taken of the area covered by ink on the stainless steel, with the camera (Nokia) located horizontally and parallel to the stainless steel plate. The contact area was determined using ImageJ 1.51j8 (National Institutes of Health, USA) software to analyze the percentage of the nominal 3 x 3 cm square that contained an ink impression.

Determination of the contact area consisted of first converting the image into grayscale (*'8-bit type image'*) and setting the scale by drawing a line of a dimension that is already known. This first step sets the threshold of the contact area for the potato to just the dark areas. This step was achieved with the tool to make the image binary. Finally, the command *'Analyze particles'* outlines the area and calculates the gray portion.

The normal pressure added on the potato sample during the “pre-compressing” procedure also contributed to increase the true contact area. The results of the contact area achieved at the different pressures used on the potato samples are summarized in Table 3.4. “Pre-compressing” the sample resulted in an increase of the true contact area of the potato from approximately 50% to 82% (Figure 3.11).

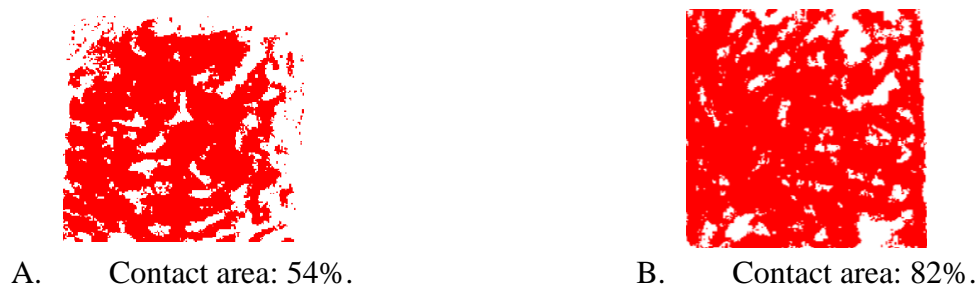


Figure 3.11 Contact area between the stainless steel plate and the potato sample determined using ink impressions and ImageJ software. (A) Contact area achieved without previous preparation (i.e., “pre-compression”) of the sample (B) Contact area of the weighted sample.

Table 3.4 True contact area obtained by inking and image analysis after different pressures applied to the potato samples.

Pressure (Pa)	Contact area (%)
8,869	82
7,473	78
5,247	74
4,487	70
2,307	61
1,217	60

3.2.8 Moisture content control on the potato surface

Two surface water contents of the potato (80% and 83%) were tested to evaluate the effect of surface water content on bacterial transfer to and from the potato. Moisture content of

the potato sample (i.e., the surface that would subsequently be contacting the stainless steel) was reduced by setting the sample on a stack of four Kimwipes folded into 3 x 3 cm squares, and placing a 50 g weight on top of the sample for 20 min, during which water diffused from the potato surface into the Kimwipes. Quantification of potato surface water content was done using the oven method (American Association of Cereal Chemists (AACC)1993a), by drying a surface slice ~2 mm thick of the potato in an oven at 105°C until the weight was constant. The surface slice corresponded to the side previously in contact with the stainless steel plate and for which moisture content was altered prior to transfer experiments.

3.2.9 Statistical analysis

Analyses of variance (ANOVA) was performed to determine the effects of variables and interactions ($\alpha = 0.05$), using SAS 9.4. Factorial design and randomized complete block design models were used. The purpose was to determine the effect of each physical variable independently, and the interaction among the physical variables present in the respective experimental designs as shown in (equation 3.1).

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2 \quad (3.1)$$

where y = the response measure of interest, x_1 , x_2 , and x_3 to the relevant experimental variables (e.g., pressure or speed), and β_1 , β_2 , β_3 are the model parameters.

The statistical models used to evaluate the effect of the physical variables and their interactions are presented in this section as examples. For a factorial design, an example of a general model used was:

```

proc mixed data = potato method=type3;

class time pressure;

model recovery = time pressure time*pressure;

run;

```

where ‘potato’ corresponded to the name of the file that compiles the data. ‘Time’ referred to the contact time between the potato sample and the stainless steel, and ‘pressure’ to the normal force on the potato sample. ‘Recovery’ was the number of bacteria transferred.

The details of the model used to evaluate the data collected from a completely randomized block experimental design were:

```

proc mixed data=potato method=type3;

class treatment distance day;

model recovery= treatment /outp=mr;

random day;

run;

```

Where ‘potato’ corresponds to the name of the file that compiles the data. ‘Treatment’ corresponds to the speed and normal force used to slide the potato sample. ‘Day’ was the day the experiment was conducted, and it was the random variable in the experiment. ‘Distance’ was the contact distance between the potato sample and the stainless steel. ‘Recovery’ was the number of bacteria transferred.

A paired comparison test was included in the analysis performed on bacteria transferred via multiple static contact (C₁ to C₁₈) to identify significant differences among normal pressure evaluated.

```
proc glm data = plate12;  
  
class pressure day contact;  
  
model transfer = pressure day contact pressure*contact;  
  
lsmeans pressure*contact/slice=(pressure contact);  
  
run;  
  
proc mixed data = plate12;  
  
class pressure;  
  
model transfer = pressure;  
  
lsmeans pressure/pdiff adjust=tukey;  
  
lsmeans pressure/pdiff adjust=scheffe;  
  
lsmeans pressure/pdiff adjust=Dunnett;  
  
run;
```

3.3 Results

3.3.1 Effect of potato surface moisture, contact time, and contact pressure on bacterial transfer from potato (3 x 3 x 1 cm) to a sterile stainless steel plate via static contact

3.3.1.1 Effect of potato surface moisture on bacterial transfer via static contact

Results in this chapter are presented as the number of bacteria transferred vs. contact number or the physical variable under evaluation. In all cases, bacterial transfer refers to Log CFU *Salmonella* transferred, as assayed on MTSA.

The surface drying treatment was applied only to the potato surface, and the statistical analysis (Table 3.5) revealed an increasing effect due to moisture content for bacterial recovery only in C₄. No significant differences ($p > 0.05$) were found in the interaction of moisture content and contact number (Table 3.6). Previous studies by Schaffner & Schaffner (2007) affirmed that differences in bacterial attachment can be due to liquid moisture present on the surface of unfrozen versus frozen products. Ak et al (1994a, 1994b) found less bacterial cross-contamination on wooden boards dried inside a hood. The conditions of the current study were different from the studies found in the literature, mostly because the surface moisture content range used on these experiments was small (~3%) to keep the potato fresh, and its characteristics close to reality. Wet potato surface corresponded to a moisture content on the surface of $83 \pm 0.48\%$, and dry potato surface corresponded to a moisture content on the surface of $80 \pm 0.32\%$.

Table 3.5 Effect of moisture content per contact number (C₁ to C₈) on bacterial transfer via static contact.

Contact	DF	Sum of Squares	Mean Square	F Value	Pr > F
1	1	0.410817	0.410817	1.57	0.2122
2	1	0.377504	0.377504	1.44	0.2317
3	1	0.264600	0.264600	1.01	0.3164
4	1	1.075267	1.075267	4.10	0.0443
5	1	0.507504	0.507504	1.94	0.1658
6	1	0.810337	0.810337	3.09	0.0804
7	1	0.579704	0.579704	2.21	0.1387
8	1	0.416067	0.416067	1.59	0.2093

Table 3.6 Effect of moisture content, contact number (C₁ to C₈), and the interaction between them on bacterial transfer via static contact.

Source	DF	Type III SS	Mean Square	F	Pr > F
Moisture content	1	4.2423	4.2423	16.19	<0.0001
Contact number	7	22.8648	3.2664	12.46	<0.0001
Moisture content *contact number	7	0.1994	0.0284	0.11	0.9977

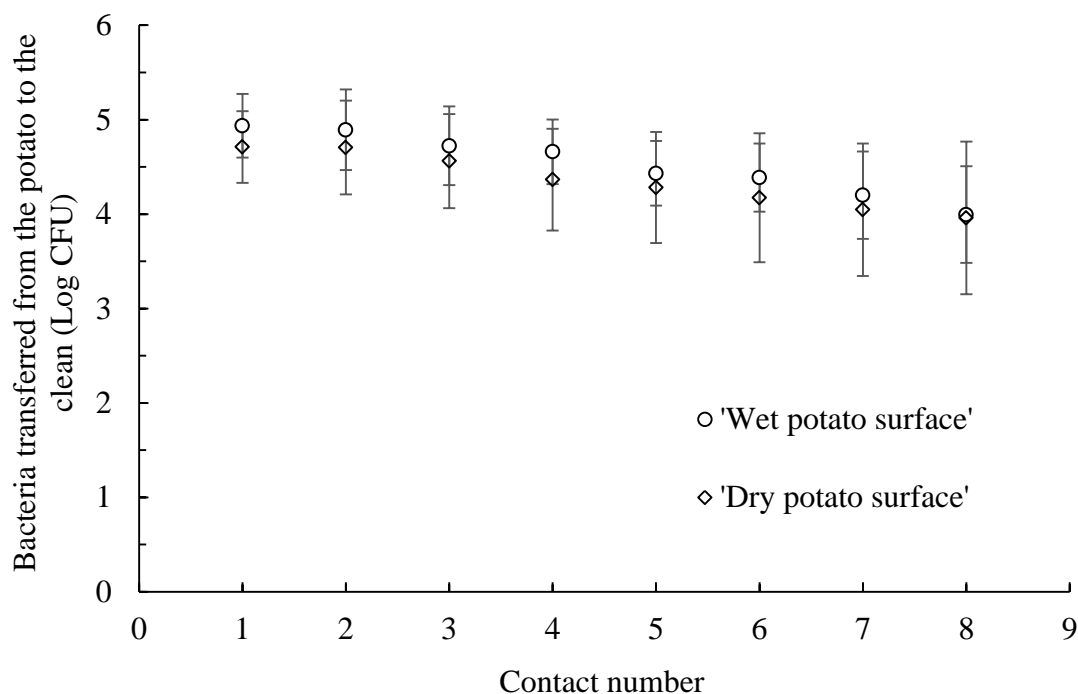


Figure 3.12 Bacterial transfer from the potato to the plate versus number of multiple contacts (8 static contacts) at two levels of moisture on the surface (means of 12 replicates).

Moisture content was evaluated in an experimental set up that quantified the number of bacteria transferred via 8 static contacts (C_1 to C_8) mainly for two reasons (Figure 3.12). Potatoes change their characteristics over a relatively short period of time. Physically, the dimensions and flat shape of the potato surface change during “dewatering”. In addition, the evaluations were done before oxidation started, because it was assumed that the results might have been affected by this process. As a result, moisture content was lowered on the potato surface using the Kim-Wipe method described in section 3.2.5. It was decided not to analyze a higher than normal surface moisture content due to potatoes characteristics. A lower moisture content was avoided to keep as constant as possible the dimensions of the potato sample and to maintain essential fresh raw potato properties. Also, the purpose of the experimental set up was to use fresh

produce as a food model, so to be applicable to real-life situations. For instance, the difference obtained in C_4 occurred in only one event (one contact) from 8. The decrease in moisture content achieved on the potato surface was only 3%, which affected the sensitivity to moisture-influenced differences in the resulting transfer. The current study was focused on physical variables instead of food composition; therefore, given the very small impact of surface moisture content, it was decided to evaluate moisture content as an independent variable in only one set of experiments instead of including this variable in all subsequent experiments (Figure 3.12).

A factor that might have added variability to this set of experiments was the mass transfer between the potato and the inoculum on the stainless steel plate. Mass transfer started when the wet potato surface contacted the inoculum on the plate, because water transferred from the potato surface to the dried plate surface. In addition, the system was dynamic because fluids were interacting, and the viscosity of that fluid might have affected the results. As a conclusion, the wet conditions of this experiment made it difficult to discern the effect of surface moisture content in the range used.

3.3.1.2 Effect of a single contact pressure for 40 s

Levels of normal pressure on the potato sample were selected to achieve maximum and uniform contact area, in order to minimize variability among samples. In addition, the detachment forces measured for 14 cells ranged from 0.11 to 2.26 μN (10^{-6}), averaging $0.59 \pm 0.62 \mu\text{N}$ (Tsang et al, 2006). The assumption that the size of a bacterium is $\sim 1 \mu\text{m}$, and the consideration that the force of a bacterium is 7.85 pN (0.11/14 μN). The pressures of the experimental design were closed to the detachment pressure of a bacterium ($\sim 7,857 \text{ Pa}$).

Bacterial transfer was assessed after single contact (C_1) for 40 s to isolate the effect of pressure, and to later compare the effect of a 5 s contact time between the plate and the potato sample (Figure 3.13). Because the contact between the plate and potato was static, the physical variables in the experimental set up were pressure and bacterial transfer from the plate to the potato and back to the plate. Bacterial transfer was not significantly affected by pressure for the single contact. However, the number of bacteria transferred from the plate (C_0) to the potato (P) were significantly higher ($p < 0.0001$) than those remaining on the plate (C_0), and those transferred to C_1 (Figure 3.13).

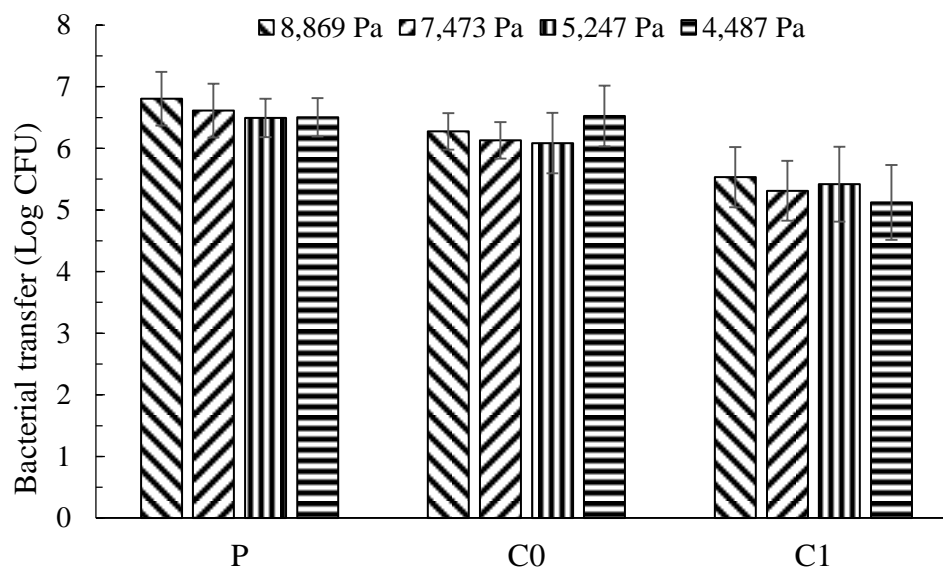


Figure 3.13 Bacterial transfer from the plate to the potato (P) and back to the plate (C_1) at 4 contact pressures (8,869, 7,473, 5,247, and 4,487 Pa) and a total contact time of 40 s. C_0 refers to the number of bacteria remaining on the plate after contacting the potato.

3.3.1.3 Effect of contact pressure for (18 multiple contacts) of 5 s

Experiments on bacterial transfer were conducted for 18 sequential static contacts (C_1 to C_{18}) at four different normal pressures (Figure 3.14). As expected, bacterial transfer decreased as contact number increased over 18 sequential contacts between potato samples and the sterile squares of stainless steel (Figure 3.14). These trends were similar to those from other studies included in the meta-analysis of bacterial transfer (see Chapter 4). Bacterial transfer was highest when the highest normal pressure (8,869 Pa) was applied to the potato, in comparison to bacterial transfer seen at the lowest normal pressure. The three pairwise comparison tests (Tukey, Scheffe, and Dunnett) gave the same results. Bacterial transfer was significantly greater at the highest compared to the lowest pressure ($p = 0.0226$). However, pressure levels of 2,307 and 4,487 Pa were not significantly different from the others (1,217 and 8,869 Pa). Overall, the physical variable of pressure affected bacterial transfer via multiple contacts.

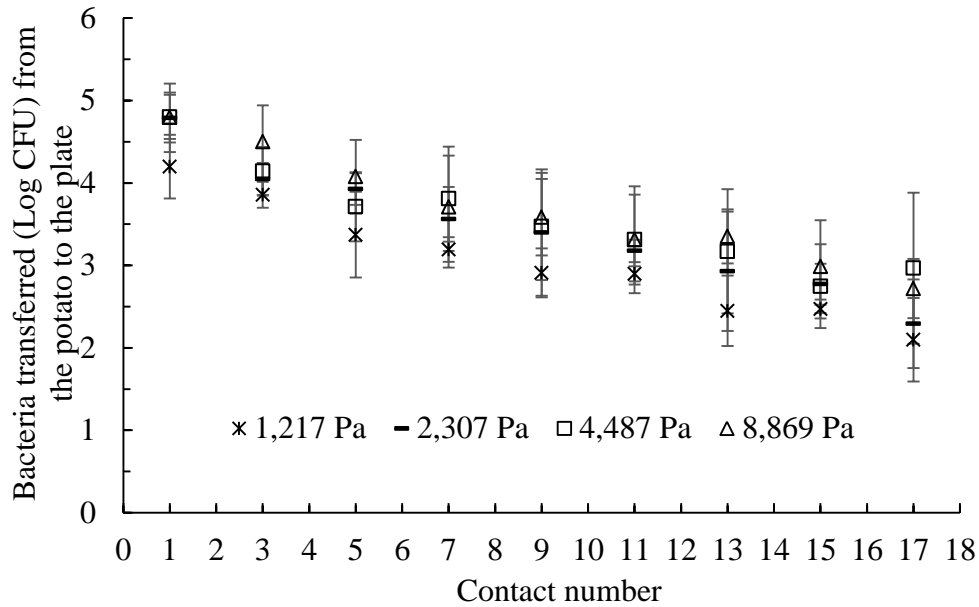


Figure 3.14 Bacterial transfer (Log CFU) from the potato to the plate via sequential static contacts (C_1 to C_{18}) applying four different contact pressures (8,869, 4,487, 2,307, and 1,217 Pa) to the potato.

3.3.1.4 Effect of contact time for a single contact

Results in this section are focused on the effect of contact time (Figure 3.15). Statistical analysis of the total number of bacteria transferred from a previously contaminated potato to a sterile 9 cm² stainless steel contact area (C_1) at two pressure levels showed that more bacteria transferred ($p < 0.0001$) after 40 compared to 5 s (Table 3.7). Previous research conducted by Miranda & Schaffner (2016) affirmed that longer food contact times result in greater bacterial transfer (stainless steel, ceramic tile, wood, and carpet). However, contact pressure was not evaluated by Miranda & Schaffner (2016), so the present data were novel in this regard. Garrood et al. (2004) found that bacterial transfer versus contact time depends on the microorganism. In an attachment study using *Listeria monocytogenes*, *Pantoea agglomerans*, and *Pseudomonas*

fluorescens, they found that *P. fluorescens* detachment was unchanged for contact times lower than 5 s or 60 min. However, *Listeria* detachment from laboratory materials (*Pseudomonas* broth F) to potato tissue decreased during the first 2 min, and then remained constant after 2 min.

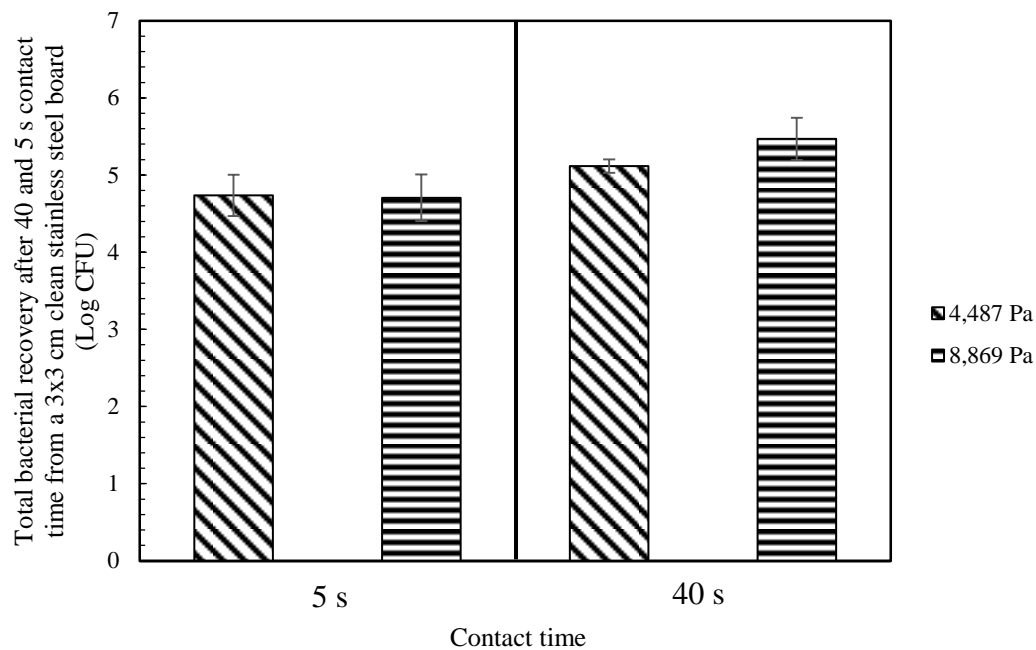


Figure 3.15 Bacterial transfer after 5 and 40 s of static contact (C_1) and at two different pressures (4,487 and 8,869 Pa).

Dawson et al. (2007) affirmed that many factors contribute to the rate of bacterial transfer from food contact surfaces, including food composition, surface type, residence time of bacteria on the surface, and contact time of the food with the surface.

For the present data, however, contact pressure on the order of magnitude of the current set of experiments did not affect total bacterial transfer via one single contact. Hypothesis #1 was ‘Bacterial transfer from food to a contact surface increases as pressure increases’. The research hypothesis therefore was rejected within the range of conditions tested (Figure 3.15) on single

contact events. The contrary was found for contact time. More bacteria were transferred to the stainless steel (C_1) at the longest contact time (40 s). These results agreed with the null hypothesis. Effect of the normal pressure was different for bacteria transferred via single contact than bacteria transfer via multiple contacts, noting that the differences might be due to the pressure range that was used.

Table 3.7 Effects of contact time (5 and 40 s), pressure (4,487 and 8,869 Pa), and the interaction between contact time and pressure on bacterial transfer from C_0 to Potato to C_1 .

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F	Pr > F
Time	1	1.9494	1.9494	Var(Residual) + Q(time,time*pressure)	MS (Residual)	20	31.88	<0.0001
Pressure	1	0.1536	0.1536	Var(Residual) + Q(pressure,time*pressure)	MS (Residual)	20	2.51	0.1287
Time*Pressure	1	0.2204	0.2204	Var(Residual) + Q(time*pressure)	MS (Residual)	20	3.60	0.0721
Residual	20	1.2229	0.0611	Var(Residual)

3.3.1.5 Bacteria remaining on the potato after C_{18} (5 s each), C_8 (5 s each), and C_1 (40 s), and bacteria transferred from an inoculated 9 cm² stainless steel area to the potato (3 x 3 cm)

These tests encompassed bacterial transfer from the plate to the potato, and the analyses were centered on the number of bacteria remaining on the potato sample after different numbers of contacts (C_0 , C_1 , C_8 , and C_{18}) with the stainless steel plate (Figure 3.16). Data presented

corresponded with recoveries from one sample that was collected at the end of the path. The initial level of bacteria on the plate also was included to verify consistency among results.

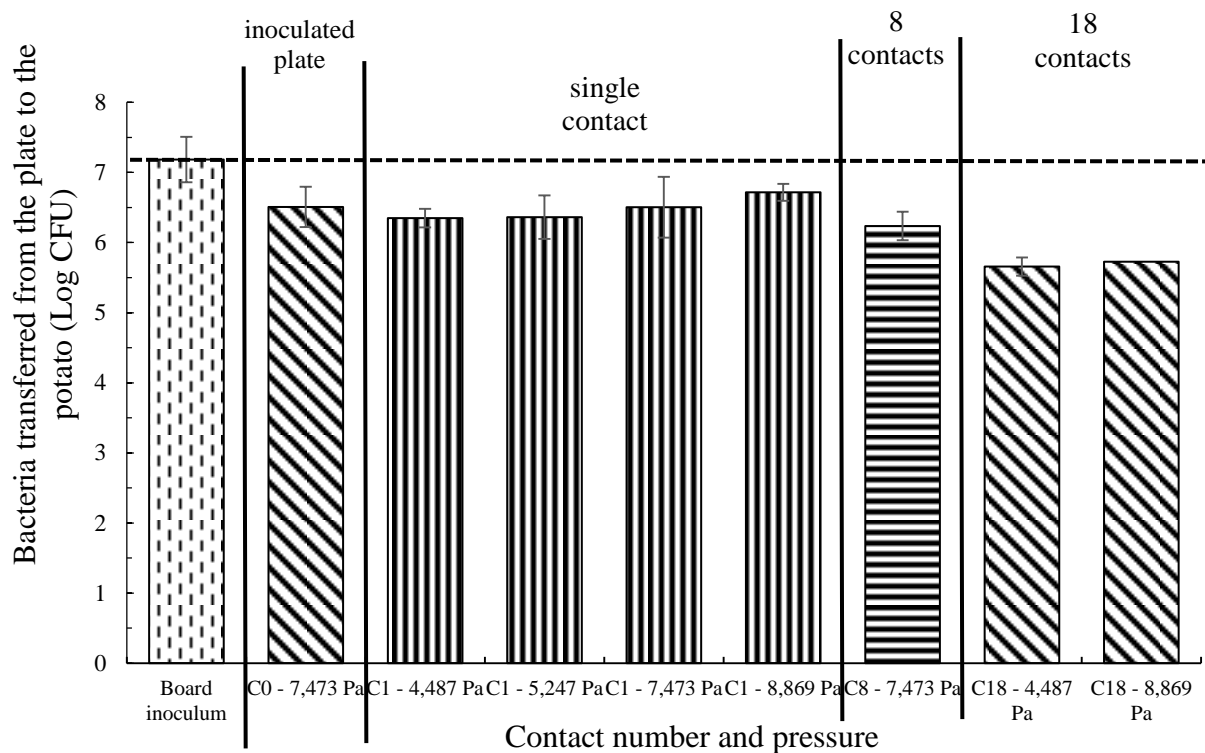


Figure 3.16 Bacteria transferred from potato samples after different static contact pressures (4,487, 5,247, 7,473, and 8,869 Pa), and comparison with the initial level of bacteria on the plate; C₀: bacteria on the potato sample after contacting a 9 cm² area for 5 s, C₁: bacteria from the potato sample after one 40 s contact, C₈: bacteria recovered from the potato sample after eight 5 s contacts, C₁₈: bacteria recovered from the potato sample after eighteen 5 s contacts.

As expected, *Salmonella* recovery from potato samples decreased with the increasing number of contacts (Figure 3.16). Bacteria were spread when the potato samples were in contact with the plate surface. The number of bacteria on the potato collected (after the initial contact with the contaminated square, C₀) was ~6 Log CFU. In addition, the number of bacteria

recovered from the potato showed that bacteria remained attached to the potato, with less bacteria transferred to the plate (C_1). Transfer preferentially occurred from the plate (C_0) to the potato, and was largely irreversible compared to transfer from the potato to the plate.

The maximum contact number used these experiments was 18, and at the end of each experiment, significant number of bacteria ($p < 0.0001$) still remained on the potato surface; 5.66 Log CFU were obtained when a pressure of 4,487 Pa was applied, and 5.73 Log CFU were obtained when a pressure of 8,869 Pa. These findings reveal that there is a risk of further cross-contamination from the potato to other contact areas due to the high number of *Salmonella* that remained on the potato even after the designated number of contacts. However, pressure ($p = 0.0937$) did not have a significant effect on bacterial transfer from the plate to the potato. For instance, independent of the pressure applied, transfer to the potato was in a range of ~5.50 to 6.50 Log CFU.

Contact number affected the number of bacteria remaining on the potato (Table 3.8). However, pressure did not affect bacterial transfer. These last results are consistent with those of previous experiments. As a result, the interaction between pressure and contact number did not affect bacterial transfer via static contact.

Table 3.8 Effect of contact number (C₀, C₁, C₈, and C₁₈) and pressure (4,487, 5,247, 7,473, and 8,869 Pa) on the bacteria recovered from the potato sample.

Effect	Num DF	Den DF	F	Pr > F
Pressure	3	64	2.23	0.0937
Contact number	3	64	20.10	<0.0001
Pressure*contact number	1	64	1.69	0.1986

3.3.2 Bacterial transfer at different speeds and pressure from a previously inoculated stainless steel plate to potato via dynamic contact

This section includes the evaluation of the physical variables and bacterial transfer direction. The first analysis focused on the effect of physical variables (contact speed, pressure, and contact time) on bacterial transfer. The same physical variables were evaluated in two bacterial transfer directions, which corresponded to bacteria transferred from a previously inoculated potato sample to sterile contact areas and from an inoculated contact area to an uninoculated potato sample. The second analysis consisted of different bacterial transfer iterations measuring bacteria transferred to one potato, 10 potatoes, and population on the potato. Results were analyzed as a completely randomized block design. The research hypothesis of the current study was that ‘Bacterial transfer from food to a contact surface increases with moisture content and pressure, and decreases with increasing speed’.

3.3.2.1 Bacterial transfer via dynamic contact evaluated at 40 s contact time, two contact speeds (3.75 and 7.75 mm/s), and three contact pressure (1,243, 2,333, and 4,513 Pa) between an inoculated potato and a sterile stainless steel surface

A completely randomized block design was used to determine if blocking the experiments per day affected bacterial transfer via dynamic contact. The goal was to evaluate the effect of the fixed variables (contact speed and pressure) on bacterial transfer from a previously inoculated potato sample to a sterile stainless steel plate (C₁ to C₅).

The completely randomized block design analysis was performed using the combination of speed and pressure as different treatment blocks, resulting in 6 different treatments (Table 3.9). Treatments corresponded to the same speeds and pressures described in Table 3.2 and in methods section 3.2.6.2. This analysis was performed to determine if the treatment had an effect, and if the random variable which was day influenced bacterial transfer from potato samples to the plate.

Table 3.9 Treatments applied to the potato sample for bacterial transfer via dynamic contact.

Treatment	Contact speed (mm/s)	Contact pressure (Pa)
1	7.75	1,217
2	7.75	2,307
3	7.75	4,487
4	3.75	1,217
5	3.75	2,307
6	3.75	4,487

There was no significant difference in transfer due to normal pressure on the potato sample, (Figure 3.17). Bacterial transfer decreased as distance increased, with a difference > 1 Log CFU between C_1 and C_5 . The speed and pressure combination also affected bacterial transfer via dynamic contact (Tables 3.10, 3.11, and 3.12). Fewer data points could be obtained at the slowest speed (3.75 mm/s) because the total contact distance was shorter.

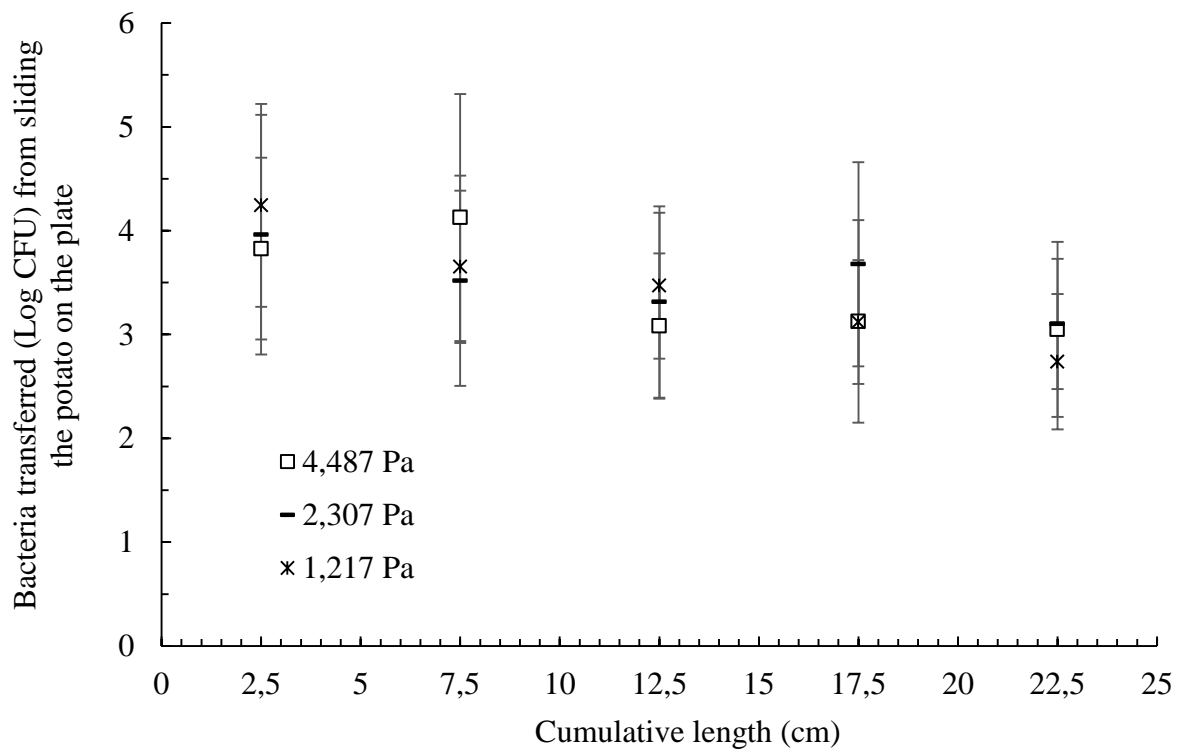


Figure 3.17 Bacteria transferred from a potato (3 x 3 x 1 cm) to the plate (C_1 to C_5) to evaluate the effect of dynamic contact at 7.75mm/s and different contact pressures (1,217, 2,307, and 4,487Pa).

Table 3.10 Effect of sliding speed (3.75 mm/s) and contact pressure (1,217, 2,307, and 4,487 Pa) on bacterial transfer from potato (3 x 3 x 1 cm) to plate (C₁ and C₂).

Pressure (Pa)	Distance (cm)	average CFU	average Log CFU
1,217	2.5	83 ± 0	1.92 ± 0
	7.5	250 ± 303	2.40 ± 0.43
2,307	2.5	1681 ± 3167	3.23 ± 0.88
	7.5	2417 ± 3345	3.38 ± 0.94
4,487	2.5	1250 ± 2436	3.10 ± 0.80
	7.5	264 ± 403	2.42 ± 0.45

Three statistical models were used. The first model evaluated the effect of the variable treatment as a fixed variable, which indicates a combination of sliding speed and normal pressure and day as a random variable. Treatment affected bacterial transfer via dynamic contact ($p = 0.0067$), which consisted of the speed and the normal pressure previously determined (Table 3.11). The blocking of the data showed that the day of experiment did not affect bacterial transfer ($p = 0.6685$) (Table 3.12).

Table 3.11 Effects of treatment, contact distance, and the random variable day the experiment was conducted on bacteria transferred to the sterile plate.

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F	Pr > F
Treatment	5	10.6467	2.1293	Var(Residual) + Q(treatment)	MS(Residual)	134	3.37	0.0067
Contact distance	4	6.4854	1.6213	Var(Residual) + Q(distance)	MS(Residual)	134	2.57	0.0409
Day	3	0.9864	0.3288	Var(Residual) + 34.222 Var(day)	MS(Residual)	134	0.52	0.6685
Residual	134	84.5654	0.6310	Var(Residual)

Table 3.12 Effect of the speed and pressure (treatment) on bacteria transferred to the sterile plate.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F	Pr > F
Treatment	5	138	2.38	0.0415

The second model evaluated the effect of treatment and distance as fixed variables, and day as a random variable (Table 3.13). Bacterial transfer decreased as contact distance increased ($p = 0.0409$). The last model separated the treatment block to allow for the evaluation of speed and pressure separately (Table 3.14).

Table 3.13 Effects of fixed variable distance and treatment on bacteria transferred to the sterile plate.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F	Pr > F
Treatment	5	134	3.99	0.0021
Distance	4	134	2.57	0.0409

Table 3.14 Effect of speed and pressure on bacteria transferred to the sterile plate.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F	Pr > F
Speed	1	116	16.43	<0.0001
Pressure	2	116	1.14	0.3233
Distance	4	116	1.89	0.1164

The number of bacteria recovered from C_1 and C_2 were summed to evaluate total bacterial transfer over a contact distance of 10 cm for all treatments, and to test if speed affected bacterial transfer via dynamic contact (Figure 3.18). Results showed that bacterial transfer via dynamic contact was higher at the highest speed ($p = 0.0098$). In addition, there were no significant differences in bacterial transfer from the potato to the clean plate at the different contact pressures (Tables 3.15 and 3.16).

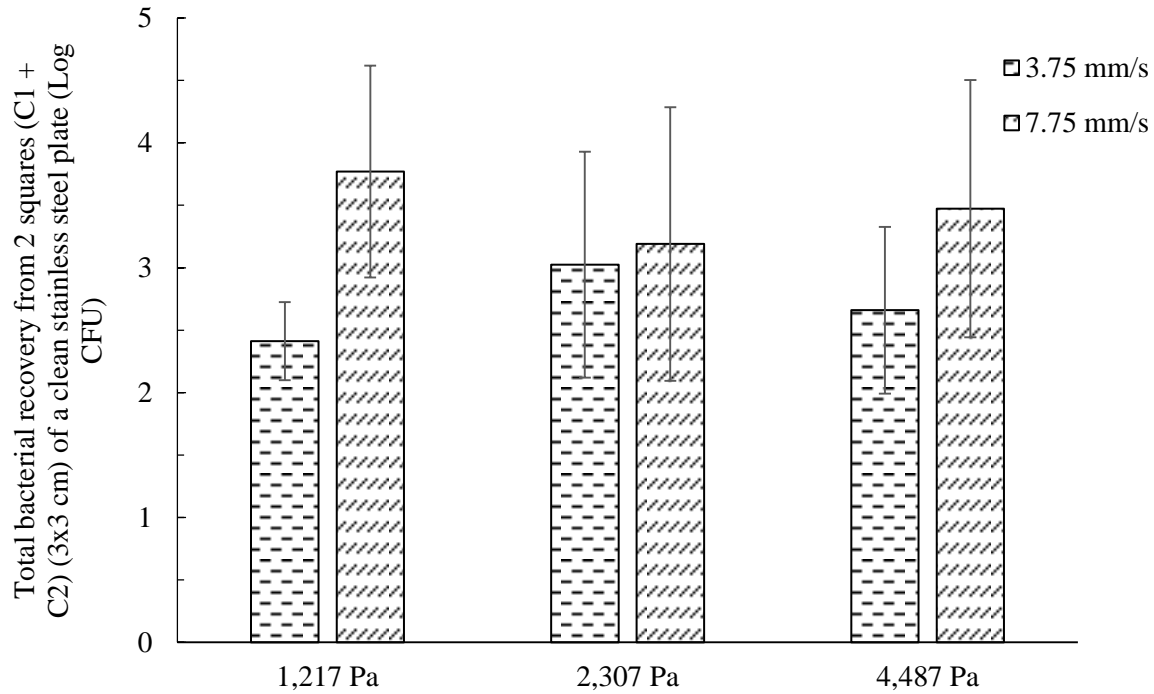


Figure 3.18 Bacterial transfer via sliding contact at three pressures (1,217, 2,307, and 4,487 Pa) and two sliding speeds (3.75 and 7.75 mm/s) from a previously contaminated potato square to C₁ and C₂ (10 cm contact distance).

Table 3.15 Effects of pressure (1,217, 2,307, and 4,487 Pa) and speed (3.75 and 7.75 mm/s) at 10 cm contact distance (C₁ = C₂) on bacterial transfer to the sterile plate.

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F	Pr > F
Speed	1	5.4990	5.4990	Var(Residual) + Q (speed,speed*pressure)	MS (Residual)	30	7.60	0.0098
Pressure	2	0.0093	0.0046	Var(Residual) + Q (pressure,speed*pressure)	MS (Residual)	30	0.01	0.9936
speed*pressure	2	2.1356	1.0678	Var(Residual) + Q(speed*pressure)	MS (Residual)	30	1.48	0.2447
Residual	30	21.7049	0.7234	Var(Residual)

Table 3.16 Effects of fixed variables speed (3.75 and 7.75 mm/s) and pressure (1,217, 2,307, and 4,487 Pa) at 10 cm contact distance (C_1 and C_2) on bacterial transfer to the sterile plate.

Effect	Num DF	Den DF	F	Pr > F
Speed	1	30	7.60	0.0098
Pressure	2	30	0.01	0.9936
speed*pressure	2	30	1.48	0.2447

Based on this analysis, pressure had no effect; however, speed did affect bacterial transfer. These results were used to decide which variables and levels to include in the next experimental design (section 3.3.2.2). From these results, one level of pressure was evaluated, and one level was added to the speed. A medium speed which corresponds to 5 mm/s was added to the next experimental design.

3.3.2.2 Effect of three contact speeds (3.75, 5, and 7.75mm/s) on bacterial transfer via dynamic contact

A randomized complete block design was used to evaluate the effect of speed on bacterial transfer versus potato number, which was the repeated measurement in this experimental design (Figure 3.19). These experiments, which were analogous to others from prior studies used to develop a meta-analysis for bacterial transfer (Chapter 4), allowed the evaluation of other transfer directions (plate to 10 subsequent potatoes). The random effect of the experimental design was the day of experimentation.

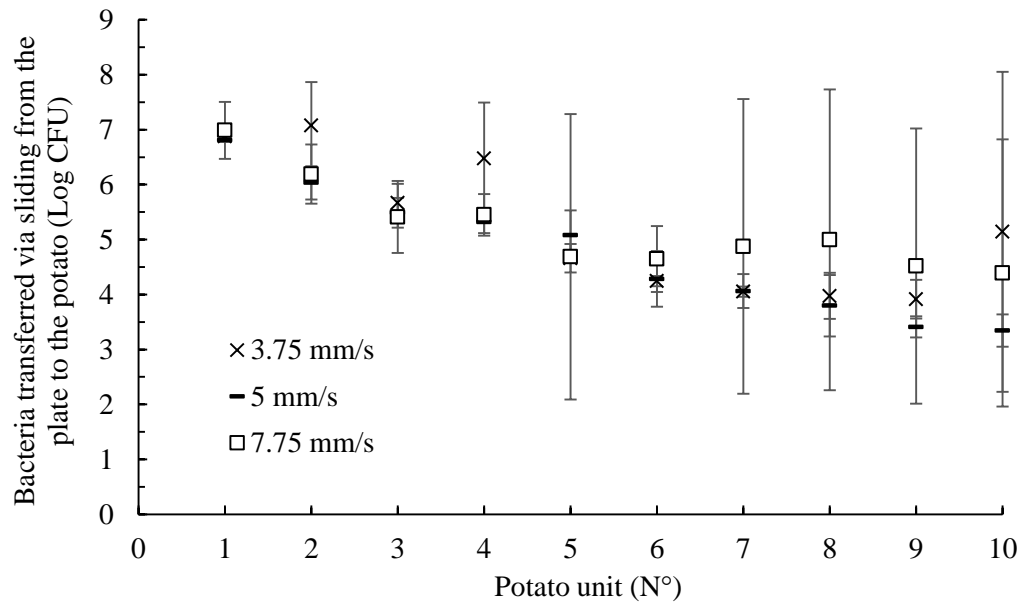


Figure 3.19 Bacterial transfer from the plate (C_0) to ten clean potato samples at three speeds (3.75, 5, and 7.75 mm/s) and a pressure of 4,487 Pa.

Contrary to previous findings, no significant differences were found among the speeds evaluated (Table 3.17). Significant differences were found among potato samples, indicating that bacterial transfer decreased along the contact surface (C_0), with fewer bacteria transferred from the plate to the potato samples. Based on these results, transfer direction affected bacterial transfer, and the effect of the physical variables was different.

Table 3.17 Randomized complete block design analysis for bacterial transfer from the plate to ten clean potatoes.

Effect	Num DF	Den DF	F	Pr > F
Speed	2	60	0.71	0.4947
sample	9	60	13.28	<0.0001
sample *speed	18	60	0.51	0.9448

3.3.2.3 Evaluation of six bacterial transfer scenarios via dynamic contact from an inoculated stainless steel plate to one and ten sterile potato samples

This study analyzed bacterial transfer to the stainless steel plate, bacteria transferred to the potato sample, and bacteria remaining on the plate, and compared the differences in the number of bacteria transferred to potato samples. In addition, the impact of the number of potato samples slid across the same previously inoculated contact surface was assessed, relative to impact on the number of bacteria remaining on the plate.

The different scenarios correspond to the direction bacteria were transferred and the number of potatoes evaluated for bacterial transfer. This analysis considered the number of potato samples slid over a previously inoculated 9 cm² stainless steel area, the transfer direction, and the effect of sliding speed. Scenario 1 corresponded to the number of bacteria transferred from a previously inoculated 9 cm² stainless steel area (C_0) to one potato. Scenario 2 corresponded to the number of bacteria remaining on a previously inoculated 9 cm² stainless steel area after sliding one potato sample over the inoculated surface (C_0). Scenario 3 corresponded to bacteria transferred to the subsequent sterile square (C_1) after one potato was

slid on a previously inoculated 9 cm² stainless steel area (C₀). Scenario 4 corresponded to the number of bacteria remaining on the plate (C₀) after sliding 10 potatoes on a previously inoculated 9 cm² stainless steel area. Scenario 5 corresponded to bacteria transferred recoveries to the first square (C₁) after sliding 10 potatoes on a previously inoculated 9 cm² stainless steel area. Scenario 6 corresponded to bacteria transferred from a previously inoculated stainless steel plate (C₀) to 10 clean potatoes (Figure 3.20).

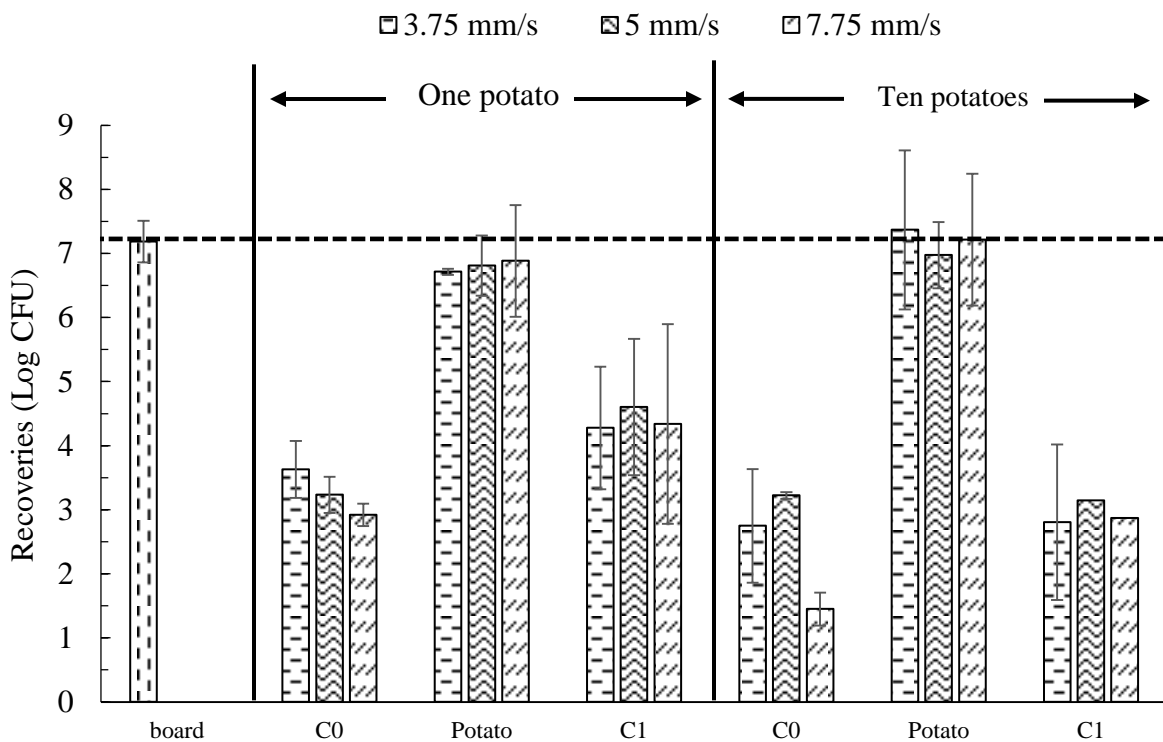


Figure 3.20 Bacteria recovered from different assays of potato or plate; C₀: bacteria remaining on the plate, C₁: bacteria transferred to a sterile 9 cm² stainless steel contact area.

Few bacteria were found on the plate (C₀) after one potato was slid. A similar result was obtained after sliding ten potatoes on a 12 cm² stainless steel contact area previously inoculated

(C₀), and bacteria transferred to the first square (C₁), with a total contact area of 15 cm² after sliding ten samples. These results showed that the potato picked up bacteria from the surface, and more bacteria remained on the potato than transferred to the plate. Ten potatoes picked up approximately twice as many bacteria (~2.45 times more; 17,030,235 ± 7,034,517 CFU) than did a single potato (6,948,663 ± 1,570,886 CFU).

After sliding 10 potatoes, ~3 Log CFU were recovered from the first square (C₁) (scenario 5), and ~7 Log CFU were recovered from potato samples (scenario 6). These results showed that the potato sample picked up more bacteria from the surface than the number of bacteria that were released to the subsequent contact area. In addition, the number of potato samples slid affected the number of bacteria remaining on the previously inoculated plate (C₀). The direction bacteria were transferred, and potato number in contact with the surface material also affected the bacterial transfer rate. More bacteria were transferred to the first square (C₁) after sliding one potato than were recovered from the plate (C₁) after sliding 10 potatoes. A possible explanation of the last observation is that each potato slid collected one portion of the bacteria that the previous potato transferred.

Based on previous analyses, speed was expected to impact bacterial transfer; however, within this portion of the study, speed did not affect bacterial transfer from the plate to multiple potatoes (Table 3.18 to Table 3.20). The variable day, which corresponded to the blocking factor, affected bacterial transfer (Table 3.18), and added variability to the results. It is recommended to block the treatments to reduce the effect of the day of experiment, which is a challenge due to the number of potato units evaluated per treatment.

Table 3.18 Six bacterial transfer scenarios from the plate to one or ten potato samples.

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F	Pr > F
Scenario	4	99.4431	24.8607	Var(Residual) + Q(scenario)	MS(Residual)	34	61.58	<0.0001
Speed	2	0.1205	0.0602	Var(Residual) + Q(speed)	MS(Residual)	34	0.15	0.8619
Day of experiment	2	8.8763	4.4381	Var(Residual) + 14.2 Var(day)	MS(Residual)	34	10.99	0.0002
Residual	34	13.7256	0.4036	Var(Residual)

Table 3.19 Test of fixed effects.

Effect	Num DF	Den DF	F	Pr > F
Scenario	4	28	36.53	<0.0001
Speed	2	28	0.26	0.7713
scenario*speed	8	28	0.84	0.5729

Table 3.20 Slice analysis for the significant differences.

Effect	scenario	speed	Num DF	Den DF	F	Pr > F
scenario*speed	1		2	28	0.01	0.9871
scenario*speed	2		2	28	0.68	0.5128
scenario*speed	3		2	28	0.11	0.8964
scenario*speed	4		2	28	1.58	0.2238
scenario*speed	5		2	28	1.16	0.3285
scenario*speed		3.75	4	28	12.16	<0.0001
scenario*speed		5	4	28	11.12	<0.0001
scenario*speed		7.75	4	28	15.74	<0.0001

These small-scale experiments were designed to control the interaction between the potato, the stainless steel surface, and *Salmonella*. Previous publications reported a higher concentration of bacteria on the first cross-contaminated surface (here C_1) in comparison to the current results (~ 4 Log CFU). For example, Vorst *et al* (2006), Benoit *et al* (2013), and Yan (data not published) recovered 6.2 (Log CFU/sample) after a single contact, but the sample collected had a higher contact area (25 cm^2) and percentage of contact was not estimated in these studies.

The current experiments (sections 3.3.2.2 and 3.3.2.3) were designed for the same transfer direction as previous studies. For example, Wang (2015) reported bacterial recoveries from different parts of a manual slicer, 1.9 ± 0.8 Log CFU/part for the blade, 2.2 ± 0.1 Log CFU/part for the back plate, and 2.3 ± 0.8 Log CFU/part on the bottom plate. In the current study, $2.06 \text{ Log CFU/cm}^2$, $2.54 \text{ Log CFU/cm}^2$, and $0.77 \text{ Log CFU/cm}^2$ were recovered after sliding 10 potatoes at 3.75 mm/s, 5 mm/s, and 7.75 mm/s, respectively. It is hard to perform a direct comparison, given that the studies resulted in different samples, which might cause differences if the data are estimated. This observation supports a need for bacterial transfer studies to quantify and report true contact areas, speeds, and forces.

Wang (2015) reported standard deviations of ~ 0.4 Log CFU in tomato recoveries and ~ 0.3 Log CFU for surface components of the blade. The same author reported that the total number of bacteria transferred was 3.4 ± 0.4 Log CFU from a contaminated blade to 20 fresh tomatoes. The results reported in Wang (2015) were less than the populations recovered in the present study after sliding 10 uninoculated potatoes on a previously inoculated 9 cm^2 stainless steel square. For example, when potato samples were slid 7.75 mm/s with a pressure of 1,217 Pa, total bacterial transfer was 4.17 ± 0.69 Log CFU. In Wang's study, the fact that a fraction of the

total area of the tomato in contact with the slicer blades was sampled for bacterial enumeration might contribute to these differences.

Finally, in a similar study conducted by Scollon (2014) on bacterial transfer from a slicer to onions, the standard deviation reported was ~1 Log CFU/onion. The standard deviation range obtained in the current study was consistent with these prior studies. Differences in results likely were due to differences in the experimental design, conditions, and variables included in the study. For example, in studies performed using tomatoes (Wang, 2015), a “wash-off” effect of the free liquid released by tomatoes was reported, which would interfere with continuous bacterial transfer.

3.3.2.4 Bacteria remaining on potato samples after dynamic contact at two speeds (3.75 and 7.75 mm/s) and three pressures (1,217, 2,307, 4,487 Pa)

Two speeds and three pressures were applied, in different combinations for each treatment (Figure 3.21). The number of bacteria transferred from the plate to the potato was not affected by sliding speed ($p = 0.1232$), pressure ($p = 0.1753$), or the interaction of both variables ($p = 0.5073$) (Table 3.21). Each contact speed had a different contact distance, and speed and distance were determined to achieve the same contact time. In addition, the data were collected from potatoes at the end of the sample path. For instance, the analysis of the variable contact distance will yield the same results as if each speed had a specific contact distance. The food component played a fundamental role in bacterial transfer, collecting and spreading the bacteria to surfaces in contact with the potato. This result was consistent at different levels of physical variables evaluated and bacterial transfer directions (Figures 3.20 and 3.21).

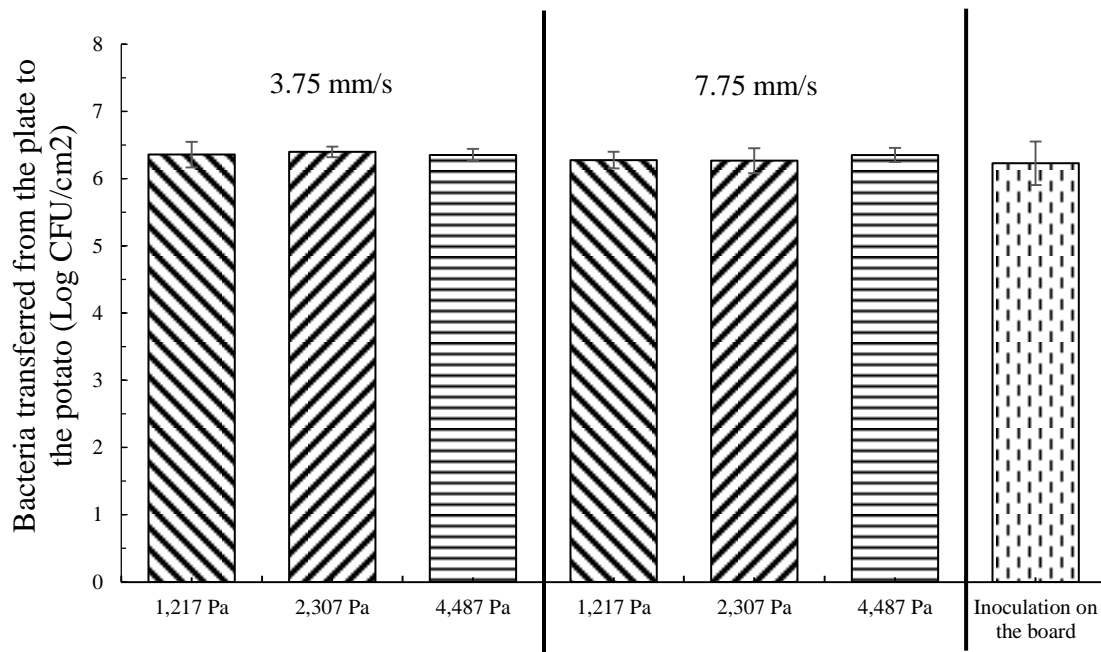


Figure 3.21 Bacterial transfer (Log CFU/cm²) to potato samples after sliding on the plate (15 and 30 cm) at different speeds (3.75 and 7.75 mm/s) and pressures (1,217, 2,307, and 4,487 Pa).

Table 3.21 Effect of each fixed variable and their interaction on bacteria transferred to potato samples.

Effect	Num DF	Den DF	F	Pr > F
Speed	1	30	2.52	0.1232
Pressure	2	30	1.85	0.1753
Speed*pressure	2	30	0.69	0.5073

3.3.3 Comparison of bacterial transfer via static and dynamic contact during 40 s of contact between a previously contaminated potato sample and sterile stainless steel

This analysis compared total bacterial transfer vs. transfer type (single contact, multiple contacts, and dynamic) to determine which interaction type facilitated bacterial transfer to

stainless steel. Type 1 bacterial transfer corresponded to a 40 s single contact time between an inoculated potato slice and a 9 cm² sterile stainless steel square (Figure 3.22). Type 2 bacterial transfer was achieved via a single contact (C₁) for 5 s. Type 3 bacterial transfer was achieved by cumulative bacterial transfer from contact C₁ to C₇. Type 4 bacterial transfer was achieved via multiple static contacts, and was estimated by interpolation (Figure 3.23). Recoveries from 4 odd numbered stainless steel squares (C₁, C₃, C₅, and C₇) of 9 cm² each were interpolated to estimate the total transfer to 8 stainless steel squares (C₁ to C₈) over 40 s contact time. Finally, Type 5 bacterial transfer was obtained via dynamic contact for 40 s and 2 speeds (3.75 and 7.75 mm/s) (Figure 3.23).

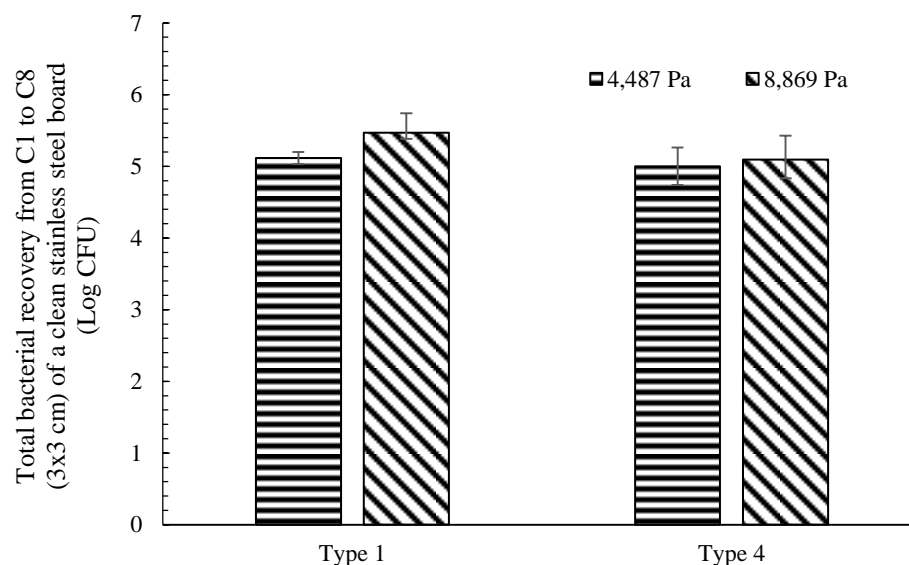


Figure 3.22 Bacterial transfer via static contact at two pressures (4,487 and 8,869 Pa) from a previously contaminated potato sample to C₁ (Type 1) single contact (40 s) and from a previously contaminated potato sample to C₁ to C₈ (Type 4) multiple contacts, 40 s total.

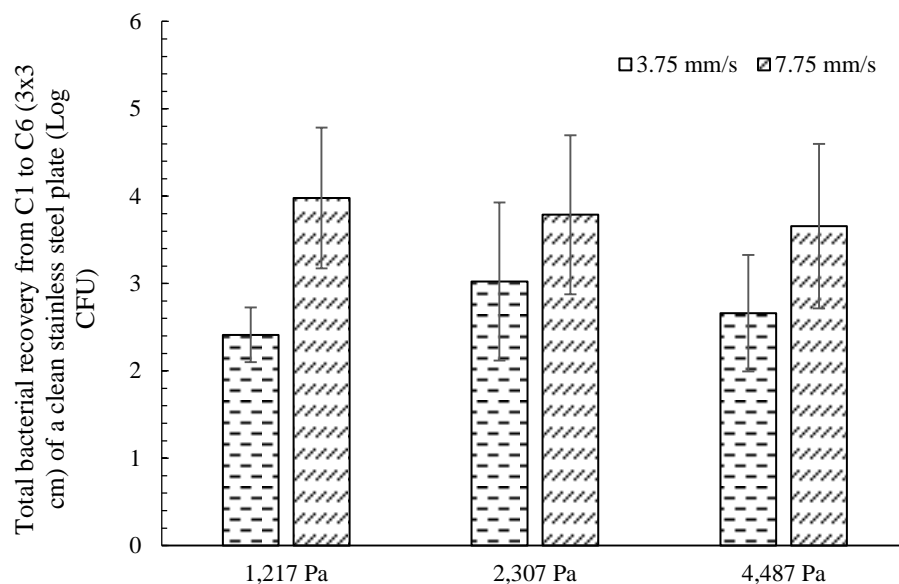


Figure 3.23 Bacterial transfer via dynamic contact at two speeds (3.75 and 7.75 mm/s) and three pressures (1,217, 2,307, and 4,487 Pa) from a previously contaminated potato square to C₁ to C₆.

Results revealed that transfer type influences the number of bacteria transferred ($p < 0.0001$). The opposite was found for the variable pressure ($p = 0.7548$). The interaction among type and pressure did not have any effect (Table 3.22).

Table 3.22 Effects of transfer type, pressure, and their interaction on bacteria transferred to sterile stainless steel.

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F	Pr > F
Type	2	22.0201	11.0100	Var(Residual) + Q(approach, approach*pressure)	MS (Residual)	53	18.19	<0.0001
Pressure	3	0.7230	0.2410	Var(Residual) + Q(pressure, approach*pressure)	MS (Residual)	53	0.40	0.7548
Type*pressure	1	0.1014	0.1014	Var(Residual) + Q(approach*pressure)	MS (Residual)	53	0.17	0.6839
Residual	53	32.0727	0.6051	Var(Residual)

Variables in the process of bacterial transfer via static and dynamic contact differed. The variable of speed was implied in the interaction via dynamic contact. In dynamic contact, potatoes were collecting bacteria along the path. This type of movement might preferentially “prevent” bacteria from remaining on the contact surface.

The total number of bacteria transferred using the static contact approach was higher than for dynamic contact, which may be due to repetitive interactions between the potato and stainless steel allowing a film of water containing bacteria to form on stainless steel during static contact.

At this point, results observed are not in concordance with the research hypothesis. Pressure did not affect bacterial transfer in an increasing trend as stated (Figure 3.22). Pressure did not affect total bacteria recovered after single, multiple, or dynamic contact. Results also refuted the research hypothesis on the variable speed (Figure 3.23). Bacterial transfer increased as speed increased ($p < 0.0001$). Fewer bacteria were found in the transfer experiments

conducted via dynamic contact. These differences can be due to the nature of the movement, which was relatively unaggressive for potatoes.

3.3.4 Model fitting of data collected

3.3.4.1 Bacterial transfer via static contact

The Weibull model was fit to data sets per methods subsequently described in Chapter 4 on bacterial transfer via static contact (Figure 3.24). Using the total of 39 data sets, the Weibull model best fit 38%, the linear model best fit 43% (Figure 3.25), and 19% did not give a good fit because recoveries from these data sets did not follow a strictly decreasing trend line.

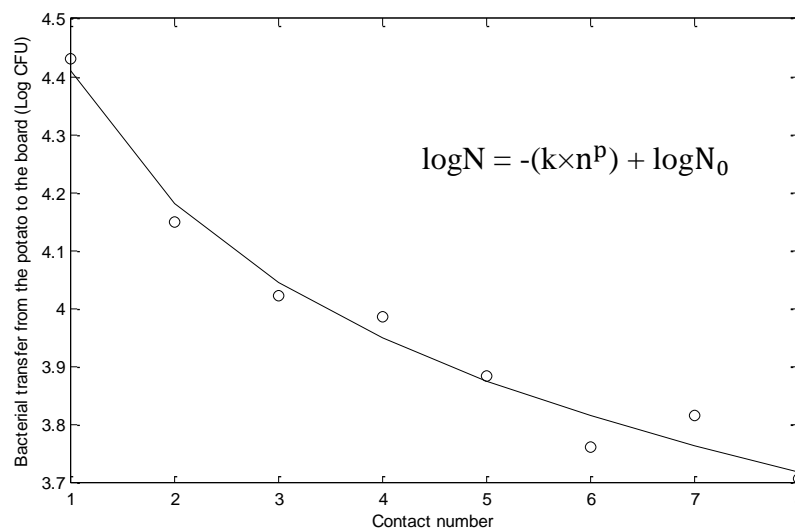


Figure 3.24 Estimated bacterial transfer via static contact (5 s) from the potato to the plate from C₁ to C₈, at a normal pressure of 7,473 Pa using the Weibull model.

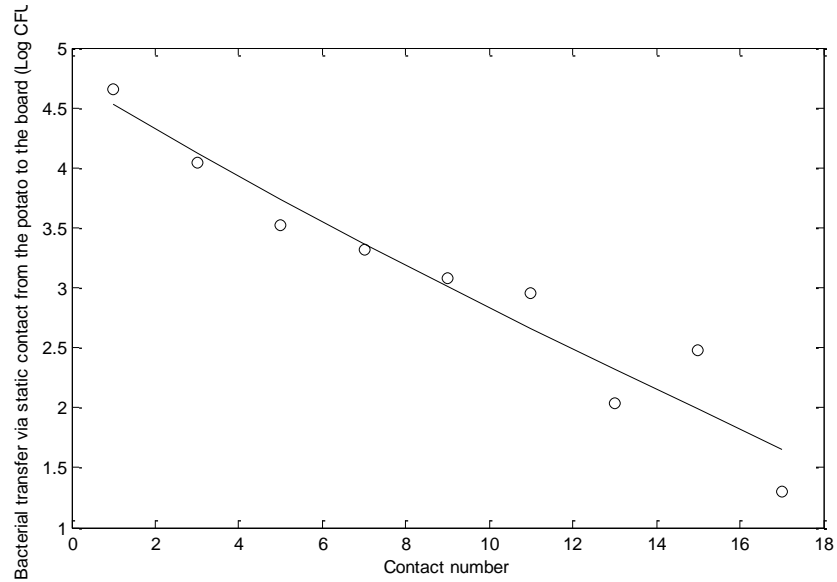


Figure 3.25 Estimated bacterial transfer via 18 static 5 s contact times from the potato to the plate (equation 4.3) using a linear model.

3.3.4.2 Bacterial transfer via dynamic contact

A Weibull model was fit to bacterial transfer data sets from dynamic contact (Figure 3.26). The challenge in fitting this model was that only 5 data points were collected per data set. Of the total 18 data sets collected, the Weibull best fit 61%, 22% fit a linear model, and 17% did not give a good fit because recoveries from these data sets did not follow a strictly decreasing trend line. Experimental results agreed with the analysis performed on data collected for the meta-analysis (Chapter 4). In both approaches, the Weibull model best fit the majority of the data sets.

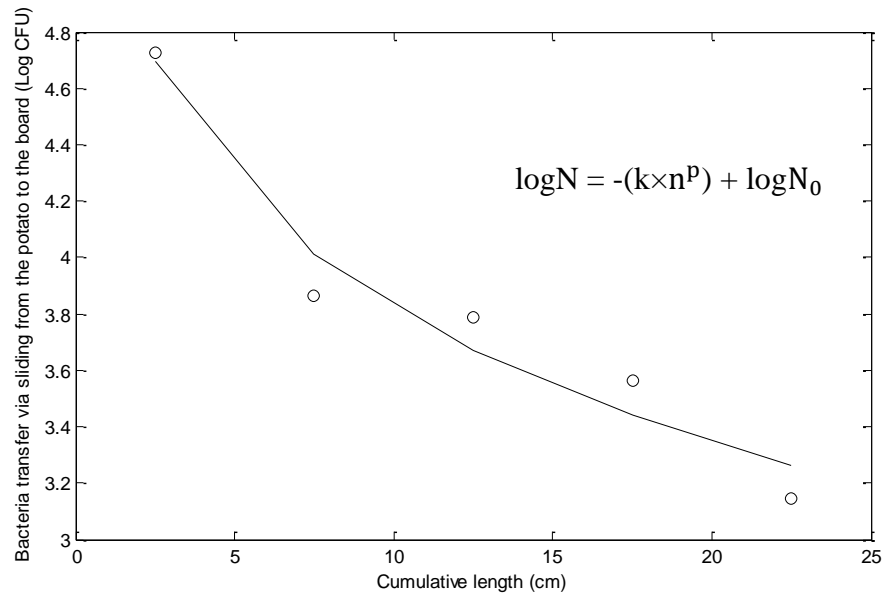


Figure 3.26 Bacterial transfer via dynamic contact from the potato to the plate (C_1 to C_5) at a contact time of 40 s, 30 cm contact distance, and 7.75 mm/s sliding speed, showing the Weibull model fit.

In the current study, it was important to include the fundamental variables and to control physical variables in the design, such as pressure, contact time, and contact area, to enable consistency among results and to improve the possibility to best fit a model. This last step of the analysis improved the understanding of bacterial transfer using a different approach. In addition, it allowed comparison of experimental results with previous studies included in a meta-analysis (see Chapter 4) focused mainly on food composition. This work identified relevant physical variables that affected bacterial transfer. From these results, it is advisable to conduct future research focused on the evaluation of physical variables, because the data trends were consistent with previous studies (see Chapter 4), and data from similar experiments will contribute to the development of new models (see Chapter 5). Predictive transfer models can be useful tools, but should ideally be based on fundamental physical variables that can be generalized across studies

and applications. Finally, the Weibull model performed similarly on both our data and data sets from a meta-analysis on bacterial transfer (See Chapter 4). This comparison allowed some limited general conclusions relative to fundamental physical variables, which was one purpose of the current dissertation.

META-ANALYSIS OF DATA ON BACTERIAL TRANSFER VIA SURFACE, SLICING, AND COMPLEX CONTACT TO FOOD PRODUCTS

4.1 Overview

This chapter encompasses a secondary analysis of data collected from previously published studies, recent collaborative work, and data previously collected at MSU. These data were compiled in a database for a meta-analysis of bacterial transfer data via static contact and dynamic contact. The bacterial transfer variables studied included food product composition, initial inoculation level, and microorganism, which were determined according to the variables available in the publications. Analyses of the data collected is presented to elucidate any generalizable trends in curves showing bacterial transfer from food contact surface to food products, which was the transfer direction evaluated in most previous studies. A meta-analysis was also performed to evaluate which variables significantly affected bacterial transfer from food to contact surfaces. This chapter is linked to the second objective of this dissertation. A quantitative meta-analysis of existing data on *Salmonella* transfer to and from food and food contact surfaces compiled in a standardized database format was conducted, to identify generalizable trends between product contact variables and the *Salmonella* transfer response.

4.2 Materials and methods

Overall, data for this study were identified via a comprehensive search of previous publications encompassing surface-to-surface transfer of bacteria in food systems. Journal articles related to the subject of bacterial transfer via surface to/from food contact surfaces were obtained followed by a determination of what data from any given study fit the selection criteria for the database (described below). If the figures and tables presented the results as repeated

measurements of bacterial transfer over several food product samples, multiple food sample units or multiple contacts, the data were collected and stored (as described below). The data came from three types of sources: previous publications, recent collaborations, and previous studies conducted at MSU. Subsequently, the meta-analysis regression consisted of the analysis of the data that were previously selected. Overall, three steps were necessary to complete the meta-analysis: model fitting, regression analysis, and categorical analysis.

4.2.1 Selection of the data

The data collection process consisted of three steps. First, published studies on bacterial transfer were selected considering the food product, the microorganism, the means of bacterial transfer, and the process type. Second, information about how the data were obtained was collected from each publication. Finally, the collected data were stored and categorized according to the variables being evaluated and the characteristics of the results of each study. Journal articles published from 1997 to 2014 were found using the Web of Knowledge. Thereafter, the data were checked to fulfill the needs for this study, preferably in units of Log CFU, Log CFU/g, or Log CFU/cm².

These data covered a range of food commodities that were sliced continuously, in contact with a surface already contaminated, and/or subjected to multiple contacts with various pieces of equipment or material types. The samples included slices obtained successively during mechanical or manual slicing, or after using a knife. Samples obtained from foods in contact with surfaces previously contaminated with a pre-inoculated product were also included. Complex contact experiments consisted of passing the clean product through multiple pieces of equipment already contaminated (e.g., grinders, or shredding, washing, conveying systems).

4.2.2 Data collection and organization

The selected data then were categorized and coded according to the publisher, number of individual data points available in figures or tables, and the variables evaluated. The data were organized in a catalogue that included the following information: data key code, year, author, title, journal, volume, page numbers, organism, product type, transfer type, surfaces, initial inoculation level, type of data, variables, # of figures, # of tables, total # of data sets, and x and y axes values.

The food items included: raw meat whole muscle, ready-to-eat-meat whole muscle, beef, tomato, onion, lettuce, cantaloupe, bologna, salami, ham, turkey, fish, and pork. These product types were grouped into the following aggregate categories: fresh produce (tomato, onion, cantaloupe, and lettuce), meat (raw meat whole muscle, cooked whole muscle meat, ready-to-eat-meat, and ground beef), sausage (bologna, salami), turkey (roasted turkey breast), fish ('gravad' salmon), pork (ham), and others (food contact materials). This classification was based on the USDA National Nutrient Database for Standard Reference Release 28.

The data included in this analysis were from studies on bacterial transfer via static contact (typically multiple contact), and dynamic contact (e.g., slicing). Data on bacterial transfer via complex contact were not included in this analysis, because the repeated events corresponded to pieces of equipment with different dimensions and different characteristics such as a grinder, shredder, or celery dicer. Also, because the focus of the current study was bacterial transfer via contact surface, studies on bacterial transfer via water were not included in this analysis.

Actual transfer data were extracted from manuscripts using Datathief software, which can identify and assign a point from an image to rectangular coordinates (Tummers, 2005). The resulting x-y data from figures were saved to a text file, while data from tables were directly

obtained. Finally, the data were imported to an Excel file for initial processing and analysis. SAS and MATLAB R2015b were used for statistical characterizations (*t*-test, proc mixed data), parameter estimation as described below, slope, and rate, and regression analysis of the parameters estimated (described below), as the key steps in the meta-analysis.

4.2.3 Data analysis and modeling

Parameter estimation, confidence intervals ($ci = nlparci(b, R, 'jacobian', J, 'alpha', alpha)$), root mean squared error (RMSE, eqn 1), R^2 , p-value, and Akaike's information criterion (AIC_c , eqn 2) were used to evaluate candidate models (described below) to describe the bacterial response data vs. discrete contact events (i.e., slices or contacts); *t* tests and regression analysis then were performed to draw general conclusions about factors affecting bacterial transfer.

$$RMSE = \sqrt{\frac{SSE}{n-k}} \quad (4.1)$$

$$AIC_c = -n \times \ln(SSE/n) + 2 \times K + (2 \times K \times (K + 1)) / (n - K - 1) \quad (4.2)$$

where:

SSE: squared residual errors

K: number of parameters plus 1

n: number of data points

One file was created per each study, with one sheet for each data set in that study. An algorithm using MATLAB R2015b was built, using command 'templist=dir('*.xlsx')'. This command allowed analysis of multiple Excel files simultaneously. Using the command line [status,sheetname] = xlsfinfo(datalist{a}) and information from the xlsread command alldata{a,i} = xlsread(datalist{a},sheet), every data set was analyzed, and the results were

reported in an output table. Parameters for the candidate models (described below) were included in the table, as estimated using the MATLAB command `nlinfit`.

Data were grouped according to three general transfer process types: transfer via slicing (dynamic contact), transfer via multiple contacts (static contact), and transfer via complex contacts or “black box” processes (in which bacterial counts are reported after samples are processed through complex, multi-step operations). Transfer via complex contacts data were grouped, but they were not analyzed. The purpose of the analysis was to determine which model best describes the transfer responses inherent in the three types of data sets.

The model fitting analysis consisted of three steps. First, a loop read all the data sets stored in Excel files. Then, parameters were estimated for three candidate models described below. Finally, a regression analysis was run to test the relationships between key physical variables and the aggregated set of model parameters for all of the transfer response curves. (The commands used for performing this analysis were: `R = corrcoef(A)`; `p1 = polyfit(x,y,1)`; `f1 = polyval(p1,x)`; `res1 = polyval(p1,x)-y`). Three models from the literature representing multiple different phenomenological outcomes in the transfer response curves were used: Log-Linear (eqn 4.3), Weibull (eqn 4.4), and Linear-Weibull (eqn 4.5). The criteria considered to determine the (i.e., “most likely correct”) best model was the AIC_c . The log-linear model was:

$$\log N = -(k \times n) + \log N_0 \quad (4.3)$$

where:

N = bacteria transferred

N_0 = initial number of bacteria

k = slope

n = number (slide or contact)

The Weibull type model was:

$$\log N = -(k \times n^p) + \log N_0 \quad (4.4)$$

where:

k = rate parameter

p = shape factor

and

The linear-Weibull model was:

$$n < n_c, \text{Log } N = \log N_0 - (k \times n) \quad (4.5)$$

$$\text{Log } N = \text{Log } N_0 - \left(\frac{k}{(p \times (n_c)^{p-1})} \right) \times (n^p) + \left(\frac{1-p}{(p \times k \times n_c)} \right) \quad (4.6)$$

n_c = critical value

4.2.4 Regression analysis

The criterion for including the parameters estimated for an individual data set in the meta-analysis regression was the difference between the parameter and the lower confidence interval:

```
for j = 1:1:length(b)
    if (b(j)-ci(j,1)) > 2*b(j)
        isError(i,1) = 1;
    end
```

The relatively loose inclusion criterion avoided discarding data that were useful even if the fit was relatively poor. The parameters were determined for each model, and the food components of the different food items in the database (meats and fresh produce) were collected (Table 4.1). The regression analysis was performed on each of the parameters of the Weibull model (intercept, “rate”, and shape) vs. each of the food components among them pH, water content (%), proteins (%), fat (%), and R_a (μm) (Table A.90).

Table 4.1 Food components of the food products collected for the meta-analysis.

food type	pH	water content (%)	proteins (%)	fat (%)	R_a (μm)
Meat	5.1 - 6.2	69.0	19.5	11.0	N
Bologna	6.22	62.4	14.8	15.9	N
Salami	5.76	43.0	17.0	36.0	8.04
Ham	5.9 - 6.1	62.7	25 - 30	5 - 20	5.19
Turkey	5.9	58.3	20.1	20.2	N
Salmon	6.6 - 6.8	63.4	17.4	16.5	N
Pork	6 - 6.5	42.0	11.9	45.0	N
Tomato	4.2 - 4.3	94.1	1.0	0.3	2.88
Onion	5.3 - 5.8	87.5	1.4	0.2	0.3
Cantaloupe	6.3 - 6.7	94.0	0.2	0.2	N
Lettuce	6	94.8	1.2	0.2	20

4.3 Results

4.3.1 Characterization of data collected

A total of 71 journal articles on bacterial transfer by 64 different authors (2002 to 2014), were collected and cataloged (Table A.89). From these 71 articles, a total of 321 data sets were coded by author, including 159 data sets on multiple static contacts and 162 data sets on slicing.

These published data sets typically represented averages from three replicates. Data sets from collaborative work and data previously collected at MSU corresponded to three to six individual replicates, depending on the study. Data collected were 76% published and 24% unpublished data. Published data came from different multidisciplinary groups and multiple co-authors. Categorized by product, 27.4% corresponded to pork (ham), 19.9% to turkey, 18.7% to meat (raw meat, cooked meat, ready-to-eat-meat), 18.1% to produce (tomato, onion, cantaloupe, and lettuce), 10.0% to sausage, 4.0% to laboratory media (non-edible materials), and 1.9% to fish ('gravad' salmon). Ultimately, 35% fit the data classification needed for the current meta-analysis (Table 4.2).

E. coli O157:H7, *Salmonella*, and *Listeria* were the bacteria used most frequently in transfer studies. *Bacillus*, *Campylobacter*, *Kocuria*, *Pseudomonas*, *Staphylococcus*, and norovirus also were reported, but used less frequently. In some cases, multiple microorganisms were used in the same study. In all, 67% used *Listeria*, 58% used *E. coli* O157:H7, 35% used *Salmonella*, and 4% of the studies used other bacteria.

The directions of transfer via to/from contact surface found in the different methodologies were mainly from surface materials to food products and from food products to surface materials. Surface materials included: stainless steel (SS) (62% of the studies), high density polyethylene (HDPE) (19%), acrylic (AC) (9.8%), polypropylene (PP) (7.6%), and glass (1.6%).

Table 4.2 Summary of the bacterial transfer data collected and stored in the database.

Author	Data sets (No.)	Food product	Category	Microorganism	Contact material	Process
Aarnisalo_1	6	Salmon fish	Fish	<i>Listeria</i>	SS	Slicing
Benoit_1	36	Turkey	Turkey	<i>Listeria</i>	SS, HDPE	Contacts

Table 4.2 Summary of the bacterial transfer data collected and stored in the database (cont'd).

Author	Data sets (No.)	Food product	Category	Microorganism	Contact material	Process
Buchholz_1	23	Lettuce	Produce	<i>E.coli</i>	SS	Complex
Chaitiemwong_1	8	Ham	Pork	<i>Listeria</i>	SS	Slicing
Danny_1	48	Meat	Meat	<i>E.coli</i>	SS, HDPE	Contacts
Flores_1	15	Meat	Meat	<i>E.coli</i>	SS	Complex
Keskinen_1	32	Turkey Salami	Turkey Sausage	<i>Listeria</i>	SS	Slicing
Kim_1	5	Glass	Others	<i>Salmonella, E.coli, Listeria</i>	Glass	Contacts
Kusumaningrum_1	4	SS	Others	<i>Staph.aureus, S.enteridis, B.cereus, C.jejuni</i>	SS	Contacts
Midelet_1	3	SS	Others	<i>K.varians P.fluorescens S.sciuri</i>	SS	Contacts
Moller_1	3	Pork	Pork	<i>Salmonella</i>	SS	Complex
Patil_1	6	Honeydew melon	Produce	<i>Listeria</i>	SS	Slicing
Patil_2	6	Cantaloupe melon	Produce	<i>Listeria</i>	SS	Slicing
Perez-Rodriguez_1	5	Cooked meat	Meat	<i>E.coli S.aureus</i>	SS	Slicing
Ren_1	12	Lettuce	Produce	<i>E.coli</i>	SS	Complex
Scollon_1	9	Onion	Produce	<i>Listeria</i>	SS	Slicing
Sheen_1	4	Salami	Sausage	<i>Listeria</i>	SS	Slicing
Sheen_2	7	Ready-to-eat-meat	Meat	<i>E.coli</i>	SS	Slicing
Sheen_3	1	Agar	Others	<i>Listeria</i>	SS	Slicing
Vorst_1	12	Turkey Bologna Salami	Turkey Sausage Sausage	<i>Listeria</i>	SS	Slicing
Vorst_2	12	Turkey Bologna Salami	Turkey Sausage Sausage	<i>Listeria</i>	SS	Slicing
Wang_1	33	Tomato	Produce	<i>Salmonella</i>	SS	Slicing
Yan_1	17	Ham	Pork	<i>Listeria</i>	SS	Slicing
Yan_2	63	Ham	Pork	<i>Listeria</i>	AC, HDPE, PP	Contacts

A total of 53 data sets were from complex systems. A few studies similar to those reported by Buchholz et al (2012a, 2012b) and Ren (2014), who tested leafy greens, were found for other products, such as ground beef. One study was performed on grinding of beef by Flores & Tamplin (2002), and another on pork (Moller et al., 2012).

4.3.2 Model fitting

After fitting the three models to every individual data set, the analysis suggested (by AIC_c) that the log-linear model was the best for approximately 35% of the data sets, the Weibull model for ~60% of the data, and the Linear-Weibull for ~5% of the data sets (Table 4.3). Similar results were obtained for transfer via multiple contacts. The linear model was best for the categories of fish and sausage, which present different characteristics in food composition among each other, but the studies that used these products as a model reported accumulation of fat on the slicer blade (Aarnisalo et al., 2007).

Overall, the Weibull model gave the best fit on the majority of the data sets analyzed (Table 4.3). For salami, the Weibull model best fit 46% of the data evaluated, the linear model best fit 27% of the data, and the same result (27%) was obtained applying the linear-Weibull model. The linear-Weibull model has the disadvantage of being a more complex model. For food products like lettuce, onion, turkey, ham, salami, and meat, the Linear-Weibull model was the most appropriate model for only ~20% of the data sets. An example of a model fit and data stored in the database is shown in Figure 4.1. Data collected for the meta-analysis follow similar decreasing relationship between bacterial transfer and contact or slice number (Figure 4.1). Data were obtained from a previous study conducted at MSU (Yan, data not published), and best fit using the Weibull model. These results and the various food products included in the meta-

analysis revealed that food composition plays a critical role in bacterial transfer via surface contact. The fresh produce data were very consistent in contrast with data collected from studies performed on sausage (Table 4.3). Lettuce was the exception among fresh produce (Table 4.3), because the results were closer to meats. Characteristic of the food items impacted the model fits. Food composition and differences in the experimental design likely had the largest impact on these results.

Table 4.3 Percentage of data according to food product type that best fit each of the models evaluated for transfer during slicing type transfer data.

Food product type	Weibull (%)	Log-Linear (%)	Linear-Weibull (%)
Fresh produce			
Tomato	77	3	19
Onion	75	0	25
Lettuce	44	35	21
Meat			
Ham	57	2	40
Turkey	51	21	28
Salmon fish	33	67	0
Meat, cooked meat, and ready-to-eat meat	64	12	24
Salami	46	27	27
Bologna	33	50	17
Others			
Agar, SS, and glass	46	46	8

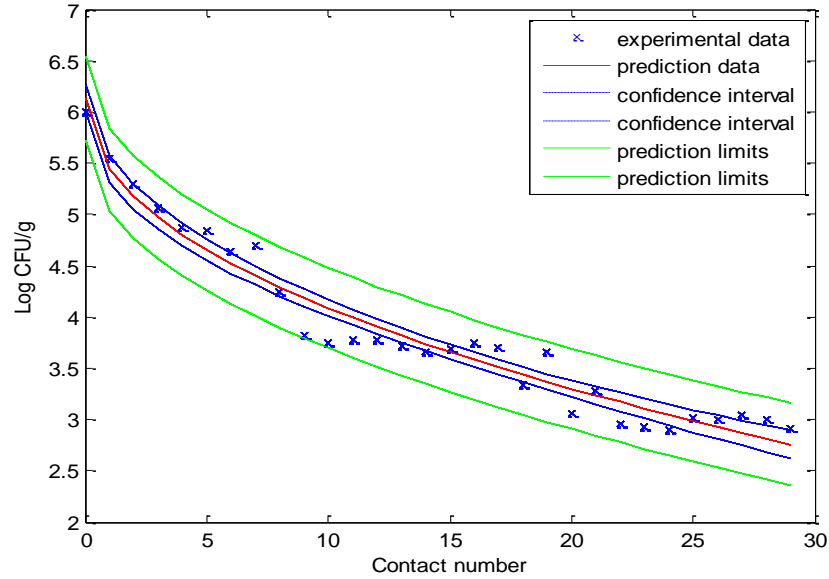


Figure 4.1 Bacterial transfer data via static contact (multiple contact) from ham to clean contact areas were fit with the Weibull model. Contact number refers to the repeated events of bacterial transfer (Yan, data not published). Experimental data, prediction data, confidence intervals, and prediction limits were estimated.

Ideally, bacterial transfer results should be reported per contact area of every slice for slicing studies, but this was not done in all the studies used. A strong recommendation is that produce contact area can be reported as an approximation of the geometric shape that is close to the product dimensions. Overall, if transfer data are analyzed and models are fit according to the process applied here, in most cases the Weibull model best fit most of the transfer data.

4.3.3 Meta-analysis results for bacterial transfer via surfaces for multiple food products

4.3.3.1 Effect of fat, protein, and moisture content on bacterial transfer via dynamic contact

Previous studies have recommended further research on the effect of strain variability, product composition, and large-scale slicers (Aarnisalo et al. 2007; Keskinen et al. 2008a; Sheen, 2008; Sheen et al. 2010; Sheen & Hwang, 2010; Vorst et al. 2006a; Vorst et al, 2006b; Wang, 2015). They mentioned solidification of fat, accumulation of fat on the slicer, and product composition (fat, protein, moisture, temperature, and initial inoculation levels) as factors that affect or prolong bacterial transfer (Aarnisalo et al. 2007).

Factors included in the meta-analysis were fat, protein, and moisture content of the food products. The regression analysis included 13 food products on bacterial transfer via slicing machines. A total of 25 studies were included from the database, including 15 on bacteria transfer via dynamic contact (slicing), 6 studies on bacterial transfer via static contact (multiple contacts), and 4 on bacterial transfer in complex systems. A summary of the regression analyses, across the full reported data and multiple product categories is in Table 4.4.

Table 4.4 Regression analysis results for the effect of moisture, fat, and protein content on the Weibull model parameters (intercept, rate, and shape) for bacterial transfer data to foods via dynamic contact (slicer machine).

Parameter	Physical variable	Slope	intercept	Coefficient of correlation	p-value	p < 0.05
Intercept	Moisture content (%)	0.023	2.645	0.178	0.1423	not significant
	Proteins (%)	-0.053	5.083	-0.262	0.0294	significant
	Fat (%)	-0.041	4.792	-0.206	0.0892	not significant
Rate	Moisture content (%)	0.014	-0.292	0.309	0.010	significant
	Proteins (%)	-0.021	1.049	-0.293	0.014	significant
	Fat (%)	-0.022	0.996	-0.303	0.011	significant
Shape	Moisture content (%)	-0.004	0.759	-0.278	0.021	significant
	Proteins (%)	0.004	0.447	0.179	0.140	not significant
	Fat (%)	0.007	0.416	0.343	0.004	significant

From the aggregate meta-analysis, a decreasing dependency was found for the intercept parameter, and an increasing dependency was found for the shape parameter as protein increased. In other words, the Weibull shape constant increased and intercept decreased with increasing protein content. The database included results from studies using both fresh produce and meats. A difference was expected due to the difference in protein content among product types, and the results of the regression analysis are in Figures 4.2 and 4.3.

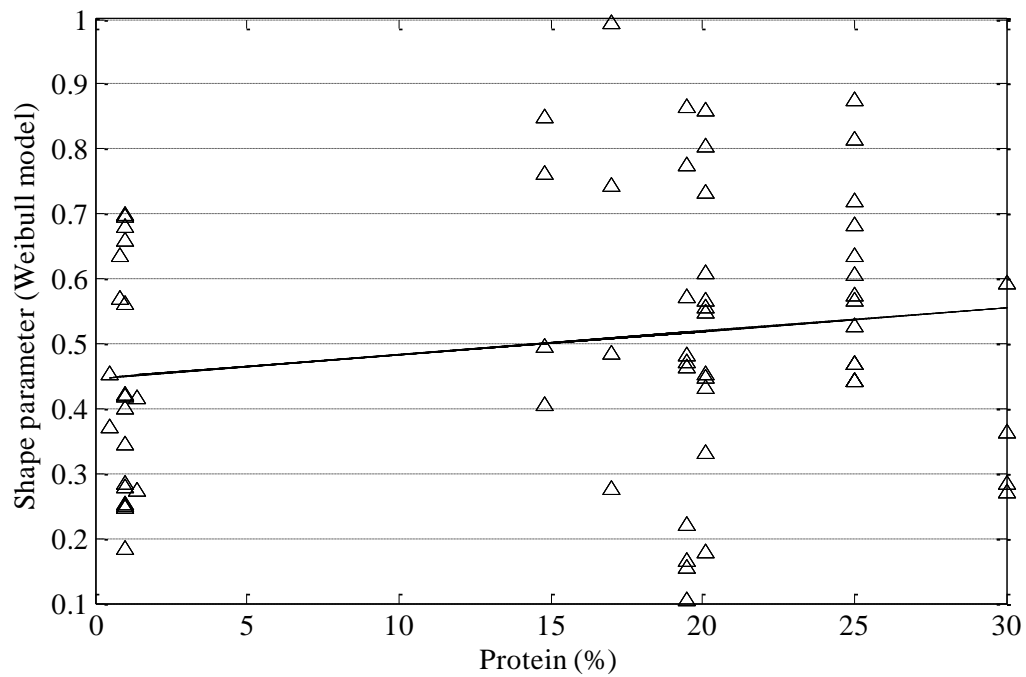


Figure 4.2 Weibull shape parameter versus protein content (%) for bacterial transfer from a slicing machine to foods (n = 70, and 13 food products).

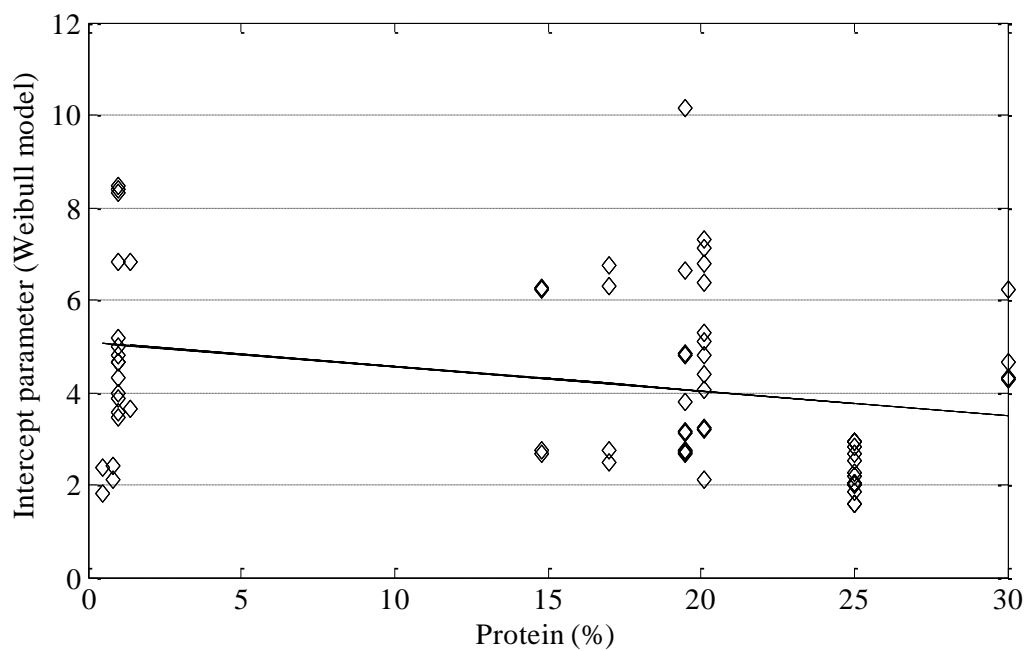


Figure 4.3 Weibull intercept estimated versus protein content (%) for bacterial transfer from a slicing machine to foods (n = 70, and 13 food products).

According to Aarnisalo et al (2007), the reduction in the number of *L. monocytogenes* transferred to smoked salmon, over multiple slicing contacts, was lower than that reported for turkey breast, bologna, and salami by Vorst et al (2006). Aarnisalo et al (2007) observed accumulation of a layer of soft salmon material that consisted mainly of protein, fat, and moisture. They showed that product components other than fat influenced bacterial transfer. Regression analysis of the current study showed significant differences between meat and fresh produce ($p < 0.05$). Results reported by Erickson et al (2015) found that the presence of food residues and bacteria type increased contamination of graters and knives. These findings are consistent with the current data collected.

Regression analysis of the data from bacterial transfer via mechanical slicer suggested an increasing dependency ($p < 0.05$) of the Weibull rate parameter (Figure 4.4) as moisture content increased. The contrary occurred in the Weibull shape parameter (Figure 4.5). For bacterial transfer via dynamic contact (slicing), a moisture and fat dependency ($p < 0.05$) was found for the Weibull rate parameter (k) and shape factor (p). The increasing or decreasing behavior of the parameters as a function of physical variables determines the behavior of the curves describing bacterial transfer. The rate parameter indicated if bacterial transfer increased or decreased as a function of slice number or contact number. The intercept gave an estimate of the initial number of bacteria from the donor surface.

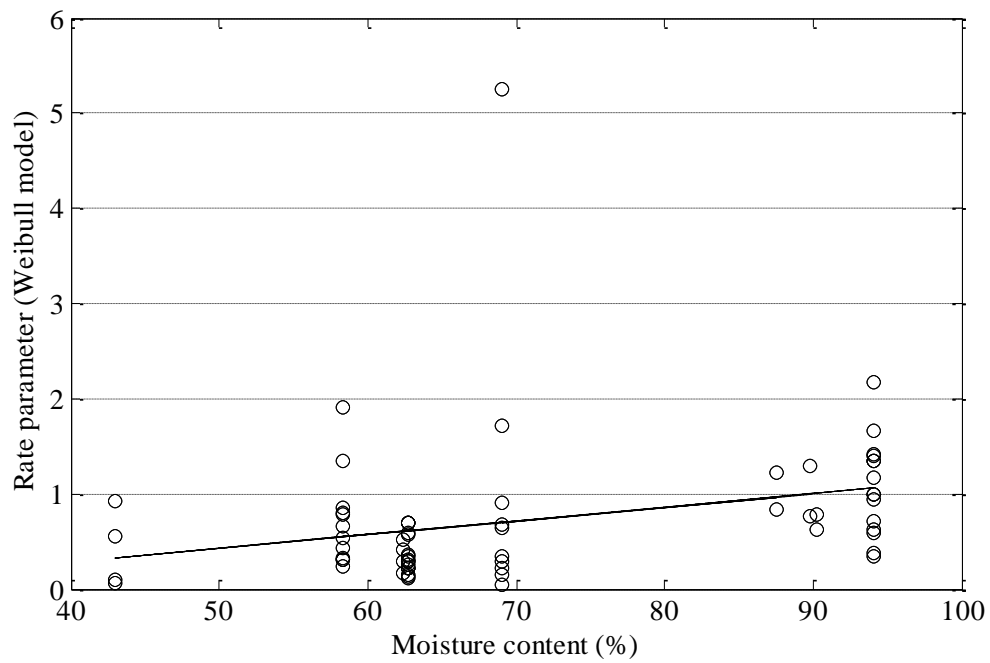


Figure 4.4 Weibull rate parameter estimation versus moisture content (%) on bacterial transfer data via dynamic contact (mechanical slicer) to foods (n = 70).

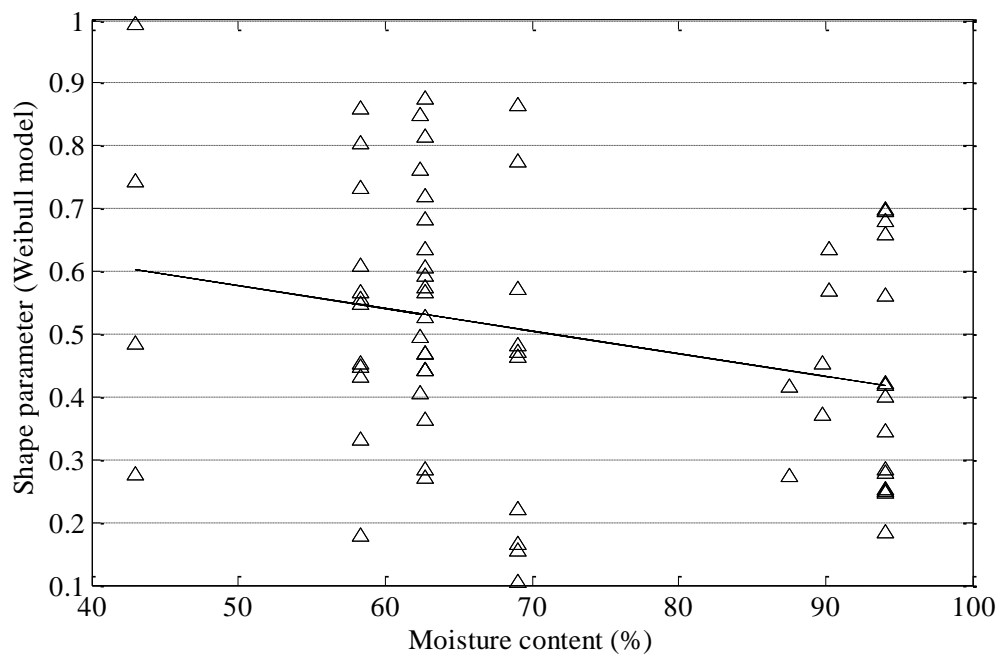


Figure 4.5 Weibull shape parameter estimation versus moisture content (%) on bacterial transfer data via dynamic contact (mechanical slicer) to foods (n = 70).

These meta-analysis results agreed with experimental data in the present study (Chapter 3), which was one purpose of the current study; there was an effect on bacteria transferred as a function of moisture content (%). Experimental results from the current study suggested that potatoes with the highest surface moisture content (%) had more bacteria transferred. These results corresponded to previous studies that suggested further research on the effect of moisture content (%) on bacterial transfer is needed (Kusumaningrum et al, 2003; Schaffner & Schaffner, 2007). Figures 4.4 and 4.5 showed an increasing trend for the rate parameter and a decreasing trend for the shape parameter with an increase in moisture content.

Aarnisalo et al (2007) affirmed that solidification of fat at lower temperatures might affect the transfer of *L. monocytogenes* at colder temperatures. Regression analysis of the Weibull rate parameter showed a decreasing dependency with fat (Figure 4.6). For the Weibull shape parameter, an increasing dependency with fat was found (Figure 4.7).

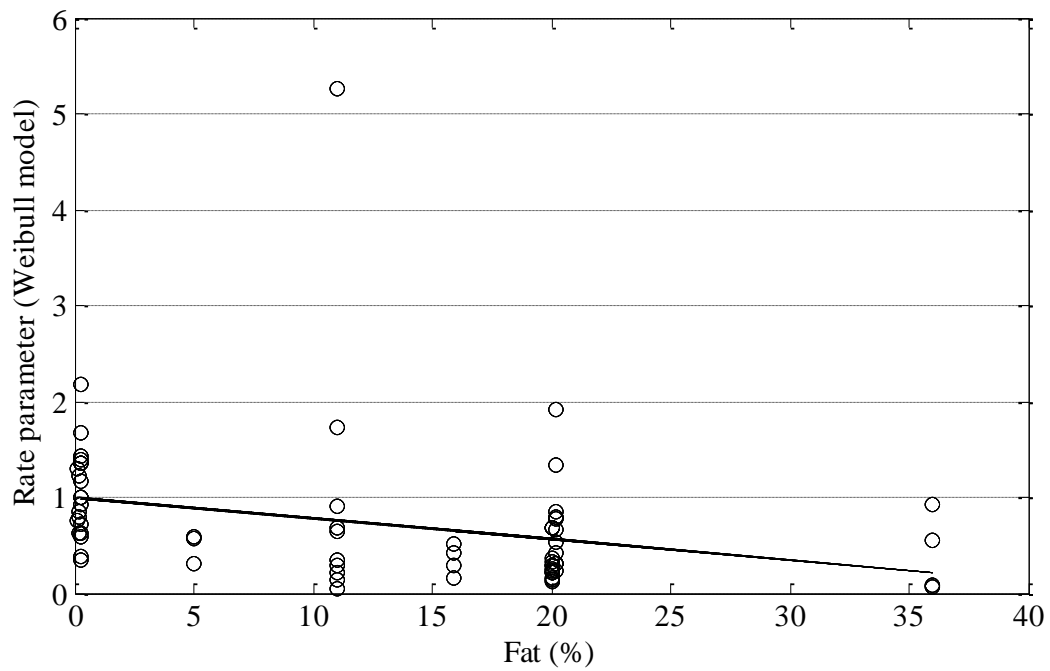


Figure 4.6 Regression analysis ($p = 0.009$) of bacterial transfer data via slicing between the Weibull rate parameter and fat (%), $n = 70$.

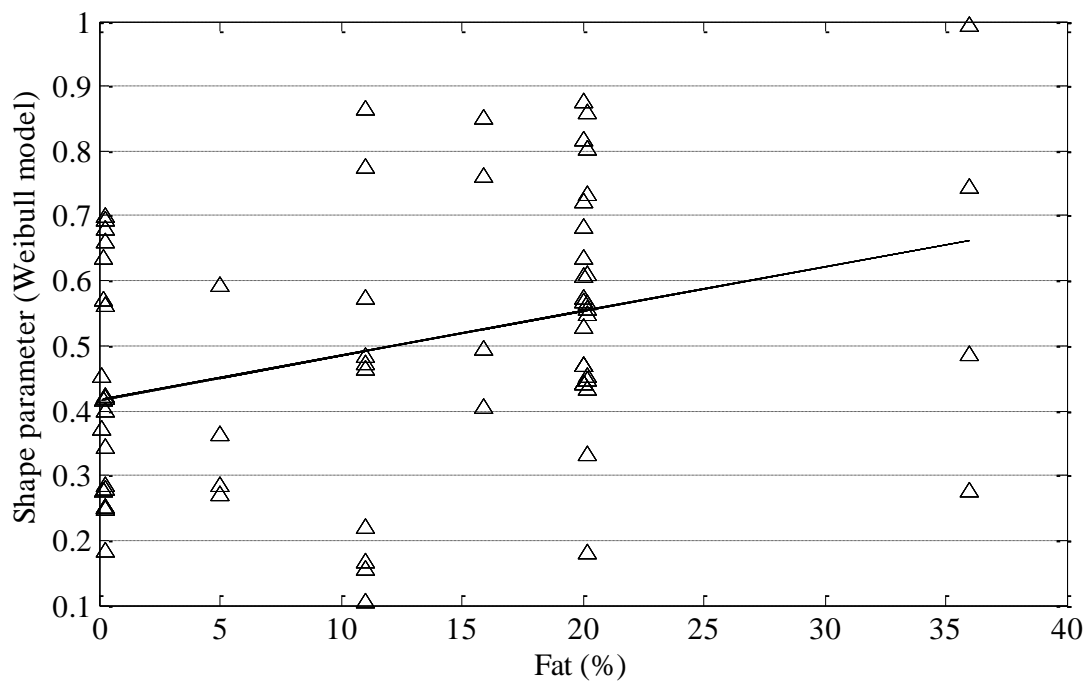


Figure 4.7 Regression analysis ($p = 0.004$) of bacterial transfer data via slicing between the Weibull shape parameter and fat (%), $n = 70$.

In addition to the meta-analysis regression, the Weibull model parameters were compared across the broad categories of produce and meat products, via *t*-tests for the two properties of model parameter values. Overall, there are significant differences in the rate parameter between the groups ($p < 0.05$) with greater values for fresh produce. Chen, Moschakis, & Nelson (2004) claimed differences between products because different proteins and polysaccharides affect the surface roughness of foods. A pure protein gel has a relatively rougher surface than protein aggregates in the presence of small amounts of polysaccharides. The presence of protein aggregates in protein gel makes the gel's surface much smoother. They also explained that the surface changes from a porous microstructure for a pure protein gel to a more sealed microstructure for a xanthan-containing gel. Although these relationships are very complex in foods, the regression categorical analyses presented suggest that general trends can be discerned across broadly aggregated transfer data sets.

4.3.3.2 Effect of fat, protein, and moisture content of foods on bacterial transfer via multiple contacts

For the static contact transfer data, regression analysis also revealed significant differences in the parameters estimated by the Weibull model, dependent on fat and moisture content (%). As moisture content increased, the intercept and shape parameter decreased, and the rate parameter increased. Changes in fat content caused an increase on the intercept. In the case of moisture content, the results (Figure 4.8) are consistent with previous analysis performed on data collected from studies developed on bacterial transfer via slicing. They are also consistent with the experimental results of the current study (Chapter 3). A total of 52 data sets were included in the regression analysis.

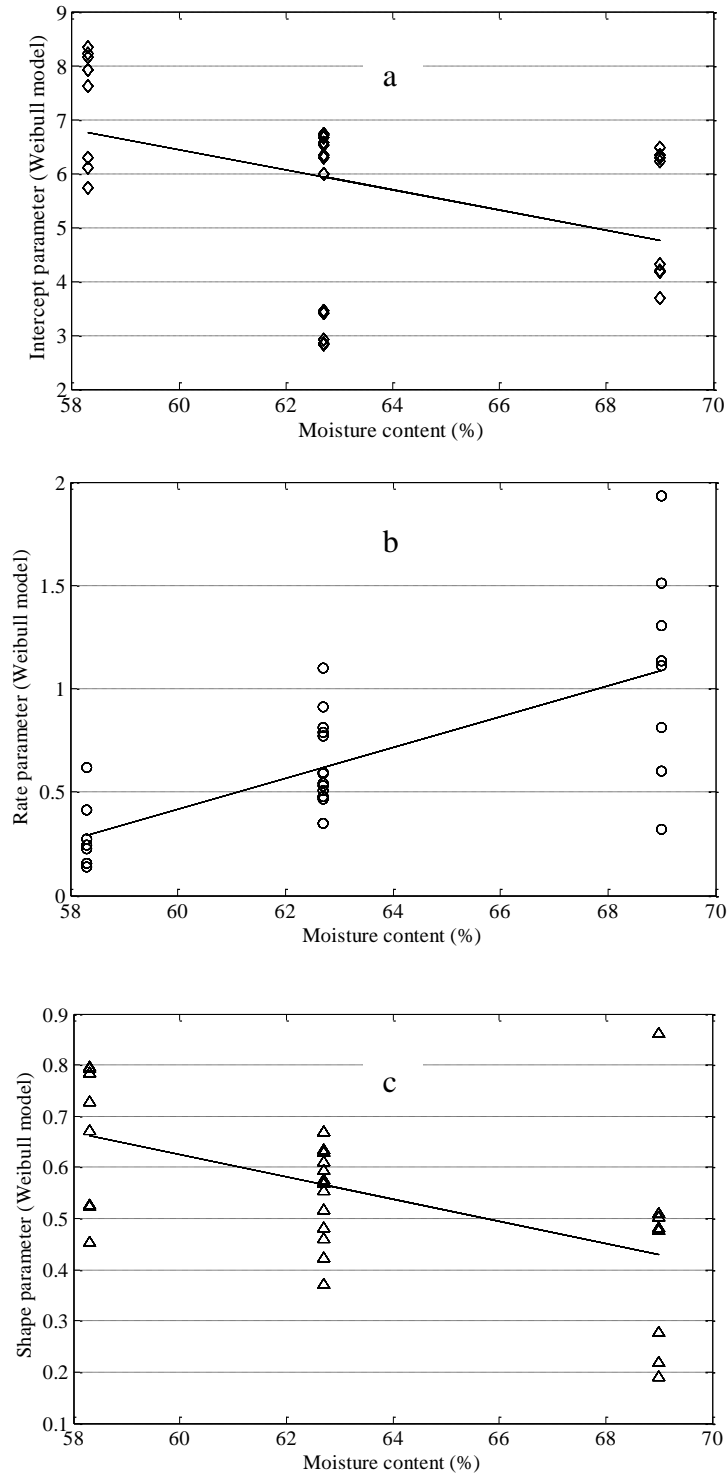


Figure 4.8 Regression analysis of bacterial transfer data via static contact (multiple contact); data correspond to the Weibull intercept (a), rate parameter (b), and shape parameter (c) versus moisture content (%) for 52 data sets, 6 studies, and 5 products.

For fat, only the Weibull intercept parameter increased ($p < 0.05$) as fat content (%) (Figure 4.9) increased for multiple static contacts. None of the Weibull parameters were significantly related to protein content. Less data were available for bacterial transfer via static contact, with fewer data points presented in the figures showing bacterial transfer versus Weibull parameters.

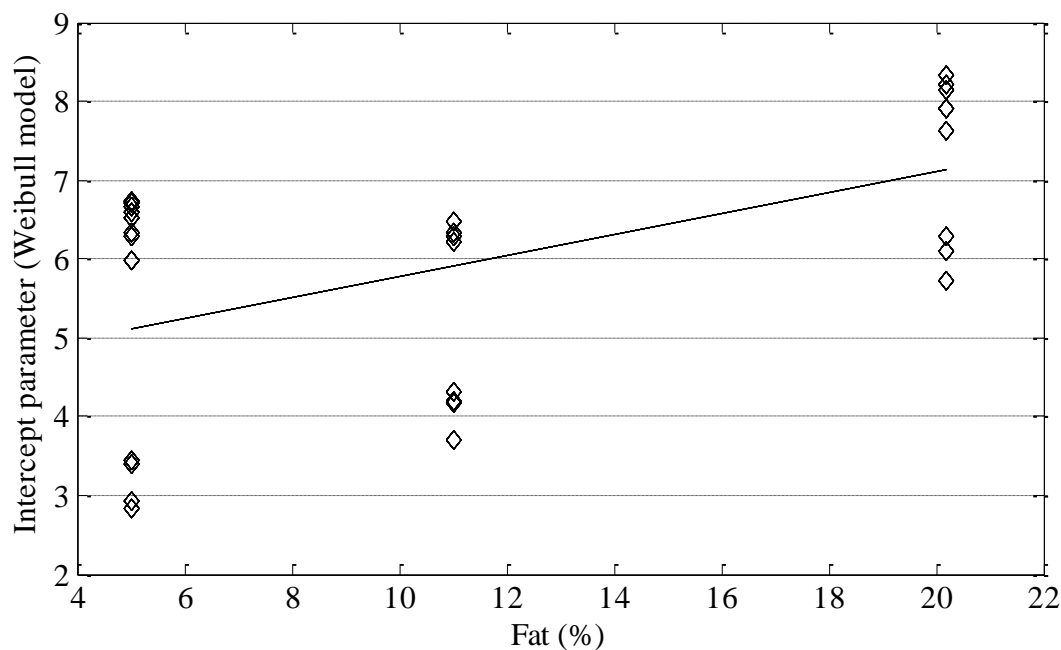


Figure 4.9 Regression analysis between the Weibull intercept parameter and fat (%) for bacterial transfer data via multiple contacts.

4.3.3.3 Effect of pH on bacterial transfer via slicing

The analysis of bacterial transfer dependency on pH included a total of 12 studies and 66 data sets. The intercept and rate parameter decreased with increasing pH (Table 4.5 and Figure 4.10). From experimental results in the present study (Chapter 3), it was observed that bacteria preferentially attached to food products. The pH might allow the microorganism to remain

attached to the product tissue than to the contact surface material. The variable of pH was available for most food products included in this meta-analysis, but no study was found that analyzed bacterial transfer as a function of pH.

Table 4.5 Regression analysis for pH on bacterial transfer data via slicing machine to foods.

Variable	Parameter	Slope	intercept	Correlation coefficient	p-value	p < 0.05
pH	Intercept	-1.041	9.979	-0.414	0.0006	significant
	Rate	-0.300	2.394	-0.327	0.008	significant
	shape	0.074	0.088	0.303	0.014	significant

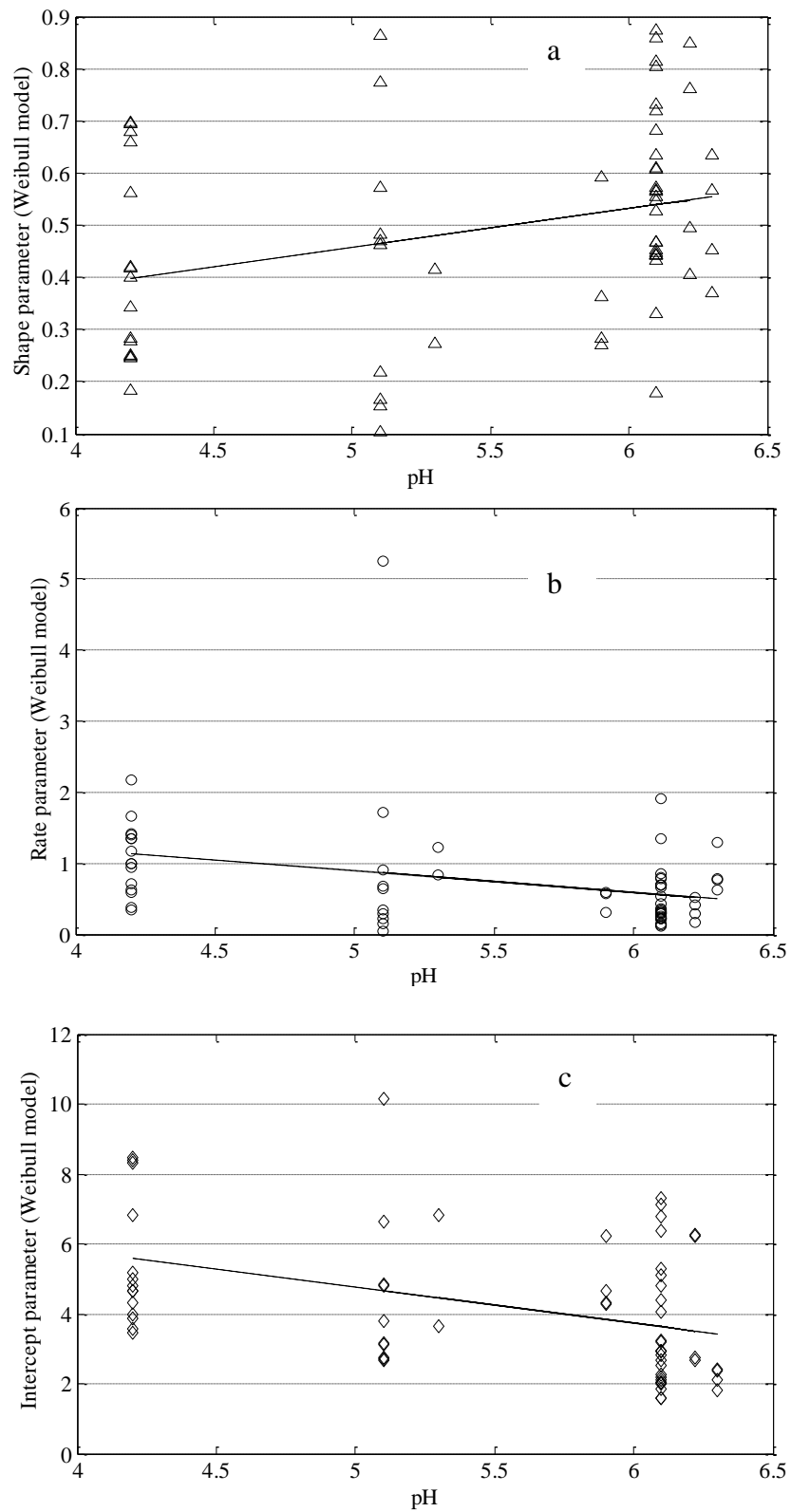


Figure 4.10 Regression analysis of bacterial transfer data via slicing machine; data correspond to the Weibull shape (a), rate (b), and intercept (c) parameters versus pH.

4.3.3.4 Effect of pH on bacterial transfer via multiple contacts

Figure 4.11 shows the relationship between the Weibull shape and rate parameter vs. pH from studies on bacterial transfer via multiple contacts (Table 4.6).

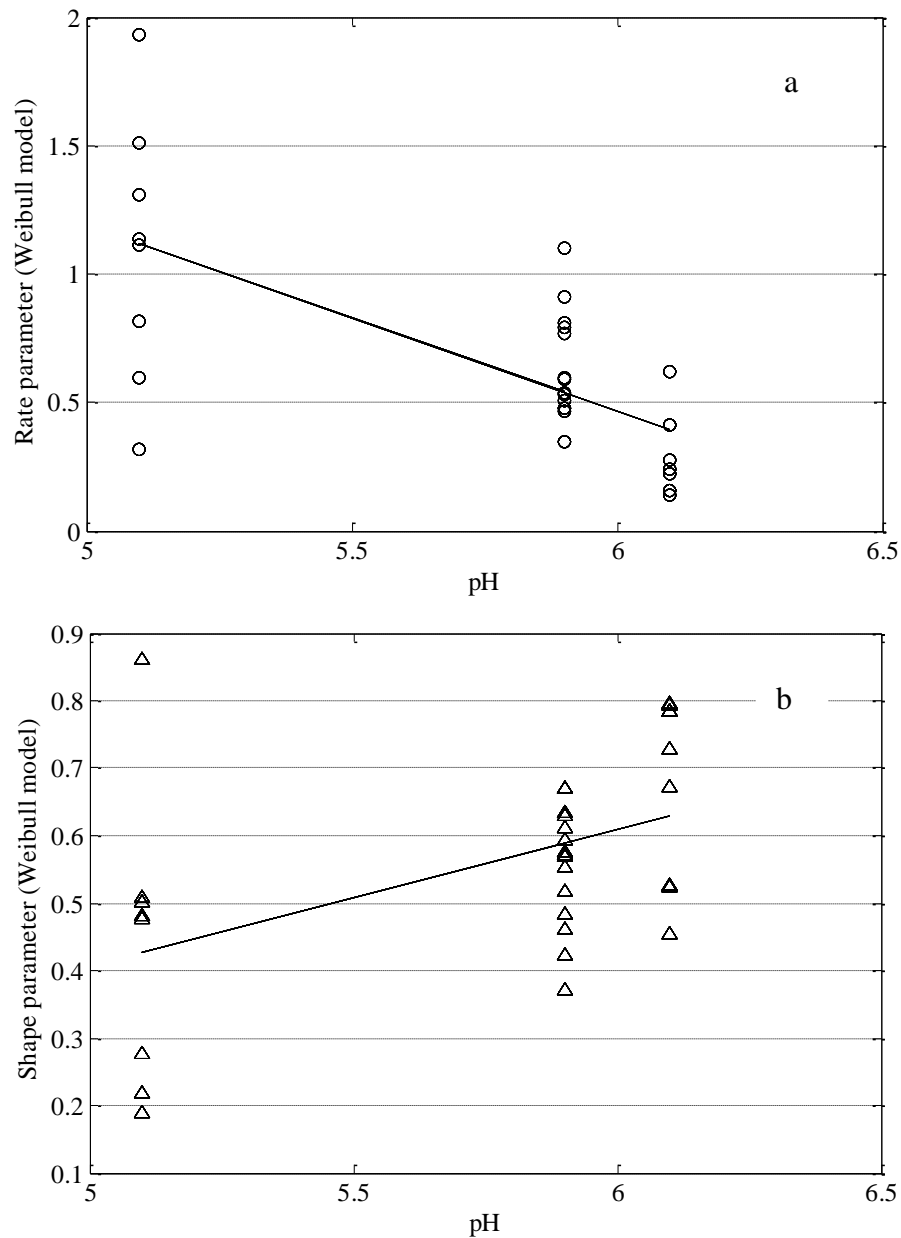


Figure 4.11 Regression analysis of bacterial transfer data via multiple contacts; data correspond to the Weibull rate (a), and shape (b) parameters versus pH.

Table 4.6 Regression analysis results for pH on bacterial transfer via multiple contacts to foods.

Variable	Parameter	Slope	intercept	Correlation coefficient	p-value	p < 0.05
pH	Intercept	1.427	-2.345	0.339	0.05	significant
	Rate	-0.724	4.812	-0.682	<0.0001	significant
	shape	0.202	-0.605	0.490	0.004	significant

This meta-analysis was based on the dependency of bacterial transfer, to meat and produce, on food components such as pH, fat, protein, and moisture content. Due to limited reporting of physical variables in bacterial transfer studies, this analysis focused on the interaction between food components and bacterial transfer.

Variable pH revealed similar behavior on the shape and rate parameters on bacterial transfer via dynamic and via static contact. On both bacterial transfer processes, shape parameter increased as pH increased, and rate parameter decreased as pH increased. The opposite behavior was observed between both processes on parameter intercept. For bacterial transfer via dynamic contact, the intercept parameter decreased as pH increased, and on bacterial transfer via static contact, the intercept parameter increased as pH increased. As a conclusion, bacterial transfer behavior was similar in both transfer types. As it was originally proposed, pH might be acting as a “surrogate” or highly correlated variable for some other product characteristic. For example, fat presented a similar behavior as pH on bacterial transfer data via dynamic contact.

4.3.3.5 Effect of the type of microorganism

The database in this study including data sets for three microorganisms: *E. coli* O157:H7, *Listeria*, and *Salmonella*. Because a majority of the studies collected used *E. coli* O157:H7, *Listeria*, and *Salmonella* as a microorganism, the data sets were categorized in three groups, and the *t*-test revealed that bacterial transfer differed among microorganisms (over all studies).

Higher bacterial transfer was found for *Listeria* and *E. coli* O157:H7 than *Salmonella* (Table 4.7) ($p < 0.05$). These results agree with Perez-Rodriguez et al (2007); they affirmed that transfer coefficients at high (10^8 CFU/cm²) and moderate (10^6 CFU/cm²) initial inoculation levels showed significant differences between *E. coli* O157:H7 and *Staphylococcus aureus*. Although the number of bacteria transferred to and from a surface depend on the microorganism, other environmental and physiological factors might also impact transfer. Among physiological factors, Sheen (2008) mentioned age, strain, inoculum size, the capability to adapt different stresses, and adhesion characteristics.

Table 4.7 Statistical analysis of three microorganisms (*E. coli* O157:H7, *Salmonella*, and *Listeria*) transfer via dynamic contact to foods.

Bacteria		Estimate	Standard Error	DF	t Value	Pr > t
<i>E.coli</i> O157:H7	<i>Listeria</i>	4.4522	0.5695	12.6	7.82	<0.0001
<i>E.coli</i> O157:H7	<i>Salmonella</i>	4.1765	0.5940	14.6	7.03	<0.0001
<i>Listeria</i>	<i>Salmonella</i>	-0.2757	0.2072	13	-1.33	0.2062

4.3.3.6 Effect of initial inoculation level

The categories used to classify the initial inoculation level on the food (donor) for meta-analysis were: low (10^3 CFU/cm²), moderate (10^6 CFU/cm²), and high (10^8 CFU/cm²). In the publications found high initial inoculation level corresponds to a bacterial population of 10^8 CFU/cm², moderate initial inoculation level corresponds to 10^6 CFU/cm² bacterial population, and low initial inoculation level corresponds to 10^3 CFU/cm² bacterial population. This classification was used to categorize the data sets collected for this meta-analysis (Table 4.8).

Higher bacterial transfer was found at the highest initial inoculation level, and no differences were obtained between high and moderate initial inoculation levels.

Table 4.8 *E.coli* O157:H7, *Salmonella*, and *Listeria* transfer data via dynamic contact to foods.

Level		Estimate	Standard Error	DF	t Value	Pr > t
High	Moderate	0.07517	0.1714	56.3	0.44	0.6626
High	Low	0.4442	0.1499	41.4	2.96	0.0050
Moderate	Low	0.3691	0.09734	27.6	3.79	0.0007

In the aggregated meta-analysis, number of bacteria transferred at high initial inoculation level on the donor were significantly different from the low inoculation level. This result agrees with previous studies, including Fravallo, Laisney, Gillard, Salvat, & Chemaly (2009), who described how percent transfer rates vary significantly depending on the initial natural contamination levels on poultry legs. Thus, the initial inoculation level can affect bacterial transfer via surface contact.

Garrood et al (2004), found that the probabilities of detachment for inoculum concentrations from 8.0×10^4 to 7.6×10^7 Log CFU/mL were not significantly different from one another. They concluded that the probability of detachment was not affected significantly by changes in inoculum concentration. That study is related to the current study, because in some processes detached bacteria can transfer to other surfaces or foods.

4.3.3.7 Effect of surface roughness

Regression analysis performed on the variable roughness considered only five studies, given that food roughness data was limited. Few studies have measured and reported this

physical variable. The variable reported is Ra, which corresponds to the arithmetic mean value of surface roughness. Studies were found for foods such as salami (8.04 μm), ham (5.17 μm), tomato (2.88 μm), onion (0.3 μm), lettuce (20 μm), and cantaloupe from this relatively limited meta-analysis (Figure 4.12). Food product roughness affected bacterial transfer. There was a decreasing dependency on the Weibull intercept versus roughness (μm) (Table 4.9). In contrast, there was no significant effect for the shape and rate parameters versus roughness (μm).

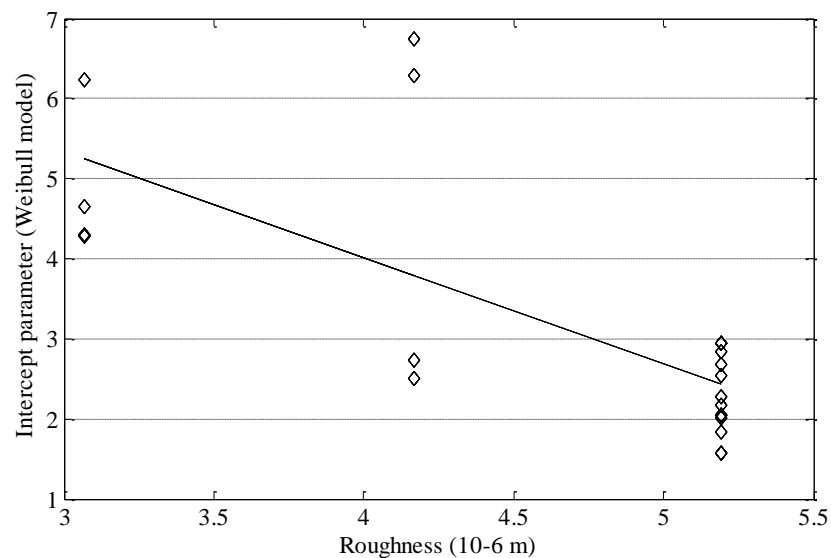


Figure 4.12 Regression analysis for bacterial transfer data via slicing contact, data corresponds to the parameter of intercept versus roughness (μm), 22 data sets were included.

Table 4.9 Regression analysis results for the impact of product roughness on bacterial transfer via slicing machines to foods.

Variable	Parameter	Slope	intercept	Correlation coefficient	p-value	p < 0.05
Roughness	Intercept	-1.325	9.311	-0.721	0.0002	significant
	Rate	-0.083	0.762	-0.291	0.189	not significant
	Shape	0.084	0.170	0.368	0.092	not significant

In terms of bacterial transfer, it is expected that bacteria get trapped on the surface topography of food contact materials and/or food products during slicing processes. As a consequence, if the bacteria trapped, they are not “available” on the surface for transfer to other surfaces. The regression analysis suggested that there is a significant dependency among bacterial transfer and roughness.

Surface roughness is different among food contact materials. Surface materials included in a *t*-test were stainless steel, polypropylene, high-density polyethylene, acrylic, and glass. Contrary to expectations, no differences were found among surface material ($p < 0.05$), in terms of bacterial transfer. Different studies report differences among the various materials evaluated. Transfer to such materials also depends on the components of the food surface structure (Chen, Moschakis & Nelson, 2004).

However, very few studies performed on fresh produce have reported food product roughness (Fernandes, 2014; Hershko, 1998). Most of the studies have reported the roughness of the surface material acting as a donor, such as stainless steel previously inoculated. The lack of information of this variable for foods makes it difficult to draw general conclusions. Also, according to Chen (2007), surface texture is frequently used to describe physical characteristics of surface materials, but no precise definition has yet been available in the literature. As roughness is not a quality criteria for evaluating foods, few bacterial transfer studies have included or reported this physical variable.

4.3.3.8 Effect of direction of transfer from the food product to the food contact material

Most of the results of this meta-analysis were based on studies where the bacterial donor was a non-food surface previously contaminated via an inoculated food product. In the case of

the current analysis, all studies (13 total) present the same direction of bacterial transfer, which is bacteria transferred from a previously inoculated slicing machine to food items. The direction of transfer goes from a donor surface to a receiver food product. From the studies on bacterial transfer via slicing, it is not possible to draw general conclusions. However, a few studies designed for multiple contacts reported data for a transfer direction of the microorganism from the food product to the surface. Therefore, the present database included only six studies with this type of data, making a general meta-analysis not feasible for this scenario (until more data of this type are accumulated in the literature).

Most of the studies in the meta-analysis were conducted with one single product and one microorganism, which made difficult to draw general conclusions or comparisons across studies. From this meta-analysis, it was possible to obtain generalizable trends, but they were focused mainly in food composition. The two current studies (Chapter 3 and Chapter 4) support recommendations for a new approach to conduct bacterial transfer studies. Many data have been obtained focused on the impact of food composition, and it was demonstrated that food components affects the Weibull model parameters. However, prior bacterial transfer studies generally have not reported the experimental treatments in terms of fundamental physical variables, such as speed, normal pressure, and contact time. Research focused on the effects of fundamental physical variables on bacterial transfer would enhance future opportunities to develop generalizable knowledge and models (Chapter 5).

COMPARISON OF EXPERIMENTAL RESULTS WITH DIMENSIONAL ANALYSIS

5.1. Overview

This chapter focuses on bacterial transfer model development, using a dimensional analysis approach. It is a synthesis of the data collection and analyses performed in previous chapters, but data from experimental work are insufficient (to date) to prove the validity of the model. The data collected in the third and fourth chapters were used to apply and conceptually test the equations developed in the present chapter. This section is linked to the third objective of the current dissertation: to propose a mathematical model for relationships between *Salmonella* transfer and fundamental physical variables, based on a dimensional analysis approach.

5.2. Methods

5.2.1. Determination of the Pi terms

This study is the first attempt to develop a bacterial transfer model based on fundamental macroscopic variables (even if not yet mechanistic). As it is known, a large volume of experimental data is necessary to develop a bacterial transfer model. Any system with different biological and physical components is complex. This complex system will be represented by a model based on physical variables.

The analysis started with the identification of the fundamental units for all variables; in this case, the fundamental variables are mass, length, and time. Additionally, CFU was also added as a fundamental unit. Subsequently, all variables involved in bacterial transfer via dynamic and static contact were listed (Table 5.1). The Buckingham Pi theorem (Kunes, 2012) provided guidance on how to group the different variables to obtain dimensionless terms.

Briefly, the Buckingham Pi theorem accounted for the fundamental units of each variable and the total number of variables in each process to reduce the model to a smaller number of dimensionless (Pi) terms. Finally, solving the system of equations determines the power of each variable for each term.

Initially, 14 candidate variables (product and process) were identified as potentially affecting bacterial transfer in equipment contact events (e.g., slicing, shredding, and conveying) (Table 5.1). Based on expert knowledge, variables unlikely to significantly affect transfer were excluded (-).

Table 5.1 Physical variables of three pilot-scale processes selected by expert criteria.

Process	Variables
Shredder and slicer	Temperature (-)
	Friction force
	Thickness (slicer) (-)
	Initial inoculation level
	Microorganism (-)
Conveyer	Contact time
	Contact pressure
	Temperature (-)
	Initial inoculation level
	Water content (-)
	Microorganism (-)
	Whole or cut product (-)
	Product roughness
	Surface roughness

Temperature, the thickness of the slice, microorganism, water content, whole product or cut product, and product roughness were considered in the first selection of the physical variables to perform a dimensional analysis (Table 5.1), but were ultimately removed for various reasons. The thickness of the slice was excluded because it represented a portion of the product that had no direct contact with transfer surfaces. Categorical variables, such as microorganism and whole product or cut product, were excluded because they possess no units in which to define a dimension. Water content usually is reported as a percentage or as a fraction, and was therefore excluded because it lacked a dimension to define it. The temperature was initially included as a fundamental variable, but previous studies (Wang, 2015) reported no effect of temperature on bacterial transfer via slicing machine.

It was not practical to include all the possible variables in the Pi terms, because the model would then be too complex to fit and to perform a regression analysis. In addition, it was impractical to evaluate all the physical variables with one experiment. Ultimately the number of variables was reduced to 6 for bacterial transfer via static process, and 7 for bacterial transfer via dynamic process (Table 5.2). As a result, 2 Pi terms were formulated for bacterial transfer via static process, and 3 Pi terms for bacterial transfer via the dynamic process. The total number of Pi terms was determined by the subtraction of the number of fundamental variables to the total number of physical variables. As the number of variables was reduced to obtain 2 and 3 Pi terms, and the model was simpler to fit, the amount of data collection needed to perform the analysis becomes more feasible. In addition, the results from the experimental work and the meta-analysis database might fulfill the key components of the model.

Table 5.2 Physical variables considered in the dimensional analysis for bacterial transfer via dynamic and static contact.

Variable number	Bacterial transfer via static contact			Bacterial transfer via dynamic contact		
	Physical variable	Symbol	units	Physical variable	Symbol	Units
1	Pressure	P	Pa	Pressure	P	Pa
2	Initial inoculation level	N_i	CFU/m ²	Initial inoculation level	N_i	CFU/m ²
3	Bacteria transferred	N_t	CFU/t	Bacteria transferred	N_t	CFU/t
4	Contact time	t	s	Contact time	t	s
5	Characteristic length of the potato	L	m	Viscosity	ν	Pa s
6	Surface tension	σ	N/m	Friction force	F	N
7				Speed	V	m/s

The procedure to obtain each Pi term consisted of solving a system of equations that yielded the power for each variable in each Pi term. Each Pi term was dimensionless. After the determination of the Pi terms, the relationship between the total number of Pi terms was determined by estimating the parameters. This last step leads to the final equation, which describes bacterial transfer as a function of physical variables (equation 5.1). The example (equation 5.1) was presented to show the shape of the general equation. One equation describes each bacterial transfer type (static and dynamic).

$$\Pi_1 = C(\Pi_2)^a(\Pi_3)^b \quad (5.1)$$

5.2.2. Determination of Pi terms for the process of bacterial transfer via static contact

Variables included in the model for static contact (e.g., like product contacting a conveyer) were pressure (P), initial inoculation level (N_i), bacteria transferred (N_t), contact time (t), characteristic length of the potato (L), and surface tension (σ) which represents influence of water on bacteria adhesion and transfer at the product surface (Table 5.3).

Table 5.3 Fundamental physical variables impacting bacterial transfer via static contact.

Variable	Symbol	Fundamental units
Pressure	P	$M \frac{L}{T^2} \frac{1}{L^2} = M \frac{1}{T^2} \frac{1}{L}$
Initial inoculation level	N_i	$\frac{CFU}{L^2}$
Bacteria transferred	N_t	$\frac{CFU}{T}$
Contact time	t	T
Characteristic length	L	M
Surface tension	σ	$M \frac{1}{T^2}$

The Pi terms were derived following two criteria. First, the units were canceled in the numerator and in the denominator in each term (equation 5.2) to obtain dimensionless terms (equation 5.3). Secondly, the variables in the Pi terms interacted following the physics of the process. As a result, the first dimensionless term was obtained (equation 5.4).

$$\Pi_2 = CFU^0 M^0 T^0 L^0 = 1 \quad (5.2)$$

$$\Pi_{c1} = CFU^0 M^0 T^0 L^0 = \frac{\frac{CFU}{T} \times T}{\frac{CFU}{L^2} \times L^2} \quad (5.3)$$

$$\Pi_{c1} = \frac{N_t \times t}{N_i \times L^2} \quad (5.4)$$

The first Pi term was a ratio between transferred bacterial number to the sterile stainless steel plate and the remaining bacterial number on the donor; which in this case is the potato sample. As these two variables were dependent on each other, they were grouped in the same Pi term. The second Pi term was obtained following the procedure previously explained (equations 5.5 and 5.6). The term obtained is dimensionless, which means that all the units cancel. The remaining physical variables (pressure, potato length, and surface tension) were grouped in Π_{c2} .

$$\Pi_{c2} = CFU^0 M^0 T^0 L^0 = \left(\frac{M \times \frac{L}{T^2} \times L}{\frac{L^2}{M \times \frac{1}{T^2}}} \right) \quad (5.5)$$

$$\Pi_{c2} = \frac{P \times L}{\sigma} \quad (5.6)$$

5.2.3. Determination of Pi terms for the process of bacterial transfer via dynamic contact

The variables included in the process of dynamic contact (e.g., slicing) were: normal pressure (P), initial inoculation level (N_i), bacteria transferred (N_t), contact time (t), viscosity (ν), friction force (F), and speed (V) (Table 5.4).

Table 5.4 Fundamental physical variables impacting bacterial transfer via dynamic contact.

Variable	Symbol	Fundamental units
Pressure	P	$M \frac{L}{T^2} \frac{1}{L^2} = M \frac{1}{T^2} \frac{1}{L}$
Initial inoculation level	N_i	$\frac{CFU}{L^2}$
Bacteria transferred	N_t	$\frac{CFU}{T}$
Contact time	t	T
Viscosity	ν	$M \frac{1}{T} \frac{1}{L}$
Friction force	F	$M \frac{L}{T^2}$
Speed	V	$\frac{L}{T}$

The Pi terms were obtained using the same procedure as the one described in section 5.2.1. The first Pi term in the fundamental variables of CFU, time, and length (equation 5.7) corresponded to the ratio between bacteria transferred and bacteria remaining on the donor, which was the potato sample. Consistency in the units of the first Pi terms for both bacterial transfer via static and dynamic was achieved to use the same units in the general equation (5.21 and 5.23).

$$\Pi_{s1} = CFU^0 M^0 T^0 L^0 = \frac{\frac{CFU}{T} \times T}{\frac{CFU}{L^2} \times L^2} = \frac{N_t \times t}{N_i \times L^2} \quad (5.7)$$

The second Pi term was obtained solving a system of equation (equation 5.9), and it was based on the fundamental units of time, length, and mass. As viscosity is a variable interacting in dynamic systems, it was included in the second and the third Pi terms (Π_{s2} and Π_{s3}) as well as

time. In the second Pi term, normal pressure was grouped with viscosity and time, and it was included only the second Pi term to describe bacterial transfer independently of other physical variables. In addition, it is possible to exclude this Pi term in a bacterial transfer scenario that does not include pressure.

$$\Pi_{s2} = CFU^0 M^0 L^0 T^0 = \left(\left(M \frac{1}{T^2} \frac{1}{L} \right)^a \right) \left(\left(M \frac{1}{T} \frac{1}{L} \right)^b \right) (T) = 1 \quad (5.8)$$

$$M: \quad a + b = 0.$$

$$T: \quad (-2a) + (-b) + 1 = 0. \quad (5.9)$$

$$L: \quad -a - b = 0.$$

This system lead to the following solution: $a = 1$, and $b = -1$.

$$\Pi_{s2} = CFU^0 M^0 L^0 T^0 = \left(\frac{P x t}{v} \right) \quad (5.10)$$

The same process was followed to obtain the third Pi term, which was developed grouping the variables of speed, friction force, time, and viscosity (equation 5.11). Speed and friction force were grouped in the same Pi term because they interact in dynamic systems.

$$\Pi_{s3} = CFU^0 M^0 L^0 T^0 = \left(\frac{v x t x (V)^2}{F} \right) \quad (5.11)$$

Together, the three Pi terms of the equation for this process are:

$$\Pi_{s1} = \frac{N_t x t}{N_i x L^2} \quad (5.12)$$

$$\Pi_{s2} = \left(\frac{P x t}{v} \right) \quad (5.13)$$

$$\Pi_{s3} = \left(\frac{v x t x(V)^2}{F} \right) \quad (5.14)$$

5.2.4. Model developed by applying Buckingham Pi theorem for simultaneous processes of bacterial transfer via static contact and bacterial transfer via dynamic contact

The analysis detailed in sections 5.2.2 and 5.2.3 result in two final equations describing bacterial transfer via static contact and dynamic contact, respectively. It was necessary to find a relationship between the Pi terms to write the equations, and to keep the units on the right and left side equivalent.

5.2.4.1 General equation for bacterial transfer via static contact

In the process of bacterial transfer via static contact, there was a dependency only between 2 Pi terms (equation 5.16). Parameters C and a were estimated using MATLAB nonlinear fitting tools (nlinfit). Data used to estimate parameters were on bacterial transfer via 18 static contacts (Section 3.3.1.3). Parameter estimates, confidence intervals, root mean squared error (1.7844) were determined as it detailed in section 4.2 (Table 5.5). From this analysis, it was determined that $C = 0.5$ and $a = 1.1222$ (5.17).

$$\Pi_{c1} \propto C(\Pi_{c2})^a \quad (5.16)$$

$$\Pi_{c1} = 0.5 \left(\frac{P \times L}{\sigma} \right)^{1.1222} \quad (5.17)$$

Table 5.5 Confidence intervals estimated for the parameters of the model for bacterial transfer via static contact.

Parameter letter	Parameter	Confidence interval low	Confidence interval upper
C	0.5000	-0.7661	1.7661
a	1.1222	0.8080	1.4364

5.2.4.2 General equation for bacterial transfer via dynamic contact

Parameters were estimated using MATLAB (nlinfit). Parameter estimation, and confidence intervals were determined as it is detailed in section 4.2 (Table 5.6). The parameters estimates were: C = 1, a = -0.8371, and b = -1.1172, which allowed the general equation for bacterial transfer via slicing contact (equation 5.19). The root mean square error was 0.1232. From the experiments conducted for the current study (Chapter 3), it was inferred that speed affects bacterial transfer according to the transfer direction. For instance, parameters changed according to the transfer direction of plate-to-potato (5.19) or potato-to-plate (equation 5.20). Data used to estimate parameters were on bacterial transfer via dynamic contact (Sections 3.3.2.1 and 3.3.2.2).

$$\Pi_s \propto C (\Pi_{s2})^a x (\Pi_{s3})^b \quad (5.18)$$

$$\Pi_{s11} = 1 x \left(\left(\frac{P \times t}{v} \right) \right)^{-1.1172} \left(\left(\frac{v \times t \times (V)^2}{F} \right) \right)^{-0.8371} \quad (5.19)$$

$$\Pi_{s12} = 1 x \left(\left(\frac{P \times t}{v} \right) \right)^{0.0014} \left(\left(\frac{v \times t \times (V)^2}{F} \right) \right)^{-0.0018} \quad (5.20)$$

Table 5.6 Confidence intervals estimated for the parameters of the model for bacterial transfer via dynamic contact (equation 5.19 and 5.20).

Parameter letter	Parameter	Confidence interval low	Confidence interval upper
Π_{s11} (Equation 5.19)			
C	1	-2.4404	4.4404
a	-1.1172	-2.2242	-0.0102
b	-0.8371	-1.8270	0.1528
Π_{s12} (Equation 5.20)			
C	1	0.9754	1.0246
a	0.0014	-1.9339 x 10 ⁻⁶	0.0029
b	-0.0018	-0.0028	-0.0008

5.2.4.3 General equation for bacterial transfer via surface

In both general equations for bacterial transfer via static contact and via dynamic contact, it was observed that the first Pi terms, which corresponds to the ratio between bacteria transferred and bacteria remaining on the donor, were the same. From these results, it was possible to combine two equations into one general equation (equation 5.23) on bacterial transfer via the combined net effect of static and dynamic contact (equations 5.17, 5.19 and 5.20).

$$\tau = \Pi_{c1} + \Pi_{s11} + \Pi_{s12} \quad (5.21)$$

$$\tau = \frac{N_t x t}{N_i x L^2} \quad (5.22)$$

$$\tau = 0.5 (\Pi_{c2})^{1.1222} + \left(\left(\frac{P x t}{v} \right) \right)^{-1.1172} \left(\left(\frac{v x t x(V)^2}{F} \right) \right)^{-0.8371} + \left(\left(\frac{P x t}{v} \right) \right)^{0.0014} \left(\left(\frac{v x t x(V)^2}{F} \right) \right)^{-0.0018} \quad (5.23)$$

The results of the experimental plan and the meta-analysis of the database on bacterial transfer help to elucidate the behavior of bacterial transfer as dependent on fundamental physical variables. In the current study, data of the physical variables in both equations were obtained by measurements (Chapter 3), such as characteristic length of the potato sample, friction force, initial inoculation level, and bacterial transfer. In addition, other variables were controlled, such as speed, contact time, and pressure. The pressure was controlled to achieve a maximum contact area between the stainless steel plate and the potato sample. Friction force was measured with a texture analyzer. Finally, the surface tension of the water was taken as a theoretical value at the test (room) temperature.

The physical variables either controlled or measured during the development of the experiments were the inputs for the equations obtained using dimensional analysis (equations 5.17, 5.19, and 5.20). In addition, the *Salmonella* recoveries obtained from the plate and from the potatoes were used to perform a bacterial count balance. Results of the count balance were used to estimate the ratio between bacteria transferred and bacteria remaining on the donor. The results of the balance indicated that the addition of bacteria transferred to stainless steel and bacteria remaining on the potato surface were on the same order of magnitude as the levels of bacteria applied as initial inoculation, but they did not add up accurately to the original inoculation level on the stainless steel.

Data for this analysis were obtained using only potato as a food model (Chapter 3). In addition, all the variables listed in the dimensional analysis were not evaluated in the experimental design. In this analysis, few data sets were used in comparison to analysis performed in the meta-analysis (Chapter 4). It was not possible to use the data in the meta-analysis to evaluate the model developed by dimensional analysis because physical variables,

such as speed, normal pressure, and dimensions of the food samples, were not included in the studies previously published. For instance, it is possible to develop a model using dimensional analysis (Hypothesis 3) but the confidence interval results (table 5.5 and 5.6) revealed that more data are necessary for further evaluation, as well as other variables, and food products.

Fundamental variables were included in the dimensional analysis, and physical variables were evaluated in the experimental designs on bacterial transfer via static and dynamic contact. The same variables are also in larger-scale processes (pilot- or commercial-scale). For example, the normal force is present on a conveyer belt or when the food products are dropped or bounced. The purpose of this study was to propose new methodologies to conduct bacterial transfer research. Such methodologies hopefully can advance the state-of-the-art in modeling approaches for bacterial transfer. However, it clearly is necessary to conduct more studies quantifying fundamental physical variables and their impact on transfer outcomes.

5.3. Results

5.3.1 Comparison of the dependency of physical variables and bacteria transferred via static contact for experimental data versus a dimensional analysis model

A direct dependency between bacterial transfer and the characteristic length of the potato sample and the pressure during contact was observed. The data (Chapter 3) were collected after performing 18 multiple contacts (Figure 5.1). Previous experimental results (Chapter 3) revealed higher bacterial transfer at the highest pressure on the potato sample, and significant differences were found between the highest and the lowest contact pressure on bacterial via 18 multiple contacts. The dimensional analysis revealed an increasing trend (Figure 5.1).

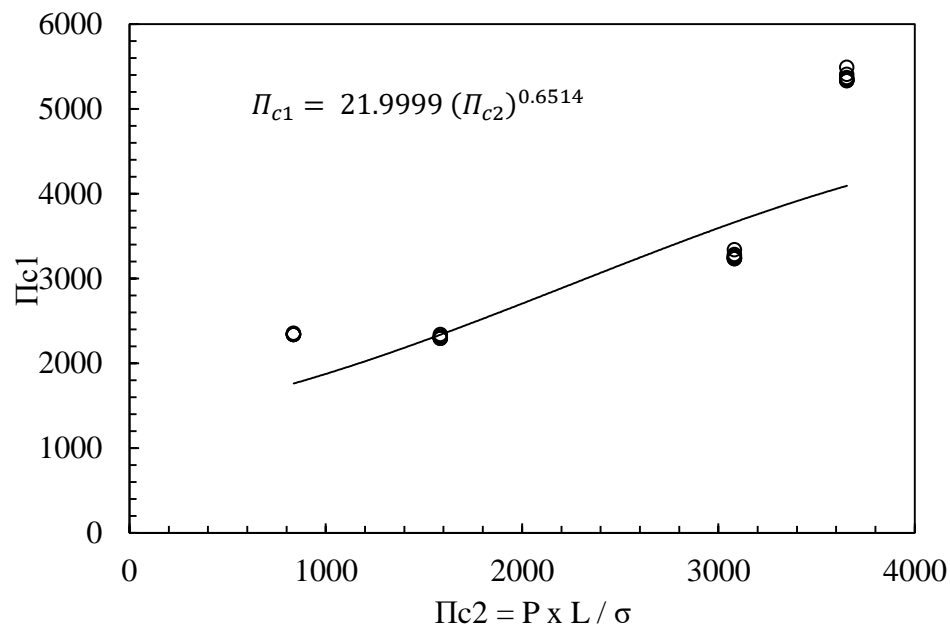


Figure 5.1 Model for bacterial transfer via static contact vs. Π_{c2} determined by dimensional analysis, which is a combination of pressure, potato length, and surface tension, based on one experimental data set (Chapter 3).

The model developed showed an increasing dependency on bacterial transfer ratio (Figure 5.1). Experimental data were grouped, and predicted data were presented as a line (equation 5.17). The results of the dimensional analysis are consistent with the null hypothesis, which was that bacterial transfer from food to a contact surface increases with pressure. However, there was not good agreement with the limited experimental data available for comparison (Figure 5.1). Predicted data were close to a line. On the other hand, experimental results revealed that lower pressures had similar bacterial transfer recoveries, and higher pressures had higher bacterial transfer recoveries. The trend of the data was difficult to model, because it was constant at the beginning and increasing at the end. This probably was due to the limited number of data and the levels of the physical variables evaluated in the experiments (Chapter 3). More data are needed, at more levels of the physical variables, to validate the model concept and form.

5.3.2 Comparison of the dependency of physical variables (friction force and pressure) and bacteria transferred via dynamic contact on experimental data versus a dimensional analysis

Results were used from experiments on bacterial transfer via dynamic contact at constant contact distance and different speed, which were analogous to the experiments conducted in prior studies collected to develop a meta-analysis on bacterial transfer. Bacterial transfer was achieved from the plate to subsequent potato samples. The equations showed that there was a dependency between friction force and bacterial transfer as the pressure was held constant (equations 5.19 and 5.20). Bacterial transfer decreases as friction force increases, a variable that was dependent on speed. The slowest speed achieved the highest friction force, for instance at the slowest speed less bacterial

transfer was found. The comparison with the limited experimental data suggest weak agreement, but again the data were limited to a fairly small portion of the potential range for the Π terms.

The data from potato pulled at 7.75 mm/s speed and 3 pressures were used as input (Figure 5.2). From these results, it can be inferred that pressure affected bacterial transfer, but as 3 pressures were applied changes on friction force should be considered. The curve was drawn considering the friction force collected from experimental data when the pressure and the speed were already selected. Friction force increased as pressure on the potato also increased according to the measurements. The ratio between pressure and friction force in equations 5.19 and 5.20 changed by the combination of these 2 variables. Higher pressure had as a result higher friction force, for instance, more bacterial transfer was found at the highest pressure and highest friction force.

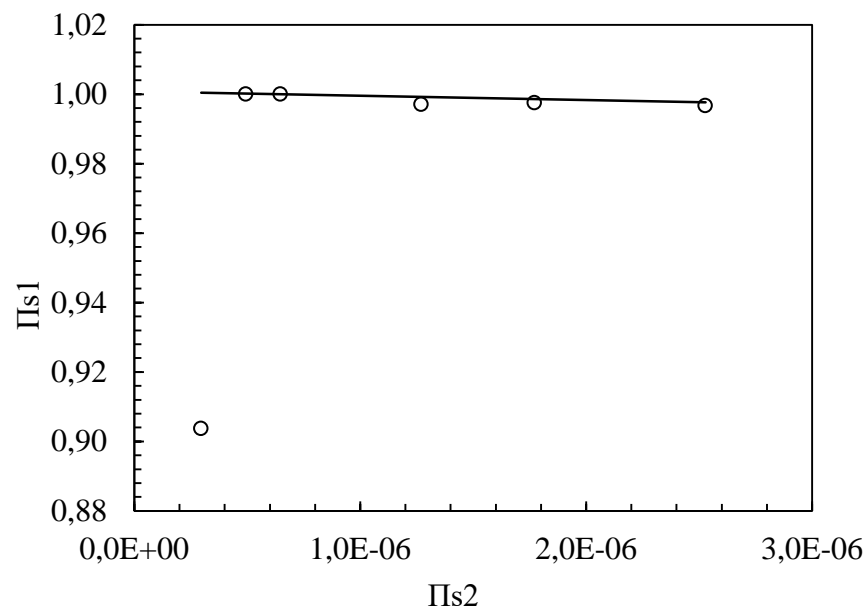


Figure 5.2 Model for bacterial transfer via dynamic contact vs. Πs_2 determined by dimensional analysis which is a combination of pressure, friction force, and length.

These analyses considered bacterial transfer via static contact and via dynamic contact. From both processes, it can be affirmed that variables of pressure (Figure 5.1) and sliding speed affected bacterial transfer. Since sliding speed and pressure directly impact friction force, it was also considered to affect bacterial transfer (Figure 5.2). Many physical variables were included in the current analysis, such as contact time, potato length, surface tension, and initial inoculation level, which were constant during the experimental work. As they were constant, no general conclusion can be drawn from them; however, the control of these variables allowed general conclusions regarding physical variables under evaluation, such as pressure and speed. Ultimately, there currently are insufficient experimental data, of the type and information needed, to rigorously test the utility of the proposed models. However, the results presented here suggest that this modeling approach might be conceptually and phenomenologically feasible (given future data designed specifically to test the proposed models).

These results were based on data collected from small-scale experiments that were designed based on approximations to reality, and only the most relevant variables were considered. Other variables, such as product roughness and microorganism, were not included in the experimental design. Moisture content was not included (as a constant) in the application of the Buckingham Pi theorem and in the resulting equations. Other steps are necessary before using the model for prediction. More data are necessary to validate the model, as well as other levels of the variables.

5.3.3 State-of-the-art of the use of the fundamental units of physics for a modeling approach

The fundamental units included in the current study were meter, kilogram, and second, corresponding to the base quantities of length, mass, and time, respectively. These variables are independent, and they define other quantities such as area, pressure, and velocity. These variables are also interacting in food processing facilities and food processing equipment in contact with foods. In addition, these variables are also interacting in processes of cross-contamination and bacterial transfer.

The units more frequently used in bacterial transfer studies are CFU, CFU/unit, CFU/g, and CFU/cm², and their respective logarithmic scale. Most of the data are reported on Log CFU/g or Log CFU/unit. There is not a standard unit used on bacterial transfer studies based on fundamental units.

For the case of bacterial transfer studies, the unit recommended is total Log CFU or Log CFU/cm². For data collected from previous publications, the values of the physical variables involved in the experimental design were difficult to obtain (Chapter 3). Furthermore, the physical variables were not included as independent variables in the experimental design, and/or they were not measured. Most of the data were reported as bacterial transfer versus the unit number. The data collected to perform a meta-analysis on bacterial transfer were focused mainly on food composition because many studies were developed using different product types. The studies used similar methods, and the results obtained are similar across studies in terms of units used to present the data and the curves to describe bacterial transfer behavior. However, there is still a gap in bacterial transfer data versus physical variables, such as contact area, contact time, pressure, and speed among others.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Results from the current study gave a new approach for conducting future experiments on bacterial transfer via surface contacts (static and dynamic). Physical variables, such as speed, normal pressure, friction force, and contact area were fundamental to the consistency among results of surface contact experiments. For example, sliding speed and contact time increased bacterial transfer from potato to plate. The meta-analysis of prior bacterial transfer studies revealed insufficient reporting of fundamental physical variables, such as quantitative roughness, contact area, and contact time between food products and cutting tools. The majority of studies collected in the meta-analysis evaluated food components instead of focusing on fundamental physical variables (e.g., force, time, contact area, speed, etc). Experiments in this study and data collected from prior studies were inputs for a new modeling approach applying dimensional analysis. The results suggested that it is possible to develop a bacterial transfer model using a dimensional analysis approach, which considered basic physical variables. This work contributes bacterial transfer modeling research, because few prior studies were conducted to obtain as end-product a model form, and most such of studies have focused on probabilistic or best-fit models. Additionally, the meta-analysis of a database of bacterial transfer data elucidated generalized conclusions about factors affecting transfer response across diverse studies. Overall, the three parts of this dissertation (i.e., bench-scale experiments, the meta-analysis, and a novel model formulation) combine to demonstrate that future bacterial transfer studies should design and report treatments based on fundamental physical variables. The net result would enhance comparability across studies and significantly enhance the potential for generalizable conclusions about bacterial transfer phenomena.

6.1 Overall conclusions

- Bacterial transfer via dynamic contact from stainless steel plate to potato increases as sliding speed increases. No effect of speed was found on bacteria transferred from the plate to the potato. Transfer direction influences bacterial transfer outcomes direction.
- Bacterial transfer via static contact was higher at 40 s than 5 s.
- Bacterial transfer on static contact increased with contact pressure.
- Bacterial transfer remaining on the originally inoculated potato surface was higher after a single static contact than after 18 multiple contacts with the inoculated surface.
- Bacterial transfer was significantly higher from the plate to the potato than from the potato to the plate. Bacterial transfer was preferential to the potato over the stainless steel.
- Bacteria remaining on the potato after dynamic contact did not affect bacterial transfer from the plate to the potato at 3.75 and 7.75 mm/s and the 3 pressures evaluated.
- The meta-analysis enabled general conclusions on the dependency between food composition, microorganism, and product type on bacterial transfer via static contact and via dynamic contact across a large group of prior studies from previous publications, collaboration work, and data collected at MSU.
- Based on the meta-analysis of the Weibull rate for parameter bacterial transfer response via dynamic and via static contact increased as moisture content increased.
- The Weibull shape parameter decreased as moisture content increased on bacterial transfer data via dynamic contact.
- The Weibull shape and rate parameters had the same behavior in bacterial transfer data via static and dynamic contact; the rate parameter decreased and the shape parameter increased as pH increased.

- Similarly, the Weibull rate parameter for bacterial transfer response via dynamic contact decreased as fat and protein decreased.
- The data collected on bacterial transfer via multiple contacts (18) and bacterial transfer via dynamic contact experiments are (to date) insufficient to prove the validity of the model developed by dimensional analysis.

6.2 Future work and recommendations

- It is advisable to report bacterial transfer data in units of Log CFU and/or Log CFU/cm², rather than Log CFU/g, which is not related to the fundamental variables affecting transfer.
- True contact area between the donor and recipient surfaces should be controlled and reported in future bacterial transfer experiments, in order to improve the generalizability of the results.
- As higher normal pressure increased bacterial transfer, it is recommended to study more deeply this variable in terms of order-of-magnitude and/or other distribution of the normal force on the food product.
- True contact area between the donor and recipient surfaces should be controlled and reported in future bacterial transfer experiments, in order to improve the generalizability of the results.
- Future studies should evaluate other physical variables, such as moisture content in a wider range, roughness, and product tissue properties that might be added to improved version of the bacterial transfer model developed by the dimensional analysis approach.

- Lastly, a future study should be designed at a microscale to quantify bacterial transfer as affected by the physical variables, such as friction force, roughness, and transfer type.

6.3 Limitations

- The effect of fundamental physical variables, such as normal pressure, speed, and contact time, were difficult to discern across diverse data sets that did not necessarily report those variables.
- All physical variables in the dimensional analysis were not evaluated due to practical constraints, as a single study cannot easily include all fundamental and physical variables affecting bacterial transfer.
- It was a challenge to collect the data appropriate for the meta-analysis, in terms of the number of data and variables described in fundamental units.

APPENDICES

APPENDIX A

Experimental data

Table A.1 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 1, day 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	18	17	10	1000	0.1	6.24
1	7473	674	11.6	174	149	10	10	0.1	5.21
2	7473	674	11.6	206	108	10	10	0.1	5.20
3	7473	674	11.6	77	98	10	10	0.1	4.94
4	7473	674	11.6	29	29	10	10	0.1	4.46
5	7473	674	11.6	202	259	10	1	0.1	4.36
6	7473	674	11.6	192	158	10	1	0.1	4.24
7	7473	674	11.6	58	69	10	1	0.1	3.80
8	7473	674	11.6	33	37	10	1	0.1	3.54

Table A.2 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 2, day 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	6	13	10	1000	0.1	5.98
1	7473	674	11.6	107	99	10	10	0.1	5.01
2	7473	674	11.6	97	137	10	10	0.1	5.07
3	7473	674	11.6	41	62	10	10	0.1	4.71
4	7473	674	11.6	49	52	10	10	0.1	4.70
5	7473	674	11.6	16	18	10	10	0.1	4.23
6	7473	674	11.6	41	50	10	10	0.1	4.66
7	7473	674	11.6	141	187	10	1	0.1	4.21
8	7473	674	11.6	74	91	10	1	0.1	3.92

Table A.3 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 3, day 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	14	13	10	1000	0.1	6.13
1	7473	674	11.6	121	127	10	10	0.1	5.09
2	7473	674	11.6	36	44	10	10	0.1	4.60
3	7473	674	11.6	39	48	10	10	0.1	4.64
4	7473	674	11.6	36	27	10	10	0.1	4.50
5	7473	674	11.6	21	29	10	10	0.1	4.40
6	7473	674	11.6	110	91	10	1	0.1	4.00
7	7473	674	11.6	18	41	10	1	0.1	3.47
8	7473	674	11.6	27	30	10	1	0.1	3.45

Table A.4 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 1, day 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	12	9	10	1000	0.1	6.02
1	7473	674	11.6	181	89	10	10	0.1	5.13
2	7473	674	11.6	45	76	10	10	0.1	4.78
3	7473	674	11.6	44	47	10	10	0.1	4.66
4	7473	674	11.6	33	36	10	10	0.1	4.54
5	7473	674	11.6	234	216	10	1	0.1	4.35
6	7473	674	11.6	107	119	10	1	0.1	4.05
7	7473	674	11.6	48	52	10	1	0.1	3.70
8	7473	674	11.6	47	67	10	1	1	2.76

Table A.5 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 2, day 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	7	9	10	1000	0.1	5.90
1	7473	674	11.6	120	149	10	10	0.1	5.13
2	7473	674	11.6	133	58	10	10	0.1	4.98
3	7473	674	11.6	205	237	10	1	0.1	4.34
4	7473	674	11.6	110	73	10	10	0.1	4.96
5	7473	674	11.6	106	83	10	10	0.1	4.98
6	7473	674	11.6	212	228	10	1	0.1	4.34
7	7473	674	11.6	192	183	10	1	0.1	4.27
8	7473	674	11.6	168	155	10	1	0.1	4.21

Table A.6 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 3, day 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	8	13	10	1000	0.1	6.02
1	7473	674	11.6	229	409	10	1	0.1	4.50
2	7473	674	11.6	169	133	10	10	0.1	5.18
3	7473	674	11.6	278	409	10	1	0.1	4.54
4	7473	674	11.6	79	95	10	10	0.1	4.94
5	7473	674	11.6	28	30	10	10	0.1	4.46
6	7473	674	11.6	76	59	10	10	0.1	4.83
7	7473	674	11.6	249	237	10	1	0.1	4.39
8	7473	674	11.6	66	56	10	1	0.1	3.79

Table A.7 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 1, day 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	19	21	10	100	0.1	5.30
1	7473	674	11.6	29	25	10	10	0.1	4.43
2	7473	674	11.6	176	107	10	1	0.1	4.15
3	7473	674	11.6	130	81	10	1	0.1	4.02
4	7473	674	11.6	74	120	10	1	0.1	3.99
5	7473	674	11.6	75	78	10	1	0.1	3.88
6	7473	674	11.6	51	65	10	1	0.1	3.76
7	7473	674	11.6	62	69	10	1	0.1	3.82
8	7473	674	11.6	61	41	10	1	0.1	3.71

Table A.8 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 2, day 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	15	8	10	100	0.1	5.06
1	7473	674	11.6	83	94	10	10	0.1	4.95
2	7473	674	11.6	107	90	10	10	0.1	4.99
3	7473	674	11.6	192	176	10	1	0.1	4.26
4	7473	674	11.6	75	57	10	10	0.1	4.82
5	7473	674	11.6	25	25	10	10	0.1	4.40
6	7473	674	11.6	56	35	10	10	0.1	4.66
7	7473	674	11.6	21	12	10	10	0.1	4.22
8	7473	674	11.6	31	37	10	10	0.1	4.53

Table A.9 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 3, day 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	46	48	10	100	0.1	5.67
1	7473	674	11.6	48	46	10	10	0.1	4.67
2	7473	674	11.6	132	118	10	1	0.1	4.10
3	7473	674	11.6	38	44	10	10	0.1	4.61
4	7473	674	11.6	33	35	10	10	0.1	4.53
5	7473	674	11.6	21	25	10	10	0.1	4.36
6	7473	674	11.6	88	103	10	1	0.1	3.98
7	7473	674	11.6	163	209	10	1	0.1	4.27
8	7473	674	11.6	126	180	10	1	0.1	4.18

Table A.10 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 1, day 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	30	25	10	100	0.1	5.44
1	7473	674	11.6	132	132	10	10	0.1	5.12
2	7473	674	11.6	53	57	10	10	0.1	4.74
3	7473	674	11.6	184	166	10	10	0.1	5.24
4	7473	674	11.6	25	47	10	10	0.1	4.56
5	7473	674	11.6	20	19	10	10	0.1	4.29
6	7473	674	11.6	269	353	10	1	0.1	4.49
7	7473	674	11.6	112	115	10	1	0.1	4.05
8	7473	674	11.6	129	150	10	1	0.1	4.14

Table A.11 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 2, day 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	83	81	10	100	0.1	5.91
1	7473	674	11.6	165	202	10	1	0.1	4.26
2	7473	674	11.6	11	12	10	10	0.1	4.06
3	7473	674	11.6	54	60	10	1	0.1	3.76
4	7473	674	11.6	6	9	10	10	0.1	3.88
5	7473	674	11.6	36	36	10	1	0.1	3.56
6	7473	674	11.6	49	56	10	1	0.1	3.72
7	7473	674	11.6	14	9	10	1	0.1	3.06
8	7473	674	11.6	23	0	10	1	0.1	3.06

Table A.12 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 83% (replicate 3, day 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	23	19	10	1000	0.1	6.32
1	7473	674	11.6	341	300	10	1	0.1	4.51
2	7473	674	11.6	118	134	10	10	0.1	5.10
3	7473	674	11.6	120	77	10	10	0.1	4.99
4	7473	674	11.6	63	80	10	10	0.1	4.85
5	7473	674	11.6	33	36	10	10	0.1	4.54
6	7473	674	11.6	212	204	10	1	0.1	4.32
7	7473	674	11.6	62	63	10	10	0.1	4.80
8	7473	674	11.6	96	138	10	1	0.1	4.07

Table A.13 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 1, day 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	13	14	10	1000	0.1	6.04
1	7473	674	11.6	15	10	10	10	0.1	4.01
2	7473	674	11.6	91	112	10	1	0.1	3.92
3	7473	674	11.6	55	62	10	1	0.1	3.68
4	7473	674	11.6	34	36	10	1	0.1	3.46
5	7473	674	11.6	10	18	10	1	0.1	3.06
6	7473	674	11.6	13	15	10	1	0.1	3.06
7	7473	674	11.6	10	12	10	1	0.1	2.96
8	7473	674	11.6	30	25	10	1	1	2.35

Table A.14 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 2, day 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	8	8	10	1000	0.1	5.82
1	7473	674	11.6	79	117	10	1	0.1	3.91
2	7473	674	11.6	23	27	10	1	0.1	3.31
3	7473	674	11.6	22	27	10	1	0.1	3.30
4	7473	674	11.6	7	10	10	1	0.1	2.84
5	7473	674	11.6	7	5	10	1	0.1	2.69
6	7473	674	11.6	19	12	10	1	1	2.10
7	7473	674	11.6	1	1	10	1	0.1	1.91
8	7473	674	11.6	14	5	10	1	1	1.89

Table A.15 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 3, day 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	11	5	10	1000	0.1	5.82
1	7473	674	11.6	24	15	10	10	0.1	4.20
2	7473	674	11.6	20	22	10	10	0.1	4.24
3	7473	674	11.6	100	176	10	1	0.1	4.05
4	7473	674	11.6	80	91	10	1	0.1	3.85
5	7473	674	11.6	121	126	10	1	0.1	4.01
6	7473	674	11.6	26	69	10	1	0.1	3.59
7	7473	674	11.6	17	32	10	1	0.1	3.30
8	7473	674	11.6	3	4	10	10	0.1	3.46

Table A.16 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 1, day 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	31	42	10	1000	0.1	6.48
1	7473	674	11.6	57	55	10	10	0.1	4.66
2	7473	674	11.6	70	70	10	10	0.1	4.76
3	7473	674	11.6	60	63	10	10	0.1	4.70
4	7473	674	11.6	240	188	10	1	0.1	4.24
5	7473	674	11.6	98	74	10	1	0.1	3.85
6	7473	674	11.6	77	72	10	1	0.1	3.79
7	7473	674	11.6	12	12	10	10	0.1	3.99
8	7473	674	11.6	32	33	10	1	1	2.43

Table A.17 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 2, day 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	39	51	10	1000	0.1	6.57
1	7473	674	11.6	55	55	10	10	0.1	4.65
2	7473	674	11.6	33	40	10	10	0.1	4.48
3	7473	674	11.6	38	34	10	10	0.1	4.47
4	7473	674	11.6	32	30	10	10	0.1	4.41
5	7473	674	11.6	119	131	10	1	0.1	4.01
6	7473	674	11.6	20	17	10	10	0.1	4.18
7	7473	674	11.6	95	96	10	1	0.1	3.89
8	7473	674	11.6	70	83	10	1	0.1	3.80

Table A.18 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 3, day 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	20	15	10	1000	0.1	6.16
1	7473	674	11.6	36	44	10	10	0.1	4.52
2	7473	674	11.6	21	28	10	10	0.1	4.30
3	7473	674	11.6	21	23	10	10	0.1	4.26
4	7473	674	11.6	22	19	10	10	0.1	4.23
5	7473	674	11.6	160	173	10	1	0.1	4.14
6	7473	674	11.6	78	81	10	1	0.1	3.81
7	7473	674	11.6	47	34	10	1	0.1	3.52
8	7473	674	11.6	31	44	10	1	0.1	3.49

Table A.19 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 1, day 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	24	36	10	1000	0.1	6.48
1	7473	674	11.6	67	69	10	10	0.1	4.83
2	7473	674	11.6	43	29	10	10	0.1	4.56
3	7473	674	11.6	21	24	10	10	0.1	4.35
4	7473	674	11.6	165	134	10	1	0.1	4.17
5	7473	674	11.6	158	96	10	1	0.1	4.10
6	7473	674	11.6	84	87	10	1	0.1	3.93
7	7473	674	11.6	29	43	10	1	0.1	3.56
8	7473	674	11.6	50	37	10	1	0.1	3.64

Table A.20 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 2, day 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	51	67	10	100	0.1	5.77
1	7473	674	11.6	70	74	10	10	0.1	4.86
2	7473	674	11.6	38	41	10	10	0.1	4.60
3	7473	674	11.6	120	146	10	10	0.1	5.12
4	7473	674	11.6	39	36	10	10	0.1	4.57
5	7473	674	11.6	46	45	10	10	0.1	4.66
6	7473	674	11.6	29	32	10	10	0.1	4.48
7	7473	674	11.6	21	25	10	10	0.1	4.36
8	7473	674	11.6	144	133	10	1	0.1	4.14

Table A.21 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 3, day 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	5	14	10	1000	0.1	5.98
1	7473	674	11.6	185	78	10	1	0.1	4.12
2	7473	674	11.6	130	149	10	1	0.1	4.14
3	7473	674	11.6	74	49	10	1	0.1	3.79
4	7473	674	11.6	78	49	10	1	0.1	3.80
5	7473	674	11.6	44	51	10	1	0.1	3.68
6	7473	674	11.6	51	46	10	1	0.1	3.69
7	7473	674	11.6	39	34	10	1	0.1	3.56
8	7473	674	11.6	32	34	10	1	0.1	3.52

Table A.22 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 1, day 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	55	50	10	1000	0.1	6.72
1	7473	674	11.6	142	174	10	10	0.1	5.20
2	7473	674	11.6	212	159	10	10	0.1	5.27
3	7473	674	11.6	37	62	10	10	0.1	4.69
4	7473	674	11.6	41	47	10	10	0.1	4.64
5	7473	674	11.6	71	52	10	10	0.1	4.79
6	7473	674	11.6	31	41	10	10	0.1	4.56
7	7473	674	11.6	69	36	10	10	0.1	4.72
8	7473	674	11.6	52	26	10	10	0.1	4.59

Table A.23 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 2, day 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	78	43	10	1000	0.1	6.78
1	7473	674	11.6	84	84	10	10	0.1	4.92
2	7473	674	11.6	106	98	10	10	0.1	5.01
3	7473	674	11.6	49	46	10	10	0.1	4.68
4	7473	674	11.6	71	87	10	10	0.1	4.90
5	7473	674	11.6	17	27	10	10	0.1	4.34
6	7473	674	11.6	29	37	10	10	0.1	4.52
7	7473	674	11.6	15	13	10	10	0.1	4.15
8	7473	674	11.6	25	19	10	10	0.1	4.34

Table A.24 Bacterial transfer from the potato to the plate via static contact (8 multiple contacts) a pressure of 7473, and moisture content of the potato of 80% (replicate 3, day 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	7473	674	11.6	127	161	10	100	0.1	6.16
1	7473	674	11.6	28	31	10	10	0.1	4.47
2	7473	674	11.6	56	79	10	10	0.1	4.83
3	7473	674	11.6	30	44	10	10	0.1	4.57
4	7473	674	11.6	95	124	10	1	0.1	4.04
5	7473	674	11.6	35	26	10	10	0.1	4.48
6	7473	674	11.6	166	339	10	1	0.1	4.40
7	7473	674	11.6	85	67	10	1	0.1	3.88
8	7473	674	11.6	74	150	10	1	0.1	4.05

Table A.25 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 8869, and 5 s contact time (replicate 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	8869	802	11.6	28	42	10	100	0.1	5.54
1	8869	802	11.6	33	34	10	10	0.1	4.53
3	8869	802	11.6	23	25	10	10	0.1	4.38
5	8869	802	11.6	70	75	10	1	0.1	3.86
7	8869	802	11.6	27	27	10	1	0.1	3.43
9	8869	802	11.6	32	33	10	1	0.1	3.51
11	8869	802	11.6	20	15	10	1	0.1	3.24
13	8869	802	11.6	23	14	10	1	0.1	3.27
15	8869	802	11.6	8	11	10	1	0.1	2.98
17	8869	802	11.6	2	4	10	1	0.1	2.48

Table A.26 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 8869, and 5 s contact time (replicate 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	8869	802	11.6	7	9	10	100	0.1	4.90
1	8869	802	11.6	18	24	10	10	0.1	4.32
3	8869	802	11.6	101	158	10	1	0.1	4.11
5	8869	802	11.6	59	65	10	1	0.1	3.79
7	8869	802	11.6	27	26	10	1	0.1	3.42
9	8869	802	11.6	59	44	10	1	0.1	3.71
11	8869	802	11.6	5	7	10	1	0.1	2.78
13	8869	802	11.6	12	9	10	1	0.1	3.02
15	8869	802	11.6	11	5	10	1	0.1	2.90
17	8869	802	11.6	4	4	10	1	0.1	2.60

Table A.27 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 8869, and 5 s contact time (replicate 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	8869	802	11.6	15	15	10	100	0.1	5.18
1	8869	802	11.6	8	5	10	100	0.1	4.81
3	8869	802	11.6	39	36	10	10	0.1	4.57
5	8869	802	11.6	23	31	10	1	0.1	3.43
7	8869	802	11.6	2	4	10	1	0.1	2.48
9	8869	802	11.6	10	5	10	1	0.1	2.88
11	8869	802	11.6	3	5	10	1	0.1	2.60
13	8869	802	11.6	19	18	10	1	0.1	3.27
15	8869	802	11.6	9	12	10	1	0.1	3.02
17	8869	802	11.6	11	8	10	1	0.1	2.98

Table A.28 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 8869, and 5 s contact time (replicate 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	8869	802	11.6	17	18	10	1000	0.1	6.24
1	8869	802	11.6	15	15	10	100	0.1	5.18
3	8869	802	11.6	87	97	10	10	0.1	4.96
5	8869	802	11.6	41	46	10	10	0.1	4.64
7	8869	802	11.6	192	183	10	1	0.1	4.27
9	8869	802	11.6	108	113	10	1	0.1	4.04
11	8869	802	11.6	85	88	10	1	0.1	3.94
13	8869	802	11.6	70	55	10	1	0.1	3.80
15	8869	802	11.6	19	19	10	1	0.1	3.28
17	8869	802	11.6	13	4	10	1	0.1	2.93

Table A.29 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 8869, and 5 s contact time (replicate 5).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	8869	802	11.6	21	25	10	100	0.1	5.36
1	8869	802	11.6	4	10	10	100	0.1	4.85
3	8869	802	11.6	171	209	10	1	0.1	4.28
5	8869	802	11.6	91	95	10	1	0.1	3.97
7	8869	802	11.6	62	57	10	1	0.1	3.77
9	8869	802	11.6	21	21	10	1	0.1	3.32
11	8869	802	11.6	5	11	10	1	0.1	2.90
13	8869	802	11.6	6	7	10	1	0.1	2.81
15	8869	802	11.6	1	0	10	1	0.1	1.70
17	8869	802	11.6	7	4	10	1	0.1	2.74

Table A.30 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 8869, 5 s contact time (replicate 6).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	4487	400	11.6	38	40	10	1000	0.1	6.59
1	4487	400	11.6	10	12	10	100	0.1	5.04
3	4487	400	11.6	13	9	10	10	0.1	4.04
5	4487	400	11.6	99	94	10	1	0.1	3.98
7	4487	400	11.6	98	86	10	1	0.1	3.96
9	4487	400	11.6	46	43	10	1	0.1	3.65
11	4487	400	11.6	40	41	10	1	0.1	3.61
13	4487	400	11.6	20	33	10	1	0.1	3.42
15	4487	400	11.6	11	17	10	1	0.1	3.15
17	4487	400	11.6	19	22	10	1	0.1	3.31

Table A.31 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 4487, 5 s contact time (replicate 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	4487	400	11.6	55	53	10	100	0.1	5.73
1	4487	400	11.6	8	13	10	100	0.1	5.02
3	4487	400	11.6	30	28	10	10	0.1	4.46
5	4487	400	11.6	142	117	10	1	0.1	4.11
7	4487	400	11.6	20	23	10	10	0.1	4.33
9	4487	400	11.6	125	87	10	1	0.1	4.03
11	4487	400	11.6	61	63	10	1	0.1	3.79
13	4487	400	11.6	35	60	10	1	0.1	3.68
15	4487	400	11.6	8	16	10	1	0.1	3.08
17	4487	400	11.6	27	30	10	1	0.1	3.45

Table A.32 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 4487, 5 s contact time (replicate 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	4487	400	11.6	38	40	10	1000	0.1	6.59
1	4487	400	11.6	10	12	10	100	0.1	5.04
3	4487	400	11.6	13	9	10	10	0.1	4.04
5	4487	400	11.6	99	94	10	1	0.1	3.98
7	4487	400	11.6	98	86	10	1	0.1	3.96
9	4487	400	11.6	46	43	10	1	0.1	3.65
11	4487	400	11.6	40	41	10	1	0.1	3.61
13	4487	400	11.6	20	33	10	1	0.1	3.42
15	4487	400	11.6	11	17	10	1	0.1	3.15
17	4487	400	11.6	19	22	10	1	0.1	3.31

Table A.33 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 4487, 5 s contact time (replicate 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	4487	400	11.6	65	44	10	1000	0.1	6.74
1	4487	400	11.6	7	4	10	100	0.1	4.74
3	4487	400	11.6	163	173	10	1	0.1	4.23
5	4487	400	11.6	16	17	10	1	0.1	3.22
7	4487	400	11.6	31	17	10	1	0.1	3.38
9	4487	400	11.6	7	15	10	1	0.1	3.04
11	4487	400	11.6	6	11	10	1	0.1	2.93
13	4487	400	11.6	10	4	10	1	0.1	2.85
15	4487	400	11.6	1	1	10	1	0.1	2.00
17	4487	400	11.6	3	1	10	1	0.1	2.30

Table A.34 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 4487, 5 s contact time (replicate 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	4487	400	11.6	10	13	10	1000	0.1	6.06
1	4487	400	11.6	2	3	10	100	0.1	4.40
3	4487	400	11.6	56	87	10	1	0.1	3.85
5	4487	400	11.6	26	41	10	1	0.1	3.53
7	4487	400	11.6	35	33	10	1	0.1	3.53
9	4487	400	11.6	10	8	10	1	0.1	2.95
11	4487	400	11.6	4	5	10	1	0.1	2.65
13	4487	400	11.6	1	6	10	1	0.1	2.54
15	4487	400	11.6	2	2	10	1	0.1	2.30
17	4487	400	11.6	3	2	10	1	1	1.40

Table A.35 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 4487, 5 s contact time (replicate 5).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	4487	400	11.6	10	8	10	1000	0.1	5.95
1	4487	400	11.6	3	3	10	100	0.1	4.48
3	4487	400	11.6	1	2	10	100	0.1	4.18
5	4487	400	11.6	12	13	10	1	0.1	3.10
7	4487	400	11.6	16	8	10	1	0.1	3.08
9	4487	400	11.6	7	6	10	1	0.1	2.81
11	4487	400	11.6	3	5	10	1	0.1	2.60
13	4487	400	11.6	3	5	10	1	0.1	2.60
15	4487	400	11.6	4	3	10	1	0.1	2.54
17	4487	400	11.6	4	4	10	1	0.1	2.60

Table A.36 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 4487, 5 s contact time (replicate 6).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	4487	400	11.6	8	6	10	1000	0.1	5.85
1	4487	400	11.6	5	6	10	100	0.1	4.74
3	4487	400	11.6	42	43	10	1	0.1	3.63
5	4487	400	11.6	29	11	10	1	0.1	3.30
7	4487	400	11.6	8	11	10	1	0.1	2.98
9	4487	400	11.6	1	2	10	1	0.1	2.18
11	4487	400	11.6	4	3	10	1	0.1	2.54
13	4487	400	11.6	3	4	10	1	1	1.54
15	4487	400	11.6	0	2	10	1	0.1	2.00
17	4487	400	11.6	2	2	10	1	1	1.30

Table A.37 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 2307, 5 s contact time (replicate 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	2307	200	11.6	38	48	10	1000	0.1	6.63
1	2307	200	11.6	13	11	10	100	0.1	5.08
3	2307	200	11.6	121	111	10	1	0.1	4.06
5	2307	200	11.6	74	86	10	1	0.1	3.90
7	2307	200	11.6	47	40	10	1	0.1	3.64
9	2307	200	11.6	33	44	10	1	0.1	3.59
11	2307	200	11.6	16	20	10	1	0.1	3.26
13	2307	200	11.6	8	13	10	1	0.1	3.02
15	2307	200	11.6	5	6	10	1	0.1	2.74
17	2307	200	11.6	1	3	10	1	0.1	2.30

Table A.38 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 2307, 5 s contact time (replicate 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	2307	200	11.6	54	40	10	1000	0.1	6.67
1	2307	200	11.6	0	2	10	100	0.1	4.00
3	2307	200	11.6	135	135	10	1	0.1	4.13
5	2307	200	11.6	117	127	10	1	0.1	4.09
7	2307	200	11.6	44	55	10	1	0.1	3.69
9	2307	200	11.6	37	34	10	1	0.1	3.55
11	2307	200	11.6	13	20	10	1	0.1	3.22
13	2307	200	11.6	12	14	10	1	0.1	3.11
15	2307	200	11.6	4	2	10	1	0.1	2.48
17	2307	200	11.6	4	5	10	1	0.1	2.65

Table A.39 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 2307, 5 s contact time (replicate 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	2307	200	11.6	16	17	10	1000	0.1	6.22
1	2307	200	11.6	5	3	10	100	0.1	4.60
3	2307	200	11.6	105	92	10	1	0.1	3.99
5	2307	200	11.6	64	68	10	1	0.1	3.82
7	2307	200	11.6	8	11	10	1	0.1	2.98
9	2307	200	11.6	1	1	10	1	0.1	2.00
11	2307	200	11.6	8	10	10	1	0.1	2.95
13	2307	200	11.6	1	0	10	1	0.1	1.70
15	2307	200	11.6	3	2	10	1	0.1	2.40
17	2307	200	11.6	1	2	10	1	0.1	2.18

Table A.40 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 2307, 5 s contact time (replicate 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	2307	200	11.6	4	7	10	1000	0.1	5.74
1	2307	200	11.6	6	5	10	100	0.1	4.74
3	2307	200	11.6	88	71	10	1	0.1	3.90
5	2307	200	11.6	43	53	10	1	0.1	3.68
7	2307	200	11.6	61	30	10	1	0.1	3.66
9	2307	200	11.6	50	41	10	1	0.1	3.66
11	2307	200	11.6	20	16	10	1	0.1	3.26
13	2307	200	11.6	26	18	10	1	0.1	3.34
15	2307	200	11.6	12	12	10	1	0.1	3.08
17	2307	200	11.6	9	3	10	1	1	1.78

Table A.41 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 2307, 5 s contact time (replicate 5).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	2307	200	11.6	36	42	10	1000	0.1	6.59
1	2307	200	11.6	14	10	10	100	0.1	5.08
3	2307	200	11.6	208	171	10	1	0.1	4.28
5	2307	200	11.6	120	160	10	1	0.1	4.15
7	2307	200	11.6	71	54	10	1	0.1	3.80
9	2307	200	11.6	19	38	10	1	0.1	3.45
11	2307	200	11.6	15	21	10	1	0.1	3.26
13	2307	200	11.6	4	5	10	1	0.1	2.65
15	2307	200	11.6	6	8	10	1	0.1	2.85
17	2307	200	11.6	3	3	10	1	0.1	2.48

Table A.42 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 2307, 5 s contact time (replicate 6).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	2307	200	11.6	25	23	10	1000	0.1	6.38
1	2307	200	11.6	5	0	10	100	0.1	4.40
3	2307	200	11.6	53	51	10	1	0.1	3.72
5	2307	200	11.6	57	45	10	1	0.1	3.71
7	2307	200	11.6	8	9	10	1	0.1	2.93
9	2307	200	11.6	1	2	10	1	0.1	2.18
11	2307	200	11.6	4	17	10	1	0.1	3.02
13	2307	200	11.6	6	4	10	1	1	1.70
15	2307	200	11.6	6	5	10	1	0.1	2.74
17	2307	200	11.6	2	1	10	1	1	1.18

Table A.43 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 1217, 5 s contact time (replicate 1).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	1217	100	11.6	11	12	10	1000	0.1	6.06
1	1217	100	11.6	1	1	10	100	0.1	4.00
3	1217	100	11.6	72	65	10	1	0.1	3.84
5	1217	100	11.6	8	22	10	1	0.1	3.18
7	1217	100	11.6	13	24	10	1	0.1	3.27
9	1217	100	11.6	18	18	10	1	0.1	3.26
11	1217	100	11.6	7	14	10	1	0.1	3.02
13	1217	100	11.6	6	3	10	1	0.1	2.65
15	1217	100	11.6	1	6	10	1	0.1	2.54
17	1217	100	11.6	2	4	10	1	0.1	2.48

Table A.44 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 1217, 5 s contact time (replicate 2).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	1217	100	11.6	25	27	10	1000	0.1	6.41
1	1217	100	11.6	1	1	10	100	0.1	4.00
3	1217	100	11.6	86	112	10	1	0.1	4.00
5	1217	100	11.6	44	31	10	1	0.1	3.57
7	1217	100	11.6	24	25	10	1	0.1	3.39
9	1217	100	11.6	1	8	10	1	0.1	2.65
11	1217	100	11.6	7	5	10	1	0.1	2.78
13	1217	100	11.6	1	0	10	1	0.1	1.70
15	1217	100	11.6	2	2	10	1	0.1	2.30
17	1217	100	11.6	1	0	10	1	0.1	1.70

Table A.45 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 1217, 5 s contact time (replicate 3).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	1217	100	11.6	5	9	10	1000	0.1	5.85
1	1217	100	11.6	0	1	10	100	0.1	3.70
3	1217	100	11.6	40	39	10	1	0.1	3.60
5	1217	100	11.6	40	43	10	1	0.1	3.62
7	1217	100	11.6	12	9	10	1	0.1	3.02
9	1217	100	11.6	5	2	10	1	0.1	2.54
11	1217	100	11.6	7	8	10	1	0.1	2.88
13	1217	100	11.6	1	2	10	1	0.1	2.18
15	1217	100	11.6	7	1	10	1	0.1	2.60
17	1217	100	11.6	1	2	10	1	0.1	2.18

Table A.46 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 1217, 5 s contact time (replicate 4).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	1217	100	11.6	22	23	10	1000	0.1	6.27
1	1217	100	11.6	0	1	10	100	0.1	3.61
3	1217	100	11.6	67	55	10	1	0.1	3.70
5	1217	100	11.6	22	23	10	1	0.1	3.27
7	1217	100	11.6	19	7	10	1	0.1	3.03
9	1217	100	11.6	4	4	10	1	0.1	2.52
11	1217	100	11.6	14	9	10	1	0.1	2.97
13	1217	100	11.6	7	0	10	1	0.1	2.46
15	1217	100	11.6	4	2	10	1	0.1	2.39
17	1217	100	11.6	4	3	10	1	1	1.46

Table A.47 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 1217, 5 s contact time (replicate 5).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	1217	100	11.6	41	59	10	1000	0.1	6.61
1	1217	100	11.6	6	1	10	100	0.1	4.46
3	1217	100	11.6	111	93	10	1	0.1	3.92
5	1217	100	11.6	2	2	10	1	0.1	2.21
7	1217	100	11.6	11	19	10	1	0.1	3.09
9	1217	100	11.6	11	12	10	1	0.1	2.97
11	1217	100	11.6	7	9	10	1	0.1	2.82
13	1217	100	11.6	12	4	10	1	0.1	2.82
15	1217	100	11.6	6	2	10	1	0.1	2.52
17	1217	100	11.6	0	5	10	1	0.1	2.31

Table A.48 Bacterial transfer from the potato to the plate via static contact (18 multiple contacts)
a pressure of 1217, 5 s contact time (replicate 6).

Contact number	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
0	1217	100	11.6	30	37	10	1000	0.1	6.44
1	1217	100	11.6	5	4	10	100	0.1	4.57
3	1217	100	11.6	111	108	10	1	0.1	3.95
5	1217	100	11.6	28	39	10	1	0.1	3.44
7	1217	100	11.6	19	23	10	1	0.1	3.24
9	1217	100	11.6	10	14	10	1	0.1	2.99
11	1217	100	11.6	8	10	10	1	0.1	2.87
13	1217	100	11.6	7	15	10	1	1	1.96
15	1217	100	11.6	2	4	10	1	0.1	2.39
17	1217	100	11.6	2	2	10	1	1	1.21

Table A.49 Bacterial transfer from the plate to the potato after a single contact, a pressure of 7473 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	7473	674	11.6	44	63	20	1000	0.1	6.60
2	7473	674	11.6	143	147	20	1000	0.1	7.03
3	7473	674	11.6	142	148	20	1000	0.1	7.03
4	7473	674	11.6	82	94	20	1000	0.1	6.81
5	7473	674	11.6	40	45	20	1000	0.1	6.50
6	7473	674	11.6	53	55	20	1000	0.1	6.60
7	7473	674	11.6	15	15	10	1000	0.1	6.57
8	7473	674	11.6	22	14	10	1000	0.1	6.17
9	7473	674	11.6	30	48	10	100	0.1	6.15
10	7473	674	11.6	36	15	10	1000	0.1	5.57
11	7473	674	11.6	32	39	10	100	0.1	5.93
12	7473	674	11.6	21	13	10	1000	0.1	6.40

Table A.50 Bacteria remaining on the plate after a single contact (C_0), a pressure of 7473 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	7473	674	11.6	15	15	10	1000	0.1	6.18
2	7473	674	11.6	22	14	10	1000	0.1	6.26
3	7473	674	11.6	30	48	10	100	0.1	5.59
4	7473	674	11.6	36	15	10	1000	0.1	6.41
5	7473	674	11.6	32	39	10	100	0.1	5.55
6	7473	674	11.6	21	13	10	1000	0.1	6.23
7	7473	674	11.6	29	30	10	1000	0.1	6.47
8	7473	674	11.6	8	5	10	1000	0.1	5.81
9	7473	674	11.6	10	8	10	1000	0.1	5.95
10	7473	674	11.6	8	14	10	1000	0.1	6.04
11	7473	674	11.6	7	8	10	1000	0.1	5.88
12	7473	674	11.6	13	17	10	1000	0.1	6.18

Table A.51 Bacterial transfer from the potato to the plate after a single contact (C_1), a pressure of 7473 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	7473	674	11.6	9	16	10	100	0.1	5.10
2	7473	674	11.6	6	5	10	100	0.1	4.74
3	7473	674	11.6	12	10	10	100	0.1	5.04
4	7473	674	11.6	4	6	10	100	0.1	4.70
5	7473	674	11.6	6	4	10	1000	0.1	5.70
6	7473	674	11.6	6	6	10	100	0.1	4.78
7	7473	674	11.6	21	18	10	100	0.1	5.29
8	7473	674	11.6	1	18	10	1000	0.1	5.98
9	7473	674	11.6	3	0	10	1000	0.1	5.18
10	7473	674	11.6	1	2	10	100	0.1	4.18
11	7473	674	11.6	3	7	10	100	0.1	4.70
12	7473	674	11.6	18	21	10	100	0.1	5.29

Table A.52 Bacterial transfer from the plate to the potato after a single contact, a pressure of 5247 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	5247	470	11.6	42	100	20	1000	0.1	6.72
2	5247	470	11.6	40	55	20	1000	0.1	6.54
3	5247	470	11.6	36	19	20	1000	0.1	6.31
4	5247	470	11.6	88	60	20	1000	0.1	6.74
5	5247	470	11.6	11	6	20	10000	0.1	6.80
6	5247	470	11.6	39	57	20	1000	0.1	6.55
7	5247	470	11.6	42	100	20	1000	0.1	6.42
8	5247	470	11.6	40	55	20	1000	0.1	6.24
9	5247	470	11.6	36	19	20	1000	0.1	6.39
10	5247	470	11.6	88	60	20	1000	0.1	6.42
11	5247	470	11.6	11	6	20	10000	0.1	6.18
12	5247	470	11.6	39	57	20	1000	0.1	5.64

Table A.53 Bacteria remaining on the plate after a single contact (C_0), a pressure of 5247 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	5247	470	11.6	23	32	10	1000	0.1	6.44
2	5247	470	11.6	2	3	10	1000	0.1	5.40
3	5247	470	11.6	13	16	10	1000	0.1	6.16
4	5247	470	11.6	50	38	10	100	0.1	5.64
5	5247	470	11.6	32	39	10	1000	0.1	6.55
6	5247	470	11.6	8	8	10	1000	0.1	5.90
7	5247	470	11.6	4	4	10	100	0.1	6.22
8	5247	470	11.6	1	3	10	100	0.1	6.06
9	5247	470	11.6	6	5	10	100	0.1	5.11
10	5247	470	11.6	3	5	10	100	0.1	6.27
11	5247	470	11.6	1	3	10	100	0.1	5.11
12	5247	470	11.6	0	1	10	1000	0.1	5.65

Table A.54 Bacterial transfer from the potato to the plate after a single contact (C_1), a pressure of 5247 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	5247	470	11.6	9	8	10	1000	0.1	5.93
2	5247	470	11.6	12	12	10	1000	0.1	6.08
3	5247	470	11.6	29	26	10	100	0.1	5.44
4	5247	470	11.6	11	6	10	100	0.1	4.93
5	5247	470	11.6	47	34	10	100	0.1	5.61
6	5247	470	11.6	10	12	10	100	0.1	5.04
7	5247	470	11.6	4	4	10	100	0.1	4.60
8	5247	470	11.6	1	3	10	100	0.1	4.30
9	5247	470	11.6	6	5	10	100	0.1	4.74
10	5247	470	11.6	3	5	10	100	0.1	4.60
11	5247	470	11.6	1	3	10	100	0.1	4.30
12	5247	470	11.6	0	1	10	1000	0.1	4.70

Table A.55 Bacteria remaining on the potato after a single contact, a pressure the potato of 8869 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	8869	802	11.6	55	51	20	1000	0.1	6.59
2	8869	802	11.6	102	107	20	1000	0.1	6.89
3	8869	802	11.6	93	86	20	1000	0.1	6.82
4	8869	802	11.6	76	61	20	1000	0.1	6.70
5	8869	802	11.6	122	102	20	1000	0.1	6.92
6	8869	802	11.6	102	80	20	1000	0.1	6.83

Table A.56 Bacteria remaining on the plate after a single contact (C_0), a pressure of 8869 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	8869	802	11.6	18	20	10	1000	0.1	6.28
2	8869	802	11.6	19	23	10	1000	0.1	6.32
3	8869	802	11.6	35	37	10	100	0.1	5.56
4	8869	802	11.6	27	17	10	1000	0.1	6.34
5	8869	802	11.6	26	14	10	1000	0.1	6.30
6	8869	802	11.6	24	32	10	1000	0.1	6.45

Table A.57 Bacterial transfer from the potato to the plate after a single contact (C_1), a pressure of 8869 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	8869	802	11.6	19	34	10	100	0.1	5.42
2	8869	802	11.6	47	51	10	100	0.1	5.69
3	8869	802	11.6	59	68	10	100	0.1	5.80
4	8869	802	11.6	18	30	10	100	0.1	5.38
5	8869	802	11.6	30	32	10	100	0.1	5.49
6	8869	802	11.6	108	103	10	10	0.1	5.02

Table A.58 Bacteria remaining on the potato after a single contact, a pressure the potato of 4487

Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	4487	400	11.6	61	48	20	1000	0.1	6.60
2	4487	400	11.6	24	26	20	1000	0.1	6.26
3	4487	400	11.6	46	59	20	1000	0.1	6.59
4	4487	400	11.6	35	35	20	1000	0.1	6.41
5	4487	400	11.6	39	41	20	1000	0.1	6.47
6	4487	400	11.6	54	52	20	1000	0.1	6.59

Table A.59 Bacteria remaining on the plate after a single contact (C_0), a pressure of 4487 Pa,

contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	4487	400	11.6	29	19	10	1000	0.1	6.38
2	4487	400	11.6	48	34	10	1000	0.1	6.61
3	4487	400	11.6	121	112	10	100	0.1	6.07
4	4487	400	11.6	55	52	10	1000	0.1	6.73
5	4487	400	11.6	18	18	10	1000	0.1	6.26
6	4487	400	11.6	57	48	10	1000	0.1	6.72

Table A.60 Bacterial transfer from the potato to the plate after a single contact (C_1), a pressure of

4487 Pa, contact time of 40 s

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	4487	400	11.6	13	16	10	100	0.1	5.16
2	4487	400	11.6	13	14	10	100	0.1	5.13
3	4487	400	11.6	14	9	10	100	0.1	5.06
4	4487	400	11.6	14	20	10	100	0.1	5.23
5	4487	400	11.6	15	12	10	100	0.1	5.13
6	4487	400	11.6	84	108	10	10	0.1	4.98

Table A.61 Bacterial transfer from the plate to the potato after a single contact, a pressure of 7473 Pa, contact time of 40 s, and a moisture content of 83 %.

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	7473	674	11.6	10	14	20	1000	0.1	5.95
2	7473	674	11.6	40	65	20	1000	0.1	6.59
3	7473	674	11.6	13	37	20	1000	0.1	6.26
4	7473	674	11.6	80	111	20	1000	0.1	6.85
5	7473	674	11.6	18	20	20	1000	0.1	6.15
6	7473	674	11.6	65	71	20	1000	0.1	6.70
7	7473	674	11.6	3	3	20	1000	0.1	5.34
8	7473	674	11.6	6	11	20	1000	0.1	5.80
9	7473	674	11.6	1	3	20	1000	0.1	5.17
10	7473	674	11.6	3	3	20	1000	0.1	5.34
11	7473	674	11.6	2	2	20	1000	0.1	5.17
12	7473	674	11.6	5	6	20	1000	0.1	5.61
13	7473	674	11.6	77	78	20	1000	0.1	6.76
14	7473	674	11.6	65	57	20	1000	0.1	6.65
15	7473	674	11.6	72	124	20	1000	0.1	6.86
16	7473	674	11.6	45	50	20	1000	0.1	6.54
17	7473	674	11.6	90	64	20	1000	0.1	6.75
18	7473	674	11.6	37	44	20	1000	0.1	6.47

Table A.62 Bacteria remaining on the plate after a single contact, a pressure of 7473 Pa, contact time of 40 s, and a moisture content of 83 %.

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	7473	674	11.6	10	12	10	1000	0.1	6.04
2	7473	674	11.6	17	22	10	1000	0.1	6.29
3	7473	674	11.6	12	17	10	1000	0.1	6.16
4	7473	674	11.6	21	28	10	1000	0.1	6.39
5	7473	674	11.6	12	18	10	1000	0.1	6.18
6	7473	674	11.6	9	16	10	1000	0.1	6.10
7	7473	674	11.6	2	2	10	1000	0.1	5.30
8	7473	674	11.6	0	2	10	1000	0.1	5.00
9	7473	674	11.6	0	1	10	1000	0.1	4.70
10	7473	674	11.6	1	5	10	1000	0.1	5.48
11	7473	674	11.6	2	3	10	1000	0.1	5.40
12	7473	674	11.6	0	2	10	1000	0.1	5.00
13	7473	674	11.6	145	133	10	100	0.1	6.14
14	7473	674	11.6	54	64	10	1000	0.1	6.77
15	7473	674	11.6	24	28	10	1000	0.1	6.41
16	7473	674	11.6	50	37	10	1000	0.1	6.64
17	7473	674	11.6	28	48	10	1000	0.1	6.58
18	7473	674	11.6	21	20	10	1000	0.1	6.31

Table A.63 Bacterial transfer from the plate to the potato after a single contact, a pressure of 7473 Pa, contact time of 40 s, and a moisture content of 80 %.

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	7473	674	11.6	20	22	20	1000	0.1	6.19
2	7473	674	11.6	39	41	20	1000	0.1	6.47
3	7473	674	11.6	49	58	20	1000	0.1	6.60
4	7473	674	11.6	42	59	20	1000	0.1	6.57
5	7473	674	11.6	28	43	20	1000	0.1	6.42
6	7473	674	11.6	29	62	20	1000	0.1	6.52
7	7473	674	11.6	10	3	20	1000	0.1	5.68
8	7473	674	11.6	2	1	20	1000	0.1	5.04
9	7473	674	11.6	1	1	20	1000	0.1	4.87
10	7473	674	11.6	2	5	20	1000	0.1	5.41
11	7473	674	11.6	4	4	20	1000	0.1	5.47
12	7473	674	11.6	4	1	20	1000	0.1	5.26
13	7473	674	11.6	21	63	20	1000	0.1	6.49
14	7473	674	11.6	64	56	20	1000	0.1	6.64
15	7473	674	11.6	75	60	20	1000	0.1	6.70
16	7473	674	11.6	20	23	20	1000	0.1	6.20
17	7473	674	11.6	20	30	20	1000	0.1	6.26
18	7473	674	11.6	26	32	20	1000	0.1	6.33

Table A.64 Bacteria remaining on the plate after a single contact, a pressure of 7473 Pa, contact time of 40 s, and a moisture content of 80 %.

Sample	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	7473	674	11.6	18	21	10	1000	0.1	6.29
2	7473	674	11.6	13	13	10	1000	0.1	6.11
3	7473	674	11.6	42	48	10	1000	0.1	6.65
4	7473	674	11.6	12	21	10	1000	0.1	6.22
5	7473	674	11.6	24	14	10	10000	0.1	7.28
6	7473	674	11.6	37	41	10	1000	0.1	6.59
7	7473	674	11.6	1	5	10	1000	0.1	5.48
8	7473	674	11.6	0	3	10	1000	0.1	5.18
9	7473	674	11.6	3	2	10	1000	0.1	5.40
10	7473	674	11.6	0	1	10	1000	0.1	4.70
11	7473	674	11.6	2	1	10	1000	0.1	5.18
12	7473	674	11.6	2	5	10	1000	0.1	5.54
13	7473	674	11.6	33	53	10	1000	0.1	6.63
14	7473	674	11.6	33	62	10	1000	0.1	6.68
15	7473	674	11.6	35	45	10	1000	0.1	6.60
16	7473	674	11.6	94	104	10	100	0.1	6.00
17	7473	674	11.6	98	73	10	100	0.1	5.93
18	7473	674	11.6	41	26	10	1000	0.1	6.53

Table A.65 Initial concentration of bacteria on the plate

Sample	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
1	92	130	10	10000	0.1	8.0
2	102	90	10	1000	0.1	7.0
3	164	135	10	1000	0.1	7.2
4	94	90	10	1000	0.1	7.0
5	13	16	10	10000	0.1	7.2
6	115	124	10	1000	0.1	7.1
7	140	126	10	1000	0.1	7.1
8	80	74	10	1000	0.1	6.9
9	145	91	10	1000	0.1	7.1
10	107	62	10	1000	0.1	6.9
11	240	462	10	1000	0.1	7.5
12	170	188	10	1000	0.1	7.3

Table A.66 Bacteria remaining on the potato after 18 multiple static contacts and 5 s contact time.

Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
8869	802	11.6	5	10	20	1000	0.1	5.74
8869	802	11.6	9	14	20	1000	0.1	5.93
8869	802	11.6	6	9	20	1000	0.1	5.74
8869	802	11.6	5	10	20	1000	0.1	5.74
8869	802	11.6	9	14	20	1000	0.1	5.93
8869	802	11.6	6	9	20	1000	0.1	5.74

Table A.67 Bacteria remaining on the potato after 18 multiple static contacts and 5 s contact time.

Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
4487	400	11.6	8	9	20	1000	0.1	5.80
4487	400	11.6	10	11	20	1000	0.1	5.89
4487	400	11.6	5	6	20	1000	0.1	5.61
4487	400	11.6	8	12	20	1000	0.1	5.87
4487	400	11.6	10	14	20	1000	0.1	5.95
4487	400	11.6	7	6	20	1000	0.1	5.68

Table A.68 Bacteria remaining on the potato after a single contact during 40 s contact time.

Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
8869	802	11.6	55	51	20	1000	0.1	6.59
8869	802	11.6	102	107	20	1000	0.1	6.89
8869	802	11.6	93	86	20	1000	0.1	6.82
8869	802	11.6	76	61	20	1000	0.1	6.70
8869	802	11.6	122	102	20	1000	0.1	6.92
8869	802	11.6	102	80	20	1000	0.1	6.83

Table A.69 Bacteria remaining on the potato after a single contact during 40 s contact time.

Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
7473	674	11.6	44	63	20	1000	0.1	6.60
7473	674	11.6	143	147	20	1000	0.1	7.03
7473	674	11.6	142	148	20	1000	0.1	7.03
7473	674	11.6	82	94	20	1000	0.1	6.81
7473	674	11.6	40	45	20	1000	0.1	6.50
7473	674	11.6	53	55	20	1000	0.1	6.60
7473	674	11.6	45	55	20	1000	0.1	6.57
7473	674	11.6	21	19	20	1000	0.1	6.17
7473	674	11.6	19	19	20	1000	0.1	6.15
7473	674	11.6	4	6	20	1000	0.1	5.57
7473	674	11.6	17	6	20	1000	0.1	5.93
7473	674	11.6	34	35	20	1000	0.1	6.40

Table A.70 Bacteria remaining on the potato after a single contact during 40 s contact time.

Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
5247	470	11.6	42	100	20	1000	0.1	6.72
5247	470	11.6	40	55	20	1000	0.1	6.54
5247	470	11.6	36	19	20	1000	0.1	6.31
5247	470	11.6	88	60	20	1000	0.1	6.74
5247	470	11.6	11	6	20	10000	0.1	6.80
5247	470	11.6	39	57	20	1000	0.1	6.55
5247	470	11.6	33	39	20	1000	0.1	6.42
5247	470	11.6	20	27	20	1000	0.1	6.24
5247	470	11.6	31	35	20	1000	0.1	6.39
5247	470	11.6	29	42	20	1000	0.1	6.42
5247	470	11.6	19	22	20	1000	0.1	6.18
5247	470	11.6	8	4	20	1000	0.1	5.64

Table A.71 Bacteria remaining on the potato after a single contact during 40 s contact time.

Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
4487	400	11.6	61	48	20	1000	0.1	6.60
4487	400	11.6	24	26	20	1000	0.1	6.26
4487	400	11.6	46	59	20	1000	0.1	6.59
4487	400	11.6	35	35	20	1000	0.1	6.41
4487	400	11.6	39	41	20	1000	0.1	6.47
4487	400	11.6	54	52	20	1000	0.1	6.59

Table A.72 Bacteria remaining on the potato after 8 multiple contacts during 5 s contact time.

Normal pressure (Pa)	Additional mass (g)	Mass (g)	Plate count A	Plate count B	Initial dilution (ml)	Plated dilution	Plate (ml)	Log CFU
7473	674	11.6	12	18	20	1000	0.1	6.04
7473	674	11.6	20	26	20	1000	0.1	6.23
7473	674	11.6	15	18	20	1000	0.1	6.08
7473	674	11.6	21	35	20	1000	0.1	6.31
7473	674	11.6	21	24	20	1000	0.1	6.22
7473	674	11.6	30	28	20	1000	0.1	6.33
7473	674	11.6	29	34	20	1000	0.1	6.37
7473	674	11.6	46	33	20	1000	0.1	6.46
7473	674	11.6	47	61	20	1000	0.1	6.60
7473	674	11.6	49	49	20	1000	0.1	6.56
7473	674	11.6	12	13	20	1000	0.1	5.96
7473	674	11.6	36	43	20	1000	0.1	6.46

Table A.73 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 3.75 mm/s and pressure of 4487 Pa.

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
0	4486	400	11.6	1	124	207
2.5	4486	400	11.6	1	34	40
7.5	4486	400	11.6	1	11	2
12.5	4486	400	11.6	1	235	292
Replicate 2						
0	4486	400	11.6	1	176	150
2.5	4486	400	11.6	1	6	6
7.5	4486	400	11.6	1	1	0
12.5	4486	400	11.6	1	184	212
Replicate 3						
0	4486	400	11.6	1	146	146
2.5	4486	400	11.6	1	1	0
7.5	4486	400	11.6	1	0	0
12.5	4486	400	11.6	1	0	0
Replicate 4						
0	4486	400	11.6	1	0	0
2.5	4486	400	11.6	1	0	0
7.5	4486	400	11.6	1	0	0
12.5	4486	400	11.6	1	0	0
Replicate 5						
0	4486	400	11.6	1	262	307
2.5	4486	400	11.6	1	1	0
7.5	4486	400	11.6	1	1	1
12.5	4486	400	11.6	1	3	6
Replicate 6						
0	4486	400	11.6	1	62	58
2.5	4486	400	11.6	1	0	0
7.5	4486	400	11.6	1	1	0
12.5	4486	400	11.6	1	85	76

Table A.74 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 3.75 mm/s and pressure of 2307 Pa.

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
0	2307	200	11.6	1	218	229
2.5	2307	200	11.6	1	0	0
7.5	2307	200	11.6	1	0	0
12.5	2307	200	11.6	1	65	43
Replicate 2						
0	2307	200	11.6	1	71	80
2.5	2307	200	11.6	1	0	0
7.5	2307	200	11.6	1	57	37
12.5	2307	200	11.6	1	15	12
Replicate 3						
0	2307	200	11.6	1	69	95
2.5	2307	200	11.6	1	48	48
7.5	2307	200	11.6	1	33	31
12.5	2307	200	11.6	1	48	35
Replicate 4						
0	2307	200	11.6	1	266	173
2.5	2307	200	11.6	1	0	0
7.5	2307	200	11.6	1	0	0
12.5	2307	200	11.6	1	27	60
Replicate 5						
0	2307	200	11.6	1	90	113
2.5	2307	200	11.6	1	10	11
7.5	2307	200	11.6	1	7	6
12.5	2307	200	11.6	1	206	169
Replicate 6						
0	0	2307	200	11.6	1	44
2.5	2.5	2307	200	11.6	1	0
7.5	7.5	2307	200	11.6	1	0
12.5	12.5	2307	200	11.6	1	10

Table A.75 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 3.75 mm/s and pressure of 1217 Pa.

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
0	1217	100	11.6	1	84	100
2.5	1217	100	11.6	1	0	0
7.5	1217	100	11.6	1	2	2
12.5	1217	100	11.6	1	9	9
Replicate 2						
0	1217	100	11.6	1	25	33
2.5	1217	100	11.6	1	0	0
7.5	1217	100	11.6	1	0	0
12.5	1217	100	11.6	1	47	46
Replicate 3						
0	1217	100	11.6	1	396	311
2.5	1217	100	11.6	1	0	0
7.5	1217	100	11.6	1	1	0
12.5	1217	100	11.6	1	157	138
Replicate 4						
0	1217	100	11.6	1	120	89
2.5	1217	100	11.6	1	0	1
7.5	1217	100	11.6	1	0	1
12.5	1217	100	11.6	1	71	93
Replicate 5						
0	1217	100	11.6	1	85	99
2.5	1217	100	11.6	1	1	0
7.5	1217	100	11.6	1	0	0
12.5	1217	100	11.6	1	52	59
Replicate 6						
0	1217	100	11.6	1	236	249
2.5	1217	100	11.6	1	1	0
7.5	1217	100	11.6	1	0	0
12.5	1217	100	11.6	1	56	37

Table A.76 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 7.75 mm/s and pressure of 4487 Pa (replicate 1 to replicate 3).

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
0	4487	400	11.6	1	22	19
2.5	4487	400	11.6	1	1	0
7.5	4487	400	11.6	1	1	1
12.5	4487	400	11.6	1	0	0
17.5	4487	400	11.6	1	8	5
22.5	4487	400	11.6	1	6	6
27.5	4487	400	11.6	1	239	266
Replicate 2						
0	4487	400	11.6	1	60	68
2.5	4487	400	11.6	1	216	233
7.5	4487	400	11.6	1	103	89
12.5	4487	400	11.6	1	43	33
17.5	4487	400	11.6	1	37	45
22.5	4487	400	11.6	1	35	36
27.5	4487	400	11.6	1	272	193
Replicate 3						
0	4487	400	11.6	1	20	31
2.5	4487	400	11.6	1	1	6
7.5	4487	400	11.6	1	0	2
12.5	4487	400	11.6	1	1	2
17.5	4487	400	11.6	1	7	9
22.5	4487	400	11.6	1	5	9
27.5	4487	400	11.6	1	311	230

Table A.77 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 7.75 mm/s and pressure of 4487 Pa (replicate 4 to replicate 6).

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 4						
0	4487	400	11.6	2	25	26
2.5	4487	400	11.6	2	1	0
7.5	4487	400	11.6	2	0	0
12.5	4487	400	11.6	2	0	0
17.5	4487	400	11.6	2	1	0
22.5	4487	400	11.6	2	1	1
27.5	4487	400	11.6	2	118	98
Replicate 5						
0	4487	400	11.6	2	50	36
2.5	4487	400	11.6	2	2	3
7.5	4487	400	11.6	2	5	4
12.5	4487	400	11.6	2	3	1
17.5	4487	400	11.6	2	42	53
22.5	4487	400	11.6	2	21	25
27.5	4487	400	11.6	2	201	240
Replicate 6						
0	4487	400	11.6	2	342	314
2.5	4487	400	11.6	2	5	6
7.5	4487	400	11.6	2	43	33
12.5	4487	400	11.6	2	2	0
17.5	4487	400	11.6	2	7	8
22.5	4487	400	11.6	2	9	15
27.5	4487	400	11.6	2	116	310

Table A.78 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 7.75 mm/s and pressure of 2306 Pa (replicate 1 to replicate 3).

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
0	2306	200	11.6	1	3	2
2.5	2306	200	11.6	1	0	0
7.5	2306	200	11.6	1	0	0
12.5	2306	200	11.6	1	0	0
17.5	2306	200	11.6	1	0	0
22.5	2306	200	11.6	1	0	1
27.5	2306	200	11.6	1	9	6
Replicate 2						
0	2306	200	11.6	1	107	117
2.5	2306	200	11.6	1	1	0
7.5	2306	200	11.6	1	0	0
12.5	2306	200	11.6	1	30	12
17.5	2306	200	11.6	1	124	172
22.5	2306	200	11.6	1	395	0
27.5	2306	200	11.6	1	69	98
Replicate 3						
0	2307	200	11.6	1	214	237
2.5	2307	200	11.6	1	24	10
7.5	2306	200	11.6	1	7	8
12.5	2306	200	11.6	1	1	4
17.5	2306	200	11.6	1	11	11
22.5	2306	200	11.6	1	15	15
27.5	2306	200	11.6	1	191	216

Table A.79 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 7.75 mm/s and pressure of 2306 Pa (replicate 4 to replicate 6).

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 4						
0	2306	200	11.6	2	131	153
2.5	2306	200	11.6	2	1	3
7.5	2306	200	11.6	2	0	0
12.5	2306	200	11.6	2	0	1
17.5	2306	200	11.6	2	5	5
22.5	2306	200	11.6	2	31	32
27.5	2306	200	11.6	2	95	61
Replicate 5						
0	2306	200	11.6	2	273	359
2.5	2306	200	11.6	2	158	146
7.5	2306	200	11.6	2	31	50
12.5	2306	200	11.6	2	28	25
17.5	2306	200	11.6	2	157	197
22.5	2306	200	11.6	2	171	197
27.5	2306	200	11.6	2	364	327
Replicate 6						
0	2306	200	11.6	2	194	194
2.5	2306	200	11.6	2	4	4
7.5	2306	200	11.6	2	2	1
12.5	2306	200	11.6	2	1	0
17.5	2306	200	11.6	2	35	28
22.5	2306	200	11.6	2	24	21
27.5	2306	200	11.6	2	238	298

Table A.80 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 7.75 mm/s and pressure of 1217 Pa (replicate 1 to replicate 3).

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
0	1217	100	11.6	1	176	235
2.5	1217	100	11.6	1	47	69
7.5	1217	100	11.6	1	27	37
12.5	1217	100	11.6	1	10	12
17.5	1217	100	11.6	1	71	75
22.5	1217	100	11.6	1	20	11
27.5	1217	100	11.6	1	108	95
Replicate 2						
0	1217	100	11.6	1	84	76
2.5	1217	100	11.6	1	12	17
7.5	1217	100	11.6	1	1	4
12.5	1217	100	11.6	1	1	2
17.5	1217	100	11.6	1	9	10
22.5	1217	100	11.6	1	5	8
27.5	1217	100	11.6	1	156	197
Replicate 3						
0	1217	100	11.6	1	139	50
2.5	1217	100	11.6	1	259	186
7.5	1217	100	11.6	1	78	79
12.5	1217	100	11.6	1	54	47
17.5	1217	100	11.6	1	124	124
22.5	1217	100	11.6	1	82	35
27.5	1217	100	11.6	1	66	42

Table A.81 Bacterial transfer via dynamic contact from the potato to the plate at a speed of 7.75 mm/s and pressure of 1217 Pa (replicate 4 to replicate 6).

Cumulative length (cm)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 4						
0	1217	100	11.6	2	211	267
2.5	1217	100	11.6	2	356	281
7.5	1217	100	11.6	2	43	45
12.5	1217	100	11.6	2	31	43
17.5	1217	100	11.6	2	19	25
22.5	1217	100	11.6	2	81	86
27.5	1217	100	11.6	2	191	196
Replicate 5						
0	1217	100	11.6	2	268	163
2.5	1217	100	11.6	2	26	31
7.5	1217	100	11.6	2	1	4
12.5	1217	100	11.6	2	5	6
17.5	1217	100	11.6	2	50	32
22.5	1217	100	11.6	2	40	23
27.5	1217	100	11.6	2	49	56
Replicate 6						
0	1217	100	11.6	2	26	39
2.5	1217	100	11.6	2	1	4
7.5	1217	100	11.6	2	2	2
12.5	1217	100	11.6	2	2	0
17.5	1217	100	11.6	2	6	9
22.5	1217	100	11.6	2	3	0
27.5	1217	100	11.6	2	70	74

Table A.82 Bacterial transfer via dynamic contact from the plate to 10 potatoes at a speed of 3.75 mm/s and pressure of 4487 Pa.

Speed (mm/s)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
3.75	4487	400	11.6	2	79	104
3.75	4487	400	11.6	2	50	40
3.75	4487	400	11.6	2	116	126
3.75	4487	400	11.6	2	34	202
3.75	4487	400	11.6	2	48	22
3.75	4487	400	11.6	2	23	17
3.75	4487	400	11.6	2	16	9
3.75	4487	400	11.6	2	22	26
3.75	4487	400	11.6	2	19	21
3.75	4487	400	11.6	2	0	0
Replicate 2						
3.75	4487	400	11.6	3	113	99
3.75	4487	400	11.6	3	14	20
3.75	4487	400	11.6	3	4	5
3.75	4487	400	11.6	3	3	3
3.75	4487	400	11.6	3	41	46
3.75	4487	400	11.6	3	26	34
3.75	4487	400	11.6	3	16	22
3.75	4487	400	11.6	3	4	3
3.75	4487	400	11.6	3	10	9
3.75	4487	400	11.6	3	61	48
Replicate 3						
3.75	4487	400	11.6	3	107	118
3.75	4487	400	11.6	3	22	24
3.75	4487	400	11.6	3	3	2
3.75	4487	400	11.6	3	1	2
3.75	4487	400	11.6	3	107	109
3.75	4487	400	11.6	3	25	18
3.75	4487	400	11.6	3	9	20
3.75	4487	400	11.6	3	10	12
3.75	4487	400	11.6	3	5	3
3.75	4487	400	11.6	3	2	2

Table A.83 Bacterial transfer via dynamic contact from the plate to 10 potatoes at a speed of 5.00 mm/s and pressure of 4487 Pa.

Speed (mm/s)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
5	4487	400	11.6	2	79	98
5	4487	400	11.6	2	28	26
5	4487	400	11.6	2	12	2
5	4487	400	11.6	2	4	5
5	4487	400	11.6	2	4	1
5	4487	400	11.6	2	5	5
5	4487	400	11.6	2	19	4
5	4487	400	11.6	2	1	2
5	4487	400	11.6	2	4	0
5	4487	400	11.6	2	3	0
Replicate 2						
5	4487	400	11.6	3	108	80
5	4487	400	11.6	3	6	14
5	4487	400	11.6	3	4	3
5	4487	400	11.6	3	1	3
5	4487	400	11.6	3	221	179
5	4487	400	11.6	3	49	45
5	4487	400	11.6	3	27	30
5	4487	400	11.6	3	22	17
5	4487	400	11.6	3	3	5
5	4487	400	11.6	3	7	4
Replicate 3						
5	4487	400	11.6	3	78	84
5	4487	400	11.6	3	5	9
5	4487	400	11.6	3	2	2
5	4487	400	11.6	3	1	3
5	4487	400	11.6	3	30	43
5	4487	400	11.6	3	29	24
5	4487	400	11.6	3	10	4
5	4487	400	11.6	3	5	4
5	4487	400	11.6	3	3	6
5	4487	400	11.6	3	0	4

Table A.84 Bacterial transfer via dynamic contact from the plate to 10 potatoes at a speed of 7.75 mm/s and pressure of 4487 Pa.

Speed (mm/s)	Normal pressure (Pa)	Additional mass (g)	Mass (g)	Day	Plate count A	Plate count B
Replicate 1						
7.75	4487	400	11.6	2	17	21
7.75	4487	400	11.6	2	23	17
7.75	4487	400	11.6	2	4	4
7.75	4487	400	11.6	2	2	1
7.75	4487	400	11.6	2	0	0
7.75	4487	400	11.6	2	0	0
7.75	4487	400	11.6	2	0	0
7.75	4487	400	11.6	2	0	0
7.75	4487	400	11.6	2	0	0
7.75	4487	400	11.6	2	0	0
Replicate 2						
7.75	4487	400	11.6	3	72	86
7.75	4487	400	11.6	3	16	24
7.75	4487	400	11.6	3	6	6
7.75	4487	400	11.6	3	4	5
7.75	4487	400	11.6	3	11	6
7.75	4487	400	11.6	3	5	13
7.75	4487	400	11.6	3	29	30
7.75	4487	400	11.6	3	41	38
7.75	4487	400	11.6	3	12	13
7.75	4487	400	11.6	3	11	8
Replicate 3						
7.75	4487	400	11.6	3	118	118
7.75	4487	400	11.6	3	18	16
7.75	4487	400	11.6	3	1	3
7.75	4487	400	11.6	3	0	1
7.75	4487	400	11.6	3	106	121
7.75	4487	400	11.6	3	35	46
7.75	4487	400	11.6	3	9	13
7.75	4487	400	11.6	3	8	11
7.75	4487	400	11.6	3	7	13
7.75	4487	400	11.6	3	4	7

Table A.85 Bacterial transfer remaining on the potato after bacterial transfer via dynamic contact

Replicate	Speed (mm/s)	Pressure (Pa)	Mass (g)	Mass (g)	Day	Plate A	Plate B
1	7.75	1217	100	11.6	1	73	66
2	7.75	1217	100	11.6	1	133	139
3	7.75	1217	100	11.6	2	114	125
4	7.75	1217	100	11.6	2	142	146
5	7.75	1217	100	11.6	3	157	149
6	7.75	1217	100	11.6	3	112	120
1	7.75	2307	200	11.6	1	60	46
2	7.75	2307	200	11.6	1	180	176
3	7.75	2307	200	11.6	2	114	125
4	7.75	2307	200	11.6	2	142	146
5	7.75	2307	200	11.6	3	157	149
6	7.75	2307	200	11.6	3	112	120
1	7.75	4487	400	11.6	1	140	112
2	7.75	4487	400	11.6	1	139	107
3	7.75	4487	400	11.6	2	220	146
4	7.75	4487	400	11.6	2	250	174
5	7.75	4487	400	11.6	3	157	170
6	7.75	4487	400	11.6	3	236	185
1	3.75	1217	100	11.6	1	92	65
2	3.75	1217	100	11.6	1	105	139
3	3.75	1217	100	11.6	2	95	122
4	3.75	1217	100	11.6	2	213	247
5	3.75	1217	100	11.6	3	166	158
6	3.75	1217	100	11.6	3	237	238
1	3.75	2307	200	11.6	1	180	176
2	3.75	2307	200	11.6	1	208	184
3	3.75	2307	200	11.6	2	140	135
4	3.75	2307	200	11.6	2	184	176
5	3.75	2307	200	11.6	3	111	132
6	3.75	2307	200	11.6	3	141	194
1	3.75	4487	400	11.6	1	180	141
2	3.75	4487	400	11.6	1	119	147
3	3.75	4487	400	11.6	2	231	265
4	3.75	4487	400	11.6	2	151	160
5	3.75	4487	400	11.6	3	158	153
6	3.75	4487	400	11.6	3	149	178

Table A.86 Results of the evaluations of three statistical tests (Tukey, Scheffe, and Dunnett) for least squares means comparisons on bacterial transfer via 18 multiple static contacts for all the treatments applied.

Pressure (Pa)		Estimate	Standard Error	t Value	Pr > t	Adjustment	Adj P
1243	2333	-0.2909	0.1597	-1.82	0.0698	Tukey	0.2658
1243	4513	-0.2972	0.1597	-1.86	0.0641	Tukey	0.2479
1243	8894	-0.4598	0.1597	-2.88	0.0044	Tukey	0.0226
2333	4513	-0.00630	0.1597	-0.04	0.9686	Tukey	1.0000
2333	8894	-0.1689	0.1597	-1.06	0.2914	Tukey	0.7155
4513	8894	-0.1626	0.1597	-1.02	0.3097	Tukey	0.7389
1243	2333	-0.2909	0.1597	-1.82	0.0698	Scheffe	0.3474
1243	4513	-0.2972	0.1597	-1.86	0.0641	Scheffe	0.3279
1243	8894	-0.4598	0.1597	-2.88	0.0044	Scheffe	0.0429
2333	4513	-0.00630	0.1597	-0.04	0.9686	Scheffe	1.0000
2333	8894	-0.1689	0.1597	-1.06	0.2914	Scheffe	0.7726
4513	8894	-0.1626	0.1597	-1.02	0.3097	Scheffe	0.7923
2333	1243	0.2909	0.1597	1.82	0.0698	Dunnett	0.1704
4513	1243	0.2972	0.1597	1.86	0.0641	Dunnett	0.1573
8894	1243	0.4598	0.1597	2.88	0.0044	Dunnett	0.0122

APPENDIX B

SAS analysis

SAS inputs for statistical analysis

```
proc mixed data=potato method=type3;  
class treatment distance day;  
model recovery= treatment distance /outp=mr;  
random day;  
run;
```

The details of the model used to evaluate the effect of the fix variables speed, pressure, and distance, and the random variable day are:

```
proc mixed data=potato method=type3;  
class speed pressure distance day;  
model recovery= speed pressure distance /outp=mr;  
random day;  
run;
```

Where ‘potato’ corresponds to the name of the file that compiles the data. Speed corresponds to the velocity used to slide the potato, and ‘pressure’ to the normal pressure used on the potato unit, ‘day’ to the day the experiment was conducted, and ‘distance’ to the contact distance between the potato unit and the stainless steel. ‘Recovery’ is the number of bacteria transferred.

The statistical model used in SAS for this analysis was:

```
proc mixed data = potato method=type3;  
class speed pressure;  
model recovery = speed pressure speed*pressure;
```

run;

The details of the analysis conducted in SAS are:

```
proc mixed data=potato;  
class unit speed day;  
model recovery= speed/unit/ddfm=kr;  
random day;  
repeated unit/type=cs subject=day*speed;  
run;
```

Where ‘potato’ corresponds to the name of the file that compiles the data. Speed corresponds to the velocity used to slide the potato, and ‘unit’ to the unit of potato used as sample, ‘day’ to the day the experiment was conducted. ‘Recovery’ is the number of bacteria transferred.

Details of the SAS code used are:

```
proc mixed data=potato method=type3;  
class scenario speed day;  
model recovery= scenario speed /outp=mr;  
random day;  
run;
```

In addition, an analysis of the least means square was also performed which helps to identify the significant differences, the details of the SAS code used are:

```
proc mixed data = potato method=type3;  
class scenario speed day;  
model recovery = scenario speed scenario*speed;  
lsmeans scenario*speed/slice=(scenario speed);
```

run;

Where ‘potato’ corresponds to the name of the file that collects the data. Scenario corresponds to the direction bacteria were transferred and the number of potatoes evaluated for bacterial transfer. Speed corresponds to the velocity used to slide the potato unit, and ‘day’ to the day the experiment was conducted.

Results are in Table 3.22 and the details of the SAS model are:

proc mixed data = potato method=type3;

class approach pressure;

*model recovery = approach pressure approach*pressure;*

run;

Where ‘potato’ corresponds to the name of the file that collects the data. Approach represents bacterial transfer type. Speed corresponds to the velocity used to slide the potato unit, and ‘day’ to the day the experiment was conducted.

SAS output for the analysis of the effect of speed and pressure on bacteria remaining on the potato after dynamic contact (1)

The Mixed Procedure

Model Information

Data Set	WORK.POTATO
Dependent Variable	Recovery
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information

Class	Levels	Values
day	13	1 10 11 12 13 2 3 4 5 6 7 8 9
pressure	4	4487 5247 7473 8869
contact	4	1 18 2 8

Dimensions

Covariance Parameters	1
Columns in X	17
Columns in Z	0
Subjects	1
Max Obs per Subject	72

Number of Observations

Number of Observations Read	72
Number of Observations Used	72
Number of Observations Not Used	0

SAS output for the analysis of the effect of speed and pressure on bacteria remaining on the potato after dynamic contact (2)

Type 3 Analysis of Variance								
Source	D F	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Erro r DF	F Valu e	Pr > F
pressure	3	0.5048 76	0.1682 92	Var(Residual) + Q(pressure,pressure*co ntact)	MS(Residu al)	64	2.23	0.093 7
Contact	3	4.5577 93	1.5192 64	Var(Residual) + Q(contact,pressure*cont act)	MS(Residu al)	64	20.1 0	<.000 1
pressure*cont act	1	0.1276 04	0.1276 04	Var(Residual) + Q(pressure*contact)	MS(Residu al)	64	1.69	0.198 6
Residual	64	4.8386 50	0.0756 04	Var(Residual)

Covariance Parameter Estimates

Cov Parm	Estimate
Residual	0.07560

Fit Statistics

-2 Res Log Likelihood	33.5
AIC (Smaller is Better)	35.5
AICC (Smaller is Better)	35.5
BIC (Smaller is Better)	37.6

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
pressure	3	64	2.23	0.0937
contact	3	64	20.10	<.0001
pressure*contact	1	64	1.69	0.1986

APPENDIX C

Journals articles on bacterial transfer used for data collection for the meta-analysis

Table C.1 Journals articles on bacterial transfer for data collection for the meta-analysis

Title	Author	Year	Journal
Modelling transfer of <i>Listeria monocytogenes</i> during slicing of gravad salmon	Aarnisalo, K	2007	International journal of food microbiology
Attachment behavior of <i>Escherichia coli</i> K12 and <i>Salmonella</i> Typhimurium P6 on food contact surfaces for food transportation	Abban, S	2012	Food microbiology
3D finite element model of biofilm detachment using real biofilm structures from CLSM data	Böl, M	2008	Biotechnology and bioengineering
Fresh fruit and vegetables as vehicles for the transmission of human pathogens	Berger, N C	2010	environmental microbiology
Transfer of <i>Escherichia coli</i> O157:H7 from equipment surfaces to fresh-cut leafy greens during processing in a model pilot-plant production line with sanitizer-free water	Buchholz, A	2012	Journal of food protection
Quantitative transfer of <i>Escherichia coli</i> O157:H7 to equipment during small-scale production of fresh-cut leafy greens	Buchholz, A	2012	Journal of food protection
Quantitative transfer of <i>Escherichia coli</i> O157:H7 between beef and equipment contact surfaces	Campos, D	2006	Institute of Food Technologists
Quantification of transfer of <i>Listeria monocytogenes</i> between cooked ham and slicing machine surfaces	Chaitiemwong, N	2014	Food control
Attachment and colonization by <i>Escherichia coli</i> O157:H7, <i>Listeria monocytogenes</i> , <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar typhimurium, and <i>Staphylococcus aureus</i> on stone fruit surfaces and survival through a simulated commercial export chain	Collignon, S	2010	Journal of food protection

Table C.1 (cont'd)

Adhesion of human pathogenic enteric viruses and surrogate viruses to inert and vegetal food surfaces	Deboosere, N	2012	Food microbiology
Transfer of bacillus cereus spores from packaging paper into food	Ekman, J	2009	Journal of food protection
Effects of gallotannin treatment on attachment, growth, and survival of escherichia coli O157:H7 and listeria monocytogenes on spinach and lettuce	Engels, C	2012	Eur food res technol
Modelling and prediction of bacterial attachment to polymers	Epa, V C	2014	Materials views
Effect of shear stress on growth, adhesion and biofilm formation of pseudomonas aeruginosa with antibiotic-induced morphological changes	Fonseca, A P	2007	International journal of antimicrobial agents
Campylobacter transfer from naturally contaminated chicken thighs to cutting boards in inversely related to initial load	Fravalo, P	2009	Journal of food protection
Salmonella typhimurium internalization is variable in leafy vegetables and fresh herbs	Golberg, D	2012	International journal of food microbiology-Israel
The use of meta-analytical tools in risk assessment for food safety	Gonzales-Barron, U	2011	Food microbiology
Surface roughness of stainless steel influences attachment and detachment of escherichia coli O157	Goulter-Thorsen, M R	2011	Journal of food protection
Adhesion of staphylococcus aureus on stainless steel treated with three types of milk	Hamadi, F	2014	Food control
Persistence of E.coli on injured vegetable plants	Harapas, D	2010	International journal of food microbiology
Adherence to stainless steel by foodborne microorganisms during growth in model food systems	Hood, K S	1997	International journal of food microbiology
Estimation of listeria monocytogenes transfer coefficients and efficacy of bacterial removal through cleaning and sanitation	Hoelzer, K	2012	International journal of food microbiology

Table C.1 (cont'd)

Effect of inoculum size, relative humidity, storage temperature, and ripening stage on the attachment of salmonella montevideo to tomatoes and tomatillos	Iturriaga, H M	2003	Journal of food protection
Impact of bacterial stress and biofilm-forming ability on transfer of surface-dried <i>Listeria monocytogenes</i> during slicing of delicatessen meats	Keskinen, L A	2008	International journal of food microbiology
Quantification of attachment strength of selected foodborne pathogens by the blot succession method	Kim, T	2005	Journal of rapid methods and automation in microbiology
Interactions of salmonella enterica with lettuce leaves	Kroupitski, Y	2009	Journal of applied microbiology
Distribution of salmonella typhimurium in romaine lettuce leaves	Kroupitski, Y	2011	Food microbiology
Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods	Kusumaningrum, H D	2003	International journal of food microbiology
A model for bacterial conjugal gene transfer on solid surfaces	Lagido, C	2003	Microbiology ecology
Attachment and growth of salmonella chester on apple fruits and in vivo response of attached bacteria to sanitizer treatments	Liao, C H	2000	Journal of food protection
Interaction between natural microbiota and physicochemical characteristics of lettuce surfaces can influence the attachment of salmonella enteritidis	Lima, M P	2013	Food control
Surface conditioning of stainless steel coupons with skim milk, buttermilk, and butter serum solutions and its effect on bacterial adherence	Manh, D N	2014	Food control
Comparison of different washing treatments for reducing pathogens on orange surfaces and for preventing the transfer of bacterial pathogens to fresh-squeezed orange juice	Martinez-Gonzales, N E	2011	Journal of food protection

Table C.1 (cont'd)

Construction and analysis of fractional multifactorial designs to study attachment strength and transfer of listeria monocytogenes from pure or mixed biofilms after contact with a solid model food	Midelet, G	2006	Applied and environmental microbiology
Growth and persistence of listeria monocytogenes isolates on the plant model arabidopsis thaliana	Milillo, R S	2008	Food microbiology
Modelling transfer of Salmonella Typhimurium DT104 during simulation of grinding of pork	Moller, C O A	2011	Journal of applied microbiology
Inoculum size influences bacterial cross contamination between surfaces	Montville, R	2003	Applied and environmental microbiology
Recovery and transfer of salmonella typhimurium from four different domestic food contact surfaces	Moore, G	2007	Journal of food protection
A poultry-processing model for quantitative microbial risk assessment	Nauta, M	2005	Risk analysis
Biofilm formation of salmonella typhimurium on stainless steel and acrylic surfaces as affected by temperature and pH level	Nguyen, H D N	2014	Food science and technology
Effects of processing and storage variables on penetration and survival of escherichia coli O157:H7 in fresh-cut packaged carrots	O'Beirne, D	2014	Food control
Adhesion of salmonella enteritidis to stainless steel surfaces	Oliveira, K	2011	brazilian journal of microbiology
Detachment of listeria innocua and pantoea agglomerans from cylinders of agar and potato tissue under conditions of couette flow	Perni, S	2008	Journal of food engineering
Modeling transfer of Escherichia coli O157:H7 and Staphylococcus aureus during slicing of a cooked meat product	Perez Rodriguez, F	2007	Meat science

Table C.1 (cont'd)

A process risk model for the shelf life of atlantic salmon fillets	Rasmussen, S K J	2002	International journal of food microbiology
Assessing the cross contamination and transfer rates of salmonella enterica from chicken to lettuce under different food-handling scenarios	Ravishankar, S	2010	Food microbiology
Effects of rhamnolipids and shear on initial attachment of pseudomonas aeruginosa PAO1 in glass flow chambers	Raya, A	2010	Environmental science
Effect of biofilm dryness on the transfer of listeria monocytogenes biofilms grown on stainless steel to bologna and hard salami	Rodriguez, A	2007	Journal of food protection
Biofilm growth on rugose surfaces	Rodriguez, D	2012	Physical review
Bacterial transport suppressed by fluid shear	Rusconi, R	2014	Nature physics
Management of risk of microbial cross-contamination from uncooked frozen hamburgers by alcohol-based hand sanitizer	Schaffner, W D	2007	Journal of food protection
Transfer and Survival of Listeria Monocytogenes during Slicing, Dicing, and Storage of Onions	Scollon, M A	2014	A thesis submitted to Michigan State University
Modeling surface transfer of Listeria monocytogenes on Salami during slicing	Sheen	2008	Food engineering and physical properties
Mathematical modeling the cross-contamination of Escherichia coli O157:H7 on the surface of ready-to-eat meat product while slicing	Sheen, C	2010	Food microbiology
Impact of mechanical shear on the survival of Listeria monocytogenes on surfaces	Sheen	2010	Food engineering and physical properties
Attachment of escherichia coli on plant surface structures built by microfabrication	Sirinutsomboon, B	2011	Biosystems engineering
Variability and uncertainty analysis of the cross-contamination ratios of salmonella during pork cutting	Smid, J	2013	Risk analysis

Table C.1 (cont'd)

Salmonella transfer potential onto tomatoes during laboratory-simulated in-field debris removal	Sreedharan, A	2014	Journal of food protection
Transfer of E.coli O157:H7 to iceberg lettuce via simulated field coring	Taormina, J P	2009	journal of food protection
Bacillus subtilis attachment, colonization, and survival on avocado flowers and its mode of action on stem-end rot pathogens	Tesfagiorgis, D B	2006	Biological control
Desiccation of adhering and biofilm listeria monocytogenes on stainless steel: survival and transfer to salmon products	Truelstrup, H L	2011	international journal of food microbiology
Bacteria-surface interactions	Tuson, H H	2013	Soft matter
Behavior of listeria monocytogenes inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces	Ukuku, O D	2002	Journal of food protection
Effects of cell charge and hydrophobicity on attachment of 16 salmonella serovars to cantaloupe rind and decontamination with sanitizers	Ukuku, O D	2006	Journal of food protection
Effect of time before storage and storage temperature on survival of salmonella inoculated on fresh-cut melons	Ukuku, O D	2007	Food microbiology
Effect of native microflora, waiting period, and storage temperature on listeria monocytogenes serovars transferred from cantaloupe rind to fresh-cut pieces during preparation	Ukuku, O D	2012	journal of food protection
Evaluation of an attachment assay on lettuce leaves with temperature-and starvation-stresses escherichia coli O157:H7 MB3885	Van der Linden, I	2014	Journal of food protection
Attachment of salmonella serovars and listeria monocytogenes to stainless steel and plastic conveyor belts	Veluz, G A	2012	Poultry science
Use of the atomic force microscope to determine the strength of bacterial attachment to grooved surface features	Verran, J	2012	Journal of adhesion science and technology

Table C.1 (cont'd)

Modeling of the effect of washing solution flow conditions on escherichia coli O157:H7 population reduction on fruit surfaces	Wang, H	2007	Journal of food microbiology
Transfer and inactivation of Salmonella during post-harvest processing of tomatoes.	Wang, H	2015	A dissertation submitted to Michigan State University

APPENDIX D

Model fitting results

Output from MATLAB of model fitting regression results for the data sets subsequently used in the meta-analysis.

Dataset	Intercept	confidence interval L	confidence interval U	Slope	confidence interval L	confidence interval U	Shape	confidence interval L	confidence interval U	RMSE	AICc	isError
Sheen_3_1	7.009916	5.756770	8.263063	1.743142	0.539351	2.946932	0.258321	0.125810	0.390831	0.626283	46.307692	0.000000
Sheen1_1	4.825445	4.614599	5.036291	0.020102	-0.024673	0.064878	0.732973	0.341800	1.124145	0.209730	156.480010	0.000000
Sheen1_2	2.988550	2.886714	3.090386	0.000000	-0.000002	0.000003	3.830651	2.437516	5.223786	0.229646	159.666447	1.000000
Sheen1_3	2.996644	2.873267	3.120020	0.000792	-0.000970	0.002553	1.813466	1.276031	2.350902	0.207580	210.718869	0.000000
Sheen1_4	2.497934	2.231265	2.764603	0.069981	-0.039760	0.179722	0.743351	0.369453	1.117249	0.192081	164.742993	0.000000
Shieh1_1	8.316246	7.606116	9.026375	1.398424	0.611631	2.185217	0.246240	0.089584	0.402897	0.329424	46.855348	0.000000
Shieh1_2	8.321955	6.974414	9.669496	1.670972	0.216835	3.125108	0.249230	0.026585	0.471875	0.637802	27.954816	0.000000
Shieh1_3	8.484572	7.955672	9.013471	0.932929	0.467977	1.397881	0.421333	0.296460	0.546206	0.264979	76.743978	0.000000
Shieh1_4	8.398201	7.918730	8.877671	1.351820	0.870912	1.832727	0.283954	0.203025	0.364884	0.234295	85.739654	0.000000
PerezRodriguez1_1	6.651918	6.204306	7.099531	0.347047	0.026619	0.667474	0.571752	0.329803	0.813702	0.084771	109.462651	0.000000
PerezRodriguez1_2	3.799443	3.339605	4.259281	0.143764	-0.083840	0.371367	0.863865	0.379968	1.347762	0.246769	66.722533	0.000000
PerezRodriguez1_3	1.527283	0.861318	2.193249	0.037768	-0.185572	0.261109	1.093211	-0.773186	2.959608	0.397651	47.637617	1.000000
PerezRodriguez1_4	4.471619	3.896502	5.046735	0.074330	-0.112510	0.261169	1.110847	0.315831	1.905863	0.346465	53.149316	1.000000
PerezRodriguez1_5	3.170786	2.608270	3.733301	0.639555	0.151155	1.127956	0.461985	0.251678	0.672291	0.270704	63.019526	0.000000

Aarnisalo1_1	5.143818	4.903363	5.384272	0.018720	-0.021836	0.059276	1.209032	0.631936	1.786127	0.229794
89.634577	0.000000									
Aarnisalo1_2	2.689466	2.097993	3.280939	0.182728	-0.188784	0.554240	0.719772	0.125771	1.313773	0.326775
51.261466	0.000000									
Aarnisalo1_3	2.788243	2.486179	3.090307	0.029172	-0.095072	0.153415	0.841786	-0.275506	1.959078	0.209573
88.472926	1.000000									
Aarnisalo1_4	2.301819	1.805484	2.798154	0.064980	-0.145835	0.275794	0.825493	-0.022904	1.673891	0.339762
64.314205	1.000000									
Aarnisalo1_5	3.475505	2.977882	3.973128	0.099150	-0.130304	0.328604	0.782888	0.182858	1.382919	0.328856
65.945527	0.000000									
Aarnisalo1_6	2.812443	2.692802	2.932083	0.002210	-0.003106	0.007525	1.762765	1.090582	2.434947	0.144211
107.162409	0.000000									
Cantaloupe1	2.113456	1.233674	2.993238	0.617424	-0.238853	1.473701	0.634777	0.078597	1.190958	0.763463
26.277366	0.000000									
Cantaloupe2	2.399002	1.564281	3.233724	0.786578	-0.062660	1.635817	0.568315	0.142313	0.994317	0.717914
29.968225	0.000000									
Honeydew1	2.372216	1.970318	2.774115	1.294433	0.839283	1.749583	0.370403	0.238116	0.502691	0.340557
74.714220	0.000000									
Honeydew2	1.800049	1.080981	2.519116	0.760628	-0.021966	1.543223	0.451855	0.057240	0.846470	0.611920
39.553133	0.000000									
Sheen2_1	10.143951	2.631238	17.656665	5.255808	-2.036595	12.548211	0.103695	-0.006427	0.213817	0.329121
111.916082	0.000000									
Sheen2_2	4.789870	4.181669	5.398070	1.723649	1.126729	2.320569	0.165832	0.114922	0.216741	0.303054
124.252372	0.000000									
Sheen2_3	2.668359	2.257683	3.079035	0.901548	0.476503	1.326593	0.153722	0.076683	0.230760	0.202490
124.870941	0.000000									
Sheen2_4	4.840282	4.385360	5.295203	0.285118	0.069739	0.500497	0.470862	0.346199	0.595525	0.275333
146.294006	0.000000									
Sheen2_5	3.130561	2.813319	3.447802	0.220136	0.040807	0.399466	0.481848	0.326105	0.637591	0.188940
169.610646	0.000000									
Sheen2_6	2.742467	2.440402	3.044532	0.046454	-0.041281	0.134190	0.774732	0.366318	1.183146	0.249232
131.971948	0.000000									

Sheen2_7 2.707756 2.331084 3.084429 0.679329 0.298946 1.059711 0.218836 0.106804 0.330868 0.178408
 76.365078 0.000000
 Chaitiemwong1_1 5.428893 5.200078 5.657708 0.003242 -0.014196 0.020681 1.482165 0.025268 2.939062
 0.265491 115.879115 1.000000
 Chaitiemwong1_2 4.302417 3.784152 4.820682 0.592884 0.098254 1.087514 0.269594 0.106636 0.432552
 0.259680 114.975171 0.000000
 Chaitiemwong1_3 6.236481 5.613195 6.859768 0.314527 -0.060103 0.689157 0.592808 0.309093 0.876522
 0.376107 88.015558 0.000000
 Chaitiemwong1_4 4.647820 4.100223 5.195418 0.576946 0.104532 1.049359 0.363254 0.188564 0.537943
 0.281810 106.086578 0.000000
 Chaitiemwong1_5 5.401082 5.184605 5.617559 0.004996 -0.018696 0.028689 1.357324 0.073899 2.640748
 0.232398 123.633049 1.000000
 Chaitiemwong1_6 4.285653 3.752747 4.818559 0.565574 0.062537 1.068611 0.284135 0.106472 0.461799
 0.267441 110.064091 0.000000
 Chaitiemwong1_7 5.309097 4.935623 5.682571 0.132894 -0.152139 0.417926 0.445354 -0.030331 0.921040
 0.201758 137.840125 0.000000
 Chaitiemwong1_8 3.876086 3.327217 4.424955 0.068573 -0.265727 0.402873 0.597311 -0.578870 1.773491
 0.327825 94.591888 1.000000
 Keskinen_1_25 7.307497 6.275233 8.339761 0.656409 -0.205481 1.518298 0.608889 0.186766 1.031012 0.507392
 33.033339 0.000000
 Keskinen_1_26 7.124616 5.949839 8.299392 1.907789 0.668924 3.146654 0.330601 0.143252 0.517950 0.547637
 30.590814 0.000000
 Keskinen_1_27 6.592830 5.007768 8.177893 1.374536 -0.442694 3.191766 0.175647 -0.183444 0.534737 0.734157
 21.211249 0.000000
 Keskinen_1_28 5.100503 3.718548 6.482458 1.345601 -0.236499 2.927701 0.178943 -0.140686 0.498572 0.640114
 25.597738 0.000000
 Keskinen_1_29 5.295679 4.285992 6.305367 0.793672 -0.179949 1.767294 0.452716 0.079637 0.825796 0.477845
 34.953250 0.000000
 Keskinen_1_30 4.393801 3.530415 5.257188 0.317382 -0.220666 0.855431 0.858231 0.279479 1.436983 0.469231
 35.535346 0.000000
 Keskinen_1_31 4.792558 4.290501 5.294614 0.774634 0.332191 1.217076 0.554332 0.373932 0.734731 0.242923
 56.602633 0.000000

Keskinen_1_32	4.044154	3.582647	4.505662	0.529741	0.120051	0.939430	0.546621	0.302987	0.790255	0.222856		
	59.361618	0.000000										
Scollon1_1	6.804446	5.807152	7.801741	1.221756	0.281764	2.161747	0.414675	0.204624	0.624726	0.884029		
	24.298057	0.000000										
Scollon1_2	3.419335	2.604009	4.234661	0.324453	-0.351720	1.000625	0.570743	-0.045329	1.186815	NaN	NaN	
	0.000000											
Scollon1_3	3.626428	2.548196	4.704660	0.842232	-0.317720	2.002183	0.273836	-0.093010	0.640683	NaN	NaN	
	0.000000											
Vorst1_1	3.216581	2.606478	3.826684	0.314633	-0.230154	0.859419	0.731795	0.044718	1.418871	0.245481		
	41.657484	0.000000										
Vorst1_2	2.758904	2.423201	3.094608	0.413765	0.093234	0.734296	0.495125	0.256392	0.733858	0.122335		
	70.604053	0.000000										
Vorst1_3	54.148672	-80490.735701	80599.033045	51.120365	-80493.838568	80596.079298	0.000804	-1.303460				
	1.305068	0.181917	112.336205	1.000000								
Vorst1_4	2.128668	1.730003	2.527332	0.233698	-0.137879	0.605275	0.565150	0.014509	1.115792	0.181735		
	53.377148	0.000000										
Vorst1_5	1.575724	1.102204	2.049244	0.003376	-0.080197	0.086949	2.344871	-10.283265	14.973006	0.277674		
	36.260932	1.000000										
Vorst1_6	1.486147	0.956113	2.016182	0.180354	-0.393868	0.754575	0.394869	-0.722420	1.512158	0.231402		
	45.130769	1.000000										
Vorst1_7	6.784707	6.237029	7.332384	0.856427	0.398046	1.314809	0.432420	0.304183	0.560657	0.281962		
	86.042886	0.000000										
Vorst1_8	6.287847	5.971984	6.603709	0.091758	-0.011313	0.194828	0.993401	0.675758	1.311044	0.238042		
	96.202448	0.000000										
Vorst1_9	6.245854	5.807198	6.684511	0.162258	-0.027914	0.352431	0.849021	0.525200	1.172842	0.297184		
	82.888217	0.000000										
Vorst1_10	3.184344	2.881558	3.487131	0.427243	0.096943	0.757543	0.447409	0.151198	0.743619	0.129049		
	54.518045	0.000000										
Vorst1_11	2.970745	2.487756	3.453735	0.098610	-0.404488	0.601708	0.368770	-1.174628	1.912168	0.223747		
	56.446120	1.000000										
Vorst1_12	2.660580	2.308570	3.012589	0.518192	0.160306	0.876078	0.404097	0.192197	0.615996	0.163674		
	65.825268	0.000000										

Vorst2_1	4.435522	3.528707	5.342336	0.138519	-0.199130	0.476169	1.141569	0.313795	1.969343	0.595847
	28.751044	0.000000								
Vorst2_2	3.866944	3.311883	4.422006	0.093421	-0.153217	0.340058	0.991620	0.141919	1.841321	0.350045
	50.753464	1.000000								
Vorst2_3	3.659762	3.124112	4.195413	0.002030	-0.007467	0.011528	2.447758	0.887529	4.007987	0.510844
	33.984332	1.000000								
Vorst2_4	1.720251	1.076320	2.364181	0.059615	-0.222902	0.342132	0.999076	-0.539309	2.537461	0.333818
	40.603568	1.000000								
Vorst2_5	1.035323	0.204517	1.866129	0.021834	-0.926927	0.970595	0.277612	-13.471025	14.026250	0.373258
	37.700025	1.000000								
Vorst2_6	2.729032	2.010692	3.447372	0.550284	-0.158702	1.259270	0.276258	-0.000701	0.553217	0.354143
	72.367140	0.000000								
Vorst2_7	6.739629	5.397122	8.082137	0.927279	-0.134736	1.989295	0.484671	0.199613	0.769728	0.703976
	30.485432	0.000000								
Vorst2_8	6.362490	5.560757	7.164223	0.313745	-0.062902	0.690392	0.802883	0.474917	1.130849	0.526312
	48.595598	0.000000								
Vorst2_9	6.224111	5.410141	7.038081	0.294577	-0.117508	0.706662	0.761564	0.382831	1.140296	0.517685
	49.587212	0.000000								
Vorst2_10	3.869802	3.347972	4.391631	0.081048	-0.136389	0.298486	1.050000	0.164461	1.935540	0.335529
	52.362988	1.000000								
Vorst2_11	3.880805	3.132296	4.629314	0.015968	-0.045724	0.077659	1.850779	0.537899	3.163660	0.705955
	24.678520	1.000000								
Vorst2_12	4.517482	3.482909	5.552055	0.155841	-0.199955	0.511638	1.137327	0.390451	1.884203	0.714285
	24.209322	0.000000								
Yan1_1	2.946065	2.429539	3.462592	0.688148	0.240001	1.136295	0.441896	0.275571	0.608221	0.260360
	77.623157	0.000000								
Yan1_2	2.536709	2.160592	2.912827	0.227278	-0.027890	0.482446	0.635334	0.324578	0.946089	0.207498
	88.970250	0.000000								
Yan1_3	1.715386	1.253350	2.177423	0.421670	-0.095317	0.938658	0.123737	-0.132402	0.379875	0.222876
	85.395775	0.000000								
Yan1_4	2.839836	2.471503	3.208170	0.336145	0.061507	0.610783	0.566599	0.345848	0.787350	0.195728
	91.890172	0.000000								

Yan1_5	1.578027	1.084216	2.071837	0.298370	-0.117588	0.714328	0.468174	0.107503	0.828846	0.251223
	79.409413	0.000000								
Yan1_6	2.051289	1.733063	2.369516	0.151073	-0.050700	0.352846	0.681570	0.306605	1.056535	0.180507
	95.938018	0.000000								
Yan1_7	1.715386	1.253349	2.177423	0.421670	-0.095318	0.938658	0.123737	-0.132401	0.379876	0.222876
	85.395731	0.000000								
Yan1_8	1.578023	1.084214	2.071832	0.298367	-0.117589	0.714323	0.468177	0.107503	0.828850	0.251223
	79.409493	0.000000								
Yan1_9	2.682735	2.284341	3.081129	0.368650	0.056293	0.681008	0.527547	0.302152	0.752942	0.207756
	88.908315	0.000000								
Yan1_10	2.946069	2.429541	3.462596	0.688150	0.240002	1.136298	0.441895	0.275570	0.608220	0.260360
	77.623117	0.000000								
Yan1_11	2.051494	1.599412	2.503577	0.137395	-0.094329	0.369119	0.815069	0.324952	1.305185	0.279881
	74.008251	0.000000								
Yan1_12	2.176562	1.847394	2.505730	0.260057	0.016535	0.503580	0.572493	0.318899	0.826086	0.175444
	97.360365	0.000000								
Yan1_13	2.273136	1.864112	2.682160	0.213793	-0.030765	0.458351	0.720127	0.395591	1.044662	0.237723
	82.171202	0.000000								
Yan1_14	2.946069	2.429541	3.462596	0.688150	0.240002	1.136298	0.441895	0.275570	0.608220	0.260360
	77.623117	0.000000								
Yan1_15	1.580777	1.203821	1.957734	0.086780	-0.099442	0.273003	0.837124	0.211207	1.463041	0.236843
	82.356503	0.000000								
Yan1_16	2.004730	1.577531	2.431930	0.115229	-0.082749	0.313207	0.874240	0.369607	1.378873	0.275248
	74.842831	0.000000								
Yan1_17	1.843348	1.375928	2.310769	0.214629	-0.115800	0.545057	0.606241	0.184550	1.027933	0.253626
	78.933345	0.000000								
Wang1_1	4.977068	4.151522	5.802615	0.583527	-0.011658	1.178711	0.659296	0.352856	0.965737	0.796324
	36.836055	0.000000								
Wang1_2	4.662497	3.741210	5.583784	2.171809	1.203309	3.140310	0.277029	0.164034	0.390024	0.801380
	36.076569	0.000000								
Wang1_3	5.188839	4.053564	6.324115	0.717029	-0.203487	1.637545	0.561673	0.187995	0.935350	1.050522
	3.591697	0.000000								

Wang1_4	6.810136	6.606216	7.014055	0.376834	0.236285	0.517383	0.694034	0.580884	0.807184	0.200049	
202.609472	0.000000										
Wang1_5	4.331876	3.454112	5.209639	1.424556	0.585690	2.263423	0.399143	0.239599	0.558687	0.775685	
39.987159	0.000000										
Wang1_6	3.559324	2.514441	4.604206	1.353151	0.235939	2.470363	0.251237	0.045087	0.457386	0.907309	
21.178849	0.000000										
Wang1_7	3.873404	2.595754	5.151054	1.001221	-0.199595	2.202036	0.417765	0.089770	0.745759	1.133284	-
5.508164	0.000000										
Wang1_8	4.798269	4.322821	5.273716	0.336901	0.010893	0.662909	0.697915	0.404014	0.991816	0.467338	
100.790579	0.000000										
Wang1_9	3.463571	2.055431	4.871711	0.995680	-0.414687	2.406046	0.342947	-0.029621	0.715515	1.233225	-
15.649707	0.000000										
Wang1_10	3.991697	2.646518	5.336875	1.177256	-0.319125	2.673637	0.182715	-0.123475	0.488905	1.164859	-
8.805804	0.000000										
Wang1_11	4.661086	3.794245	5.527928	0.617072	0.008770	1.225375	0.680285	0.382331	0.978238	0.844635	
29.768249	0.000000										

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