ABSTRACT

Title of Document: The Development of a Qualitative Risk Assessment and Targeted Storage Decline Kinetics Data as Critical Components for Developing a Full Quantitative Risk Assessment of *Salmonella* Contamination in Milk Chocolate

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Salmonella enterica infections and outbreaks have been associated with chocolate consumption over the last four decades. The source of contamination for these occasional salmonellosis outbreaks are often unidentified, and typically the level of contamination is only a few *salmonella*e per serving. The main goals of this dissertation were to collate relevant scientific information regarding microbial safety of milk chocolate, conduct a qualitative assessment of risk factors for *Salmonella* contamination encountered during the complex processes of cocoa bean cultivation and the subsequent process of milk chocolate manufacturing, and to generate targeted data and survival models for kinetics of *Salmonella* stored in milk chocolate crumb; all components critical to the development of a stochastic quantitative microbial risk assessment.

The farm-to-packaging qualitative assessment provided categorizations of risk for relevant activities and ingredients, identified critical data gaps and “risk spots” and culminated in an Excel-based risk rating tool used to illustrate the usability of the qualitative assessment. Results indicate an overall low residual risk of *Salmonella* contamination of a packaged milk chocolate product for a base model, provided dictates of process control measures are rigorously adhered to, and the risk rating tool enables the assessment of what-if scenarios for deviations from optimal practices.

One of the data gaps identified in the qualitative risk assessment led to investigation into the use of milk chocolate crumb, an intermediate product during milk chocolate processing, and its potential association with *Salmonella* risk. Evaluation of the survival kinetics of *S. enterica* in milk crumb showed a significant ($p<0.05$) dependence of survival on storage temperature, strain and crumb type. Due to the manner in which crumb is generally utilized during milk chocolate processing, findings from this study are the first to link the use of crumb and *Salmonella* risk, and presents promising opportunities for risk reduction which can be explored through further research into optimization of crumb storage parameters. This study serves as a valuable resource to food safety stakeholders in the chocolate industry as it builds the foundation and provides much-needed data for a quantitative microbial risk assessment model that can be used to optimize food safety control programs.

THE DEVELOPMENT OF A QUALITATIVE RISK ASSESSMENT AND TARGETED STORAGE KINETICS DATA AS CRITICAL COMPONENTS FOR DEVELOPING A FULL QUANTITATIVE RISK ASSESSMENT OF *SALMONELLA* CONTAMINATION IN MILK CHOCOLATE

by

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Dedication

To Zoey and Nathan: both of you were born during my PhD journey.

You are emblems of what can be accomplished when passion and grit walk hand in hand on the often-uphill climb to success.

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"If my ship sails from sight, it doesn't mean my journey ends; it simply means the river bends." – John Enoch Powell

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Chapter 1: Introduction

1.1. Chocolate

Chocolate is a traditionally safe product enjoyed by a wide variety of consumers including young children. It has been touted as the world's most favorite confectionary product, with global chocolate markets estimated to have reached $98 billion in 2016, up from $83 billion in 2010 (Markets & Markets, 2017). The United States leads the market in North America with retail sales of chocolate hitting the $18 billion mark in 2011, and a net world cocoa import of about 21%. Europe accounts for approximately half of global chocolate consumption, followed by North America (24%), Asia (15%), South America (9%) and Africa (3%) (Afoakwa, 2016). Chocolate is a rather dense suspension of fine, solid particles of cocoa, sugar and milk (depending on type) with about 60 – 70% solid particles dispersed in a continuous fat phase mostly composed of cocoa butter (Afoakwa, 2010). The major types of chocolate are dark, milk and white, categorized based on their varying cocoa solid, cocoa butter and milk fat contents. Chocolates are generally solid at ambient temperatures of 20 – 25 °C and will melt rapidly at oral temperature of 37 °C, an attribute highly desirable because it provides the smooth, pleasant mouthfeel associated with chocolate during consumption. Out of the three types of chocolate generally available, dark and white chocolate account for an estimated market share of 31% and 18% respectively, while milk chocolate is said to be the most popular worldwide accounting for a share of 51% (Afoakwa, 2016).

1.2. Food Safety Concerns Associated with Chocolate

From a public health and safety standpoint, the microbial hazards associated with chocolate that represent the greatest threats to public health are toxigenic fungi and *Salmonella* (Nascimento et al., 2010). Out of these two organisms, the scientific literature suggests that *Salmonella* is of greater concern to microbiologists since the other is largely classified as a chemical hazard. Since the first discovery of *Salmonella* in cocoa (Depew, 1968), and subsequently in chocolate (D'Aoust, 1977; Gästrin et al., 1972), it has been and still is the most important public health microbial risk associated with chocolate and its related products. The European Commission (2003) opinion on public health status listed chocolate among food products associated with major human salmonellosis outbreaks that spread across a number of countries and affected large populations. Although classified as a chemical hazard, it should be noted that mycotoxins (e.g. Ochratoxin A (OTA) or aflatoxin), toxic substances produced by some fungal molds, can present some challenges to the chocolate industry. Moldy beans are highly undesirable at any stage of production. (Cordier, 1994) however suggests they may not pose a significant problem because mycotoxinogenic molds hardly grow or produce toxins inside the cocoa beans. Other than causing problems during storage, mycotoxigenic fungal species have not been reported in chocolate (Copetti et al., 2014), and where found, have only been in low levels (ICMSF, 2005).

In spite of the use of improved production technologies and increased expertise, the chocolate industry continues to deal with a small risk of *Salmonella* contamination in their products(Werber et al., 2005). Available scientific and epidemiological literature indicate that this pathogen may be introduced at various stages during the production of chocolate.

From a microbial food safety perspective, the farm-to-retail process of chocolate production can be better understood by splitting in two: 1) on-farm processing of cocoa beans and 2) industrial manufacture of chocolate from cocoa beans. Details of these two processes are discussed under literature review. Literature highlights several factors which are thought to play a role in *Salmonella* contamination as well as protection of its resilience during the manufacturing process. There are also other factors which sometimes interact to affect its inactivation. However, there is a considerable gap in the scientific data and information available concerning contamination of raw materials used in chocolate production, the points of entrance for this pathogen into the food chain, as well as its fate through the production process. Currently, the many individual steps associated with the cultivation of cocoa beans and the subsequent production of chocolate have not been integrated quantitatively to develop rigorous evaluation of risks and the identification of new approaches for optimizing the prevention of *Salmonella* contamination in chocolate products at any point during production. The use of risk analysis tools can help identify, define and quantify the effects and interactions of the factors that affect the frequency and extent of *Salmonella* contamination. Risk assessment can be instrumental in providing critical and objective evaluations of the relative impact of prevention and intervention strategies. Furthermore, investment in a microbial risk assessment can provide a cost effective *in silico* means of exploring and identifying additional strategies for pathogen management before investing the substantial resources needed to validate new food safety mitigation strategies.

Chapter 2: Literature Review

This review section examines publicly available information associated with *Salmonella* as a microbial contaminant in the cocoa-to-chocolate production continuum, a detailed description of the entire production process, and an overview of the use of risk assessment tools in food safety.

2.1. The Pathogen: *Salmonella*

Salmonella enterica is the primary cause of non-typhoidal salmonellosis. The signs and symptoms associated *S. enterica* infections are well established, with the primary sign being gastroenteritis that typically includes diarrhea, fever, abdominal cramps and headaches. A small percentage of cases progress to septicemia, with the major sequella being reactive arthritis. The gastroenteritis is typically self-limiting, with recovery occurring within 12 to 72 hours (CDC, 2015). The relative virulence of *Salmonella* serotypes and strains can vary substantially, depending on the virulence factors acquired by the microorganism. The susceptibility among consumers can vary substantially depending on age, health and immune status (Cordier, 1994). Although, *Salmonella* is considered to be a mesophilic bacteria which can survive and replicate between 15°C and 45°C (Montville et al., 2005), several strains have been found to grow at extremes of extrinsic factors such as temperature, pH or water activity.

2.2. *Salmonella* in Chocolate: the Food-Pathogen Pair

The type of food which serves as a vehicle for *Salmonella* can have a significant impact on dose-response relationships, with chocolate outbreaks being associated with remarkably low infective doses; 0.005 to 2 cells/g (Hockin et al., 1989). Tamminga et al (1976) and D'Aoust (1977) hypothesized that these low infective doses may reflect the high percentage of fat in chocolate, which serves to protect the bacteria from gastric acids, combined with a short gastric-residence time. This increases the probability that the *Salmonella*e survive passage through the stomach and reaches the gastrointestinal tract, allowing the cells to colonize and produce clinical symptoms (Hiramatsu et al., 2005; Podolak et al., 2010) also reported that *Salmonella* might survive for months in the presence of sucrose in food matrices like chocolate because the combination of low moisture and high fat levels might have a synergistic effect on survival. There are also suggestions that the reduced inactivation of *Salmonella* cells during heat processes in low-moisture foods is related to water activity and the interaction of the cells with water (Santillana-Farakos et al., 2013).

The potential for the presence of *Salmonella*e in chocolate and its products is not a recent development: salmonellosis infections and outbreaks have been associated with chocolate consumption since the early seventies. These outbreaks (Craven et al., 1975; D'Aoust et al., 1975; Gästrin et al., 1972; Gill et al., 1983; Harker et al., 2014; Kapperud et al., 1990; Werber et al., 2005) have dramatically emphasized the importance of this and other low-moisture foods that were previously considered low-risk as a vehicle of exposure to *Salmonella* (Beuchat et al., 2013). In addition to outbreaks, *Salmonella* has also been isolated from retail chocolate during routine inspections (Food Quality News, 2015a, Guardian, 2015; Torres-Vitela et al., 1995), thereby prompting recalls. Routes of contamination and cross-contamination of chocolate during production are not well understood. Control of *Salmonella* contamination in the production process is challenging because incoming raw materials such as cocoa beans, and ingredients such as cocoa powder and powdered milk, some of which have been incriminated as sources of outbreaks (Bell & Kyriakides, 2002; Cordier, 1994; McDonough & Hargrove, 1968), may carry the pathogen. Inadequate hygiene practices at the manufacturing plant can also facilitate contamination. In the production environment, for example, cross-contamination can occur via contact with contaminated equipment, surfaces, or airborne particulate (Carrasco et al., 2012). Also, cross-contamination of batches and persistence of *Salmonella* in protected niches in various production equipment is of particular concern, as some of the equipment may not be readily accessed for cleaning purposes.

Several other factors contribute to the difficulty the chocolate industry faces regarding this food/pathogen combination. Chocolate production, for the most part, is a dry operation allowing for little to no moisture. In outbreak cases where cocoa beans, the major raw material, or cocoa powder were suspected as vehicles of contamination, *Salmonella* may have survived adverse processing conditions such as heat treatment and desiccation, as well as long shelf-lives before final consumption. As a result of this possibility, it is pertinent that the quality of the raw materials used is optimal as it may ultimately determine the microbial quality of finished products. While *Salmonella* cannot grow in finished chocolate, its ability to survive for a long time in this product, even at low levels of contamination, represents a significant public health risk, especially given that most retail chocolate require no further cooking before consumption. Literature presents evidence that ingestion of fewer than 10^3 *S. enterica* cells can lead to illness, as documented in a 1973 outbreak (Craven et al., 1975). Research has shown that although the low water activity (Aw) and high fat content of chocolate would not favor bacterial proliferation, these conditions can significantly increase thermal resistance so that temperatures reached during chocolate production may not necessarily destroy *Salmonella* cells (D'Aoust, 1977). Furthermore, after ingestion, the fatty matrix of chocolate, particularly milk chocolate, has been hypothesized to protect the *Salmonella* cells from the acidic environment in the gut, thereby enabling colonization of the lower gastrointestinal tract (Craven et al., 1975; D'Aoust, 1977).

2.2.1. Salmonella in Low-moisture Foods and Matrices

Although more prevalent in high-moisture foods, *Salmonella* infections and outbreaks have been increasingly associated with low-moisture foods including but not limited to herbs and spices, milk powder, infant formula, nuts, flour, dry cereal and peanut butter (Beuchat et al., 2013, CDC, 2017). Low-moisture foods may be categorized as having water activity of 0.70 and below (Blessington et al., 2013), although some classifications include foods with a_w up to 0.85 (Beuchat et al, 2013). The survival of *Salmonella* under dry, unfavorable conditions, irrespective of the contamination route, have been determined to be the cause of the numerous documented outbreaks in many low moisture foods (Podolak et al., 2010). Although strain-dependent, *Salmonella* in these type of foods can survive for up to 13 years at ambient temperature (Hockin et al, 1989). *Salmonella* has a minimum water activity growth limit of 0.94 – 0.95 (Fontana, 2008, Mattick et al, 2000), however, it is able to survive in matrices with water activities as low as 0.3 for extended periods (Beuchat et al., 2013). This is because *Salmonella* is regarded as having the physiological mechanisms that facilitate long-term survival in desiccated environments (Mondal et al., 2014). *Salmonella* has been shown to survive in chocolate whose water activity is typically between 0.37–0.5 (Beckett, 2009; Simonsen et al., 1987); and such low a_w contributes to protection against the inactivation of *Salmonella* cells in this food. *Salmonella* is able to persist in certain matrices at low levels for many months at low water activity (Juven et al., 1984; Oni et al., 2015; Uesugi et al., 2006) and particularly at low temperatures (Hiramatsu et al., 2005), demonstrating its ability to resist various stresses. It has been observed that *Salmonella* cells which have undergone desiccation appear to be more resistant to heat treatments and are potentially more infectious than cells that persisted in high-moisture conditions (Barrile et al., 1970; Podolak et al., 2010). Hence, not only initial contamination levels but also the history of exposure to variable temperatures and moisture levels, in addition to individual factors such as serotype and strain used, determines whether *Salmonella* will be present in a final food product. Survival or inactivation of *Salmonella*e in low-moisture foods cannot be predicted solely on the basis of water activity; several other factors are known to play a role. This concept has been explained using the “synergistic effect” theory. In chocolate for example, the synergistic or interactive effect of low water activity and high fat content is said to possibly increase heat resistance of *Salmonella* in this type of food (Cordier, 1994; Hiramatsu et al., 2005), and increased heat resistance in low-moisture foods is thought to be the result of the interaction of *Salmonella* cells with components in a food matrix (Podolak et al., 2010). Therefore, interactions between low water activity, acidity, fat and solute content, composition and structure of food matrix and environmental factors such as temperature and storage conditions, appear to play a significant role in

Salmonella survival (Simonsen et al., 1987). To date however, the synergistic effect theory and its associated mechanisms remain to be fully elucidated.

2.3. History of Outbreaks and Recalls

One of the first recorded *Salmonella* outbreaks associated with chocolate occurred in the 1970s when a *S.* Durham strain was linked to an epidemic that caused infections in 110 people (Gästrin et al., 1972). Another major outbreak occurred a few years later in North America involving a different serotype: *S.* Eastbourne contaminated chocolate candy and resulted in an infection of about 200 people, most of whom were children (Craven et al., 1975; D'Aoust et al., 1975). Over the next decade, several other outbreaks were recorded. **Table 2.1** shows the various strains of *Salmonella* which have been found to contaminate chocolate and chocolate products over the past forty years.

Table 2. 1. Worldwide Outbreaks of Illnesses from *Salmonella* Associated with Consumption of Chocolate/Chocolate Products

Salmonella strain	Region identified	Year	Specific product implicated	Affected population	Contamination source	References
S. Cubana	USA	Mid 1960s	Pink chocolate summer coating	Varied	Unknown	(Cordier, 1994)
S. Durham	Sweden	Early 1970s	Cocoa powder/chocolate	110 individuals	Cocoa powder	(Gästrin et al., 1972)
S. Eastbourne	USA/Canada	Mid 1970s	Milk chocolate candy	200 individuals; mostly children	Raw cocoa beans	(Craven et al., 1975; D'Aoust et al., 1975)
S. Napoli	UK/Italy	Early 1980s	Chocolate bars	245 individuals; mostly children	Unknown	(Gill et al., 1983)
S. Nima	Canada/ Belgium	1980s	Chocolate coins	24 individuals	Unknown	(Hockin et al., 1989)
S. Typhimurium	Norway	Late 1980s	Kids chocolate	Children (>300)	Avian wildlife suspected	(Kapperud et al., 1990)
S. Oranienburg	Germany/ Canada	2001/02	Varied chocolate products	462 individuals; patients age range 0 - 92	Unknown	(Werber et al., 2005)
S. Montevideo	UK	2006	Chocolate bars	56 individuals	Chocolate crumb (via leaky pipe)	(Food Standards Agency, 2006; The Guardian, 2006)

The first recall ever documented associating *Salmonella* with chocolate occurred in the 1960s and prompted the US Food and Drug Administration (FDA) to begin investigating *Salmonella* in confectionery products (Cordier, 1994). In more recent times, there have been a handful of recalls due to *Salmonella* contamination in chocolate products both in the

US and across Europe (FDA, 2012; Food Standards Agency, 2006; The Guardian, 2015; Food Quality News, 2015a). A recent voluntary recall was made “out of an abundance of caution” and occurred as a result of concern over *Salmonella* contamination of milk powder, an ingredient used in the confection’s coating (FDA, 2016). In a few of the cases mentioned here, both cocoa beans and cocoa powder were suspected to have been contaminated with *Salmonella* prior to their use in chocolate production. One of the incidents was attributed to cross-contamination during production (Food Quality News, 2015b), while the source of another was traced to a leaky pipe within the production facility (Guardian, 2006). Yet, a historical analysis of these outbreaks reflect the challenges in pinpointing a specific source along the production chain where contamination occurred. These outbreaks also reflect the adaptability of *Salmonella* to different food processing environments.

2.4. From Cocoa to Chocolate – Overview of the Production Process

2.4.1. Introduction to Cocoa

Cocoa beans are the seeds of the fruit from cocoa trees (*theobroma cacao*) which grow in tropical regions around the equator but are native to the Amazon basin of South America. The major growing regions of the world are: West Africa, South East Asia and South America, with the five largest producing countries being Cote d'Ivoire, Brazil, Ghana, Indonesia and Malaysia (Afoakwa, 2010; Beckett, 2009). Cote d'Ivoire, a small country in West Africa, is known to be the largest producer and exporter of cocoa worldwide, followed by Ghana and Indonesia (ICCO, 2015). West Africa and Indonesia are the origins of significant volumes of cocoa exports; while other large producing regions have internal markets that absorb cocoa products.

2.4.2. Cocoa Cultivation

Cocoa cultivation requires an appropriate climate – generally high humidity (70 – 80% day, 90 – 100% night), temperature ranging between 18 – 32 °C (65-90 °F), and yearly well-distributed rainfall (Afoakwa, 2010). Harvesting of ripe cocoa fruits, also known as pods, is a process carried out over a period of days or weeks depending on the size of the plantation. Sometimes harvesting is staggered when the crops do not all ripen at the same time (Beckett, 2009). Climate and variety of cocoa usually determine timing of harvest in different countries. In Indonesia, for example, cocoa harvest is not confined to one short period but is often spread over several months once or twice a year – March-July and September-December (ICCO, 2015). Although cocoa fruits vary in physical appearance – shape, size color etc., the major classification that has been used to distinguish varieties is flavor quality. The two main varieties of cocoa fruits are *Criollo* - a white-seeded variety native to South and Central America, and *Forastero* – a purple-seeded variety native to the Amazon. *Forastero* has been deemed more prolific, and hence accounts for about 95 % of the world production (Fowler, 2009; Thompson et al., 2013). *Trinitario* is a third and less common variety that is said to be a hybrid of *Criollo* and *Forastero* trees.

2.4.3. Health Benefits derived from Cocoa

Cocoa is known to be rich in polyphenols, and possible health benefits from consumption of food products made using cocoa have been suggested as cocoa beans and its derivatives are rich in antioxidants.

2.5. Steps in Chocolate Production

The farm-to-retail process of making chocolate is outlined in the steps below:

1. Cocoa pod harvest
2. Cocoa beans fermentation
3. Drying and storage of beans for transportation
4. Receiving, cleaning and quality assessment
5. Sterilization, alkalization and roasting beans
6. Shelling and winnowing beans
7. Grinding nibs, mixing and refining of chocolate mass
8. Conching of chocolate paste
9. Pressing, tempering of semi-finished chocolate product
10. Packaging and distribution

As mentioned earlier, this farm-to-retail process of chocolate production can be better understood by splitting it in two parts:

Primary processing - on-farm pre-processing of cocoa beans which consists of the first three steps above; also referred to in this manuscript as 'pod to bean'.

Secondary processing - industrial manufacture of chocolate from cocoa beans which entails steps four to ten above; also referred to as 'bean to bar'.

2.5.1. Part 1: Primary Processing – pod to bean

2.5.1.1. Harvesting

Harvesting cocoa pods is a manually labor-intensive process which involves removal of pods from trees by hand or tools such as hooked knives. After removal, the pods may be gathered into heaps and allowed to sit for a few days, a practice said to be beneficial to the flavor and quality of the beans during subsequent fermentation (Fowler, 2009). The pods are manually or mechanically split open; an action that marks the end of sterility of the beans in the pod. The approximately 30-40 beans enveloped in the sweet, white, mucilaginous pulp inside each pod are scooped out either manually or mechanically, and are immediately exposed to microorganisms from sources including the fruit's exterior surface, workers' hands and tools and the immediate environment (dust, transportation containers, insects, birds, rodents etc.). This inadvertent inoculation initiates microbiological bombardment of the sugar-rich, acidic pulp by various microorganisms including yeasts, and initiates the natural fermentation process (Afoakwa, 2010). The pulp is an excellent medium for microbial growth as it contains about 10–15% sugars, and moisture content of freshly harvested beans is about 65% (Fowler, 2009).

2.5.1.2 Fermentation

This is a key processing stage that facilitates removal of the pulp and prevents germination of the beans. The primary purpose of fermentation of cocoa beans is to induce several biochemical transformations within the beans and subsequently induce development of aroma, color and flavor precursors of chocolate (Thompson et al., 2013). It is also during fermentation that the extremely bitter and astringent taste of cocoa beans are significantly reduced. The fermentation process vary according to geographic and traditional practices around the world. After removal of beans from pod, the beans and its adhering pulp are collected in heaps, boxes or baskets for fermentation which lasts anywhere between 2-3 days (*Criollo* variety beans) or 5-8 days (*Forastero* beans)

(Thompson et al., 2013). In small-scale or traditional settings as seen in West Africa, cocoa beans undergoing heap fermentation are left in a pile or a hole in the ground and covered with banana or plantain leaves. Basket and box ("sweat boxes") fermentation are done in a similar fashion with plantain leaves lining and covering the containers. The fermenting mass is regularly turned to ensure even fermentation. On bigger plantations or fermentaries, large wooden boxes are designed to hold the mass during fermentation, with provisions made for the liquefied pulp to drain away and for good aeration (Fowler, 2009; ICCO, 2015).

The microbial population during fermentation is often variable in quantity and type, but largely consist of yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB), all of which develop in succession (Thompson et al., 2013). Based on this, fermentation can be described as occurring in roughly three phases.

i. ***Anaerobic hydrolytic phase (yeast phase)***: During the first 24 h, natural yeasts multiply in the pulp, causing breakdown of the sugars and conversion into carbon dioxide and ethanol under conditions of low oxygen and high acidity – pH 3.4 – 4.0 (Fowler, 2009, Thompson et al., 2013). Increasing yeast populations produce pectinolytic enzymes which degrade the adhering pulp and cause it to liquefy. The resulting juice known as "sweatings" is drained off and this process is normally accompanied by a gradual rise in temperature (Afoakwa, 2010). Bean death usually occurs within 48 h and is initiated by the anaerobic environment created by the buildup of acetic acid and ethanol. The rising pH creates a self-limiting factor on further yeast growth (Afoakwa, 2016). After the bean dies, its soluble components seep out through the skin and are drained off.

Thus, although the cocoa bean itself does not undergo microbial fermentation, the products of the fermenting pulp induce important chemical changes within the bean (Thompson et al., 2013). Mixing the beans at this stage as practiced by many farmers has the immediate effect of promoting aeration and subsequent bacterial activity (Burndred, 2009).

ii. ***Oxidative condensation phase (bacteria phase)***: as yeast population declines under relatively aerobic conditions, this phase is initially dominated by LAB which convert sugars and the organic acids present into lactic acid. Temperature of the fermenting pulp mass increases as microbial activity increases, creating an environment suitable for growth of AAB, and these bacteria eventually replace LAB as dominant microflora (Afoakwa, 2010). AAB are responsible for converting alcohol into acetic acid, a strongly exothermic reaction responsible for a significant rise in temperature – up to 45 - 50°C or higher (Fowler, 2009; Thompson et al., 2013) and increased aeration.

iii. The final phase of cocoa bean fermentation is marked by increased aeration, increase in pH (up to 3-5 – 5.0) (Afoakwa, 2016), as well as the aforementioned temperature rise. These conditions encourage the growth and dominance of *Bacillus* spp., the aerobic, spore-forming and mostly thermo-tolerant bacteria see **Fig. 2.1**). Bacillus production in cocoa fermentation is not necessarily desirable as they are said to produce compounds that can adversely affect the flavor of chocolate, and their production is mostly attributed to over-fermentation (Schwan & Wheals, 2004). Some of these bacteria can be isolated from cocoa beans which have been dried and roasted at temperatures of up to 150 °C

(Afoakwa, 2010). After all the ethanol present has been oxidized to acetic acid, and subsequently to carbon dioxide and water, fermentation subsides and the temperature of the bean mass rapidly decreases (Thompson et al., 2013).

Throughout fermentation, concentrations of ethanol, lactic acid and acetic acids successively increase and decrease, resulting in an excess of acid remaining in the beans post-fermentation. Fermented beans with lower pH (4.75 -5.9) are considered to be properly fermented, while beans with relatively high pH (5.5-5.8) are considered unfermented (Afoakwa, 2010). Enzymic and microbial fermentations post-harvest introduce both physical and chemical changes in cocoa beans over 5-7 days. The fermentation process has to be well monitored and controlled as immature or unfermented beans yield very little chocolate flavor after roasting, while too much fermentation produces off, putrid flavors (Beckett, 2000).

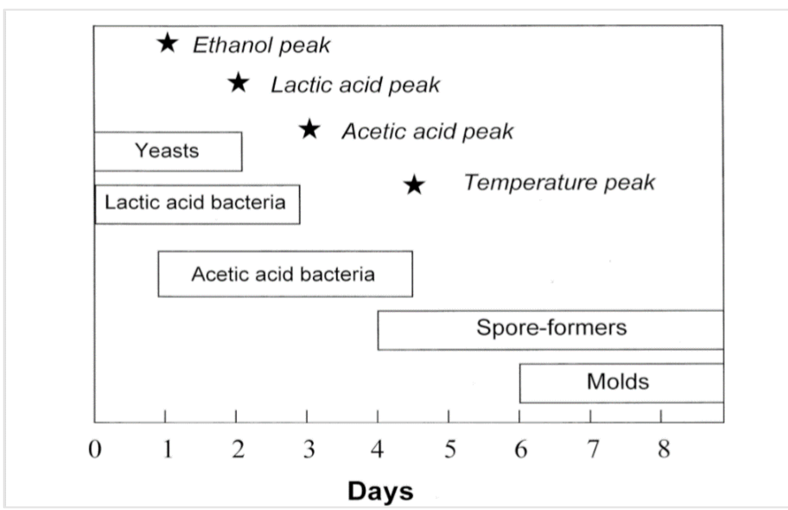


Figure 2. 1. Schematic of a microbial succession during cocoa bean fermentation in Bahia, Brazil. The rectangular boxes indicate the periods during the fermentations when a particular microbial group is most abundant and/or important. The stars indicate the timing of peaks of metabolites and temperature. (Source: Schwan & Wheals, 2004).

Chocolate flavor is largely dependent on the enzymatic formation of flavor precursors within the bean cotyledon that are unique to cocoa (Thompson et al., 2013). Subsequent roasting of these dried beans containing precursors further develop the flavor, aroma notes and color of the cocoa beans in readiness for chocolate manufacture.

2.5.1.3 Post-fermentation – drying, transportation and storage

At the end of fermentation, the beans are removed from the heaps, boxes or baskets and dried on mats or polypropylene sheets spread on the ground or on raised platforms. Their moisture content at this point is about 40 – 60% (Afoakwa, 2010). Drying usually takes about 8 days of sunlight, or up to 4 weeks although mechanical driers are used in some parts of the world for convenience or lack of adequate sunlight (Fowler, 2009; Schwan and Wheals, 2004). The moisture content target post-drying is between 6 and 8% - a level that is microbiologically optimal for safe storage and mold prevention (Burndred, 2009; Copetti et al, 2014). Ultimately, the efficiency (or lack) of the drying process can influence the shelf life and microbiological quality of the cocoa beans. Although drying practices vary across regions, traditional sun-drying has been identified as that which generates optimal chocolate flavor, when compared to other natural or artificial drying methods. This is because it allows for slow migration of moisture throughout the bean, which facilitates transportation of the flavor precursors formed during the fermenting process (Thompson et al., 2013). In addition to limiting mold growth during storage and transportation, drying also helps reduce acidity levels and astringency in cocoa beans.

Current marketing practices dictate that fermented and dried cocoa beans might remain in storage for anywhere between 3 to 12 months (Thompson et al., 2013). Storage locations might include farm or plantation warehouses, shipping docks during import or export and warehouses at manufacturing facilities prior to further processing. Optimal transportation conditions are necessary for prevention of moisture buildup as well as mold growth. If properly dried and stored, cocoa beans are quite stable and will not deteriorate in quality for several years (Fowler, 2009).

2.5.2. Part 2: Secondary Processing – bean to bar

The complexity of chocolate making among manufacturers need be acknowledged as processes and methods are highly varied around the world. This is due in part to unique industry practices, specific type of end-product and targeted consumer preferences. There are however basic steps generally employed by all chocolate manufacturers (see **Fig. 2.2**), and these steps are discussed below.

2.5.2.1. Bean receipt, cleaning and quality assessment

On arrival at the manufacturing factory, the beans must be cleaned by sorting, de-stoning and metal-detection procedures in order to remove potential physical hazards such as metal pieces, stones, wood and other foreign materials. Standards for cocoa bean quality have been set by major cocoa trading markets, and are expected to be adhered to by manufacturers (Afoakwa, 2010). This is in addition to, or in combination with, any internal criteria put in place by producing countries or recipient manufacturing companies. In the US for example, the FDA set standard trade contracts for cocoa bean quality (ITC, 2001). In order to assess quality, proper sampling and evaluation must be carried out to assess characteristics such as defect levels and degree of bean fermentation, and identify beans that are moldy, flat or shrunken (contain no nibs), germinated or infested (Fowler, 2009).

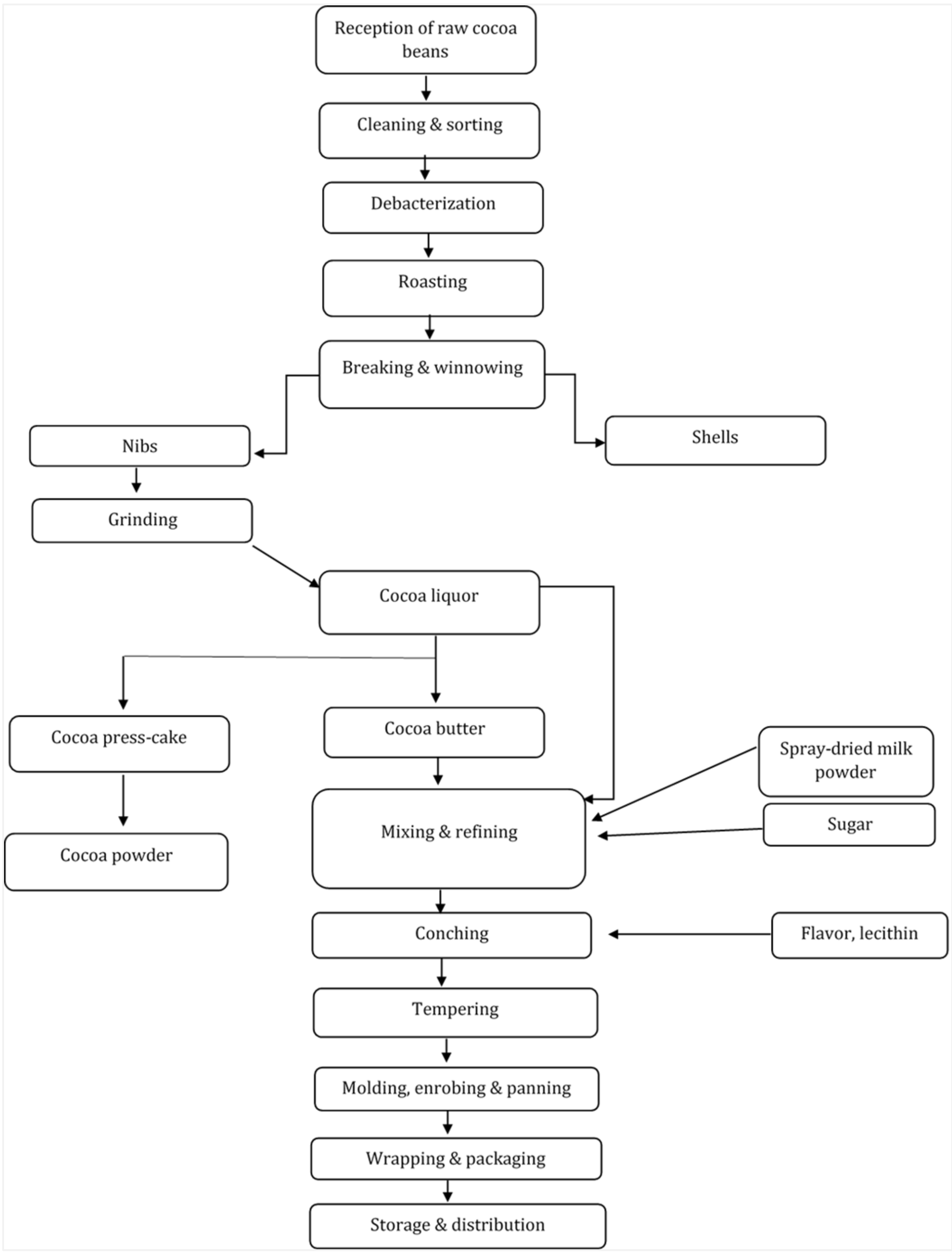


Figure 2. 2. Schematic representation of industrial chocolate production – bean to bar

2.5.2.2. Roasting, Debacterization and Alkalization

Depending on manufacturer practices, these three procedures can be carried out in alternating order, i.e., debacterization and/or alkalization can be done before or after the roasting step, and vice versa. In many cases, debacterization, also referred to as sterilization or steam-treatment, is carried out during roasting (Burndred, 2009). It is used as a treatment process for whole cocoa beans or nibs (broken bean pieces) and involves exposing them briefly to a combination of high temperature and steam to aid microbial inactivation (Afoakwa 2010; Burndred, 2009). Heat and humidity can help destroy bacteria and spores and reduce total plate count to less than 500 per gram (Afoakwa, 2016). Interestingly, the process of steam-treating the nibs prior to roasting was apparently introduced to improve flavor, rather than for safety reasons (Ziegleder & Oberparleiter, 1996). Initial sterilization is commonly carried out at 40–60°C for 10–15 min, and subsequently, drying at 98–110°C to achieve a moisture level of 3% completes the sterilization process (Burndred, 2009). If sterilization is carried out after roasting, a fine spray of steam is introduced into the roasting chamber for about 20 seconds (Awua, 2002). This post-roasting sterilization is done to further destroy any heat-resistant bacteria or spores that may have survived roasting (Afoakwa, 2010). Both heat processes also help to loosen the bean shells. Based on available information in literature, it is unclear how common the practice of debacterization is among chocolate manufacturers.

Alkalization or Dutching, a process that was introduced by a Dutchman in the early nineteenth century, is mostly used in cocoa powder production. Carried out before or during roasting, this process involves the treatment of cocoa beans or nibs with an alkali solution such as potassium carbonate in order to modify the color and flavor of cocoa powder or liquor (Ziegleder, 2009). This artificial pH change is said to reduce the acidity and astringency of cocoa and enhance its solubility particularly in powdered products such as drink mixes (Miller et al., 2008).

Roasting achieves two important things: completes the chemical reactions needed for the development of chocolate flavors, and reduces microbial contamination (Copetti et al, 2014). A study conducted in the seventies suggests that optimal roasting is best achieved by a slow reduction in moisture content to about 3% followed by rapid heating to the final roast temperature (Mohr et al, 1978). Different roasting temperature ranges are reported in literature: Burndred (2009) reports a roasting temperature between 110 and 140°C, while Afoakwa (2010) advises that temperatures could go up to 170°C, depending on the roasting method used (dry or moist). The duration of the roasting process can vary between 15 – 45 min, and sometimes up to 2 h (Burndred, 2009; ICMSF 2005; Stobinska, 2006). These temperature/time combinations (roasting profiles) often depend on the desired flavor profile of the final product as well as the type of equipment being used. The final moisture content of roasted beans is reported to be 1-2 % (Simonsen et al., 1987).

Microbiological safety considerations

1. After receipt of cocoa beans at the manufacturing facility, the presence of *Salmonella* up to a certain level, detected during testing, is not a suitable reason to reject a shipment of cocoa beans, mainly because it is expected that high microbial levels come with the fermentation and drying processes that took place on the farm. Total bacterial load on cocoa beans received could range between 1 to 10 x 10^6 CFU/g

(Beckett, 2009), but subsequent processing steps involving high temperatures are expected to achieve pathogen destruction. Specifically, the roasting step is regarded as the major lethal step for microbial contaminants including *Salmonella* during chocolate production (Cordier, 1994).

2. From a microbiological safety perspective, it is useful to note that practices regarding roasting and an earlier winnowing step vary during processing:
    a. Whole bean roasting – this traditional method involves pre-heating whole bean shells to about 100°C to make them brittle and easier to remove. The beans are then roasted, de-shelled and winnowed.
    b. Nib roasting – here the whole cocoa beans are first broken, after which the shell and nibs are separated by winnowing. The nibs are treated by alkalization (if preferred) and then roasted.
    c. Liquor roasting – cocoa beans are thermally pre-treated before winnowing, and the nibs are ground to liquor prior to roasting.
3. Studies have shown that the type of matrix - cocoa beans or nibs – processed during roasting plays a significant role on the heat resistance of *Salmonella* cells (Nascimento et al, 2012).
4. According to ICMSF (1986), unless cocoa bean roasting is satisfactorily done and subsequent handling of the roasted beans is well controlled, intermediate chocolate products such as cocoa powder, butter or liquor may be contaminated with *Salmonella*.
5. The combination of thermal and alkalization treatments of cocoa nibs, liquor or powder is said to produce a strong sterilizing effect (Minifie, 1999). This lends

credence to the probability that any contamination observed beyond this stage is a result of cross-contamination within the facility.

6. It is expected that microbiological testing is carried out after any roasting profile; this is to ensure total plate count reduction and destruction of microorganisms of concern, especially *Salmonella*.

2.5.2.3. Shelling and winnowing

The cleaned, roasted beans are cracked and broken into pieces (nibs) and the loosened, relatively lighter shell particles are removed during the following step, a mechanical process known as winnowing. The efficiency of winnowing is said to be a critical point for reducing the level of physical contaminants still possibly present in the nibs before subsequent processing steps (Beckett, 2000; Copetti et al., 2014). These nibs are the most valuable part of bean as they are the raw material actually used in production. After winnowing, the nibs are stored for further processing.

2.5.2.4. Grinding, Mixing and Refining

After the roasting step, the cocoa nibs are ground into a thick liquid known as cocoa mass or liquor, a homogenous form that contains roughly 50% cocoa solids suspended in about 50% cocoa butter, with particle sizes up to 30 μm (Afoakwa, 2016). This liquor is the basis for all chocolate and cocoa products. The temperature and degree of grinding varies based on the type of cocoa nib used and the desired end product. However temperature needs to be at or above 35°C, the melting point of cocoa butter (Beckett, 2009). Sometimes, manufacturers will blend beans from different origins into a unique formula in order to achieve consistency in quality of their final product; a step which can also be carried out prior to roasting (Fowler, 2009).

The cocoa liquor is mechanically compressed using hydraulic presses to extract the cocoa butter, leaving a solid mass called cocoa press-cake. Depending on the time and settings of the hydraulic press, this press-cake can have fat content ranging between 10 and 24 % (Afoakwa, 2010). The processing now takes two different directions: the press-cake is prepped for the production of cocoa powder, while the extracted cocoa butter is pumped into a holding tank for further processing (see **Fig. 2.2**). The press-cake is kibbled into smaller pieces, blended and finely pulverized to produce cocoa powder. A wide variety of cocoa powder products are manufactured worldwide depending on ingredients combination: flavorings, emulsifiers such as lecithin, sugar and other desired ingredients can be added for a customized taste. To proceed with chocolate production, cocoa liquor is combined with ingredients such as cocoa butter, sugar, skimmed, spray-dried or non-fat dry milk (NFDM) powder or milk fat, depending on the type of chocolate being made, and thoroughly mixed for about 15 min at 40 – 50°C (Afoakwa, 2016).

****Basic ingredients in major types of chocolate***

Dark Chocolate – cocoa liquor, sugar, cocoa butter

Milk Chocolate – sugar, milk (spray-dried or full cream powder), cocoa liquor, cocoa butter, milk fat

White chocolate – sugar, milk (spray-dried or full cream powder), cocoa butter (deodorized), milk fat

(*ingredients listed in decreasing order of magnitude. Recipes adapted from (Beckett, 2008)

Emulsifying agents such as lecithin, and flavor can also be added now or at a later stage. The mixing is largely mechanical, with some large-scale manufacturers using automated kneaders to carry out continuous mixing (Awua, 2002). Next, the mixture undergoes refining, a texture-enhancing process, by travelling through a series of rollers until a smooth paste is formed. The main aim of refining is to ensure proper grinding of particles such as sugar and, for milk chocolate, solid milk particles, with final particle sizes usually measuring less than 30 µm (Beckett, 2000). This step is important for the sensorial quality of chocolate as final products should have a smooth, not gritty, mouthfeel.

Microbiological safety considerations

1. In many cases, chocolate manufacturers would purchase cocoa mass or liquor from third-party cocoa processors, some of whom may be situated in the originating country. The danger inherent in this practice is that the higher likelihood of poor hygiene standards can increase the possibility of *Salmonella* contamination in the cocoa mass (Burndred, 2009).
2. Some manufacturers are known to store refined cocoa liquor in heated tanks at a temperature of about 90 – 100°C. This practice is said to serve a dual purpose of additional microbial destruction and ageing (Awua, 2002).
3. To prevent cross- or re-contamination at this critical pathogen reduction step or at later stages in production, very rigorous hygienic practices must be put in place.

2.5.2.5. Conching

Conching can be described as a kneading process that marks the second and only other step involving high heat in chocolate production. It is an essential stage that further develops flavor and texture while removing undesirable chemical compounds, and reduces viscosity and particle size of the final chocolate product (Beckett, 2009). After the previous grinding and refining steps, the chocolate mass still presents as a dry and crumbly powder. The conching process involves prolonged mixing and agitation at elevated temperatures, typically between 50 and 80°C for anywhere between 6 – 24 h (Beckett, 2008; Krapf & Gantenbein-Demarchi, 2010). The shearing and mixing action or mechanical energy provided by the conches, the equipment in use, help break up any fat-trapping agglomerate and work the flaky crumbs into a fluid paste. Moisture of up to 30 % and other undesirable volatile flavors like acetic acid are reduced in the process via evaporation. Beckett (2009) suggests that conching temperature for milk chocolate should not exceed 55°C, while that of plain chocolate can go up to 80°C. To optimize viscosity, additional cocoa butter and lecithin can be added toward the end of conching to liquefy the thick chocolate paste and produce a good flow before the next step (Afoakwa, 2010).

Microbiological safety considerations

1. In addition to roasting, the conching step is also thought to play a role in the inactivation of *Salmonella* (Krapf & Gantenbein-Demarchi, 2010). Despite the high temperatures involved in the roasting and conching, past studies including those on thermal inactivation of *Salmonella* in chocolate (Barrile et al., 1970; Cordier, 1994; Goepfert & Biggie, 1968; Lund & Eklund, 2000; Nascimento et al., 2012; Peñaloza-Izurieta et al., 2008; Rieschel & Schenkel, 1971) have shown that the thermal inactivation of *Salmonella* cannot be assured. This especially rings true in the 1975 *S*. Eastbourne outbreak (Craven et al, 1975), where it was indicated that raw cocoa beans were the apparent source of *Salmonella* which must have survived the heating

steps during production. Some of these studies also document concern about increased resistance of *Salmonella* cells after either of these two heat-inducing steps (Nasciemento et al, 2012). Thus, while the conching process has the ability to reduce microbial load, it cannot be relied upon particularly in two cases: presence of heat-stressed and/or desiccated *Salmonella* cells which have already developed some thermal resistance (Geopfert and Biggie, 1968; Krapf et al, 2010), and in instances of high *Salmonella* contamination (Nasciemento et al, 2012).

2. In cases of recontamination (Izurieta & Komitopoulou, 2012), all manufacturing steps post-roasting are not guaranteed to have lethal effects on *Salmonella* cells, due in part to the combination of the low water activity and high proportion of fat of chocolate products (Cordier, 1994; Hiramatsu et al., 2005). The low water activity of intermediate products is known to increase *Salmonella*'s resistance to heat, such that small numbers of *Salmonella* spp. have been shown to survive typical temperatures reached during the milling, refining, or conching steps of chocolate processing (Lund et al., 2000; Simonsen et al., 1987).

4. It is unclear if decontamination procedures are attempted in the last stages of chocolate production, or how practicable they are, as only one study (Barrile et al., 1970) made a reference to possible product recovery at this stage, and another study evaluated the efficacy of heat, ultrasonic and ultraviolet methods for the decontamination of milk chocolate in thin films (Lee et al., 1989). However, if thermal decontamination efforts are made, such procedures would have to be meticulously validated using both microbiological and organoleptical parameters

(Cordier, 1994), given that thermal resistance can increase at such low water activities.

2.5.2.6. Tempering

Tempering is the last crucial stage of chocolate making where the mixture goes through a heating, cooling, mixing and reheating process under carefully controlled conditions. It helps ensure that the fat in the chocolate crystallizes in its most stable form, with the unstable form melting out (Afoakwa, 2010). This ultimately prevents fat bloom and discoloration. Properly tempered chocolate has good color and snap, a smooth, glossy surface, and is thermally stable.

2.5.2.7. Packaging and distribution

A series of automated high-speed machines receive the tempered chocolate mixture, now in molten form, for molding into desired shapes and sizes. Finally, depending on the desired end product, other processes could include enrobing - a process where the chocolate is used to cover a center filling, or panning – a process of using the chocolate as coating for hard centers such as nuts and dried fruits (Beckett, 2009, Afoakwa, 2010). The molds are passed through a cooling chamber, de-molded and continue on to high speed wrapping equipment after which it is packaged for retail distribution.

Microbiological safety considerations

1. Extra ingredients such as coconut, peanut, hazelnut, some dried fruits, egg powder and other similar inclusions which are known to sometimes contain *Salmonella* must undergo rigorous testing if contamination is to be avoided in the final product.

2. Burndred (2009) suggests that besides validated processes, robust sampling and analysis schemes on the final product for *Salmonella* are highly recommended. And in problematic cases of non-homogenous distribution in contaminated ingredients, an FDA-proposed sampling scheme (Andrews et al., 2007) is advised.
3. The most stringent hygienic practices under prerequisite programs should be enforced following the major pathogen reduction step to prevent recontamination during subsequent manufacturing and packaging.

2.6. Identification of Data Gaps

Available information as well as data gaps identified during literature review are summarized and are presented in **Table 2.2** below. Efforts were made to include only information relevant to the food and pathogen of concern in this study, while acknowledging that data gathered may not necessarily be comprehensive. For ease of assessment and presentation, the chocolate production process is categorized into four modules: Farm, Storage and Transportation, Processing and Post-processing.

Table 2. 2. Presentation of available data and identification of data gaps to address risk assessment of *Salmonella* contamination in milk chocolate.

	Available data	***Key Points***	***References***	***Data Gaps***
Module				
Farm	Microflora of cocoa beans; presence of enteropathogens: - pre- and post-fermentation - during drying and storage	No *Salmonella* or *E. coli* detected pre-fermentation (single study) *E. coli*, detected during fermentation; but no *Salmonella* *Salmonella* contamination and proliferation can occur in later fermentation stages due to reduced pH Potential for increased presence of coliforms and *E. coli* during and after drying Large presence of diverse fungi during drying and storage Coliform and *E. coli* contamination highest during drying and storage Both low and high incidences of *Salmonella* detected in dried beans during storage (2 studies) Drying is probably most critical stage for introduction of	(Copetti et al., 2011; De Smedt et al., 1991; Nascimento et al., 2010)	Initial *Salmonella* contamination levels, prevalence in dried, fermented cocoa beans What happens in case of a fermentation failure? Use of fermented vs un-fermented cocoa beans – impact on viability and survival of *Salmonella* cells Low incidence of *Salmonella* at farm-level storage of dried cocoa beans; more studies needed for risk assessment

		Salmonella (air, soil, dust, insects, fecal contamination, workers' feet or hands etc.) Prolonged storage and storage conditions are possible risk factors		
	Implementation and effectiveness of pre-requisites (GAPs) on farm, or lack thereof	*Salmonella* contamination during on-farm processing of cocoa not unexpected, due to poor sanitary conditions, heavy handling	(Cordier, 2000; Nascimento et al., 2010)	
	Salmonella behavior during fermentation	*Salmonella* can grow during fermentation, although affected by yeast, acetic acid bacteria and pH If contamination occurs during fermentation, growth increases during drying; if it occurs drying, growth is varied – can increase or decline	(Nascimento et al., 2012; Nascimento et al., 2013)	More data on influence of fermentation on *Salmonella* needed
	Salmonella behavior during drying and storage	Decrease in a_w reduces growth during storage at room temp. Pathogen still detectable after 120-day storage	(Komitopoulou & Peñaloza, 2009)	
Storage & Transportation	Fate of *Salmonella* in dry, raw confectionery materials during storage	*Salmonella*e can survive storage up to 4 weeks in dry raw materials (cocoa beans and crushed shells); survival is dependent on strain source, method of cell prep and inoculation, and storage temperature	(Izurieta & Komitopoulou, 2012)	More data needed on survival during storage and transportation
Processing Facility	*Salmonella* incidence and/or survival pre- and post- bean roasting	1-2 log reduction of natural microbial contamination of cocoa	(Barrile et al., 1971; Stobińska et al., 2006)	Initial *Salmonella* contamination levels and prevalence data

	beans obtained in some studies using temperatures up to 150°C		on raw cocoa beans (pre-roasting step) is largely unknown
Thermal resistance during roasting and conching	High thermal resistance of *Salmonella* in various chocolate products – due to low Aw, food composition and structure etc. Conching might increase thermal resistance Roasting might not guarantee thermal inactivation; might increase thermal resistance Establishment of D and z values Most thermally resistant *Salmonella* strains in milk chocolate – *S*. Typhimurium & *S*. Senftenberg	(Barrile et al., 1970; D'Aoust, 1977; Goepfert & Biggie, 1968; GMA 2009; Lund & Eklund, 2000; Silva & Gibbs, 2012; Simonsen et al., 1987) (Krapf and Gantenbein-Demarchi, 2010) (Izurieta & Komitopoulou, 2012; Nascimento et al., 2012; Peñaloza-Izurieta et al., 2008; Silva & Gibbs, 2012; van Asselt & Zwietering, 2006) (Goepfert & Biggie, 1968; Lee et al., 1989)	Other factors affecting *Salmonella* thermal resistance – strain variability, pH, Aw, fat content, microstructure or composition of chocolate. Possible synergistic effect and how impact can be modeled. Thermal inactivation data - models needed (log-linear regression, Weibull survival model etc)
Long term viability of *Salmonella* in finished chocolate	Up to 19 months storage History of exposure to variable temperatures and moisture levels determines whether	(Tamminga et al., 1976, 1977) (Barrile et al., 1970; Hiramatsu et al., 2005; Podolak et al.,	

		Salmonella survive heat treatments and are present in the final product	2010; Waldner et al., 2012)	
	Other factors to consider on *Salmonella* survival	Survival is strain-dependent	(Doyle & Mazzotta, 2000; Komitopoulou & Peñaloza, 2009; Santillana Farakos et al., 2014; Tamminga et al., 1976)	
	Potential contamination from other ingredients	Dried milk powder	(Cari & Potter, 1970; McDonough & Hargrove, 1968)	Equipment and surface decontamination protocols
		Nuts – hazelnuts, almonds (when used)	(Lambertini et al., 2012)	Likelihood and degree of cross-contamination and microbial transfer within facility? Regrowth? How can it be modeled? Little information on other points of entrance for *Salmonella* during facility processing
	Effectiveness of debacterization (steam treatment) to decrease thermal resistance	Increase in moisture enhances *Salmonella* inactivation Calls for debacterization to be embraced as standard practice	(Afoakwa, 2016; Beckett, 2009; Krapf & Gantenbein-Demarchi, 2010)	Debacterization of cocoa beans - influence of increased moisture on stressed *Salmonella* cells *Salmonella* reduction after debacterization: logistic regression models

Post-Processing	Sparse information on epidemiological and consumption practices		Refer to Fig. 1	Exposure assessment: anticipated human exposure? Consumption patterns and practices? Serving size? Hazard characterization: dose-response models Risk characterization: probability of infection/serving Effect of rapid, extreme temperature changes on physiological state of *Salmonella* in milk chocolate bars, e.g. during transportation, storage, weather changes

2.6.1. Available Thermal Inactivation Data

Thermal inactivation trials have been conducted for raw materials and intermediate products like cocoa beans, cocoa liquor, cocoa butter, chocolate masses and for finished products like milk and dark chocolate, with these Thermal Death Time (TDT) studies conducted before, during and after processing **(Table 2.3).** Decline kinetics of a microorganism can significantly impact exposure estimates in a risk assessment. Assuming log linear kinetics, survival of *Salmonella* post heat inactivation treatments is often estimated using the traditional D/z concept as its broad applicability makes it the most appropriate to initially observe the performance of an inactivation process (van Asselt and Zwietering, 2006, Santillana Farakos et al., 2014). D-value or decimal reduction time is a measure of the heat resistance of a microorganism and is described as the time in minutes at a given temperature required for one decimal reduction (1-log or 90 %) of an exposed microbial population. On the other hand, the z-value (decimal reduction temperature) reflects the temperature dependence of the thermal process, and refers to the temperature change (in °C) required to change the D-value tenfold, or simply, a measure of the change in inactivation rate with a change in temperature. Thus, while D-value provides the time needed at a certain temperature to kill an organism, z-value relates the resistance of such organism to differing temperatures. A plot of the log of D values against temperature provides a prediction of the survival rate of a microorganism at intermediate temperatures; i.e. the slope of the resulting curve.

D- and z- values are described by the equations below:

$$D = t/(\log N_0 - \log N_t) \qquad (1)$$

$$z = T_1 - T_2/(\log D_1 - \log D_2), \qquad (2)$$

where Nt is the concentration of cells at time t

N_0 is concentration at time 0

t is duration of heat treatment in minutes

D is the decimal reduction time also in minutes

T1 and T2 are the change in temperature values associated with D1 and D2.

Most of the publications found in the literature on thermal resistance of *Salmonella* have been analyzed by assuming first-order death kinetics. For low-moisture foods however, *Salmonella* survival curves generally do not follow log-linear kinetics; instead they tend to show a rather rapid initial decline followed by a slow inactivation over a longer period, thereby displaying bi-phasic curves or asymptotic tails (Abd et al., 2012; Doyle & Mazzotta, 2000). To this end, the Weibull survival model, (equation below) including its secondary derivations, known for its ability to model asymptotic curves with tails, has been proposed as the most accurate for describing *Salmonella* survival in low moisture foods (Abd et al., 2012; Beuchat & Mann, 2010; Mattick et al., 2001; Santillana Farakos et al., 2014).

$$\log Nt = \log N_0 - (t/\text{delta})^{\beta} \qquad (3)$$

where Nt, N_0, and t are defined as in equations (1) and (2) above

delta is the time required for the first log reduction,

and β is a fitting parameter that defines the shape of the inactivation curve.

Although none has been developed for *Salmonella* in chocolate, literature review shows that at least two survival models have been developed for use in a quantitative risk assessment of *Salmonella* in almonds, a low-moisture food (Danyluk et al., 2006; Lambertini et al., 2012). Both models do assume log-linear regression of *Salmonella* in almonds. Data from product-specific TDT studies published in literature may be used in modeling for risk assessment, provided reasonable precautions are made. For this objective, data needs will be fulfilled using a combination of selected literature data and data from the chocolate industry (see *Data Sources* section below). The table below provides some published thermal inactivation data associated with cocoa beans a raw material, semi-finished products and finished chocolate products.

Table 2. 3. Published thermal inactivation data associated with cocoa beans a raw material, semi-finished products and finished chocolate products

Salmonella Serotype	Matrix tested	Production Step	D values (min) at temp. indicated in °C								*Z* value (°C)	References
			50	**60**	**70**	**71**	**80**	**90**	**100**	**110**		
S. Typhimurium	Molten milk chocolate	End product			816		222	75			18	(Goepfert and Biggie, 1968)
S. Typhimurium	Dark chocolate	Conching	1570	1008	600		142	25			14	(Krapf and Gantenbein-Demarchi, 2010)
S. Typhimurium	Cocoa butter	Conching	245	306								
S. Typhimurium	Cocoa liquor	Conching	999	760	248		70	26			20	
S. Typhimurium	Molten milk chocolate					396						(Lee *et al.,* 1989)
S. Senftenberg	Molten milk chocolate	End product			440		116	36			19	(Goepfert and Biggie, 1968)
S. Senftenberg	Molten milk chocolate	End product				276						(Lee *et al.,* 1989)
S. Eastbourne	Molten milk chocolate	End product				270						
S. Anatum	Molten milk chocolate (0% moisture)	End product				1200						(Barrile and Cone, 1970)
S. Anatum	Molten milk chocolate (1% moisture)	End product				510						
S. Anatum	Molten milk chocolate (2% moisture)	End product				240						
S. Anatum	Molten milk chocolate (4% moisture)	End product				210						
S. Oranienburg	Cocoa bean shells (4% moisture)	Roasting						6.7	2.6		15.4	(Izurieta and Komitoupolou, 2012)
S. Oranienburg	Cocoa bean shells (7% moisture)						7.7					
S. Enteritidis PT30	Cocoa bean shells (4% moisture)							8.9	2.8		17.4	
S. Enteritidis PT30	Cocoa bean shells (7% moisture)						5.4					
Cocktail	Cocoa beans	Roasting								4.8	102	(Nasciemento *et al.,* 2012)
Cocktail	Cocoa nibs	Roasting								8.9	50	
Cocktail	Molten milk chocolate (1-180 mins)	Conching	217	102	51							
Cocktail	Molten milk chocolate (180-1440 mins)	Conching	1077	482	702							

2.7. Risk Analysis and its application to food safety

Risk analysis can be described in simple terms as the systematic use of available information to determine how often certain identified events may occur as well as the magnitude of their consequences. It involves gathering and evaluating data related to a hazard, and subsequently using the knowledge obtained to develop and implement programs which will help manage the risks associated with the hazard.

Primarily developed in the last two decades, risk analysis within the food safety system has been defined as a systematic, disciplined approach to making food safety decisions (FAO/WHO, 2006). Risk analysis is a powerful problem-solving tool which can be used to produce reliable solutions to food safety problems through the use of science-based analysis. Risk, by itself, has been defined as the chance of an undesirable outcome, described mathematically by the product of probability and consequence. Although, risks are characteristically defined as negative events, the process of risk analysis can also disclose potential positive outcomes. By exploring the full space of possible outcomes for a given situation, a good risk analysis process can do two things simultaneously: identify pitfalls and uncover new opportunities for risk mitigation. For a typical food safety problem to be analyzed, a risk manager will initiate a risk management process which can be accomplished based on a risk management framework (RMF), a generic version of which is shown in **Fig. 2.3**. The process of risk analysis is generally iterative as parts of it can be repeated as more analytical work is done and new information becomes available.

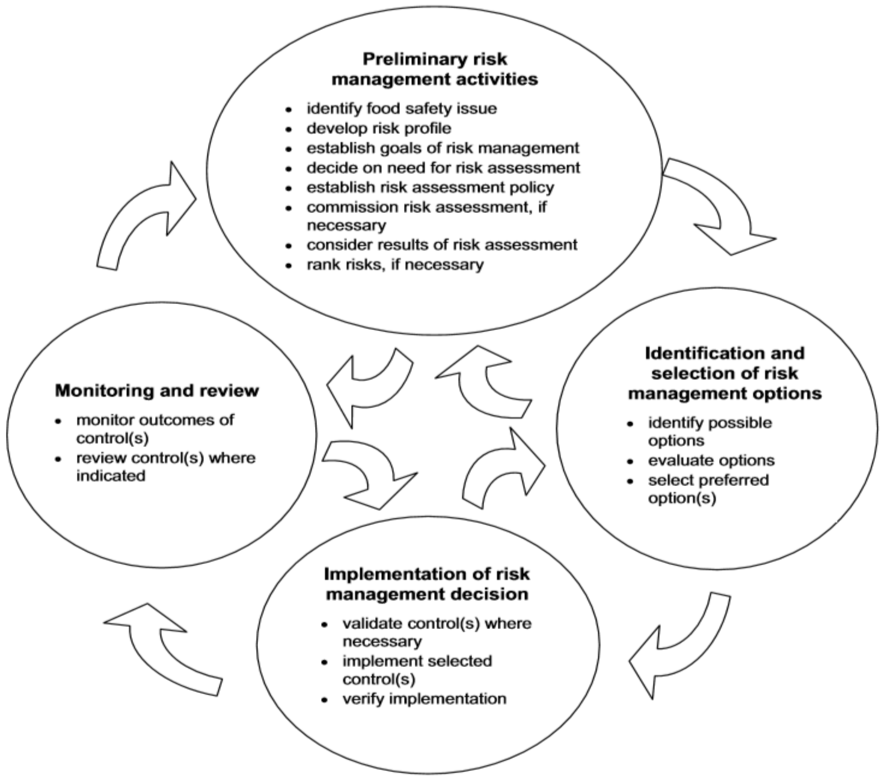


Figure 2. 3. A generic risk management framework (Source: FAO/WHO 2006)

According to Codex Alimentarius Commission, the inter-governmental body that sets guidelines and standards to protect consumers and ensure fair trade practices in relation to global food trade, risk analysis consists of three components: risk assessment, risk management and risk communication. These components which are essentially complementary to each other have been developed, refined and designed to be integrated into food safety systems. The risk assessment phase is the scientific foundation of risk analysis and uses scientific inputs to measure and describe the nature of the risk being analyzed, while the risk management process carefully considers results from the risk assessment, and weighs policy alternatives with the goal of implementing feasible outcomes. These tools emerged primarily because of the need to make decisions to protect public health in the face of scientific uncertainty. Increasingly, food companies are expected to conduct an assessment of risk to support and justify established food safety management systems.

2.7.1. Overview of Microbial Risk Assessment (MRA)

MRAs rely heavily on scientific analysis and are currently regarded as the most practical method for assessing microbial risks associated with contamination of foodstuffs including their manufacturing processes (Codex, 1999). Microbial Risk Assessments (MRA) are used by US regulatory agencies to establish performance standards that, if meticulously adhered to, are assumed to provide a safe food product (Juneja & Marks, 2006). Risk assessments can be performed either qualitatively or quantitatively with various intermediate formats, e.g. semi-quantitative assessment (FAO/WHO 2006). Qualitative risk assessment generally involves describing, compiling and presenting evidence to support prior information about a risk. Tools used in qualitative assessments include risk matrix, risk rating, risk ranking (with types including paired ranking, criteria-based ranking and evidence-based ranking), multi-criteria decision analysis (MCDA), and qualitative risk assessment models. Generally, outputs are expressed in descriptive terms such as high, medium or low. Although a qualitative assessment is formal in its organization in that it deals with broadly-defined problems, it is often flexible and reproducible (Schaffner, 2008). In instances where data, time and resource constraints make it implausible to conduct a full and immediate quantitative assessment, a qualitative assessment is valid and could be sufficient for decision making when conditioned by prior expert knowledge (Codex, 1999).

2.7.2. Steps in a Microbial Risk Assessment

According to Codex, four systematic steps are important when conducting microbial risk assessments:

i. hazard identification
ii. hazard characterization
iii. exposure assessment
iv. risk characterization

The first step involves identification of the pathogenic microorganism which may be present in the food product. After this step, the order in which subsequent steps are carried out is not rigid as the entire process is usually iterative, with steps repeated as data and assumptions are refined (FAO/WHO, 2006). Hazard characterization is a qualitative and/or quantitative assessment of the pathogen and the nature of adverse health effects associated with it. An important concept of hazard characterization is dose-response assessment which describes the quantitative relationship between the level of pathogen exposure (dose) and the likelihood and severity of adverse effects (response). Exposure assessment is carried out to determine the level of the microorganism likely to be present in the food at the time of consumption (likely intake). This step takes into account potential contamination routes of the pathogen during various processing stages and the impact of processing parameters on microbiological state and levels. The final risk characterization step is a measure of the risk assessed and involves an estimation (including uncertainties) of the probability of occurrence and severity of known or potential adverse health effects in a given population. This last step is generally based on the three preceding steps.

Uncertainty and variability are two important factors which should be considered in a risk assessment. Uncertainty indicates how much information or knowledge the risk assessor has regarding the risk and can often be reduced through engaging in more research. Variability on the other hand is an inherent phenomenon that is mostly irreducible, and is the result of the random effects of chance that might occur in the system being modeled However, variability may be managed by allowing alterations in the system, for instance by introducing or eliminating a critical control point during food processing (Vose, 1998). Whenever possible, a two-dimensional model is a good way to separately evaluate these two components of a system, although this is often a complex attempt (Vose, 1998).

2.7.3. Quantitative Microbial Risk Assessment (QMRA)

A QMRA attempts to assign numeric values to risks, either by using empirical data or by quantifying qualitative assessments. Outputs are expressed mathematically and may include a numerical description of uncertainty. A QMRA can be either deterministic or probabilistic in nature. It is deterministic when it uses fixed values (single point estimates) to obtain a probability profile, and assumes that its outcome is certain when inputs to the model is fixed, i.e. no random elements. On the other hand, the assessment is probabilistic or stochastic when it involves variations and uses mathematical models, and as such, the final risk estimate is a probability distribution. The stochastic models, although more complex, are considered to be more informative because they account for uncertainty due to varying behavioral characteristics. QMRA models have been successfully applied in the food industry to systematically understand sources and fate of potential contaminants, their effects on the microbial quality of finished products, and the risk of infection associated with the consumption of such products (FAO/WHO, 2006). Regulations and industry standards continue to embrace risk-based strategies and tools to understand critical system vulnerabilities and quantify the cost-effectiveness of risk-control efforts. QMRA modeling tools can assist both manufacturers and regulatory agencies in:

i. providing a means to consider both the variability of the overall assessment as well as the impact of individual steps
ii. quantifying the magnitude of a projected public health burden associated with a process
iii. identifying crucial factors associated with risk of illness
iv. comparing risk mitigation strategies, thereby providing rigorous evidence for subsequent decision-making

2.8. Research Overview and Objectives

2.8.1. Overview: Framework for Using Risk Assessment to Enhance Safety in Chocolate Production

There have been no risk assessment efforts to address potential risks associated with *Salmonella* contamination and interventions during chocolate production, and some researchers in this field have underscored the need for it (Nascimento et al., 2012). The absence of a comprehensive qualitative or quantitative risk profile limits the ability to assess the effectiveness of current and proposed *Salmonella* control measures in a chocolate food safety plan.

As an illustration, qualitative and quantitative assessment of the risks associated with the ingestion of chocolate products contaminated with *Salmonella* may be built on the following framework:

1. An analysis of the prevalence of *Salmonella* in the major unprocessed ingredients – cocoa beans, powdered milk, extra ingredients such as nuts - leading to estimates of the probability of any one item being contaminated, as well as the level of *Salmonella* cells that would be present.
2. Modeling of the various processes on the farm-to-table continuum that the major ingredient (cocoa beans) is subjected to before consumption. These include harvesting, transportation, manufacturing, packaging, distribution, storage, and cooking. This leads to a revised probability that the final chocolate product remains contaminated, taking into account possible cross-contamination during processing in the plant, or from other ingredients. This further leads to a revised distribution of the number of *Salmonella* cells (dose) that will be potentially present at the time of

consumption. This revision may include the reproduction and attrition effects of desiccation, heat stress, acidity levels etc., resulting from the various identified processes that the product undergoes. Analysis would also include an acknowledgement of the dry nature of the food and the possibility that the pathogen's cells might not be evenly distributed in the final chocolate product.

3. Lastly, the response that an individual in a given population will have to consuming a specified dose of *Salmonella* present in the chocolate product. This would be done by estimating the probability (P) that any one cell will cause the identified response – in this case, exhibit symptoms of salmonellosis. The estimation of P is produced from dose-response data, and different values of P are determined corresponding to different responses, typically infection and various levels of morbidity and mortality.

2.8.2. Research Objectives

This dissertation encompasses the following specific research objectives:

a. Development of a comprehensive reference document that collates relevant scientific and regulatory information regarding microbial safety of chocolate, and evaluates risk factors, specifically for *Salmonella* contamination during farm-to-packaging of milk chocolate products. This would serve as a dossier for future reference and to inform further risk assessment. This objective is embedded within literature review (Chapter 2) and portions of the qualitative risk assessment in Chapter 4.

b. Evaluation of HACCP (Hazard Assessment and Critical Control Points) and HARPC (Hazard Analysis and Risk-based Preventive Control) food safety management systems in chocolate processing (Chapter 3).

c. Development of a qualitative assessment of risk factors for *Salmonella* contamination during farm-to-packaging milk chocolate production (Chapter 4).

d. Targeted experimental data on storage kinetics and parameters for milk chocolate crumb, an intermediate product during processing, and development of survival models to estimate risk reduction during storage (Chapter 5).

Chapter 3: Examining food safety management systems - HACCP and HARPC - in milk chocolate processing

This chapter provides an examination of the interface between HACCP, HARPC and risk assessment, and how their integration can help to optimize food safety for milk chocolate processing.

3.1. HACCP Food Safety Management System

Hazard Analysis and Critical Control Points (HACCP) plans have previously been developed for the chocolate industry (Cordier, 1994). However, such protocols have not been based on qualitative nor quantitative systematic assessments of risk. The HACCP system is a science-based, global, food safety standard designed to be proactive and preventative because it helps identify potential problems before their occurrence, while establishing control measures at specific stages in the production process that are critical to the safety of the finished product. The flexibility of the HACCP system allows for changes and updates such as advances in equipment design, processing procedures, or technological developments (Codex, 1999). This feature is an important one in the ever-evolving world of food safety and makes a good case for integrating risk assessment concepts into HACCP.

In terms of structure, the food safety and quality management system in the industry can be addressed based on two major components: 1) Prerequisite Programs (PrPs), and 2) a well-established food safety management system like HACCP (Buchanan & Williams, 2013). A HACCP plan is only as effective as its prerequisites and any other food safety programs that support it, and as such, food safety risks can often be significantly reduced through strict adherence to both systems. PrPs are a compilation of best practices and procedures that serve as a general guide to providing basic environmental and operating conditions required to be incorporated into the food production chain for the production of safe and wholesome foods (FDA, 1997). Practices found under the PrP umbrella include Good Manufacturing Practices (GMPs) - used specifically for the food processing sector, Good Agricultural Practices (GAPs) - used specifically for the primary production (on-farm) sector, and Good Hygiene Practices (GHPs) (Buchanan & Williams, 2013).

A comprehensive Food Safety Management System (FSMS) must be put in place to help manage all food safety-related efforts. A FSMS functions like an all-inclusive system that comprise moving parts such as procedures, processes, specifications, verifications, validations and documentations. It is basically the manufacturer's formal plan to ensure proper food safety and quality management. The Codex Code of General Principles on Food Hygiene (Codex, 2001) underscores the importance of GMP/GAP/GHP as important foundations needed to effectively implement a good HACCP plan and develop a user-friendly FSMS. International standards such as ISO 22000 or ISO 9001 help specify the requirements for a FSMS.

Nonspecific steps in a generic HACCP model may be based on foundational principles such as:

- Product Description Process
- List of Product Ingredients and Incoming Materials
- A Process Flow Diagram
- Plant Schematic

- Biological Hazards Identification
- Chemical Hazards Identification
- Physical Hazards Identification
- Critical Control Point (CCP) Determination
- Hazards not controlled by the Operator

(Adapted from Canadian Food Inspection Agency, CFIA, 2014)

HACCP best practices may involve an initial risk assessment to understand the product and process for line operations within the manufacturing facility (Corlett and Stier, 1991).

3.2. HARPC (Hazard Analysis and Risk-based Preventive Control)

In 2011, the US government signed the Food Safety Modernization Act (FSMA) into law which mandates the FDA to implement new food safety regulations. The portion of the law in focus here is generally referred to as Preventive Controls for Human Food (PCHF), embedded within Section 103(c) of FSMA (FDA, 2017), and was proposed to make a fundamental shift in approach to food safety – from reactive to science-based actions that would strengthen accountability for prevention throughout the entire food system. The regulation is defined in Title 21 of the Code of Federal Regulations [21 CFR 117.26(a)]. The rule created a set of new requirements for production of human food by registered food manufacturing facilities and revised previous requirements.

According to the FDA, one of the reasons for introducing FSMA was the increasing scale and complexity of the food system and the dramatic changes globalization has brought to the system, and consequently to the way public health is addressed (FDA, 2014). A significant hallmark of the FSMA law is that it provides the FDA with increased authority to inspect food products and authorize mandatory recalls for contaminated foods (Grover et al., 2016). The Preventive Control Rule requires that all food facilities which fall under the FSMA act must establish and implement a food safety system that includes an analysis of hazards and risk-based preventive controls (FDA, 2014). A Food Safety Plan (FSP) must be developed, written and implemented under the guidance of "qualified individuals" using this HARPC approach, one which will establish science-based, preventive control measures to reduce the risk of food contamination. While this preventive approach to food safety is not a new development, the HARPC approach represents a new paradigm shift. HARPC moves out of the prescriptive seven-step thought process, commonly associated with HACCP, to a risk-based thinking and analysis, with the emphasis on "risk-based". This approach helps strengthen the accountability for prevention efforts.

3.3. HACCP vs HARPC

Production facilities with existing HACCP systems are now tasked with morphing their current food safety system into the more robust HARPC-based food safety plan, as HACCP training or compliance no longer meets the new FSMA requirements. While some see HARPC as an extension of HACCP, to understand how the risk-based rules compare to HACCP principles, it is important to compare their basics. Both plans were not designed to be stand-alone programs but are product and process specific. They also both require some form of hazard analysis. Traditionally, guidelines of agencies such as the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), and the FDA, define a hazard as a biological, chemical, or physical agent that is reasonably likely to cause illness or injury in the absence of its control. The HARPC approach is based on a preventive framework designed to identify specific hazards in addition to traditional HACCP critical control points (CCPs), and proactively take corrective actions to counter such hazards and prevent contamination before a food-safety event occurs (FDA, 2017a, Sherod, 2016). The HARPC-based food safety plan focuses on known hazards that could "reasonably occur" in the absence of their control: categorized under hazards inherent to the raw materials, typical manufacturing hazards that occur through error during processing, hazards that are deliberately introduced – also known as food fraud or bioterrorism (FDA, 2017a).

HARPC can broadly be regarded as an enhanced, risk-based HACCP program. In the same vein, HARPC can serve as a good link between an established HACCP program and risk assessment, because, while not exactly considered a risk assessment activity, its governing principles are "risk-based" and are meant to be operated as such. This approach brings flexibility to the level of risk assessment rigor needed by food manufacturers in low-risk situations (FAO/WHO, 2006).

The HACCP approach is based on seven principles as follows:

(1) Conduct hazard analysis

(2) Determine Critical Control Points (CCPs)

(3) Establish Critical Limits for CCPs

(4) Establish processes for monitoring CCPs

(5) Establish corrective actions

(6) Establish Verification Procedures

(7) Establish Documentation and Record-keeping

Comparable HARPC requirements are as follows:

(1) Conduct hazard analysis

(2) Identify and add preventive controls (PCs)

(3) Validate preventive controls and establish parameters

(4) Have a written recall plan for foods requiring preventive controls

(5) Establish a preventive control management system

(6) Implement monitoring and record systems

(7) Implement corrective actions

(8) Conduct verification activities - verify and validate implementation and effectiveness of controls

(9) Reanalyze food safety plan at least once every three years or as needed

At a glance, HACCP and HARPC appear conceptually similar, but an in-depth analysis shows fundamental differences. The details on the Table 3 below compares the guiding principles of both systems and outlines the differences in approach.

Table 3. 1. Detailed Comparison between HACCP and HARPC Food Safety Plans

HACCP	Highlighted comparison	HARPC
General Features of Plan		
A global food safety standard; not always mandatory		Not global standard, but mandated by US law
Requires at least one HACCP-certified individual, but trained team can implement		Requires one or more preventive control qualified individual(s) (PCQI) to implement
Hazards do not include food defense/bioterrorism plans. HACCP systems refer to hazards as ``biological, chemical and physical agents	Unlike HACCP, HARPC identifies food bioterrorism as a potential hazard	Includes plans for potential terrorist acts, food fraud and/or intentional adulteration. Hazards refer to biological, chemical, physical, and radiological hazards'
Assessment focused on defining critical control points (CCPs)	CCP vs. PC	Assessment focused on defining preventive controls (PCs)
Prerequisites		
Prerequisites programs: - various types can be used - established and managed separately from HACCP plan		Does not specifically call for prerequisites, but cGMPs are expected
Preliminary Tasks in Development of Plan		
Assemble HACCP team ↓ Describe Food and its Distribution ↓ Describe Intended Food use & Consumers ↓ Develop Flow Diagram to Describe Process ↓ Verify Flow Diagram	The five preliminary steps mandatory under HACCP, but only recommended under HARPC	Identify a PCQI ↓ Identify and describe food product ↓ Describe intended use of food and consumers ↓ Have a written food safety plan

Should be based on PrPs such as GHPs	GMPs would not be taken into consideration when carrying out the hazard analysis for HARPC, as pre-requisites would when conducting HACCP	N/A
Plan Implementation		
<u>Principle 1</u> Hazard analysis: - identification - evaluation - determine hazard control measures	Both identify hazards, carry out hazard analysis using severity and likelihood to determine significance. Key difference – HARPC hazard analysis is carried out **without** taking current controls into consideration as PrPs would be considered under HACCP	<u>Step 1</u> Hazard Analysis: - identification (environment, recipe, raw materials) - evaluation: 1) identify source: - inherent to raw material - introduced via process error - due to adulteration 2) determine if significant: - if significant, must have a preventive control (PC) - if non-significant, control with cGMPs

<u>Principle 2</u> Determine CCPs - advisory use of decision tree - expert knowledge useful	CCPs not required in HARPC but can be included. If HACCP and HARPC are combined, 3 levels of control are produced: PrP/cGMPs, CCPs and PCs (**see Fig. 3.1** below)	<u>Step 2</u> Identify and add PCs - added to manage each significant hazard identified during analysis
<u>Principle 3</u> Establish critical limits for each CCP - determine critical limits to control each CCP - validate critical limits	Critical limits are comparable to parameters. In HARPC, not all PCs would require critical limits	<u>Step 3</u> Validate PCs - establish parameters (temperature, time, pH etc)
<u>Principle 4</u> Establish monitoring system for each CCP - generate records for verification	Comparable steps	<u>Step 4</u> Implement monitoring system for each PC - generate records for verification
<u>Principle 5</u> Establish corrective actions - steps to be taken where monitoring shows deviation from established critical limits	Comparable steps	<u>Step 5</u> Implement corrective actions/corrections - where monitoring reveals deviation from parameters
<u>Principle 6</u> Establish verification procedures	Verification includes validation of CCPs & PCs, verifying monitoring and corrective actions, product and environment testing,	<u>Step 6</u> Conduct verification activities

	review of complaints etc.	
<u>Principle 7</u> Establish documentation and record keeping - provides evidence that system is under control		No specific applicable step, but strong emphasis is placed on record keeping, especially written ones, to prove adherence to all safety measures
No applicable step	HARPC expects food facilities to have records to prove protection measures have been applied and adhered to and these must be available on request of an audit	<u>Step 7</u> Reanalyze the system - at least every 3 years - modify as needed with addition of new product lines, equipment etc.
	Other comparisons	
Recall plans not mandatory		Written recall plans required as a preemptive tool

3.3.1. Controls in Food Safety Systems

With the advent of HARPC, three types of food safety systems control may now be identified:

(1) Prerequisite Programs or Controls (PrPs)

(2) Critical Control Points (CCPs)

(3) Preventive Control (PCs)

The diagram in **Fig. 3.1** below illustrates how these three controls measure up against each other.

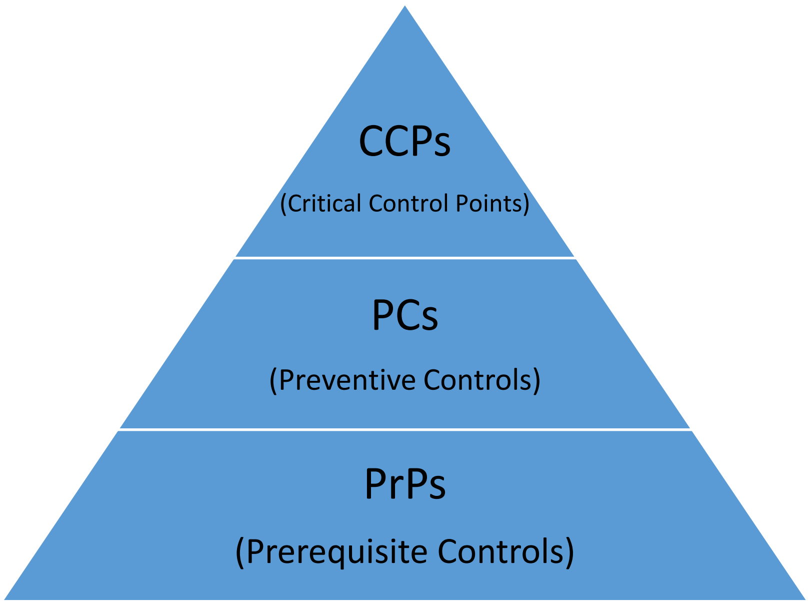


Figure 3. 1. Illustration of the hierarchy of Controls within a food safety system. (Adapted from Techni-K Consulting, 2016)

The pyramid shows a certain hierarchy to the controls: at the bottom of the pyramid, PrPs and cGMPs form the basic but important building blocks for generic controls throughout the processing facility. PCs, according to the guiding principles of HARPC, are reasonably-appropriate measures put in place to ensure that significant ("reasonably likely to occur") hazards requiring a preventive control will be minimized, prevented or eliminated. Preventive controls, according to the FDA (2017a), are broadly categorized under HARPC as follows:

- Supply-chain controls
- Process PC

- Sanitation PC
- Food allergen PC
- Recall plan
- 'Other' preventive controls

PCs are more encompassing and should be identified and implemented throughout the production process, including at critical control points, if any exist. Their position on the control hierarchy indicate that PCs may be required at points other than at CCPs, and they can be identified without establishing critical limits. In other words, critical limits would not be required for all identified PCs, and so PCs can be identified without specifying that the preventive controls establish critical limits. This is an important point of contrast between HACCP and HARPC. Critical limits are the operating parameters within which a CCP is controlled, and have been described by Codex (2001) as the criteria that separates acceptable (safe) from unacceptable (potentially unsafe) food products. Critical control points sit at the top of the pyramid, indicating they are process-specific. They are positioned at critical steps in the production process where an absence of their control creates hazards that are reasonably likely to cause illness or injury (FDA, 1997), and are thus serving the purpose to prevent, eliminate or reduce a food safety hazard to an acceptable level (Codex, 2001). Examples of CCPs are: specific thermal processes designed to destroy a specific pathogen, chill temperatures, and ingredient or product testing for chemical residues or metal contaminants (FDA, 1997). Every CCP must have at least one critical limit in place. After identification, preventive and critical control points would need to be verified and validated on a scientific or technical basis to show that they are performing as expected. In this regard, both CCPs and PCs have specific values (critical limits and parameters) that must be achieved and maintained during processing. An important aspect of verification is evaluating whether the manufacturing facility's HACCP system is functioning in accordance with the established HACCP plan, such that, end-product testing is not relied on to ensure pathogen-free products.

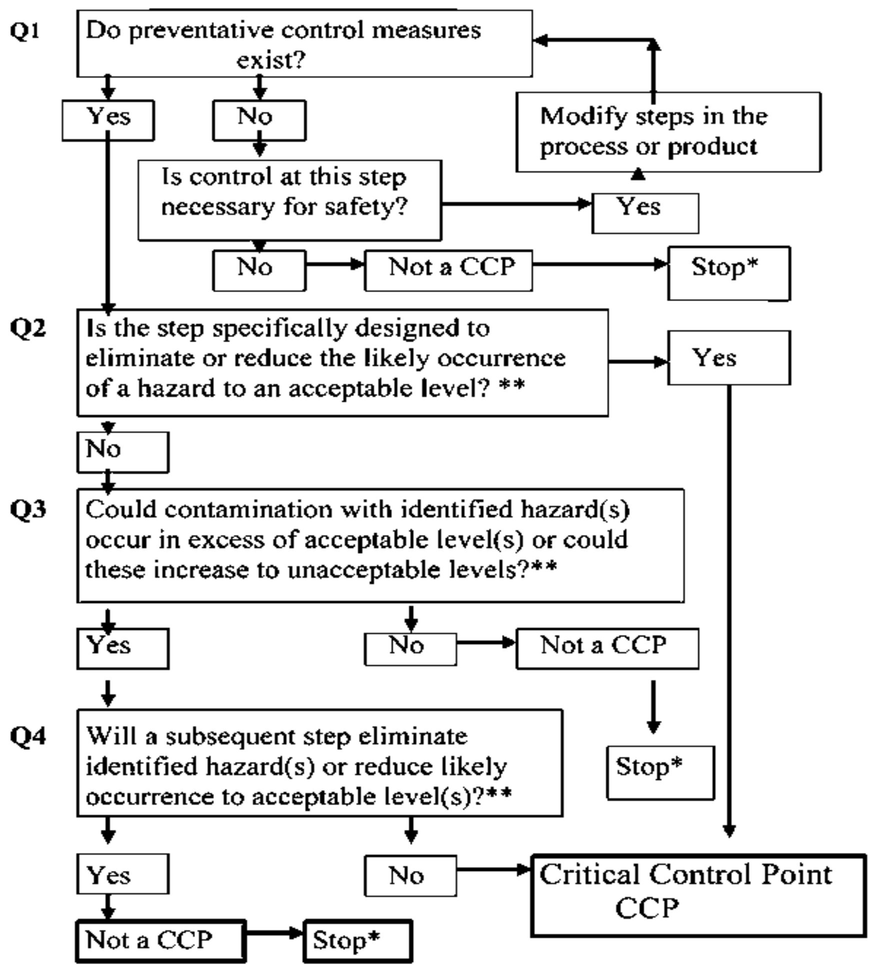
To present a clear summary of the relationship between all three controls, it is helpful to state one of the fundamental reasons behind the establishment of HARPC. The FDA had stated that a good number of foodborne outbreaks and recalls were caused by implementation failure of PrPs, and as a result decided that PrPs which control a significant hazard should have documentation similar to a CCP (US GPO, 2016). A move to create a broader safety net effectively birthed the concept of risk-based preventive controls.

3.4. Application of HACCP and HARPC in the Chocolate Industry

Guidance on establishing GMPs and HACCP plans in chocolate manufacturing have been issued by the International Commission on the Microbiological Safety of Foods (ICMSF, 1988) and by the International Office of Cocoa, Chocolate and Confectionery Products (IOCCC, 1991 & 1993). However, there does not seem to be a consensus in literature on what processing steps should be classified as CCPs. Only generic HACCP models for chocolate manufacture are publicly available since customized plans are categorized as proprietary; this makes it difficult to assess common practices in the industry.

3.4.1. Hazards associated with chocolate processing

Hazards - physical, chemical or biological – are identified as CCPs with the aid of the Codex-recommended decision tree (**Fig. 3.2**) which sequentially answers several questions. The model flow diagram below can determine CCPs in the production process where GMPs in place at the manufacturing facility are unable to control a potential hazard.



*Proceed to next identified hazard in the described process
** Acceptable and unacceptable levels need to be determined within the overall objectives in identifying the CCPs of the HACCP plan.

Figure 3. 2. Example of a decision tree used to identify CCPs. (Source: Codex, 1999).

Hazard categories commonly associated with chocolate processing are identified in the Table 3.2 below.

Table 3. 2. Hazard categories commonly associated with chocolate processing

Category	Physical	Chemical	Microbiological
Source			
Incoming raw materials (cocoa)	Mostly extrinsic materials e.g. pieces of stick, stones, glass, wood, metal, and insects	Mycotoxins, pesticide residues	Enterobacteriaceae, (*Salmonella*)
During processing	Both intrinsic and extrinsic materials: bean shells, processing equipment failures, human hair etc.	Heavy metals e.g. lead	*Salmonella*, Staphylococcus aureus
Post-processing	-	-	*Salmonella* (influence of environmental, storage & handling parameters)

3.4.2. Identification of CCPs and PCs during milk chocolate processing

During primary production of cocoa beans on the farm, food safety is generally taken care of by prerequisites such as GAPs and are not included in the CCP identification process, as is done during chocolate processing in the facility. It is recommended that risk-based analysis of any hazard identified should be retrospective, evaluating hazards that may come with raw materials or ingredients, and prospective, assessing contamination risks that might arise post-production, e.g. via the environment. During milk chocolate production, the main retrospective risks include those associated with microbial contamination of incoming raw cocoa beans as well as dry milk powder. Prospective risks would include those associated with post-roasting environmental contamination.

There does not seem to be a consensus in literature on what chocolate processing steps should be classified as CCPs. For this study, both the CCPs and PCs recognized for milk chocolate manufacture are identified on the basis of literature (Cordier, 1994; Simonsen et al., 1987) and observations during personal visit to a chocolate manufacturing facility. In the process flow chart (**Fig. 3.3**) below, rationale is provided for the steps identified as CCPs, and those suggested to be classified as PCs. They can be grouped into three broad categories:

1. Thermal process steps
2. Raw material(s) that must exhibit the absence of a hazard
3. Environmental control

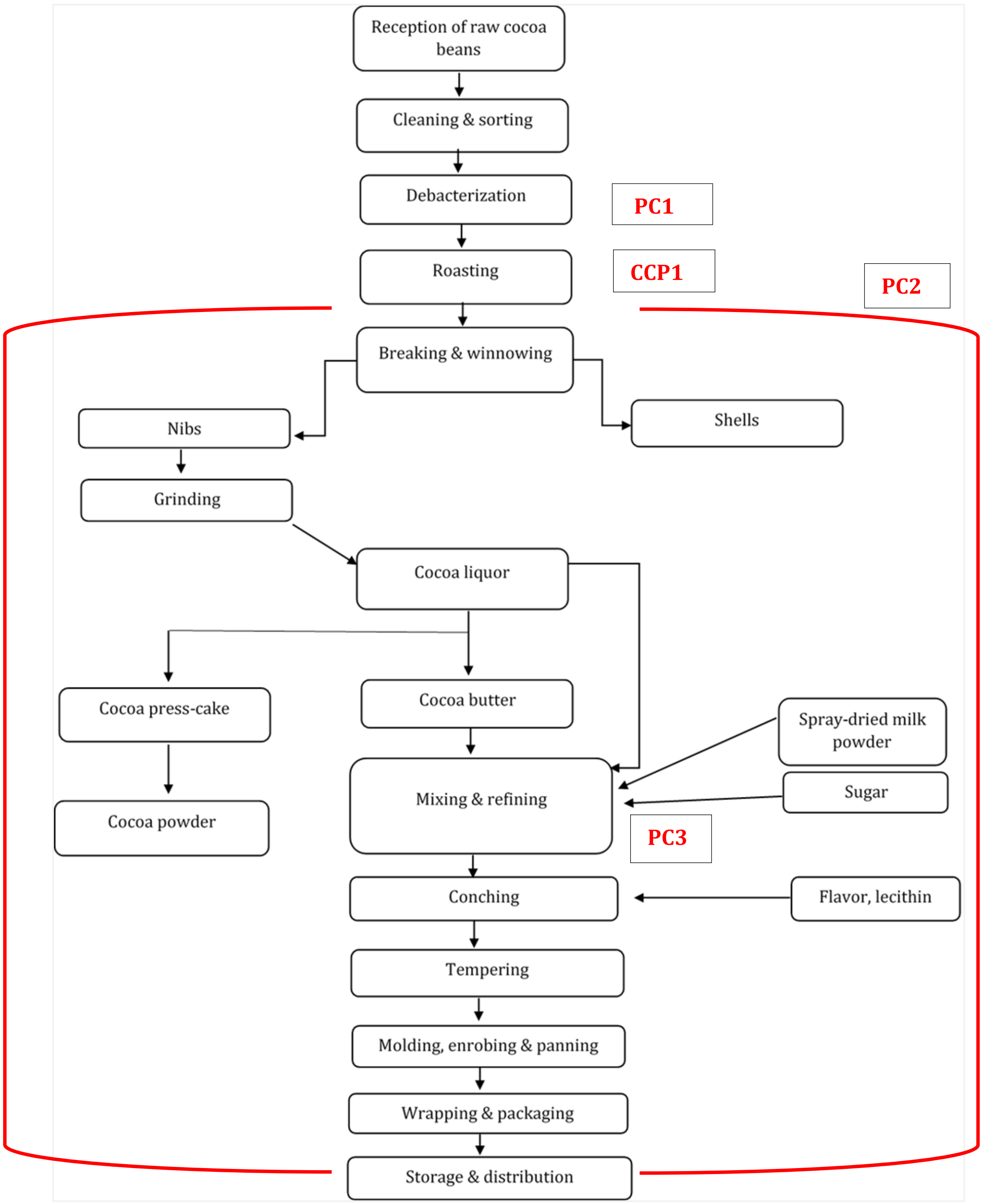


Figure 3. 3. Schematic representation of industrial milk chocolate production showing identified Critical Control Points (**CCPs**) and suggested Preventive Controls **(PCs)**. The area bounded by red brackets – [ ] – represents the environment post bean-roasting up until packaging.

The singular CCP identified here is the **roasting step** because it is considered to be the only pathogen-reduction step during milk chocolate production. In the CCP tree in **Fig. 3.2**, the response to Q2 for the roasting step is in the affirmative, clearly identifying it as a CCP. Subsequent heat-application steps such as conching and refining cannot be relied upon as inactivation steps for *Salmonella* or similar organisms, as previously discussed in literature review. Roasting must be meticulously monitored by time and temperature checks, and may depend on equipment type being used. An example of a critical limit provided in literature is a roasting profile of at least 105°C for 15 min (Simonsen et al., 1987, Stobinska et al., 2006). Roasting profiles are traditionally driven by the need to develop the correct flavor for a specific chocolate product, and not necessarily by food safety. This should be put into consideration when conducting risk assessment. Monitoring procedures for adherence to critical limits, e.g. continuous monitoring such as thermal recording charts during roasting, must be robust and effective.

Debacterization, another thermal process, is categorized as a preventive control (PC1) in this assessment. There is consensus among expert opinion (see survey in **Appendix B**) that this step should be added as a CCP in processing operations provided controllable and measurable parameters exist. In the CCP tree (**Fig. 3.2**), evaluation and justification of the debacterization step is not so clear cut. This is because classification as CCP or PC may depend on sequence of heat application, manufacturer practices or type of equipment being used. In the chocolate industry, the processes that are almost always validated in support of a Food Safety Plan (5-log reduction of *Salmonella*) are the bean or nib roaster and the debacterization system used (personal communication). It is evident from literature and feedback from industry experts that the type of roasting equipment, the type of matrix been roasted (whole bean or nibs), as well as the manufacturer's unique practices generally determine whether or not debacterization is employed alongside roasting as a kill-step, and in what sequence. When used, this process can be classified as a PC if roasting already meets critical parameters for inactivation, and the additional heat application step plays a supplemental role. Chapter 4 (Qualitative Risk Assessment) of this dissertation provides further explanation of this subject.

The **environment** and area where roasted beans are processed (area bounded by brackets in **Fig. 3.3** above) although identified as a second CCP by a few authors (Cordier, 1994; Simonsen et al., 1987), is classified as a preventive control (**PC2**) in the current analysis. Further rationale for this is presented in **Table 3.3**. It is not regarded as a CCP mostly due to the inability to apply a specific critical limit. However, avoiding recontamination after the only kill-step during processing is critical and measures must be put in place to assure this, particularly given that controls applied here could reduce but not eliminate contamination risk. Controls include strict enforcement of zoning, airflow and traffic provisions, constant visual monitoring and environmental microbial analyses: the physical areas where unprocessed beans are handled must be totally separate from areas of further processing of roasted beans; source and movement of air (airborne particulates) as well as direction of traffic throughout production areas must be strictly controlled (Cordier, 1994; Simonsen et al., 1987). Other potential recontamination and cross-contamination routes and opportunities to be closely monitored include condition of air filters, persistence of *Salmonella* in protected niches in various production equipment (Breuer, 1999; Craven et al., 1975; GMA, 2009). Appropriate environmental sampling sites for *Salmonella* or indicator organism assays include air filters, vacuum systems, floor sweepings, although such analyses might only provide information of limited value if not complemented by visual inspections, and moisture or any form of condensation in the processing environment – pipes, valves etc. - must also be strictly controlled as they can provide opportunity for microbial proliferation (Beckett, 2009, Cordier, 1994). In dry food manufacturing facilities, wet cleaning or sanitation are mostly avoided, and when absolutely necessary, measures must be put in place to ensure thorough and rapid drying (Simonsen et al., 1987).

The **mixing and refining** step where some of the other ingredients – spray-dried or non-fat dry milk (NFDM) and sugar - are added, is the third suggested preventive control (PC3) for the milk chocolate production process, and would be specifically classified as a Supplier-controlled PC. Dry, powdered milk in particular has been contaminated with *Salmonella* in the past (Collins et al., 1968; Craven et al., 1975; ICMSF 2002). Although, suppliers must meet certain specifications as well as present a Certificate of Analysis (COA), appropriate sampling plans must be in place to monitor these incoming semi-finished ingredients. As a case reference, a food company recently issued a recall for "nonfat high heat milk powder" sold to companies that use NFDM as an ingredient in their products (FDA, 2016). The precautionary recall was due a positive *Salmonella* test during environmental sampling performed by the FDA at the facility, although not found in the tested finished product. This scenario would have created problems for companies who used the milk powder in a ready-to-eat product, due to the lack of a kill-step before final consumption.

Table 3. 3. Comparative presentation of microbial CCPs and suggested PCs under HACCP and HARPC food safety management systems. Based on HARPC guidelines and review of existing literature, CCPs and PCs were identified for milk chocolate processing and rationale provided for each selection.

Production Step	CCP (HACCP)	Suggested PC (HARPC)	Rationale	Critical Limit or Parameter
Roasting	Yes	Possibly	A process control that requires critical limits and validation. However, classification as CCP or PC may depend on manufacturer practices, product specification, and equipment type. Sequence of application would also influence classification.	Roasting profiles are subject to variation in processing equipment, manufacturer practices and product specification. Example: (105–170)°C; (15–120) min
Debacterization	Possibly	Yes	Not always used; classification as CCP or PC may depend on manufacturer practices, product specification, and equipment type. Sequence of application would also influence classification.	
Environment post-roasting	No	Yes	Failure in PrPs can lead to re-contamination; no further kill-step. A risk-based analysis can be documented to justify this PC	Strict enforcement and monitoring of zoning, airflow and traffic boundaries
Mixing step (addition of '*Salmonella*-sensitive' ingredients)	No	Yes	A supply-chain control to monitor failure in supplier standard; no other kill-step after addition to chocolate mix A risk-based analysis can be documented to justify this PC	Suppliers must document proof that applied preventive control is effective, and receiving facility must verify and approve documented controls

In summary, in addition to established prerequisite programs, validation of the major thermal inactivation step, the post-roasting environment, and incoming high-risk ingredients such as milk powder as an added ingredient, are critical to the microbial safety of a finished chocolate product. These critical and preventive controls would be important in a risk assessment. The information available in literature to aid assessment and validation of HACCP plans for chocolate manufacture are vague and are assumed to be dependent on manufacturer practices, product specification and equipment type.

3.4.3. The interface between HACCP, HARPC and risk assessment

The gradual move towards evaluating food safety on a risk analysis framework rather than a hazard-control approach has been evident in the food industry and in regulatory circles within the last decade. The incorporation of risk assessment techniques into HACCP programs in order to enhance outcomes has been advocated (Serra et al., 1999; Whiting & Buchanan, 1997). Although there were initial handicaps in linking food safety risk assessments to traditional food safety management systems like HACCP mostly due to data gaps, the introduction of FSMA into the US food safety system is beginning to bridge that gap. Using qualitative risk assessment tools to develop risk-based HACCP plans, which in effect is partly embodied by the principles of HARPC, is a good start to providing an interface for these systems. A qualitative evaluation is able to provide a bedrock for further risk assessments by 1) screening risks to determine whether they merit further investigation, 2) facilitating appropriate data selection and collection, 3) exploring the effectiveness of established mitigation measures and 4) providing insights into previously unidentified but possible pathways associated with the hazard of concern. The link between established CCPs, critical limits, PCs and the risk assessment framework relies on the basis that an effective HACCP plan depends on a thorough and science-based knowledge of the food production chain. And if a good risk assessment can use scientific and risk-based tools to identify and assess critical pathways in processing, then a more effective food safety plan is attainable. If assessment is deemed necessary to move from qualitative to a quantitative analysis, a more robust platform becomes available for stronger links between HACCP/HARPC and risk assessment. Advances in food safety risk analysis are beginning to establish a relationship between food safety systems and public health outcomes, especially using quantitative means. These advances would mean getting into statistical probabilities and mathematical modeling of scenarios rather than the simplistic frequency and severity exercise afforded by HACCP.

Parameters used in food safety assessments require validation, thus, it is optimal to utilize a risk-based approach to establish parameters for PCs and CLs, and then further demonstrate how such risk-based parameters can be directly linked to risk management metrics such as Food Safety Objectives (FSOs). As an illustration, during thermal treatment to achieve *Salmonella* lethality, a risk-based approach will place emphasis quantitative estimates of inactivation that can be obtained by specific roasting profiles, rather than simply acknowledging that a thermal control (CCP) was applied within certain critical limits.This holistic approach to process hazards and associated risks, ultimately produces a more meaningful risk assessment.

Chapter 4: From Farm to Packaging: A Qualitative Microbiological Risk Assessment for *Salmonella enterica* during Milk Chocolate Production

4.1. Abstract

Routes of contamination or cross-contamination for *Salmonella* spp. during chocolate production as well as its persistence in the processing environment are currently not well understood or documented. The objective of this study was thus to provide a farm-to-packaging qualitative assessment of *Salmonella* risk factors encountered during the complex processes of cocoa bean cultivation and the subsequent manufacture of milk chocolate. The qualitative risk assessment was built using a modular framework that categorizes all steps in milk chocolate production into four modules – farm, storage and transportation, processing, and post-production. A set of criteria was created defining risk categories as high, medium, and low based on available information, assumptions, and expert opinion. A risk-rating tool was also developed in Microsoft Excel and used to illustrate the usability of the qualitative assessment analyzing the level of risk of a hypothetical milk chocolate product. Risk was qualitatively assessed within each module, and thereafter integrated, leading to the characterization of a final risk estimate and the likelihood of the presence of *Salmonella* in milk chocolate after final packaging. Although preliminary results indicated an overall low risk, "what-if" scenarios were created to enable the analysis of events and ingredients considered to be "high risk" to assess the effectiveness of current controls and evaluate the likelihood of process failures along the production chain. This study provides preliminary data and serves as a framework upon

which a subsequent stochastic quantitative microbial risk assessment (QMRA) model can be developed.

4.2. Introduction

Outbreaks and incidences of contamination reflect the adaptability of *Salmonella* to different niches in the food processing environment, and more importantly, the challenge in pinpointing specific source or entry points along production chain. To our knowledge, no comprehensive risk assessment efforts have been made to address *Salmonella* contamination and interventions during chocolate production, and the need for it has been underscored (Nascimento et al., 2012). It is expected that use of risk analysis tools can be instrumental in providing critical and objective evaluations of the microbial safety of a food product.

Increasingly, food companies are expected to conduct risk assessment to support and justify established food safety management systems. Microbial Risk Assessments (MRA) are used by US regulatory agencies to establish performance standards that, if meticulously adhered to, are assumed to provide a safe food product (Juneja & Marks, 2006; Ruzante et al., 2013). Risk assessments can be performed either qualitatively or quantitatively with various intermediate formats, e.g. semi-quantitative assessment (FAO/WHO 2006). Qualitative risk assessment generally involves describing, compiling and presenting evidence to support prior available information about a hazard and can be employed when inadequacy of data and other resources make it implausible to conduct a full and immediate quantitative assessment. In this case of assessing milk chocolate products, literature review revealed a dire lack of knowledge and data about *Salmonella* contamination during chocolate processing, and so a qualitative approach was applied as a first step in formally assessing risk.

The qualitative risk assessment developed has the following objectives:

1. create a framework that will identify critical steps associated with risk of *Salmonella* contamination along the farm-to-packaging chain
2. present a qualitative characterization of the significant risk factors for *Salmonella* contamination, identify risk reduction strategies and provide preliminary evaluation of priorities in risk-based microbial safety efforts in milk chocolate production
3. provide a framework for subsequent development of a stochastic quantitative microbial risk assessment.

4.3. Materials and Methods

This study utilized the Codex Alimentarius Commission (CAC) recommended methodology (Codex, 1999) for conducting a qualitative food safety risk assessment. A modular approach as described below was used to assess risk at four major stages in the farm-to-packaging continuum namely: 1) farm 2) storage and transportation 3) processing 4) post-processing. For each itemized step in the module, risk was evaluated via stepwise analysis of the chocolate production process and supporting answers to each question using appropriate data and expert opinions, and estimating likelihood where relevant, and finally, making reasonable conclusions for each modular assessment.

4.3.1. Gathering Data and Expert Opinion

Available data and relevant, published studies were identified in literature using web-based, scholarly search engines such as Google Scholar and PubMed, among other electronic research databases. Key words employed in various combinations include "cocoa", "cocoa beans", "*Salmonella*", "chocolate" and "risk". Other sources of information include expert opinion, government online reports, and publicly available theses or dissertation documents. Expert opinions were obtained by means of a survey constructed for the purpose of this study (see **Appendix B**), in addition to personal communications.

4.3.2. Qualitative Risk Assessment Tools

Risk tools used in this qualitative assessment include:

- Risk Rating (estimates of "increase" or "decrease" along continuum)
- Risk categorization (status in these categories include: "high", "medium", "low")
- Risk Narrative and tabulation
- Scenario Analysis

A set of criteria (see Section 4.3.5.) was created by adapting methods utilized in a qualitative risk assessment conducted by the FDA (2015). Risk categories were defined as high, medium, and low based on available information, assumptions, and expert opinion. The qualitative risk assessment culminates in a risk rating tool (see **Appendix C**) where, for each module in a base model, changes in risk status as product moves through the processing continuum were estimated. For the study, a base model is defined as one where it is assumed that all known controls are in place and all process parameters are achieved.

4.3.3. Modular Framework

A modular framework (Fig. I) was constructed using guidance provided by the Food and Agriculture Organization and the World Health Organization, FAO/WHO (Codex 1999), Wooldridge (2008) and Vose (1998); and provides details of the categorization of risk associated with the activities that make up each module. A combination of tables and narratives as well as a tabular summary adopting a model by Wooldridge (2008) is provided at the end of each module. The modular framework, modified from Guo et al (2015) is outlined below:

1) **Farm Module**: analysis of the prevalence of *Salmonella* in the major raw ingredient – cocoa.
2) **Storage and Transportation Module**: examination of effects of storage and transportation conditions on viability of *Salmonella*.
3) **Processing Module**: assessment of effects of key processing steps on the viability of *Salmonella* cells during chocolate manufacture.
4) **Post-Production Module**: consideration of the effects of post-production activities - handling and storage conditions during final production steps until packaging.

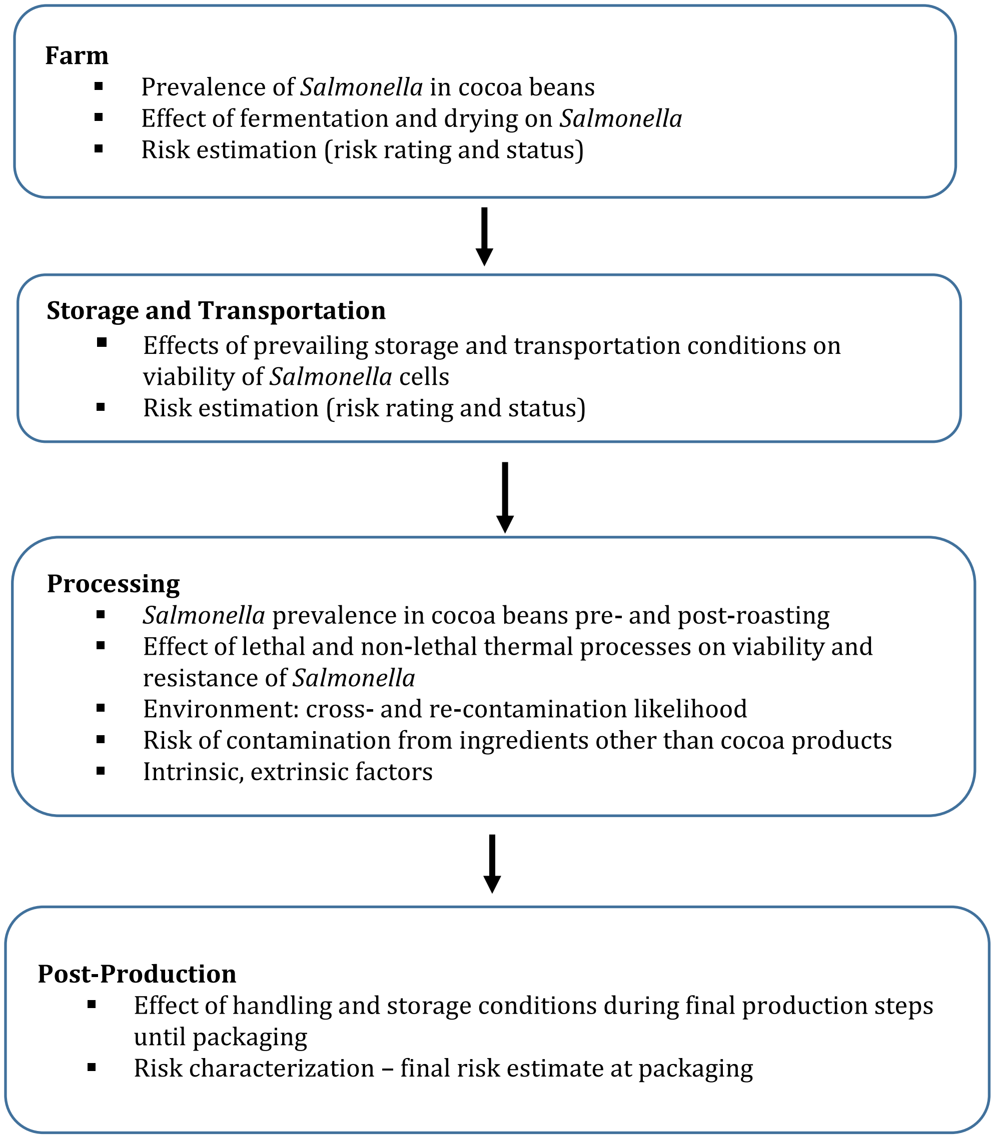


Figure 4. 1. Modular framework of farm-to-packaging qualitative risk assessment

4.3.4. Risk Questions

After the literature review, selected data on the following areas were collated and categorized for each module: *Salmonella* prevalence and behavior in cocoa and chocolate products, effects of fermentation and thermal processing on viability and physiological state of *Salmonella* cells and its survivability in finished chocolate products, and recontamination or cross-contamination incidences during production. These data were used to identify risk factors associated with *Salmonella* contamination of milk chocolate products.

Relevant questions were generated to facilitate the framework for the qualitative risk assessment. For each module, each of the questions below were discussed in narrative format, and answers were provided based on assignment of risk rating and status, and reasoning provided as appropriate.

1. At what point(s) along the cocoa-to-chocolate production chain is *Salmonella* contamination, cross-contamination or recontamination most likely to occur? Also, what specific ingredients or intermediate products carry the most significant risks?
2. What are the activities that may influence *Salmonella* contamination at the various stages of cocoa bean-to-milk chocolate production? What inherent control measures are in place to significantly minimize or prevent contamination while these activities are taking place during processing?
3. Should the thermal processes during roasting be considered the only major critical control point for the inactivation of *Salmonella*, or should considerations be given to other heat-application activities such as debacterization, conching, refining and tempering?

4. What is the likelihood that, under current manufacturing practices, milk chocolate can be a vehicle for *Salmonella* contamination during production and up to the point of final packaging?
5. What data gaps are critical for the implementation of a full QMRA?

Also, factors which are estimated to have a significant impact on contamination risk were grouped into the following categories:

- Pre lethal-step activities
- Thermal applications
- Addition of ingredients
- Production environment

For each factor, the following questions were categorized in each module:

- What controls are available?
    - Intrinsic controls?
    - Intervention control measures?
- What testing or validation procedures are in place?
- Storage conditions, location, time factor?
- What may go wrong – scenarios for when a specific control fails?
- Likelihood of control process failure?
- What are the key assumptions and limitations?

4.4. Results and Discussion

4.4.1. Risk Criteria and Classification Tables

The tables (4.1 and 4.2) below present criteria and other information used in assessing and classifying risk within this chapter.

Table 4. 1. Heat-application and heat-generating activities during milk chocolate processing and effectiveness on *Salmonella* inactivation. Table shows critical parameters and level of inactivation that must be achieved at the critical control points (CCP) during processing.

Matrix	*Activity involving Heat*	*Temperature/Time*	*Achieve lethality*	*Reference*
***Cocoa beans**	Roasting	**(105 – 140)°C; range up to 2 h (if used as critical parameter, must achieve 5-log reduction)	Yes, CCP	Beckett 2009; ICMSF 2005; Industry source 2018; Simonsen et al. 1987; Stobinska et al. 2006
***Cocoa beans**	Debacterization	**(220-240)°C; range up to 20 s (internal bean (134-140)°C) (if used as critical parameter, must achieve 5-log reduction)	Yes, but not established as CCP	Afoakwa 2010; Beckett 2009; Industry source 2018
Cocoa liquor	Grinding	**Exit temp of liquor ~120°C; range up to 60 m	No, not CCP	Beckett 2009; Industry source 2018
Milk crumb	Caramelization, crystallization & drying	**Up to 100°C; range up to 5h	No, not CCP	Miller, 1995; Wells, 2009
Milk chocolate liquor	Conching	**(50-80)°C; range up to 24 h	No, not CCP	Beckett, 2009; Krapf & Gantenbein-Demarchi, 2010
Molten milk chocolate	Tempering	**(30 – 50)°C	No, not CCP	Talbot, 2009

**Whole beans or nibs – depends on roaster-type. **Range is due to variation in processing equipment, manufacturer practices and product specification such as desired flavor profile.*

Table 4. 2. Risk classification of ingredients used in milk chocolate production.

Risk Classification	**Ingredients**	**Rationale**	**References**
Low (L)	• Sugar	Not typically associated with *Salmonella* contamination	Cordier, 1994
	• Natural flavorings, Vanillin	Risk classification can depend on supplier assessment (L or M)	Cordier, 1994; Industry source (personal communication)
Medium (M)	• Lecithin	Isolated occurrences of *Salmonella* has been reported in lecithin; risk classification can depend on supplier assessment (L or M)	Reynolds, 2006; Industry source (personal communication)
	• Cocoa butter	Risk classification can depend on supplier assessment (L or M)	D'Aoust, 1977; Industry source (personal communication)
High (H)	• Milk powder	"*Salmonella*-sensitive" ingredient	Collins et al., 1968; Craven et al., 1975; ICMSF, 2002
	• Milk fat (if used)	"*Salmonella*-sensitive" ingredient	Cotton and White, 1992
	• Lactose (sweetener)	"*Salmonella*-sensitive" ingredient	Cotton and White, 1992
	• Cocoa Liquor	"*Salmonella*-sensitive" ingredient – due to cocoa bean origin	Komitopoulou et al, 2012; Industry source (personal communication)

**Salmonella*-sensitive ingredients are regarded as having historical association with *Salmonella* and thus potential for contamination.

4.4.2. Set of criteria defining risk categories

For the purpose of this risk assessment, a set of criteria was created as shown below to define risk categories as high, medium, and low based on available information, assumptions, and expert opinion (see Section 4.3.2.).

1. Low risk is defined as an activity that:
    a. occurs prior to the kill-step or critical control point(s) during chocolate production; AND is not regarded as a potential source of *Salmonella* exposure, OR
    b. meets critical parameters in place for *Salmonella* inactivation (see **Table 4.1**), OR
    c. meets both of these requirements:
        i. occurs post-lethal step but not considered likely to introduce or increase potential for *Salmonella* contamination; e.g. involves low-risk ingredient (see **Table 4.2**).
        ii. is designed to significantly minimize, prevent or detect *Salmonella* contamination
2. Medium risk is defined as an activity that:
    a. involves heating, but not at a time/temperature combination known to be lethal to *Salmonella*, i.e. does not meet critical parameter criteria, OR
    b. has a reasonably and relatively low likelihood of introducing *Salmonella* into process, e.g. involves medium-risk ingredient
3. High risk is defined as either of these two classifications:

a. an activity that occurs prior to the kill-step, is regarded as a potential source of *Salmonella* exposure or is conducive to *Salmonella* survival; OR

b. an activity that meets all three of these criteria:

   i. occurs post-lethal step and does not meet the critical parameter criteria for *Salmonella* inactivation, AND

   ii. is considered likely to introduce or increase potential for *Salmonella* contamination (e.g. involves "*Salmonella*-sensitive" ingredients), AND

   iii. does not and is not designed to significantly minimize or prevent *Salmonella* contamination.

4.4.3. Presentation of Modules

The modules are presented following the modular framework presented in **Fig. 4.1**.

4.4.4. Farm Module

4.4.4.1. Stepwise analysis

There is significant data gap regarding prevalence and on-farm contamination. Literature has only a few pointers to entry points for *Salmonella* into cocoa beans and the microbial load typically encountered during the major stages of cocoa processing on the farm – harvesting and pod-opening, fermentation, drying and storage. These steps which make up the farm module have been presented in greater detail under literature review. Here, qualitative assessment of the farm module uses available information, perspectives from industry experts, as well as reasonable assumptions to present an evaluation of the likelihood of contamination during cocoa bean processing on the farm. The major stages of cocoa processing on the farm which include fermentation, drying and storage are discussed below.

Harvesting and breaking cocoa pods

After harvest, the process of breaking open the cocoa pods, and exposing the hitherto sterile beans to various microorganisms from sources including but not limited to worker's hands and utensils, the fruit's exterior surface, and ambient air (Afoakwa, 2010, Nascimento 2010, Schwan and Wheals 2004) is considered a potential entry point for pathogens. Information regarding presence of *Salmonella* at this stage is scarce, and only one source - a 2010 prevalence study by Nascimento et al – was found to investigate the presence of enteropathogens during farm processing of cocoa beans. This study, although limited in scale, presented data which provides some insight into the possibility of contamination at this stage. A total of 30 cocoa bean samples from three different Brazilian farms were analyzed. Neither *Salmonella* nor *E. coli* was detected in any of the pre-fermentation samples, however total coliforms averaging 1.2 CFU/g were detected in up to 70% of samples from one farm (Nascimento et al, 2010). The authors reasoned that the presence of *E. coli* would have indicated fecal contamination, and since this was absent in all samples tested after pod-opening but prior to fermentation, it was concluded that this stage was an unlikely entry point for *Salmonella*. However, in evaluating risk of contamination at this step in the farm module, it would be preemptive to make general inferences from this study given scope and data limitations. Our knowledge of ubiquitous pathogens informs the perspective that general contamination from the environment is not unexpected at this stage, particularly when considering that inadequate sanitary conditions and heavy handling are commonplace (Cordier 1994, 2000). Hence, for the purpose of this risk assessment, we assume cocoa pod-opening as the first step that can introduce contamination.

Fermentation

Although the fermentation process varies according to geographic and traditional practices around the world, the basic steps are similar. After removal of beans from pod, the beans and its adhering pulp are collected in heaps, boxes or baskets for fermentation which lasts anywhere between 2 - 8 days (Thompson et al., 2013). In small-scale or traditional settings as seen in West Africa, cocoa beans undergoing heap fermentation are left in a pile or a hole in the ground and covered with banana or plantain leaves. The fermenting mass is regularly turned to ensure even fermentation. At this stage, further environmental contamination can occur from surrounding air, dust, insects, soil, plantain leaves used to build heaps and cover boxes, or the materials used to transport pulp and beans (Beckett, 2009; Cordier, 2000; de Smedt et al, 1991; Nascimento et al., 2010). As a result, risk of contamination with *Salmonella* cannot be ruled out at this point.

The natural fermentation process is initiated when the mucilaginous, sugar-rich pulp inside the bean pods attract various microorganisms (Afoakwa, 2010). This pulp is an excellent medium for microbial growth given that it contains about 10–15% sugars and coupled with the high moisture content (~ 65%) of freshly harvested beans (Fowler, 2009). Microbial population during fermentation is often variable in quantity and type, but largely consist of yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB), all of which develop in succession (Thompson et al., 2013). These microbial species are known to tolerate the low pH - 3.0 to 3.5 - of the initial cocoa-pulp mass (Illeghems et al., 2016). Under fermentation conditions which include high temperatures of up to 50°C and accumulation of by-products such as ethanol, lactic acid, and acetic acid, *Salmonella*, if present, is not known to proliferate, but can survive (ICMSF, 2005). In the 2010 prevalence study by Nascimento et al, salmonellae were not detected either before or during fermentation. However, it was reported that 23% of 30 samples examined were contaminated with *E. coli* during the fermentation process, with counts ranging from 1.0 to 2.0 log CFU/g, and total coliform counts ranging from 0.6 to >3.0 log CFU/g. It was inferred that this *E. coli* contamination most likely occurred during fermentation, rather than being an indication of proliferation of existing cells. However, in further experiments set up to determine the behavior of *Salmonella* during cocoa bean fermentation, the same researchers presented data to suggest that *Salmonella* can grow during fermentation, although growth may be hampered by yeast and other acid-producing bacteria (Nascimento et al 2013). It was also demonstrated that *Salmonella*'s behavior was significantly influenced by the fermentation stage in which it was inoculated. For instance, in the early stages of fermentation, it has been shown that the microbial actions of yeasts multiplying in the pulp typically create conditions of low oxygen and high acidity (pH 3.4 – 4.0) (Fowler, 2009, Thompson et al., 2013), conditions unsuitable for *Salmonella* growth. Under comparable experimental conditions in Nascimento's study, *Salmonella* counts (ca. 4 CFU/g) were observed to remain stable during the first 24 h at a temperature of about 23°C (Nascimento et al, 2013). In the intermediate stages of fermentation, a strongly exothermic reaction initiated by AAB, produces increased aeration and a temperature rise of up to 50°C (Fowler, 2009; Thompson et al., 2013). Again, at this intermediate stage and under the designed experimental conditions, *Salmonella* counts fell below the detection limit of 0.48 log MPN/g (Nascimento et al, 2013), predictably due to prevailing conditions. During the final stages of fermentation when the fermenting mass would typically experience a rise in pH of up to 6.5, and a drop in temperature, conditions once again becomes relatively favorable for bacterial growth. This was observed in Nascimento's study where samples inoculated on Day 6 of fermentation showed significant growth within 24 h after inoculation. The growth dynamics of the other microorganisms present – yeast, LAB and AAB – were not affected by the presence of *Salmonella* (Nascimento et al, 2013). In addition, it was observed that if contamination occurs during fermentation, growth increases during drying, and if it occurs during drying, growth is varied and can increase or decline (Nascimento et al, 2013). This is the only study found to provide an indication of the effect of cocoa bean fermentation on *Salmonella* growth or survival. An older study (Camu et al., 2007) which assessed cocoa fermentation on a large scale in Ghana observed a pH of 4.3 in the final stages of fermentation, conditions not favorable for proliferation. Although salmonellae are considered mesophilic in nature, several strains have been found to grow or survive at extremes of extrinsic factors such as temperature, pH or water activity (Beuchat et al., 2010; Mattick et al., 2001; Scouten et al., 2002; Shachar et al., 2006; Uesugi et al., 2006). Fowler (2009) also affirms that the fermentation process is one that can result in high microbial levels, with *Salmonella* being a possible contaminant.

Studies of *Salmonella*'s behavior during fermentation in other food matrices present varying results, and could be considered a source of uncertainty. In a recent study investigating *Salmonella*'s behavior during the fermentation process of yogurt (pH 4.2-4.5), it was observed that cells can survive fermentation even at low contamination levels (3 log CFU/ml) (Savran et al., 2018). Also, the susceptibility of S. Typhimurium to inactivation by various LAB during skim milk fermentation was found to be proportional to the amount of inoculum used and was dependent on the type of dominant LAB. These observations do not present strong evidence regarding *Salmonella*'s behavior during fermentation, rather they indicate outcomes can vary depending on several factors.

In assessing risk therefore, it must be considered that inhibition or inactivation of salmonellae by lactic acid bacteria can be affected by factors including type of LAB, food matrix type, temperature and water activity levels. Although the limited data available indicates fermentation is not unequivocally known to act as an antimicrobial process for *Salmonella*, a handful of studies are inadequate to draw meaningful inferences on risk during the fermentation stage. For the purpose of this assessment, fermentation is estimated to increase or decrease the risk rating, depending on aforementioned factors.

Drying

At the end of fermentation, the beans are dried either naturally (sunlight) or mechanically, with the aim of reducing their moisture content from 40 – 60% to 6 - 8%, a level that is microbiologically optimal for safe storage and mold prevention (Burndred, 2009; Copetti et al, 2014). Drying methods and conditions will mostly determine contamination or re-contamination of fermented beans, as there may be little to no environmental control. Methods range from sun drying on tarps for small scale farmers to mechanical drying on racks as used on larger plantations. In the 2010 prevalence study by Nascimento et al, *Salmonella* was not detected during the drying stage, but E. coli and coliform contamination had significantly increased in comparison to the fermentation stage (Nascimento et al, 2010). This study inferred that the potential for presence of coliforms and *Salmonella* is increased during and after drying of cocoa beans, with drying suggested to be the most critical stage for introduction of *Salmonella* due to easy access to air, soil, dust, insects, birds or other animals, fecal contamination, and workers' feet or hands during this period. In observing prevailing conditions on the farms used in their study, Nasciemento's group noted the easy access of vectors such as birds, rodents and insects to cocoa beans, both during drying and storage, and suggested that environmental storage conditions as well as length of storage may be strong indicators of the occurrence of contamination. However, it was reported that no correlation was observed between the presence or counts of Enterobacteriacea and drying time.

Ultimately, the mode and efficiency of the drying process can significantly influence the shelf life and microbial quality of the cocoa beans. Based on available information, risk at this stage would depend on the drying method, with the natural drying process presenting a greater risk than mechanical drying. In this assessment, *Salmonella* as well as Enterobacteriacae can be expected to be present during drying.

On-farm storage of dried beans

Dried beans are typically sorted and graded by hand before being packed away for storage. Current marketing practices dictate that fermented and dried cocoa beans may remain in storage for anywhere between 3 to 12 months (Thompson et al., 2013). Storage locations might include farm or plantation warehouses, shipping docks during import or export and warehouses at manufacturing facilities prior to further processing. Information on the occurrence of salmonellae during the post-drying stage is limited to only a couple of studies. Nascimento's group (2010) found a low prevalence of *Salmonella* – a single positive out of 29 samples (3 %) from cocoa beans in storage on three different Brazilian farms. An environmental study of dust residue from warehouses and areas where raw beans were handled and stored indicated a low level but relatively high incidence of *Salmonella* (79 % - 258 of 325 samples) (De Smedt et al., 1991). For the latter study, the specific location of the warehouse where the natural contamination occurred was unclear, with storage possibilities being on the farm site or in the manufacturing facility. Generally, salmonellae are known to be good survivors and can persist in dry raw materials. A study by Izurieta and Komitopoulou (2009) which examined the survival of salmonellae on dry confectionery materials found salmonellae to survive storage up to 4 weeks on inoculated cocoa beans at ambient conditions, with survival being dependent on strain-type, method of cell preparation and inoculation, and storage temperature.

Given the scarcity of data for salmonellae prevalence in raw materials used in chocolate production, we examined the occurrence of *Salmonella* in similar dry matrices such as tree nuts. A study by Danyluk et al. (2007) reported a prevalence rate of 0.87% in Californian raw almonds sampled over 5 years, while some other studies reported higher prevalence rates of various *Salmonella* serotypes in the same matrix: 1.6% and 0.83% in two separate but consecutive years (Bansal et al., 2010). (Brar, 2015) reported a total *Salmonella* prevalence rate of 0.95% over a four-year period obtained from naturally contaminated in-shell pecans. For the current assessment, we assume that whichever microflora was introduced during drying will likely persist during storage at the same or at a decreased level, given that microbial decline over time is expected in dry matrices (Oni et al, 2015; Podolak et al, 2010).

Lot or batch homogeneity is rare, and the non-homogeneous distribution of pathogens in matrices, particularly dry foods, is expected (Kamphuis, 2009). Reasons for the nature of distribution of microbes within a matrix include but are not limited to the structure of the food matrix and the mode of contamination (Bassett et al., 2010; Jongenburger et al., 2012). In the case of *Salmonella* distribution in cocoa beans, low prevalence and high doses or vice versa, may be significant factors which determine risk rating during storage.

4.4.4.2. Summary Table for Farm Module

The table below concisely presents the farm module, an overall risk status for the module for a base model, as well as rationale for such risk categorization based on the pre-defined criteria (See section 4.4.2).

Table 4. 3. Summary table for risk assessment of farm module

What is the likelihood of *Salmonella* contamination of dried cocoa beans during farm processing?			
Processing step/scenario	**Risk Rating (change in risk level)**	**Risk categorization based on criteria**	**Rationale for risk categorization**
Harvesting and pod-opening	Increase	High (criteria 3a)	Step known to be first to introduce potential contamination into sterile bean pods. Handling and access to environmental contamination increases risk.
Fermentation	Increase or Decrease	Inconclusive based on available scientific evidence	If present, *Salmonella* behavior may be dependent on time of contamination; increased pH at end of fermentation period may increase chances of proliferation or survival.
Drying	Increase	High (criteria 3a)	a_w during initial drying period can favor *Salmonella* proliferation or survival.
Storage of dried beans	Unchanged or Decrease	High (criteria 3a)	Prolonged storage and storage conditions are possible risk factors for survival.
Summary & Conclusions: Scarce information on *Salmonella* prevalence on farm. Limited data suggest a high likelihood of exposure to *Salmonella* during on-farm processing of cocoa beans, considering the various ways cocoa beans are handled. Also, several steps in the farm module could be possible sources of environmental contamination. However, such contamination may be sporadic by nature, occur in clusters and therefore quite heterogeneous. It is also possible that prevalence remains the same during most farm steps, with distribution varying slightly after fermentation and drying, due to the biochemical and pH changes previously described. Generally, *Salmonella* behavior appears to be dependent on the stage and time at which contamination occurs. Limited indication exist that fermentation may minimally contribute to inactivation under certain conditions. End of fermentation and initial drying stages are probably the most critical periods for contamination. The utilization of Good Agricultural Practices (GAPs) on farms would decrease contamination risk as they serve as the first line of defense to minimize natural contamination on cocoa bean farms. However, lack of enforcement of monitoring is to be expected on many plantations where cocoa beans are cultivated. Data available does not provide an unequivocal indication of prevalence at major stages in farm module, however experts generally agree that if *Salmonella* contamination occurs on the farm, chances of survival, but not proliferation, are significant as bagged beans get ready to be stored or transported. Uncertainty and variability are characterized based on the availability or lack of data as well as inherent differences among factors being assessed. For example, uncertainty in *Salmonella* prevalence levels in dried cocoa beans is significant due to scarce data and comparison with similar matrices such as almonds. The lack of data on prevalence and concentration levels of *Salmonella* during various stages of farm production informs the need for more research data. For a base model, the overall risk at the farm level is **high**.			

4.4.5. Storage and Transportation Module

4.4.5.1. Stepwise Analysis

Once filled in bags, the likelihood of changes to prevalent microflora in the cocoa beans is largely dependent on storage conditions during transportation. In tropical regions, storage can present unique challenges such as re-humidification, among other issues (Hii et al., 2019). To combat this, it is recommended that storage periods not exceed 3 months except precautions are taken to control temperature and humidity (Hii et al., 2019). Optimal transportation conditions are essential to prevent moisture buildup and mold growth in cocoa beans. If properly dried and stored, cocoa beans are quite stable and will not deteriorate in quality for several years (Fowler, 2009), and the likelihood of microbial changes are low as raw beans remain bagged until further processing steps at the factory. However, in terms of microbiological quality, given the high likelihood (see farm module) that salmonellae is already present in bagged cocoa beans, an assessment of the transportation and accompanying storage period is important to aid understanding of the potential behavior of the pathogen cells and ultimately the contamination risk presented at this stage.

Risk factors to consider during transportation and storage include:

- Mode of transportation
- Prevailing physiological characteristics of cocoa beans -- a_w, pH, moisture level
- Prevailing storage conditions in transporting vessel – temperature, relative humidity
- Transit duration

- Physiological state of *Salmonella* if present

Although no scientific studies were found specifically addressing risk factors for pathogen survival during cocoa bean transportation and storage, general information from literature as well as cocoa shipping companies do provide some insight. The cocoa beans supply chain is complex as numerous small-scale farmers, often situated in developing countries, have to transport their products to chocolate manufacturers usually located in distant, temperate regions (Fowler, 2008). Cocoa beans, mostly transported on cargo ships, can also be transported locally and internationally via railroad or large trucks, using standard containers such as jute bags – which are durable, breathable and adaptable containers that facilitate easy sampling during inspection. According to the Transport Information Service of a German shipping company, GDV, cocoa beans is classified as cargo that requires specific temperature, humidity, moisture and ventilation conditions to minimize deterioration in quality (Transportation Information Service, n.d.). Various methods of cocoa shipment including but not limited to break-bulk (loading bagged cocoa beans into ship holds), mega-bulk (loading cocoa beans directly into ship hold with the ability to transport several thousand tons in one hold), or the use of containers which can either be loaded with sacks or loose-filled (Fowler, 2009). Although shipping system used would depend on factors such as quantities shipped, destination and facilities available, it is estimated that up to 70% of cocoa beans shipped to northern European ports utilize bulk-shipping methods (Fowler, 2009).

Physiological characteristics of the beans such as the moisture level is a critical parameter during shipping as it can have significant effects on quality and microbial activity. It is recommended that moisture level of bagged cocoa beans remain between 6 and 8 % to prevent mold problems. Cocoa transported within this range will correspond to an equilibrium relative humidity range of 70 – 85 % at prevailing temperatures which may fluctuate between 15 – 30°C depending the originating region and destination (Fowler, 2009; Transportation Information Service, n.d.). Improperly dried beans (>8 %) present major problems during shipping. Cocoa beans, while highly hygroscopic, are known to also release significant amounts of water vapor during transportation, conditions which can be mitigated by proper ventilation. In many cases, desiccants are used for condensation control within the transporting vessels (Cargo Handbook, n.d.). Some transporters indicate that further drying can occur during shipping, as made evident by 1-3% shrinkage of the carriage bags particularly during extended voyages (Transportation Information Service, n.d.). This shrinkage can also be interpreted as moisture loss and can be factored into assessing risks of salmonellae proliferation or survival. In cases of inadequate drying or accidental wetting of cocoa bean bags, the risk of proliferation increases significantly. There is no data to assess the likelihood or frequency of this occurrence, thus assessment here is limited.

Water activity can be expected to vary based on its close association with moisture levels, and pH levels are not expected to change from the post-fermentation levels of approximately 6 - 7.

Transit duration may also play a role in salmonellae behavior. Several studies have shown that *Salmonella* is able to survive for extended periods under low moisture conditions (Beuchat et al., 2013; Lambertini et al, 2016; Oni et al, 2015; Podolak et al., 2010). It is therefore expected that, under transportation conditions, salmonellae may either decline or die off completely. If, for example, a container in ideal shipping conditions has contamination levels of 10,000 CFU/g, and shipping were to take a total of three months, *Salmonella* levels may reasonably be expected to decline to 1,000 CFU/g upon arrival at a manufacturing facility (expert opinion). In this case, the cells are possibly stressed and may have acquired some form of resistance, a possibility that may have consequences down the line. The estimates of prevalence during transportation provided by experts are at best, educated guesses or reasonable approximations, and not data from specific testing. Inferences from our knowledge of possible shipping conditions suggest that salmonellae, if present, are likely to either decline or remain at prevalent levels during shipping – outcomes which will largely depend on a combination of prevailing conditions.

4.4.5.2. Summary Table for Storage and Transportation Module

The table below concisely presents the storage and transportation module, an overall risk status for the module for a base model, as well as rationale for such risk categorization based on the pre-defined criteria (See section 4.4.2).

Table 4. 4. Summary table for risk assessment of storage and transportation module

What is the likelihood of *Salmonella* contamination of dried cocoa beans during transportation, or if already present, what is the likelihood of proliferation, survival or decline during transportation?				
Process step/scenario	**Likelihood of contamination/ proliferation/survival/decline**	**Risk rating (change in risk level)**	**Risk categorization based on criteria**	**Rationale for risk categorization**
During transportation, if salmonellae not present in bagged cocoa beans	Likelihood of contamination - Low	Unchanged	High (Criteria 3a)	To our knowledge, there are no mitigating circumstances for *Salmonella* contamination during shipping
During transportation, if salmonellae already present in bagged cocoa beans	Likelihood of proliferation – Low Likelihood of survival –High Likelihood of decline – Medium	Unchanged or decrease	High (Criteria 3a)	Behavior of salmonellae cells, if already present in bagged cocoa beans, will be largely dependent on prevailing conditions in shipping vessel (a_w, moisture level, RH), as well as duration of transportation. Proliferation, decline or no change may occur depending on storage conditions and moisture levels (inadequate drying or accidental wetting)

Summary & Conclusions:
No specific scientific information related to the behavior or physiological characteristics or kinetics of salmonellae that may be present during cocoa beans transportation. The information presented in this module bears significant uncertainty due to the lack of supporting data from literature, however prevailing knowledge and expert opinion augments our assessment.

Given the high likelihood of contamination prior to transportation (see Farm Module), it can be inferred with a reasonable level of confidence that cocoa beans bags are already contaminated, although there are no indications as to the levels that may be present.
Expert opinion presumes that there is very little probability that contamination would occur during transportation, or that, if already present, salmonella cells would proliferate. *Salmonella* cells already present would either survive shipping conditions and maintain dormancy, or decline. And accordingly, risk rating would decrease or remain unchanged. Risk outcomes are largely dependent on the following factors: 1) prevailing condition in shipping vessel – RH and temperature; 2) transit duration; 3) cocoa bean physiological characteristics (a_w, moisture level, pH); and 4) state of existing salmonellae.

In this base model assessment, the overall risk status of cocoa beans is not changed as a result of transportation, although some reduction in existing microbial levels is expected. Thus, risk is estimated as medium to high.

4.4.6. Processing Module

4.4.6.1. Stepwise Analysis

This module is the most important in our assessment as it includes steps which directly affect *Salmonella* contamination risk. A good amount of information about steps in milk chocolate processing is available in literature. The risk-based assessment of hazards in this module is designed to be retrospective - evaluating risks that may come with raw materials or ingredients, and prospective - assessing risks that may arise post-processing. **Fig. 4.2** shows a schematic representation of the process while identifying CCPs and PCs as discussed in Chapter 3.

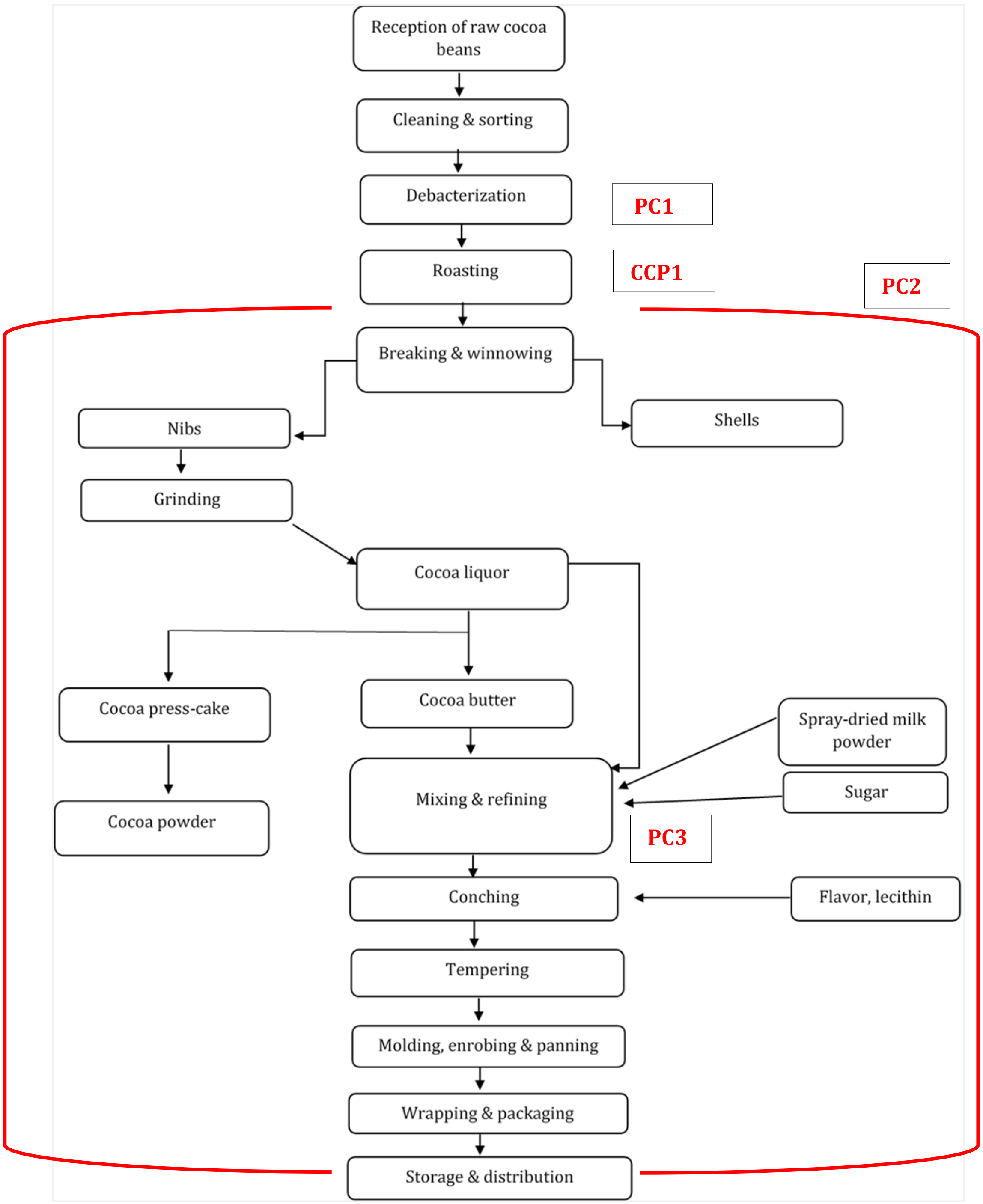


Figure 4. 2. Schematic representation of industrial milk chocolate production showing identified Critical Control Points (**CCPs**) and suggested Preventive Controls **(PCs)**. The area bounded by red brackets represents the environment post bean-roasting up until packaging.

A conceptual model was also developed (See **Appendix A**) to aid the development of a baseline risk estimate. The Table 4.5 below (an abbreviated version of Table 3.3 in Chapter 3) provides rationale for CCP and PCs designated in **Fig 4.2** above.

Table 4. 5. Comparative presentation of microbial CCPs and suggested PCs under HACCP and HARPC food safety management systems

Production Step	CCP	Suggested PC	Rationale
Roasting	Yes	Possibly	A process control that requires critical limits and validation. However, classification as CCP or PC may depend on manufacturer practices and equipment type
Debacterization	Possibly	Possibly	Not always used; classification as CCP or PC may depend on manufacturer practices and equipment type
Environment post-roasting	No	Yes	Failure in Prerequisite Programs (PrPs) can lead to re-contamination; no further kill-step. A risk-based analysis can be documented to justify this PC
Mixing step (addition of '*Salmonella*-sensitive' ingredients)	No	Yes	A supply-chain control to monitor failure in supplier standard; no other kill-step after addition to chocolate mix. A risk-based analysis can be documented to justify this PC

Reception of cocoa beans, cleaning and quality assessment

Cocoa beans, upon receipt, can be stored in silos or similar containers at the manufacturing facility prior to roasting. The beans are typically cleaned by sorting, de-stoning and metal-detection procedures to remove potential physical hazards. On a microbial level, it is not a common practice to determine *Salmonella* levels for incoming raw cocoa beans because contamination is expected. Total bacterial load on cocoa beans received is oftentimes unknown, but has been estimated to range from 1-10 x 10^6 CFU/g (Kamphuis, 2009). Data on *Salmonella* prevalence at this stage is mostly unavailable as it is not considered beneficial to make quantitative determination of *Salmonella* prevalence prior to roasting (personal communication, 2018). However, a few estimates of possible levels in raw cocoa beans are available in the scientific literature. Smedt et al (1991) reported a 79.4% incidence of *Salmonella* in naturally contaminated cocoa bean dust samples, and a reference by the same authors to unpublished data indicates that raw cocoa beans upon arrival at the manufacturing facility frequently contain low levels of salmonellae between 1 and 10 CFU/25 g. These counts were said to have been obtained from dust gathered during a bean-cleaning operation. Barrile et al (1971) characterized the microflora of raw cocoa beans and roasted cocoa beans and reported that all isolates identified in unroasted beans were either Bacilli spp. or Micrococci spp., with only bacilli isolated post-roasting. Concentrations of total bacteria load up to 10^5 -10^7 CFU/g, similar to levels estimated by Kamphuis (2009), were detected prior to roasting while counts fell to between 10^3 to 10^5 CFU/g after a 40-min roasting period (Barrile et al, 1971). Although no salmonellae contamination was identified in the study by Barrile, it is assumed that contamination incidences may be sporadic, depending on the origin of the beans among other factors.

As previously stated, occurrences of contamination may be highly heterogeneous given the commonly reported non-homogeneous distribution of pathogens in dry matrices. Cleaning and quality assessment are procedures which may lead to cross-contamination within the production facility, particularly given the airborne-prone dust from the cocoa beans (Bell & Kyriakides, 2002; Smedt et al, 1991). PrPs and HACCP guidelines regarding zoning and continuous environmental monitoring are generally in place at production facilities to mitigate this risk. For these reasons, risk rating at this step for a base model is assessed to remain unchanged from the preceding storage and transportation module.

Roasting and Debacterization

Roasting, the application of dry heat, achieves two important things: foster the chemical reactions needed for the development of chocolate flavors, and serve as the major lethal step and critical control point (CCP) for controlling microbial contaminants including *Salmonella* (Copetti et al, 2014; Cordier, 1994). Roasting profiles are traditionally driven by the need to develop the correct flavor and desired organoleptic properties for a specific chocolate product, and not necessarily by food safety. This should be put into consideration when assessing risk. Debacterization, also referred to as sterilization or steam-treatment, was largely introduced as a method of preliminary humidification to facilitate removal of cocoa shells prior to roasting whole beans and to improve flavor in cocoa nibs (Burndred, 2009; Ziegleder & Oberparleiter, 1996). However, the process has also become recognized as a treatment for whole cocoa beans or nibs to aid in microbial inactivation (Afoakwa, 2010; Burndred, 2009). Inclusion of the treatment allows the subsequent use of lower roast processes that preserve polyphenols and create a variety of flavors. It involves brief exposure to a combination of high temperature and super-heated steam and can be carried out prior to, during or after roasting. The introduced moisture is said to significantly increase the lethality of the process. When done before roasting, reported heating profile is about 60°C for 10–15 min with subsequent drying at 98–110°C to achieve a moisture level of 3% for optimal roasting (Burndred, 2009). When carried out during or after roasting, steam treatments are done in such a way that moisture of the beans is not significantly increased (Motarjemi & Lelieveld, 2013), with temperatures that could go up to 240°C for 3-5 s (Afoakwa, 2010). Post-roasting sterilization can further destroy any heat-resistant bacteria or spores that may have survived roasting (Afoakwa, 2010, Stehli et al, 2002). It is unclear how common the use of the debacterization system is among chocolate manufacturers, as only a handful of sources refer to it as an adopted practice (Afoakwa, 2010; Awua, 2002; Burndred, 2009; Krapf and Gatenbein, 2010). In addition to the aforementioned thermal inactivation procedures, other processes such as Near Infrared Roasting exist that utilize superheated steam during roasting of whole beans, nibs or cocoa liquor.

There was consensus among expert opinion that debacterization should be added as a CCP in processing operations provided controllable and measurable parameters exist, although some experts indicate that debacterization should not necessarily be separated as a CCP from roasting. Either way, monitoring procedures for adherence to critical limits must be effective because of the potentially serious consequences of a deviation from this critical limit. Whatever step is referred to as a CCP will largely depend on the position of the major kill-step within the process, and whether there are subsequent or combined steps. This should be determined during the HACCP process.

Intrinsic and Extrinsic Factors Affecting Effectiveness of Heat Treatment

Possible synergistic effects of intrinsic factors such as water activity, pH and fat content of chocolate, or other factors such as history and physiological state of *Salmonella* cells, the microstructure and amphipathic nature of chocolate molecules – may influence thermal resistance and kinetics of *Salmonella* in chocolate during processing.
Heat resistance of *Salmonella*e in dry environments and observed reductions on adding moisture, has been reported for chocolate and cocoa liquor matrices (Barrile & Cone, 1970; Davies et al., 1990; Goepfert & Biggie, 1968; Krapf & Gantenbein-Demarchi, 2010). Goepfert and Biggie (1968) conducted experiments which demonstrated the high heat resistance of *Salmonella*e cells in cocoa liquor, while Barrile and Cone (1970) showed that heat resistance could be considerably lowered in this type of matrix, by addition of small amounts of water – about 1–4 % w/w.

To our knowledge, either roasting alone, or roasting in addition to debacterization can serve as lethality steps. It is apparent that the type of roasting equipment being used, the matrix been roasted (whole bean or nibs), the desired end product, and the manufacturer's unique practices generally determine whether or not debacterization is employed alongside roasting as a kill-step, and in what sequence. For example, a Barth roaster, typically used for low-roast applications, would employ a micronizing or pre-roast step where infra-red energy of up to 700°C, in addition to a flash of moisture applied to whole cocoa beans for a few minutes. This surface heat treatment is used to facilitate easy shell removal thereby concentrating heat on the surface rather than the inside of the bean (Kamphuis, 2009), although internal bean temperature can reach 115°C.

The subsequent winnowing step removes the shell and breaks the bean into pieces (nibs). Nibs are typically roasted at a time-temperature combination that meets the critical parameter for pathogen inactivation (see **Table 4.1**). Although roasting is the only step considered to meet CCP parameters here, the micronizing step can also be regarded as an inactivation step due to the added moisture that can increase lethality. This is supported by studies such as Izurieta and Komitopoulou (2012) whose experiments to investigate the heat resistance of salmonellae in crushed cocoa bean and hazelnut shells, including the effect of moisture content, found that increasing the moisture level of cocoa or nut shells just before or during heating significantly decreased microbial thermal resistance. Other important factors to consider include the physiological characteristics of the matrices before, during and after heat application. With incoming cocoa beans having moisture content of approximately 6 – 8 %, the roasting process can effectively reduce this to a final level of between 1 – 2 % (Nascimento et al. 2012; Simonsen et al., 1987), while corresponding water activity levels decreased from 0.75 to 0.50 (Nascimento et al. 2012). This decrease in both moisture and water activity levels could significantly contribute to increased thermal resistance of salmonellae. Therefore, depending on the initial pathogen load and process parameters, roasting may not guarantee complete thermal inactivation and may even increase thermal resistance (Krapf and Gantenbein-Demarchi, 2010). It is expected, however, that chocolate producers would rely on validated thermal processes parameters.

A Buhler hybrid roaster, as another example, carries out debacterization using super-heated steam which achieves the critical parameter for inactivation shown on **Table 4.1**, followed by roasting and winnowing. For this roaster, the debacterization step may be regarded as the major kill-step, rather than the ensuing roasting step. Buhler roasters employ a continuous roasting process where beans may be roasted across several temperature zones for a specified time.

The type of matrix roasted – whole bean or nib – is another intrinsic factor which may also have an impact on effectiveness of roasting. In the study done by Nascimento et al (2012), matrix type influenced heat resistance as the D-value of *Salmonella* was slightly greater in cocoa nibs compared to cocoa beans upon exposure to temperatures ranging between 110 and 130 °C, an observation that was attributed in part to the greater fat content of nibs. By comparison, thermal resistance observed in similar matrices such as hazelnut shells and almonds show that the D-values calculated by Nascimento (2012) for cocoa beans at 110 and 120 °C (4.79 and 3.62 min respectively) were higher than those reported by Izurieta and Komitopoulou (2012) for cocoa bean shells ($D_{105\,°C}$=0.72 to 1.0 min) and hazelnut shells (D105 °C=2.0 to 2.5 min), and also higher than the values documented by Harris et al (2012) who reported a D-value of 0.8 min at 121°C in almonds. These range of values support the assessment that, besides other possible factors at play, the matrix type influences thermal resistance. Internal bean temperature is another factor to be considered. Although it is unknown whether salmonellae cells burrow into the center of the bean or largely remain on the bean or outer shell surface, the internal bean temperature is said be controlled mostly for flavor development purposes, rather than microbial inactivation. Also worthy of note here is the common practice of some manufacturers to blend beans from different origins or sources to achieve specific desired qualities in the end product (Fowler, 2009). Ideally, this should be factored into a risk assessment.

Shelling, Winnowing, and the Role of Sequence of Heat Treatment

Generally, microbial contamination on shell-intact nuts similar to cocoa beans is likely to be restricted to the shells' outer surface (Grocery Manufacturers Association, 2009). In cases where whole-bean roasting is carried out, shell-surface microbial contaminants are expected to be inactivated, and the risk of dissemination of these microbes from shell to nibs is apparently minimized (Barrile et al., 1971). As such, if cocoa beans are thermally treated using both debacterization and roasting prior to shelling and winnowing, risk can be estimated to be further reduced than when a single thermal process is used. On the other hand, the likelihood of cross-contamination from shell to nibs may increase if nib-roasting is the preferred pathway. As a result, the type of roaster being used as well as the sequence of thermal application may play a role in how risk is evaluated at this step.

Critical parameters for both roasting and debacterization are shown on **Table 4.1**. Recommended reduction levels during the roasting step is specified as 4-5 log by the National Confectioners Association Chocolate Council (NCACC, 2011). This is similar to the minimal reduction level (5-log) recommended by the FDA for other dry products such as peanuts and pistachios (FDA, 2009 & 2011) and almonds (4-log) (Almond Board of California, 2017). Risk is estimated to be reduced to a low, or at a minimal level, if roasting and debacterization, the two thermal processing events identified as critical control points, meet the critical parameters identified on **Table 4.1**. In our assessment, we suggest that the definition of a particular process as a "CCP" may vary depending on the position of the kill-step within the process – consideration being given to whether subsequent or combined steps are possible which meet critical parameter for inactivation. For instance, a debacterization system is the primary CCP when used to apply a 5-log pasteurization kill step. The roasting process which follows is primarily used to dry the beans to an appropriate moisture level. In this case, the post-debacterization process adds an additional pathogen reduction step, and may not necessarily be referred to as the primary CCP. Therefore, the two processes can be validated as one contiguous process or as separate components of the pasteurization process (see **Table 4.5** for rationale on CCP and PC identification).

Additional *Salmonella* survival and thermal resistance data would be helpful for cocoa matrices such as whole cocoa beans, nibs and liquor. Data on *Salmonella* behavior in milk crumb, the intermediate product during milk chocolate production, is currently lacking.

Post-Roasting Activities

No post-roasting step is guaranteed to have lethal effects on *Salmonella*. Microbiological safety considerations during post-roasting activities up until the point where chocolate is packaged for storage and retail distribution generally fall into three categories: i) if complete pathogen destruction is not achieved, either due to a failure in the thermal process, the presence of excessive levels of salmonellae or existence of highly resistant *Salmonella* (e.g., *S.* Seftenberg 775W); ii) if re- or cross-contamination from the environment occurs at any stage in between post-roasting and packaging; or iii) the post-thermal treatment addition of high-risk ingredients.

The low water activity of intermediate products such as cocoa liquor, cocoa butter or milk crumb is known to increase *Salmonella*'s resistance to heat, such that small numbers of *Salmonella* have been shown to survive typical temperatures reached during the milling, refining, or conching steps of chocolate processing (Lund et al., 2000; Simonsen et al., 1987). It is likely that these cells are already protected by the fatty phase of the cocoa liquor matrix (fat content ~ 50%). The combination of low water activity and high fat content can significantly increase thermal resistance so that subsequent temperatures reached during chocolate production may not ensure the destruction of *Salmonella* (Cordier, 1994; Hiramatsu et al., 2005; D'Aoust, 1977). This becomes even more problematic in subsequent steps after the addition of ingredients that may increase fat and sugar content. High sucrose content, for example, has been shown to enhance survival in food matrices like chocolate, possibly due to the combination of low moisture, high sugar and high fat levels having a synergistic effect on survival (Hiramatsu et al 2005; Podolak et al, 2010). Risk is estimated to significantly increase in the post-roasting stages under any of these scenarios.

Post-roasting: Mixing and Risk Classification of Added Ingredients

The cocoa liquor obtained after milling the nibs form the base of most chocolate products. Additional ingredients enter the ensuing mixing step either as dry mix (dairy powder, sugar, miscellaneous ingredients) or as milk chocolate crumb, a vacuum-cooked, caramelized intermediate product made from a blend of sugar, dry milk powder and sometimes cocoa liquor. Milk chocolate crumb is used in specific products formulated to use the crumb, and the resulting chocolate product usually has a different flavor profile. It should be noted that not all chocolate manufacturers use the crumb process.

An assessment of the risk of contamination coming from added ingredients during the mixing step justifies a closer examination of those ingredients.

i. ***Non-fat dry milk (NFDM) and other diary-based ingredients***: Milk powder, milk fat (if used) and lactose (used as sweetener) all carry a significant contamination risk due to their historical association with *Salmonella* (Cotton and White, 1992), and are classified in our assessment as high risk "*Salmonella*-sensitive" ingredients (**Table 4.2**). Dry, powdered milk in particular has been contaminated with *Salmonella* in the past (Collins et al., 1968; Craven et al., 1975; ICMSF, 2002, Weisman et al., 1977). Although, this does not currently seem to be a major issue in the food industry due to improved production practices, precautionary measures are needed as occasional *Salmonella* detection are still being recorded. For instance, the FDA in 2016 reported an incidence of positive *Salmonella* test during environmental sampling at a facility that produces "nonfat high heat milk powder" (FDA, 2016). Even though the pathogen was not detected in the final product, a precautionary recall was made since this "nonfat high heat milk powder" was sold to companies where the end-use involves ready-to-eat products. Although, suppliers must meet certain specifications as well as present a Certificate of Analysis (COA) to buyers, appropriate sampling plans may also be put in place by manufacturers to act as a safety net, a “trust-but-verify” approach, for these incoming semi-finished ingredients.

ii. ***Sugar***: as an ingredient not typically associated with *Salmonella* contamination, nor identified as such in literature, sugar is classified as low risk (Cordier, 1994).

iii. ***Cocoa butter***: no documented incidence of *Salmonella* in cocoa butter was found, and literature points to the apparent absence of microbes in this medium (D’Aoust, 1977). However, depending on the supplier, cocoa butter may have been obtained from origin processors where cocoa nibs are pressed and milled, processes that can carry a risk of contamination during handling and packaging whose outer surface may have been cross-contaminated (personal communication, 2018). The percentage of cocoa butter obtained from unroasted beans is unknown. Such information could provide a clearer picture of potential risk from this ingredient. Komitoupoulou (2009) studied the behavior of salmonellae in cocoa butter oil and reported that viable salmonellae were recovered over the course of the 21-day storage period at 5 and 21°C. Our assessment categorizes cocoa butter/oil as medium risk for the above reasons.

iv. ***Milk Crumb***: crumb production generally involves a caramelization process which yields a distinct ‘cooked milk’ flavor in milk chocolate products. A vacuum crumb oven could be used to heat the mixture of largely sugar and dairy solids (little added moisture), with or without cocoa liquor. The resulting crumb (powder-like) is passed through the refiner and comes out looking quite dry and crumbly. It can either be used right away or molded into huge slabs and stored for up to 12 months before further use. When ready for use, the refined powder-like crumb is re-liquefied by conching. Milk crumb is thus desirable because, in addition to its desired flavor profile, it can quickly and economically be incorporated into the manufacturing process. As there are currently no studies investigating *Salmonella* contamination or kinetics of crumb, our assessment classifies crumb as high risk, since if used, this intermediate product would carry risk synchronous to its compositional ingredients namely NFDM, sugar and, in case of brown crumb, cocoa liquor.

v. ***Lecithin***: used as an emulsifier, risk classification would primarily depend on supplier assessment considering the isolated occurrence of *Salmonella* in soy lecithin. Reports document a 2006 incident where a manufacturer received and used an allegedly tainted shipment of soy lecithin from the supplier (Reynolds, 2006). Note: lecithin is generally added post-conching.

None of these added ingredients allow outgrowth of *Salmonella*, however persistence is the hazard. Of all the ingredients mentioned here, only NFDM and *Salmonella* as a food-pathogen pair has been studied to any extent. Investigations into data on the kinetics of *Salmonella* in the various matrices would be of immense benefit to subsequent risk assessments. For instance, is milk crumb a hygroscopic matrix which would permit redistribution of moisture during extended storage?

Post-roasting: Conching

Carried out at temperatures between 50-80°C for up to 24 h, depending on the equipment, conching helps to liquefy the chocolate paste obtained after mixing, and achieve further flavor development. In addition to roasting, the conching step is also thought to play a role in the inactivation of *Salmonella* (Krapf & Gantenbein-Demarchi, 2010; Nascimento et al, 2012). Despite the high temperatures involved in the roasting and conching, past studies including those on thermal inactivation of *Salmonella* in chocolate (Barrile et al., 1970; Cordier, 1994; Goepfert & Biggie, 1968; Lund & Eklund, 2000; Nascimento et al., 2012; Peñaloza-Izurieta et al., 2008; Rieschel & Schenkel, 1971) have shown that the thermal inactivation of *Salmonella* cannot be assured. This was especially true in the 1975 S. Eastbourne outbreak (Craven et al, 1975) where raw cocoa beans were the apparent source of *Salmonella* that survived the heating steps during processing. Some of these studies also document concern about increased resistance of *Salmonella* cells after either of these two heat-inducing steps (Nasciemento et al, 2012). Thus, while the conching process has the ability to reduce microbial load, it cannot be relied upon, particularly in two cases: presence of heat-stressed and/or desiccated *Salmonella* cells which have already developed enhanced thermal resistance (Geopfert and Biggie, 1968; Krapf et al, 2010), and in instances of high *Salmonella* contamination (Nasciemento et al, 2012).

Based on assessments from available literature, it is quite common that chocolate manufacturers would purchase cocoa mass or liquor from third-party cocoa processors, some of whom may be situated in the originating country. The risk inherent in this practice is that the higher likelihood of poor hygiene standards that increase the risk of *Salmonella* contamination in the cocoa mass (Burndred, 2009).

It may be assumed that, for all high risk ingredients added prior to and during conching, risk remains the same or is reduced, but not eliminated. This is because the conching profile is not a CCP and does not meet critical parameter for inactivation (**Table**

4.1). Furthermore, assessment of the risk rating at this point can be complicated by possible heat-resistance acquisition due to the prior sublethal temperatures being applied.

Conching equipment is generally known to have an open design such that chocolate being conched may be susceptible to environmental contamination in form of aerosolized particles. Moisture or any form of condensation in the processing environment – leaky pipes, valves, dirt from ceilings etc. may contribute to contamination, thus risk rating at this conching stage can increase, decrease or remain unchanged.

Post-roasting: Tempering

Tempering is another heat application step used toward the end of production to ensure a thermally stable product. During this process, chocolate undergoes alternate heating and cooling periods, with heating temperatures as high as 50°C, depending on manufacturer's practices (**Table 4.1**). This step is not recognized as contributing to microbial inactivation, however, similar to conching, it may either achieve a degree of inactivation, or induce increased thermal resistance due to the application of sublethal temperatures. There are no studies evaluating the effect, if any, of tempering on inactivation or resistance of *Salmonella*, hence risk at this step is not assessed in this study.

Post Roasting: Production Environment and Cleaning Procedures

Considering that there are many sources of contamination in the production environment, post-lethality exposure should be considered as a major focal point of risk assessment.

PrPs and HACCP guidelines are expected to be in place and rigorously enforced in any food production facility. The relevance of a risk assessment however is to utilize a risk-based paradigm to further evaluate environmental monitoring programs and identify potential 'blind-spots'. Airflow, as an example, may be considered an ingredient for the purpose of this risk assessment, since unfinished products such as cocoa liquor may come in contact with atmospheric air during conching in open equipment (Burndred, 2009; Personal Communication, 2017). Air quality should thus be assessed with as much integrity as any other ingredient, and measures such as collecting air samples can help evaluate risk.

Additionally, opportunities for moisture accumulation and consequent condensation in the production environment is a scenario that is sometimes overlooked in hazard analysis. Condensation on the ceiling or on top of closed systems, for example, may constitute a hazard point when that moisture is redistributed as evaporation occurs. Assessment of risk in the production environment can be complicated given the "hot-spots" that may exist in various niches, and therefore an evaluation of risk would largely be facility-specific; for example, the justification of environmental monitoring zones and their influence on risk rating.

Efforts to curtail introduction or spread of pathogens due to moisture within the facility must be properly defined in existing GMPs and PrPs as the presence of moisture anywhere within the production environment constitutes significant risk of bacteria proliferation. Cleaning and sanitation procedures need strict precautions and monitoring particularly if cleaning agents are water-based; for example, equipment requiring wet cleaning must be designed to drain freely, and sound insulation around the facility must be ensured to prevent condensation.

4.4.6.2. Summary Table for Processing Module

The table below concisely presents the processing module, an overall risk status for the module for a base model, as well as rationale for such risk categorization based on the pre-defined criteria (See section 4.4.2).

Table 4. 6. Summary table for risk assessment of processing module

What is the likelihood of *Salmonella* re- or cross-contamination during milk chocolate processing within the manufacturing facility?			
Process Step/Scenario	**Risk rating (change in risk level)**	**Risk categorization based on criteria**	**Rationale for risk categorization**
Reception, storage and cleaning of raw cocoa beans	Unchanged	High (Criteria 3a)	Unprocessed cocoa beans have high likelihood of being already contaminated
Heat treatment – roasting	Decrease	Low (criteria 1b)	Major kill-step for *Salmonella* cells (CCP). Factors which may impact roasting effectiveness, heat resistance and thus how risk is evaluated: - matrix roasted: whole beans or nibs - initial pathogen load - type of roaster and sequence of thermal application - consideration of existence of highly resistant salmonellae cells Possibility of cold spots
Heat treatment – steam-based debacterization	Decrease	Low [criteria 1b, 1c(ii)]	- Equipment used and specific manufacturer practices determine if Dbac employed; unclear how common practice is among manufacturers. - Addition of moisture before or during roasting may decrease thermal resistance.

Heat treatment – conching	Increase or decrease	Medium (criteria 2a)	Conching profile does not meet critical parameter for inactivation; indications are that it can achieve some inactivation, however, sublethal temperatures could possibly contribute to heat-resistance of any surviving cells
Addition of Ingredients & Mixing: a. Addition of '*Salmonella*-sensitive" ingredients: non-fat dry milk powder (NFDM), lactose, milk fat, lecithin, cocoa butter/oil b. Addition of medium and low-risk ingredients	Increase	High (criteria 3b) Medium, Low [criteria 2b, 1c(i)]	- For ingredients regarded as "*Salmonella*-sensitive" - NFDM and other dairy ingredients, *Salmonella* has been identified as significant microbial hazard to be controlled; - largely subject to supplier control under HACCP plan; - no further kill-step applied
Production environment: risk of cross-contamination via air, condensation	Increase or unchanged	Medium (criteria 2b)	- Improper implementation of zoning restrictions and cross-traffic flow, lack of appropriate room designs, inadequate insulation, integrity of air-flow systems, inadequate employee hygiene training, methods of cleaning processing equipment, condensation in production areas such as ceilings, pests or rodents etc.
Milk crumb (if used)	Increase or unchanged	High (criteria 3b)	If not used right away, storage and handling

			could be potential contamination risks, since no further kill-step before final packaging.
Intrinsic and extrinsic factors (Aw, pH, moisture level, matrix impact)	Increase or unchanged	N/A	- Synergistic effect of low water activity and high fat and sugar content may significantly increase thermal resistance. - History and physiological state of *Salmonella* cells impact survival.

Summary & Conclusions:

Given the significant likelihood of contamination of incoming raw cocoa beans, measures such as strict zoning enforcements mediate cross-contamination within the production facility. After cleaning operations, the thermal processes of roasting and debacterization are identified by several studies in literature as the only lethality-steps, thus CCP, guaranteed to inactivate pathogenic bacteria, although debacterization, when used, may only be regarded as a supplemental kill-step depending on equipment and manufacturer practices.

Salmonella-sensitive ingredients such as NFDM present significant risk given that they are added to the process after the critical control point. Several studies in literature present data to investigate the effect of conching, carried out at sublethal temperatures, on chocolate matrix, with results indicating possible outcomes ranging from total inactivation to increase in thermal resistance. Data gap exists about milk crumb, an intermediate product, which when used, could be stored for extended periods before further use. Studies on the storage kinetics of *Salmonella* in this crumb matrix is ongoing and data obtained would be used in the quantitative risk assessment.

The major "risk spots" identified in this module include: i) the addition of *Salmonella*-sensitive ingredients to the production line, and, ii) the post-roasting production environment.

For a base model, the risk status of chocolate changes drastically from the preceding module and is categorized as low primarily due to the lethality step and the environmental controls in place.

4.4.7. Post-Processing Module

4.4.7.1. Stepwise Analysis

This module encompasses all post-thermal downstream activities, i.e. after last step involving any heat application or generation, until packaging.

Molding, enrobing, panning, rework, and final packaging

Depending on the desired end product, milk chocolate production may employ steps such as molding, enrobing and panning prior to final packaging. The tempered chocolate mixture in molten form is passed through series of high-speed machines to be molded into desired shapes and sizes. Other processes at this final stage of production could include enrobing - a process where the chocolate is used to cover a center filling, or panning – a process of using the chocolate as coating for hard centers such as nuts, spices, dried fruits, coffee (Afoakwa, 2010; Beckett, 2009). Similar inclusions, if added to molded chocolate, are critical and would affect risk rating. Addition of dehydrated coconut, for example, a product known to be associated with *Salmonella* concerns (FDA, 2018; Schaffner et al., 1967) would increase contamination risk at this step. And if no further ingredients are added, risk would remain unchanged from preceding conching step.

Contamination from the production environment presents another important consideration of risk, particularly in cases where product is being held or a particular step is being carried out in open containers and tanks. Risk rating can either increase or remain unchanged. Rework, as defined by the US Code of Federal Regulations, is "clean, unadulterated food that has been removed from processing for reasons other than insanitary conditions or that has been successfully reconditioned by reprocessing and that is suitable for use as food" (Title 21 eC.F.R. § 117.3, 2016). If not properly managed, rework can pose a significant hazard in the post-processing module for various reasons including but not limited to the possibility of cross or re-contamination from handling during removal and subsequent addition back to the production line (Cordier, 1994; Minson, 2009). Also, microbial quality of added ingredients, storage practices, traceability implementation and record keeping would have a major influence on how well contamination risk is managed. Prerequisite programs are likely to include the GMPs and Rework Policy for a facility and must be closely monitored, with revisions made as needed. The most important consideration here is that downstream processing of rework does not involve a kill-step. Hence, risk rating is increased if rework is incorporated during the post-processing stage.

4.4.7.2. Summary Table for Post-Processing Module

The table below presents a concise assessment for the post-processing module as well as rationale for risk categorization based on the pre-defined criteria.

Table 4. 7. Summary table for risk assessment of post-processing module

What is the likelihood of *Salmonella* contamination post-conching up until final packaging of milk chocolate product?			
Processing Step/Scenario	**Risk Rating (change in risk level)**	**Risk Categorization based on criteria**	**Rationale for risk categorization**
Post-conching downstream activities: - **Rework** - **Enrobing inclusions (nuts, raisins etc.)** - **Molding, packaging**	Increase or unchanged	High (criteria 3b)	- 'Rework' - risk of recontamination downstream; no kill-step. - Enrobing may involve '*Salmonella*-sensitive' ingredient
Risk of non-homogenous distribution		Low (criteria 1c)	- Sampling of final packaged product carry an inherent risk of heterogeneous distribution of bacteria cells
Summary & Conclusions: As a post-process activity, 'rework' poses one of the biggest downstream risk of recontamination due to increased handling. When chocolate is used to enrobe ingredients prior to molding, risk increases if such additional ingredient is *Salmonella*-sensitive. Also, the risk of non-homogenous distribution in final packaged products which may undermine sampling schemes must be factored into a risk assessment given the pattern of historical occurrences. The stringency of cGMP implementation in final product handling must also be recognized. For a base model, we conclude that the overall risk of contamination in the post-process module is low.			

4.4.8. Relative risk assessment in absence of mitigation steps

Integrating all modules, **Table 4.8** below provides a characterization of relative risk for a base model (i.e. when effective mitigation steps are assured), as well as relative risk in absence of effective mitigation. This way, comparison of risk levels can be made for each step in the process.

Table 4. 8. Summary table of risk categorization of processing steps in milk chocolate production.

Module	Processing Step/Scenario	*Risk Rating (impact or change in risk level)	Risk Categorization based on defined criteria	Relative Risk Characterization <u>with</u> Effective Mitigations
Farm	Harvesting and pod-opening	Increase	High (criteria 3a)	**High** (No mitigation identified: handling practices, access to environmental contamination)
	Fermentation	Increase or Decrease	Inconclusive based on available scientific evidence	N/A
	Drying	Increase or Unchanged	High (criteria 3a)	**High** (Limited mitigation: suboptimal controls, farming practices)
	Storage of dried beans	Decrease or Unchanged	High (criteria 3a)	**High** (Limited mitigation: suboptimal farming practices)
Storage & Transportation	During transportation, if salmonellae not present in bagged beans	Unchanged	High (Criteria 3a)	N/A
	During transportation, if salmonellae already present in bagged beans	Decrease or Unchanged	High (Criteria 3a)	**Medium** (Limited mitigation: largely dependent on prevailing conditions in shipping vessel - a_w, moisture level, RH)
Processing	Heat treatment – roasting	Decrease	Low (criteria 1b)	**†Low** (Mitigation: Major kill-step for *Salmonella* cells [CCP])
	Heat treatment – steam-based debacterization (Dbac)	Decrease	Low [criteria 1b, 1c(ii)]	**†Low** (Mitigation: supplemental kill-step, if used)
	Heat treatment – conching	Increase or Decrease	Medium (criteria 2a)	**†Low** (Some mitigation: can contribute to inactivation, but also possibly contribute to heat-resistance)

	Mixing & Additional Ingredients: a. addition of '*Salmonella*-sensitive" ingredients: b. addition of medium and low-risk ingredients	Increase	High (criteria 3b) Medium, Low [criteria 2b, 1c(i)]	**†Low** (Mitigation: supplier control, Certificate of analysis (COA), additional in-house testing)
	Production environment: risk of cross-contamination via air, condensation, other means	Increase or Unchanged	Medium (criteria 2b)	**†Low** (Mitigation: proper implementation of zoning restrictions, appropriate room designs, adequate insulation, air filters & cleaning methods etc.)
	Milk crumb (if utilized)	Increase or Unchanged	High (criteria 3b)	**†Low** (Mitigation: supplier control, Certificate of analysis (COA), additional in-house testing)
	Intrinsic and extrinsic factors (a_w, pH, moisture level, matrix)	Increase or Unchanged	N/A	**†Low** (Mitigation: depends on control of preceding steps)
Post-processing	Post-thermal downstream activities: - Rework - Enrobing inclusions (nuts, raisins etc.) - Molding, packaging	Increase or Unchanged	High (criteria 3b) Low (criteria 1c)	**†Low** (Mitigation: stringency of cGMP implementation in final product handling)

**Risk rating is subject to change depending on specific practices employed in either primary (bean-to-cocoa) or secondary (cocoa-to-chocolate) processing, thereby impacting risk status of final product; recommend use of criteria definitions to categorize risk.*

†With effective mitigation, relative risk in processing steps are decreased and low if all process and safety metrics are rigorously adhered to and safety deviations or failure do not occur.

As illustrated in **Table 4.8** above, if all dictates of process and safety measures discussed under each module are rigorously adhered to, overall residual risk of *Salmonella* contamination is estimated to be low. However, what-if scenarios, as presented in the risk rating tool in the next section, can provide categorical risk estimates for potential deviations along the production spectrum.

4.4.9. Risk Rating Tool

A risk rating tool, a metric for qualitatively assigning risk categories to stepwise activities during milk chocolate production, is presented on a Microsoft Excel spreadsheet (see **Appendix C** for screenshot). It can be used to make qualitative estimates on how risk changes throughout the farm-to-packaging production continuum by generating relevant questions at each step. The rating tool supports answers to each question by referencing appropriate peer-reviewed data, expert opinion and assumptions, and provides a final risk characterization. This rating tool was developed for assessing residual risk for a base model where it is assumed that all controls were put in place and all process parameters were achieved. Options are however provided for scenarios where controls are subpar, or failure occurs.

4.5. Study Assumptions

This qualitative analysis assumes that processing (cleaning, roasting, milling) of raw cocoa beans, and production of intermediate components such as chocolate liquor, milk crumb, are carried out within the same facility. Not all manufacturers are known to do this – some may purchase or transport cocoa liquor from a different facility or third party supplier – this scenario was not necessarily included in our assessment.

4.6. Study Limitations and Data Gaps

The major limitation to the qualitative assessment were the significant data gaps and lack of vertical studies on several processing steps which require an assessment of risk. The study identified critical data gaps including *Salmonella* prevalence on farm, during storage and transportation to processing facility. In order to characterize risk, there was a need for reliance on prevailing understanding of hazards and processes representative of *Salmonella* association with dry foods similar to chocolate. Little is known about initial contamination levels in raw cocoa beans immediately prior to the kill-step, or cocoa nibs that have been de-shelled in readiness for roasting. Recommended reduction levels during the roasting step is specified as 4-5 log by the National Confectioners Chocolate Council (NCACC, 2011), however, there is no formal legislation for cocoa in this regard. Another source of uncertainty is the unknown proportion of cocoa butter that is obtained from unroasted beans but utilized in milk chocolate production. This is important due to the inherent risk of *Salmonella* from unroasted cocoa beans that could cross-contaminate and possibly persist in the butter, an ingredient that does not undergo a kill-step.

Also lacking is knowledge about survival kinetics in intermediate matrices such as milk chocolate crumb. Such data is important in evaluating risk of process failure and additional risk presented by high-risk ingredients. Likewise, there is little knowledge about other points of entry of *Salmonella* into the processing continuum, particularly cross-contamination within the environment. As a result, estimation of risk in these data-scarce areas were largely based on expert opinion, assumptions and comparison studies. Significant data gaps and lack of vertical studies may complicate further quantitative risk assessment due to uncertainty, and in such cases, informed decisions must be made on how best to proceed with quantitative risk assessment.

4.7. Conclusions

Given that milk chocolate processing is a blending operation with no validated kill-steps post-roasting, assuring ingredient quality and the hygienic integrity of the manufacturing environment up until the final packaging, are the only real controls in terms of microbial risk. The overall residual risk of exposure – likelihood of *Salmonella* in a packaged milk chocolate product – is evaluated as low in this qualitative risk assessment, provided dictates of process and safety control measures are rigorously adhered to, as illustrated on **Table 4.8**. In addition to identification of important data gaps, this assessment systematically identifies "high risk spots" during milk chocolate processing and provides metrics for assessing effectiveness of some risk mitigation strategies on the predicted risk. Finally, the qualitative assessment provides an important framework upon which a stochastic microbial risk assessment can be built and developed to provide quantitative estimates of risk.

4.8. Future Directions

Risk assessments require a good amount of data to provide robust analyses. As such, in order to further streamline risk assessment, scientific research geared towards generating more data about risk encountered during chocolate production should be encouraged. For instance, investigations into the integrated effect of sub-lethal temperatures on the resistance of *Salmonella* during heat-generating activities such as bean-grinding, and heat-inducing activities such as conching and tempering, would provide a metric for probability of *Salmonella* survival in post-lethal contamination scenarios. Also, very little is known about milk crumb, the intermediate product sometimes used in milk chocolate production. Its extended storage and downstream use provides a possible pathway for contamination, and studies into pathogen kinetics given storage parameters are worth implementing.

Chapter 5: Evaluation and modeling the effect of temperature on the survival kinetics of *Salmonella enterica* in milk chocolate crumb and the identification of a potential risk reduction intervention

5.1. Abstract

Despite *Salmonella enterica* infections and sporadic outbreaks which have been associated with chocolate consumption over the last four decades, routes of contamination during chocolate production as well as persistence in the processing environment are currently not well understood. Very little data are available to aid farm-to-fork microbial risk assessment efforts in this regard, particularly, the kinetics of *Salmonella* survival and persistence in the various matrices associated with chocolate processing, such as "milk chocolate crumb". A hitherto unexplored matrix, crumb is a dry, powder-like intermediate product that can be stored for extended periods prior to downstream processing steps which do not provide any further lethality. As a means of assessing contamination risks associated with milk chocolate manufactured using crumb, this study was undertaken to evaluate the decline kinetics of preadapted *S. enterica* strains in milk crumb and subsequently develop predictive models for *Salmonella* survival in crumb under three isothermal conditions. Three strains of *S. enterica,* used both individually and as a cocktail, were pre-adapted in crumb and stored for an initial 90-day period, and subsequently followed for up to 280 days. A dual media system was used to determine extent of survival and injury, with the following variables were assessed for effects on survival: crumb type

(white vs. brown), storage temperature (15°, 24° and 35°C), storage time (0, 7 21, 35, 54, 70 90, 230, 265, 280), and strain identity.

Survival of *S. enterica* was dependent on strain identity, crumb type and storage temperature, and with the latter found to be a primary predictor of survival. Across strains and crumb type and over a 90-day period, mean reduction based on log-transformed microbial counts were < 0.84 and 1.01-log CFU/g for crumb stored at 15°C and 24°C, respectively, while a 2.04-log CFU/g was observed when crumb was stored at 35°C. Reduction by day-280 in the most resistant strain and across all other variable combinations ranged from 1.15 to 4.16-log CFU/g. The Weibull model was found to provide the best fit for all survival curves obtained up till day-280. This study establishes for the first time the potential use of crumb in milk chocolate manufacturing as a means for reducing *Salmonella* levels and provides a survival model that can be incorporated into quantitative risk assessment efforts to quantify its impact. Promising opportunities for risk reduction are discussed in relation to future research to optimize crumb storage parameters.

5.2. Introduction

Salmonella is well-known as a pathogen of concern in low moisture foods and is recognized as being highly effective in adapting to extreme environmental conditions such as desiccation, pH conditions, thermal resistance (Podolak et al 2010). *Salmonella enterica* infections and sporadic outbreaks have been associated with chocolate consumption over the last four decades, despite the inability of the bacterium to grow in milk chocolate. The routes of contamination during chocolate production as well as persistence in the processing environment are currently not well understood. Typically, contamination levels are only a few *Salmonella*e per serving, a low infective dose which reflects the low-moisture state of these chocolate products and the pathogen's ability to survive for extended periods. *Salmonella* contamination during a chocolate manufacturing and subsequent survival in a product until consumption could affect thousands of consumers, potentially including vulnerable children.

In vulnerable populations such as young children, salmonellosis can be life threatening. Since the first discovery of *Salmonella* in cocoa (Depew, 1968), and subsequently in chocolate (D'Aoust, 1977; Gästrin et al, 1972), it has been and still is the most important microbiological public health risk associated with chocolate and its related products. *Salmonella* contamination of chocolate products has been the bane of the chocolate industry, and there have been several efforts to find effective ways to reduce the risk of this pathogen during production. Available scientific and epidemiological literature indicate that *Salmonella* may be introduced at various stages during the production of chocolate (Bell & Kyriakides, 2002; Cordier, 1994; McDonough & Hargrove, 1968).

Milk chocolate crumb is an intermediate product used in commercial milk chocolate processing that can be stored for several months before being processed downstream into final milk chocolate products. Crumb is a compound ingredient which consists of sugar and milk solids (white crumb) or sugar, milk solids, and cocoa mass or liquor (brown crumb). These components are vacuum cooked to less than 1% moisture (Doust 1977), a process that involves Maillard reactions and caramelization given the protein and sugar content. Large chunks of the resulting matrix are coarsely ground into 'crumb nuggets' which are subsequently dried and milled into a fine, powdered 'crumb' product. The caramelization process used during crumb production is not necessarily considered a lethality step as the vacuum oven heating profile is not seen as effective to achieve a 5-log inactivation. Milk crumb could either be used right away or stored for further use. Chocolate manufacturers typically receive milk powder, also known as non-fat dry milk (NFDM), via the supply chain from trusted suppliers. And typically, the only control over *Salmonella* contamination risk from this incoming raw material is a supplier-verified system such as a Certificate of Analysis (COA), and possible in-house testing and verification procedures. The concern in terms of food safety is that no substantial heat treatments or lethality steps are involved in the any of the downstream processing steps such as conching or refining. Hence, the risk of re- or cross contamination should be considered.

Effects of environmental conditions such as temperature and its impact on survival kinetics of *Salmonella* in intermediate products which may impact risk (e.g. cocoa butter or chocolate crumb), have been studied less extensively. This includes investigations into the utilization of crumb during milk chocolate processing and its potential influence on *Salmonella* contamination. The survival of *S. enterica* in crumb does not appear to have been examined nor reported in literature, hence our study chose to explore this matrix and its potential effects on the risk of *S. enterica* during milk chocolate production. Specifically, the effects of storage duration and temperature on the survival of *S. enterica* in white and brown crumb were investigated.

5.3. Materials and Methods

5.3.1. Bacterial Strains

Three *Salmonella* serotypes were used for this study – *S*. Eastbourne, *S*. Limete, and *S*. Typhimurium. All strains were acquired from the culture collection of the United States Food and Drug Administration (FDA), College Park, MD. Two of the strains, *S.* Eastbourne and *S*. Limete, were chosen because they were isolated from chocolate, while *S*. Typhimurium, also isolated from a dried food matrix, was chosen for its relatively high thermal resistance in dried state and its association with low-moisture foods. The strains were tested individually and as a cocktail to determine their survival characteristics.

5.3.2. Preparation of inoculated milk crumb

Individual frozen stock cultures of the strains were activated by thawing and streaking onto tryptic soy agar (TSA) plates (BD, Sparks, MD) and incubated at 37°C for 18 to 24 h. A single colony was selected from each plate and streaked onto separate plates of xylose lysine deoxycholate (XLD) agar (BD) and also incubated at 37°C for 24 h. Strains were individually grown by picking one black colony from each XLD plate to inoculate 40 ml tryptic soy broth (TSB) which was incubated at 37°C for 18 h to reach stationary growth phase. Cultures were harvested individually by centrifugation (4500 rpm for 15 min), and resulting pellets were washed twice in 3 mL of sterile peptone water (0.1%). For cocktails, equal volumes of each strain were combined and re-centrifuged. Final pellets were re-suspended in 250 ul of sterile peptone water to produce inocula with final concentrations of ca. 10^9 CFU/ml, which were then stored on ice until used to inoculate milk crumb. A method to avoid altering the moisture content of the milk chocolate crumb and prevent osmotic shock of the cells upon inoculation was developed to dehydrate and preadapt the *Salmonella* inoculum as described below. Working under a laminar flow biosafety cabinet, the pelleted suspension was pipetted in drops into a sterile petri dish and allowed to dry for 2 – 3 h until it formed thin, translucent flakes. This dried inoculum was crushed with a spatula, transferred to a WhirlPak bag and carefully mixed with a measured portion of milk crumb. After a 24-h preadaptation period at ambient temperature, the inoculum subset was mixed into a larger batch of milk crumb. Uniform distribution was achieved by vigorous mixing and blending in batches. A 1-g aliquot was used to measure viable cell concentration in the inoculated crumb. All milk crumb inoculation procedures (weighing, mixing and blending) were done under a class IIA biosafety hood using sanitary precautions to prevent aerosolization and atmospheric contamination.

5.3.3. Measurement of water activity and moisture content of milk crumb

Water activity (a_w) and moisture levels were periodically measured in triplicate control samples throughout the duration of the study. A moisture analyzer (HE53, Mettler-Toledo, Australia) was used to assess moisture levels while a water activity meter (Novasina IC-500, AW-LAB, Switzerland) was used to measure a_w using manufacturer's specifications. The crumb was also visually inspected at regular intervals for any visible changes in quality.

5.3.4. Storage Conditions

Each crumb type (brown and white) was evenly divided into sub-batches such that all variables being tested were represented in sufficient combinations to enable triplicate sampling. Inoculated crumb samples were stored in compact, airtight plastic bags at 15±1°C, 24±1°C (measured ambient lab temperature) and 35±1°C and were assayed for *Salmonella* for up to 280 days.

5.3.5. Microbial Analysis

Levels of *S. enterica* were determined using a dual media system of tryptic soy agar and XLD agar to determine extent of survival and injury and viable counts of bacterial composites in the crumb samples were obtained at 0, 7, 21, 35, 54, 70 and 90 days (n = 9). Additional tests for survival were also conducted on days 230, 265 and 280 (n = 3). At designated sampling times, sample bags were removed from storage and assayed for *Salmonella*: 1-g of crumb was weighed and transferred into a sterile 9.0-ml dilution blank of 0.1% peptone water with additional serial 10-fold dilutions made as needed. After vortexing, 50-ul aliquots of appropriate dilutions were spiral plated (Eddy Jet 2W, IUL Instruments, Spain) on tryptic soy and XLD agar plates and incubated at 37°C for 48 h, with enumeration at 24- and 48 h using an automated colony counter (Neutec Group Inc., Farmingdale, NY). Three independent trials were carried out with sampling done in triplicates for each trial. Survivor curves were generated in Excel using log-transformed microbial counts.

The degree of non-lethal injury, i.e. proportion of injured cells was calculated according to the equation below:

Proportion of injured cells = $\frac{A-B}{A} \times 100\%$ Equation (1)

where:

A is count on non-selective TSA media (injured + non-injured cells)

B is count on selective differential XLD media (non-injured cells)

5.3.6. Confirmation of Positive Samples using Enrichment

A two-step enrichment method as described by Oni et al (2015) was used when counts fell below the limit of detection (LOD) of 2 log CFU/g (<1 CFU per 0.05 ml for a 10^{-1} dilution) for the direct plating method. The LOD for enrichment (after culturable organisms were unrecoverable on selective XLD media) was ~1 log CFU/g.

5.3.7. Statistical Analysis and Model fitting

Salmonella populations for each replicate were log-transformed to Log CFU/g before being used as the dependent variable of interest. Quantitative data from the independent triplicate experiments with three samples per replication (n = 9), except where otherwise stated, were statistically analyzed and ANOVA tests for the response variable was performed using the GLM procedure of SAS software version 9.2 (SAS Institute, Cary, NC, USA). *Post hoc* pairwise comparisons of treatment group means were performed with the Tukey adjustment (Tukey's honestly significant difference) to correct for type I experiment-wise error and differences between means were considered significant at an alpha level of 0.05. To model survival kinetics, populations of *S. enterica* cocktail obtained from the three independent experiments were log-transformed and normalized: [(Log (Y(t) – log(Y_0)], where log(Y_0) is the initial *Salmonella* population and log Y(t) is the survivor population at time t. The best-fit models were generated and compared using the Integrated Pathogen Modeling Program (IPMP) 2014 software (USDA/ARS, Wyndmoor, PA) and GInaFiT (Geeraerd et al., 2005). The Weibull model (Huang, 2009; Peleg, 1999) which is essentially an empirical model of distribution of inactivation times (van Boekel, 2002), was found to provide the best fit for the data:

$$Y(t) = Y_0 - kt^{\alpha} \qquad \text{Equation (2)}$$

where:

t is the time of isothermal treatment

Y(t) and Y_0 are the log-transformed and normalized decimal reduction populations of *Salmonella* (CFU/g) at time t and 0 respectively

k is the scale factor determining the overall steepness of the slope of the curves

α is the shape parameter which determines the shape of the curves

The decline rate of *Salmonella* was considered significant at $P < 0.05$.

To predict microbial reduction times in crumb, the Weibull model was rearranged and expressed as:

$$time\ (t) = \frac{\left(Y_0 - Y(t)\right)^{\frac{1}{\alpha}}}{k}$$

- Equation (3)

where Y (t) = desired reduction in log (CFU/g), and Y_0 is initial log population; and all other parameters are as defined above.

5.4. Results

5.4.1. Overview

Levels of preadapted *Salmonella* in initial crumb inoculum (Day 0) were approximately 6 log CFU/g across the three trials (standard deviation ≤0.3 log CFU/g). Homogeneity of distribution was checked and verified in pre-trials by triplicate sampling of 1-g inoculated crumb. Analysis beyond the initial 90-day study period tested survival at Day 230, 265, and 280 and showed that by Day 280 (~9 months), the general inactivation trend showed a temperature-dependent decline as seen in **Fig. 5.1**.

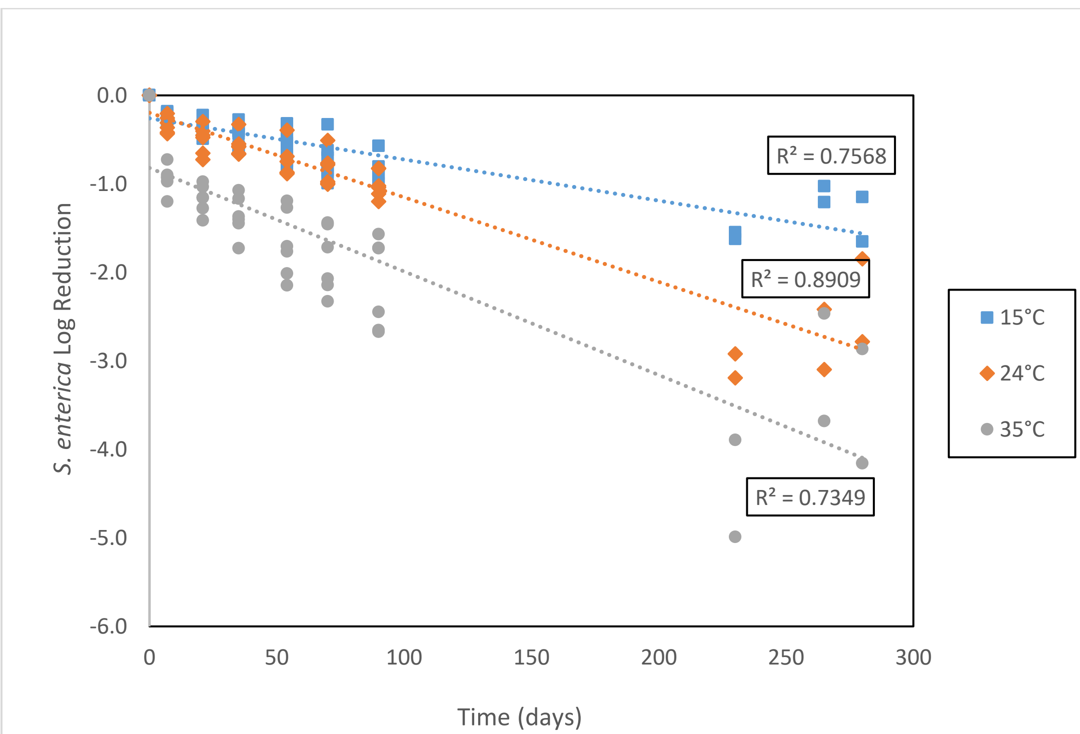


Figure 5. 1. Graph showing general inactivation trend of *S. enterica* cocktail (Log CFU/g) in milk crumb stored at three isothermal temperatures (15, 24 and 35°C) over 280 days.

Three out of the four predictor variables were found to have a significant effect ($p<0.001$) on *Salmonella* survival in crumb: strain identity, storage time, and storage temperature. The fourth variable, crumb type had a marginally significant influence on survival ($p = 0.046$). Over an initial 90-day storage period across strain and crumb types, mean reduction based on log-transformed microbial counts were < 0.84 and 1.01-log CFU/g for crumb stored at 15°C and 24°C respectively, and 2.04-log CFU/g for crumb stored at 35°C.

5.4.2. Measurement of Degree of Injury during Storage

As determined by analysis of differences in microbial plate counts between selective XLD and non-selective TSA media, the degree of injury sustained by cells stored under 35°C was significantly greater than observed in survivors recovered from 15 and 24°C storage ($p<0.001$). Mean percent injury observed during study duration (calculated using Equation 1) is as follows: 0.67 ± 0.23, 0.73 ±0.20 and 0.76 ±0.37 for cells recovered from storage at 15°C, 24°C and 35°C respectively, indicating relatively greater injury at higher temperature (**Fig 5.2**).

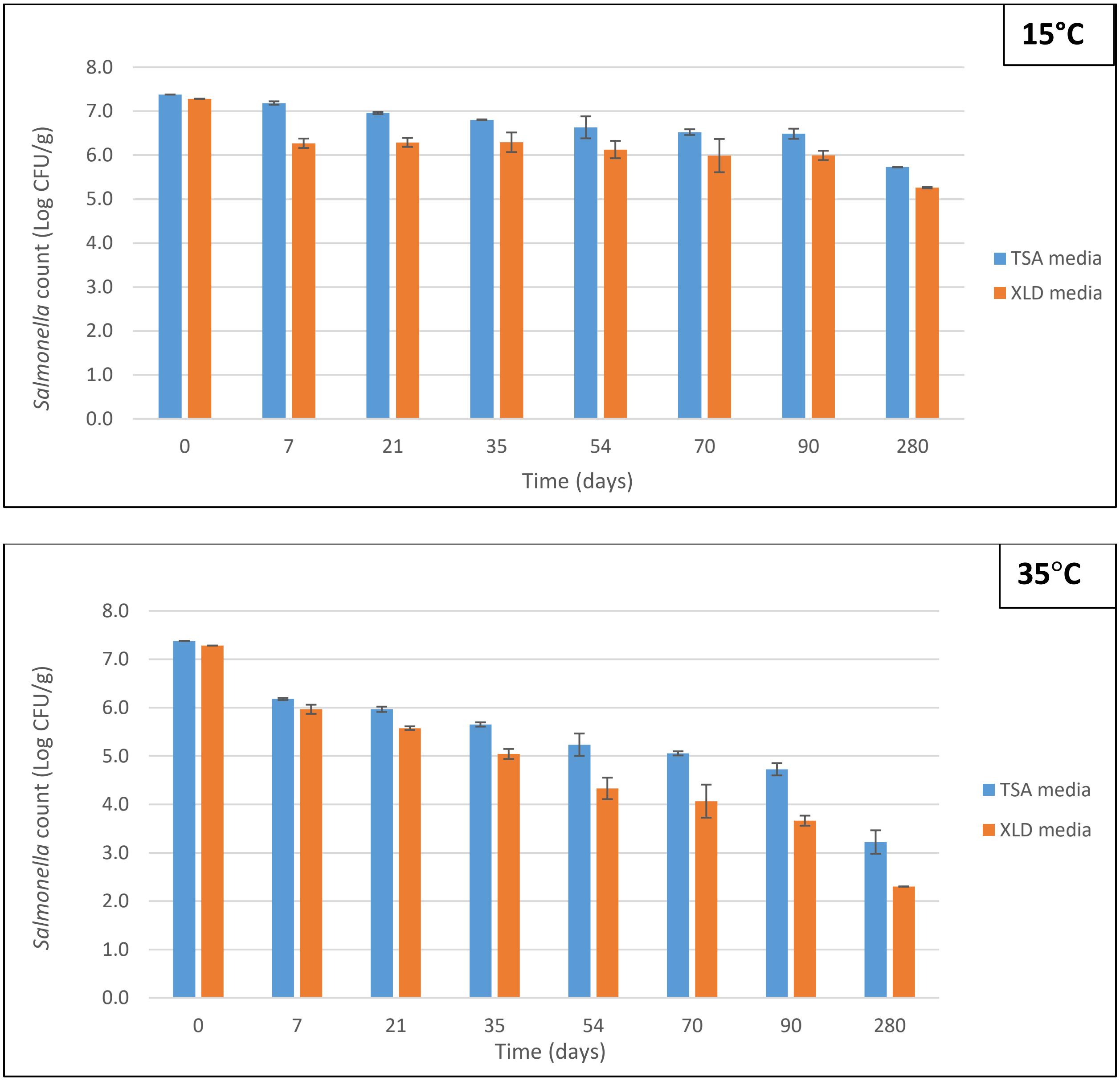


Figure 5. 2. Snapshot of degree of non-lethal injury of *S*. Limete showing difference between recovery on TSA and XLD media in brown crumb stored at 15°C and 35°C.

Analysis also showed that the degree of injury became more obvious the longer the crumb was stored. An illustration of this can be seen in **Fig. 5.2** where recovery of *S.* Limete on selective and non-selective media at either 15 or 35°C displayed a difference that increased with time. Similar trend was observed with other strains, but with the weaker strains showing greater susceptibility.

5.4.3. Water Activity and Moisture Content of Inoculated Crumb during Storage

Water activity and moisture measurements taken before, during, and after experiments indicated there were no significant changes in these intrinsic properties throughout the study duration, since the inoculum preparation method was designed to avoid altering moisture or water activity levels. The average water activity values for brown and white crumb were 0.15 ± 0.01 and 0.23 ± 0.02 respectively. Generally, a_w of white was slightly higher than that of brown crumb: brown crumb had an average moisture level of 0.83 % both before and after trials, while white crumb moisture content averaged at 0.64. No physical changes in the appearance or texture of the crumbs either during or after 90 days was observed at any of the storage temperatures.

5.4.4. Predictor Variable: Strain Identity

Substantial differences between strains were observed when individual inactivation trends were compared: *S*. Eastbourne was the least resistant strain, declining more rapidly than did the other strains regardless of storage temperature, while *S.* Limete was the most resistant. Thermal resistance tests were also conducted to verify strain sensitivity by using a submerged coil apparatus to test thermal inactivation at 58°C. *S*. Limete was still detectable and quantifiable in both crumb types at all temperatures, with the degree of log reductions being significantly higher at 35°C than the other temperatures. For example, in brown crumb, *S*. Limete levels at 35°C storage had reduced by 4.16 log CFU/g by Day-280, while levels at 24°C and 15°C had declined by 2.78 and 1.65 log CFU/g respectively (**Table 5.1**). *S. Limete* levels remained above the limit of detection (2 log CFU/g) throughout the 280-day storage period in both crumb types. In contrast and to further illustrate strain-type effect: for the same time period of 280 days in brown crumb, *S*. Eastbourne and *S*. Typhimurium were undetectable by enrichment in crumb stored at 24°C and 35°C, while levels in 15°C storage had merely reduced to 4.45 and 2.43, indicating declines of 2.29 and 4.37 log CFU/g in *S*. Eastbourne and *S*. Typhimurium respectively.

Table 5. 1. Observed population reductions (Log N/N_0) of *S.* Limete by Day 280 showing geometric means ± standard deviations (n=3). Values represent enumeration on non-selective media (TSA).

		Brown Crumb[a]	**White Crumb**[a]
Storage	15°C	-1.65 ± 0.00 A	-1.15 ± 0.03 A
Temperature	24°C	-2.78 ± 0.22 B	-1.85 ± 0.11 B
	35°C	-4.16 ± 0.24 C	-2.87 ± 0.07 C

[a] Categories with different letters represent significant differences within columns using Tukey-Kramer test (P < 0.05)

5.4.5. Predictor Variable: Storage Temperature and Time Effect

Storage temperature and time had an interactive effect on inactivation of *Salmonella* in crumb as all pairwise comparisons were significant (p < 0.001). In all three trials, regardless of crumb type or strain, microbial death was most rapid in crumb stored at 35°C compared with storage at 15°C or room temperature. For both brown and white crumb, analysis of survival by day 90 or 280 indicated that the average decline at 35°C storage was significantly greater ($p < 0.001$) than decline at the other two temperatures. However, survival measured via selective XLD media clearly distinguished the storage temperature effect as decline at each temperature were significantly ($p < 0.001$) different from each other. In other words, the longer the crumb was stored, the degree of injury became more attributable to storage temperature (**Fig. 5.2**), and the clearer it became that storage temperature largely predicted the survival of *Salmonella* in crumb.

5.4.6. Predictor Variable: Crumb effect

Salmonella populations declined significantly ($p < 0.001$) in both brown and white milk crumb at each isothermal temperature tested. However, irrespective of strain type, *S. enterica* declined faster in brown than in white crumb, although it is worth noting that this difference was borderline significant ($p=0.046$) at the chosen alpha level of 0.05. This may indicate that crumb type was not necessarily the best predictor of survival.

5.4.7. Modeling of S. enterica survival in milk crumb matrix

For both crumb types, *Salmonella* inactivation at all temperature levels demonstrated a non-linear survival kinetics (i.e., Log CFU/g vs. time) with a substantial tailing effect observed, particularly by day-280 (**Fig. 5.3**). Evaluation of several mathematical models (using GiNaFIT) found that the Weibull model satisfactorily fit the survival trends observed and could be used to provide good predictive models (**Fig. 5.4**).

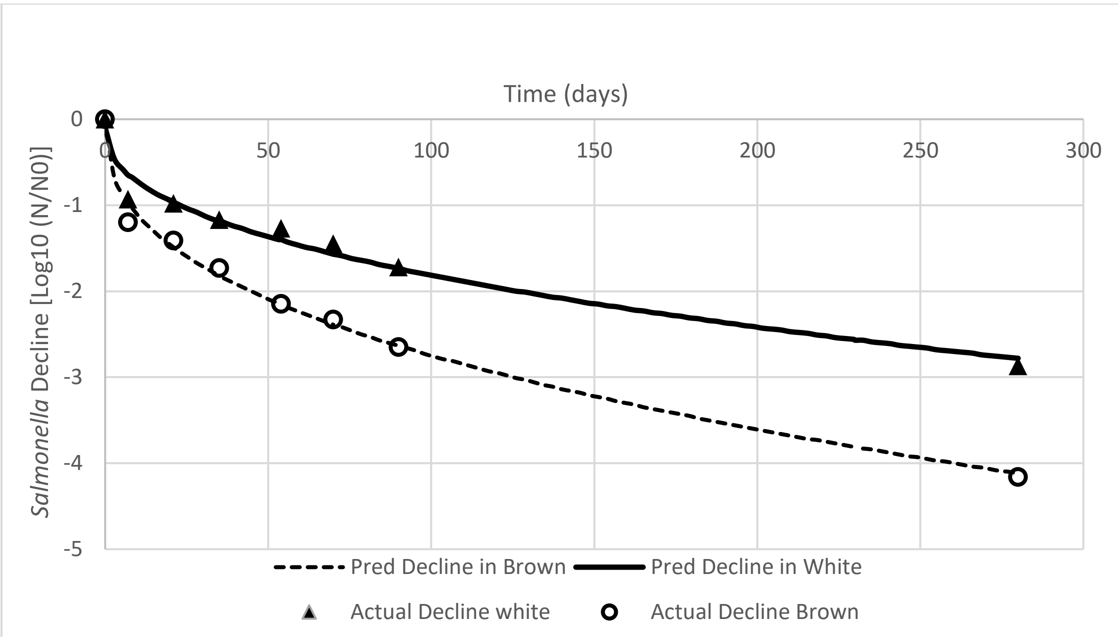


Figure 5. 3. Survival of *S. enterica* in inoculated milk crumb stored at 35°C for 280 days. Solid line represents fitted Weibull function for white crumb, dotted line represents fitted Weibull for brown crumb. Symbols represent experimental data points in white (▲) and brown (○) milk crumb. Values are from a single trial with three replicates (n = 3).

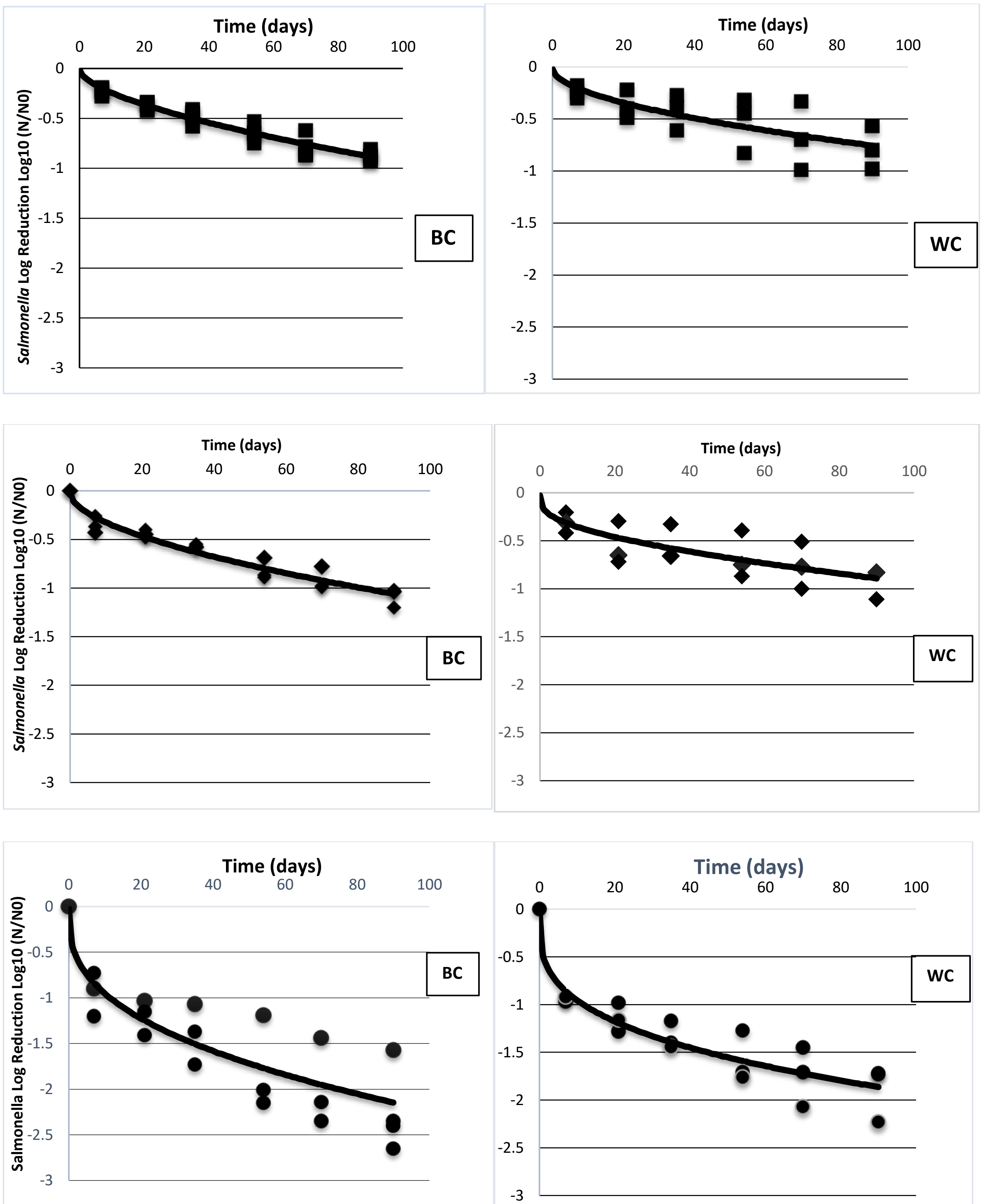


Figure 5. 4. Graph showing fitted Weibull distribution of three independent trials of *S. enterica* decline in Brown Crumb (panels labeled BC) and White Crumb (panels labeled WC) over 90-day

storage at 15°C (■), 24°C (♦) and 35°C (●). Solid, thick line represents average of the fitted distributions, and solid symbols represent empirical distribution (observed individual data).

Both the predicted and observed inactivation levels of *S. enterica* at the 90-day mark based on Weibull model parameters is depicted on **Table 5.2**. There were no significant differences between predicted and observed values. The table also shows that for both brown and white crumb, decline at 15 and 24°C were not significantly different at the end of 90 days.

Table 5. 2. Predicted and observed population reductions (Log N/N_0) of *S. enterica* by Day 90 showing geometric means ± standard deviations (n=9). Values represent enumeration on non-selective media (TSA).

		Brown Crumb[a]		White Crumb[a]	
		Predicted	**Observed**	**Predicted**	**Observed**
Storage Temperature	15°C	-0.96 ± 0.13	-0.88 ± 0.06A	-0.81 ± 0.28	-0.79 ± 0.21A
	24°C	-1.14 ± 0.13	-1.09 ± 0.10A	-0.89 ± 0.18	-0.92 ± 0.16A
	35°C	-2.30 ± 0.60	-2.19 ± 0.56B	-1.96 ± 0.32	-1.89 ± 0.29B

[a] Categories with different letters represent significant differences within columns using Tukey-Kramer test ($P < 0.05$)

For both brown and white crumb, slope increased with increase in storage temperature for all trials (**Fig 5.5**). This slope steepness is also as described by the scale factor in the Weibull function in Equation (1).

The shape parameter α for the survivor curves generated in this study was generally < 1 and displayed mostly upward concavity (**Fig. 5.4** & **5.5**), indicating that the most sensitive

S. enterica cells were inactivated toward the beginning of the storage period. Again, this was more pronounced at 35°C for both crumb types.

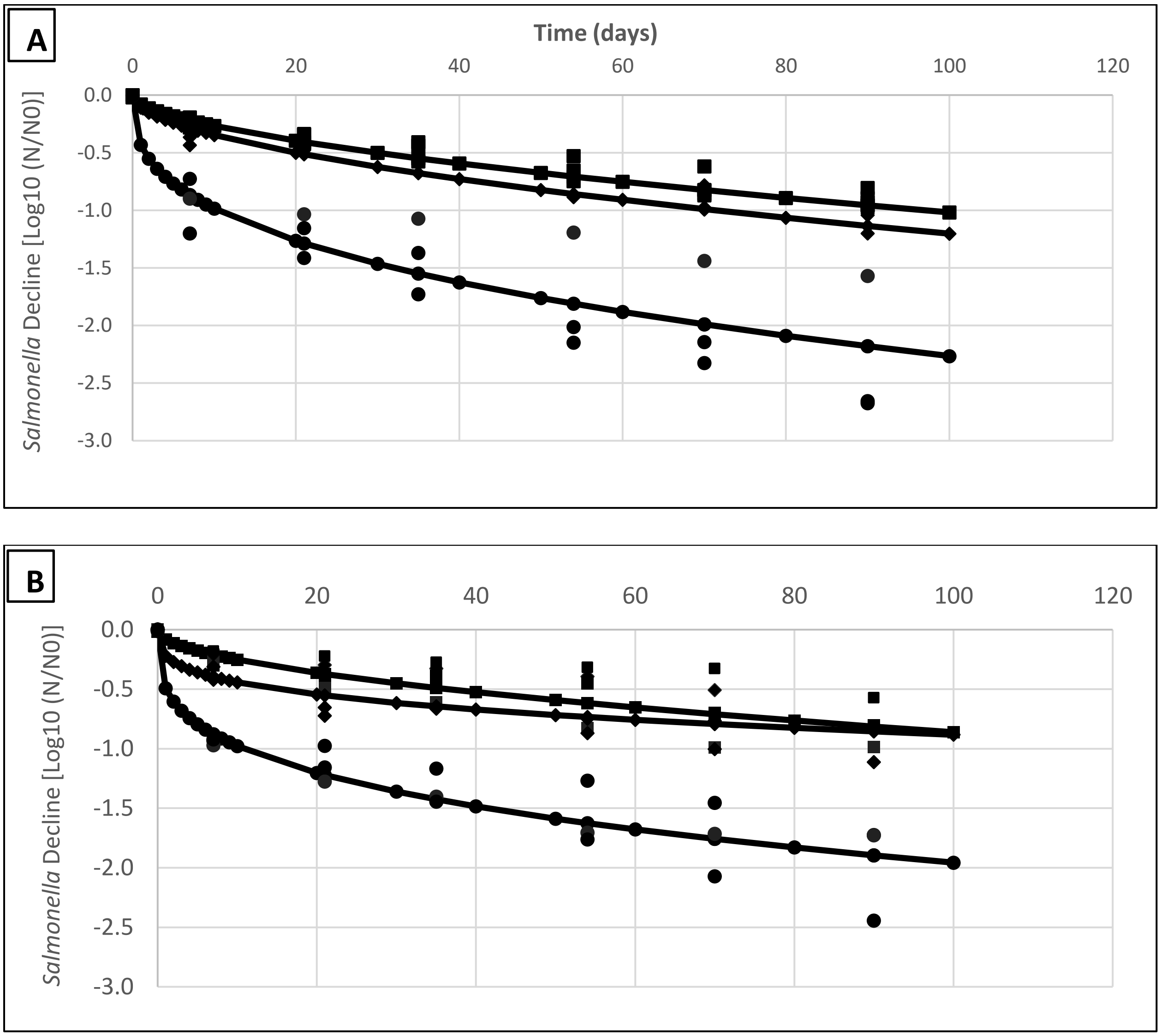


Figure 5. 5. Inactivation curves of predicted decline of *S. enterica* cocktail [*S.* Eastbourne, *S.* Limete, *S*. Typhimurium] in brown milk chocolate crumb [A] and white milk chocolate crumb [B] stored at different temperatures. The solid lines represent the fitted Weibull model using average parameters (data from each of three trials), and the symbols around each line represent observed decline at 15°C (■), 24°C (♦) and 35°C (●) for all three trials (n=9).

The few curves which indicated α >1 were observed for individual trials, but the mean values were < 1 (**Table 5.3**).

Table 5. 3. Weibull survival model parameters obtained for *S. enterica* decline in milk crumb stored under three isothermal conditions over a 90-day period. Values are mean parameter estimates from three independent trials with n = 9. Weibull equation used: $Y(t) = Y_0 - kt^{\alpha}$

	Brown Crumb			**White Crumb**		
	15°C	24°C	35°C	15°C	24°C	35°C
Parameters						
Initial reduction Y_0 [Log (CFU/g)]	-0.019	-0.017	-0.013	-0.016	-0.024	-0.012
Shape parameter (α)	0.604	0.557	0.365	0.554	0.564	0.304
Scale factor (k)	0.062	0.091	0.419	0.066	0.145	0.480

The results highlight the temperature dependence of the Weibull parameters k and α for microbial inactivation.

5.5. Discussion

Several studies have been done to evaluate the survival of *Salmonella* in low-moisture foods such as chocolate and dry milk powder during long-term storage. For instance, Barrile and Cone (1970) reported that after 15 months of room temperature storage, lyophilized cells of *Salmonella* Anatum which were inoculated into milk chocolate at levels of 50 cells/100 g were detected at a level of 14 (MPN)/100 g. Also, Tamminga et al (1977) demonstrated that S. Eastbourne could survive up to 19 months in a milk chocolate product, while Day et al (2011) reported that *Shigella dysenteriae* could remain viable for up to 12 weeks in dehydrated infant formula stored at in ambient atmosphere. Therefore, in the current study, survival of *Salmonella* in milk crumb for up to 280 days was not unexpected. The details provided in the results analysis, however, paint an interesting picture regarding storage of crumb as an intermediate product during milk chocolate production and the potential impact that could be applied to risk assessments.

The experiments for this study were designed based on the hypothesis that contamination could potentially occur anywhere from incoming raw materials, e.g., NFDM, up through post-crumb production and storage which could be > 6 months. While the vacuum-oven treatment of crumb is not designed to act as a lethality step, the crumb emerging from the oven has been assumed to be virtually bacteria-free (D'oust 1977). It does not appear that this assumption has been rigorously tested; however, the current study indicates that the levels of *Salmonella* would be expected to decline with storage. And while the greatest contamination risk is anticipated after the crumb is made, the potential for contamination from incoming raw materials, specifically milk solids, or the ability of *Salmonella* to survive the crumb-making process, is not ruled out.

Low-moisture food matrices generally follow either a log-linear or non-log-linear survival kinetics (Hiramatsu et al 2005; Podolak 2010). The present study consistently demonstrated non-linear survival kinetics for *Salmonella* in both brown and white milk chocolate crumb matrix. The survival data obtained could be effectively modeled using the Weibull model which has been used by researchers to empirically model of distribution of inactivation times in various foods and experimental systems (van Boekel, 2002). Three types of microbial inactivation can be described by the alpha (α) shape parameter of the Weibull model. Alpha can be less than, equal to, or greater than 1: $\alpha < 1$ indicates upward curve concavity or decreasing inactivation with time, $\alpha > 1$ mirrors the convex nature of the curve or increasing inactivation rate, and $\alpha = 1$ suggests probability of inactivation rate is not time-dependent (i.e., log-linear) (van Boekel 2002). Most of the survival curves obtained from our experiments demonstrated an upward concavity (Fig. 1, 4 and 5).

The effect of strain-type on survival was evident at the beginning of the storage period. Within the first 21 days, *S*. Eastbourne and *S*. Typhimurium showed comparatively poorer survival irrespective of crumb type, but followed temperature-dependent trends and declined at levels significantly greater than *S*. Limete. By Day-54, both strains were observed to be below the limit of detection for the non-selective media plates. And stabilization of strain differences were observed over the longer periods of >200 days. Effect of strain identify was highlighted in the study done by Tamminga et al (1977) where it was found that *S*. Eastbourne, isolated from a Canadian chocolate outbreak, had a better survival rate than an *S*. Typhimurium strain. Given results from the current study, one may assume, as suggested by Tamminga et al (1977), that strains differ in susceptibility to storage in low water activity environments.

Although statistical analysis results point to crumb-type as having a significant effect on survival, the marginal p-value of 0.046 (α level at 0.05) to support this alternative hypothesis may be interpreted to mean that crumb type may not necessarily the best predictor of survival. However, the possibility that cocoa-containing matrices may support microbial inactivation is not isolated and finds some backing in literature. The differences in survival observed based on crumb type could be based on a number of possibilities. First is the obvious compositional difference between the two crumb types: brown crumb contains cocoa mass, an ingredient absent in white crumb. Tamminga et al (1976) included compositional difference as a variable in their study of *Salmonella* survival in dark and milk chocolate bars. Greater *Salmonella* inactivation in dark chocolate was reported with a difference of approximately 1-log, a value higher than the mean difference of <0.5-log (Minimum Significant Difference from Tukey-adjusted means test) observed between brown and white crumb in the current study. The observation that brown crumb supported slightly faster inactivation may suggest a protective effect associated with white crumb or an antimicrobial effect associated with brown crumb. Flavonoids such as anthocyanins naturally present in cocoa or other plant sources can act as an antimicrobial that contributes to greater inactivation in brown crumb, and some studies have specifically demonstrated their bactericidal effect against *Salmonella* (Busta and Speck, 1968; Puupponen-Pimiä et al, 2005). The other possibility is the synergistic, protective effect of sugar and milk constituents against the antimicrobial effects of low moisture and/or cocoa constituents (Hiramatsu et al, 2005; Tamminga et al, 1976). One of the fundamental principles of predictive microbiology is that the combination of the conditions of the food (moisture and a_w levels, matrix-type etc), and the ecology of the specific pathogen of concern, are among the key determinants that must be considered when assessing microbiological risk.

The effect of storage temperature on pathogen decline in chocolate crumb was evident throughout the study and became even more evident past the day-200 mark. The less resistant *Salmonella* strains - *S*. Eastbourne and *S*. Typhimurium were no longer present by Day 280 in crumb stored at room temperature and 35°C, but were still present at 15°C at levels as high as 4.45-log CFU/g, well above detection limits. If the general inactivation trend is assumed, then these strains achieved faster inactivation and eventual die-off due to storage at higher temperatures. To reinforce this hypothesis, tests of cocktail-inoculated crumb at Day 230 (~7.5 months) indicate that normalized survivor population at 35°C were generally lower by at least 1.0-log in white crumb and 1.8-log in brown crumb. Predictive modeling analysis using existing literature data and carried out by Santillana-Farakos et al (2014) indicates that temperature is one of the most important factors to be considered in the survival kinetics of *Salmonella* in low a_w foods. It has been documented that added moisture can increase the susceptibility of *Salmonella* in dry matrices such as cocoa or milk powder to thermal treatments or during storage (Archer, Jervis, Bird, & Gaze, 1998; Barrile & Cone, 1970; Goepfert & Biggie, 1968). Also, while it is possible to inactivate *Salmonella* in chocolate or other low-moisture matrices using more intense thermal treatments as low as 50°C (Krapf and Gantenbein-Demarchi, 2010), the sensitive nature of this powder-like, milk crumb matrix tested in our study would make any added moisture or thermal treatment highly undesirable. The water activity and moisture content values reported in this study indicate that there were no significant changes in water content throughout the study duration. This is essential to note since the integrity of milk crumb as a dry matrix must be preserved during storage. It is also noteworthy that there were no differences in either water activity or moisture content among samples stored at different temperatures (data not shown).

A study that examined survival of *Salmonella* in milk chocolate (finished product) recovered salmonellae cells after 15 months storage at room temperature (Barrile, Cone & Keeney (1970), but did not test survival at any other temperature. Another study of *Salmonella* Typhi and *Shigella dysenteriae* in dehydrated infant formula showed survival at ambient temperature and in the presence of nitrogen for up to 84 days (Day et al, 2011). Lian et al (2015) examined a_w and water mobility as variables in the survival of *S. enterica* in skim milk powder stored at room temperature and confirmed the dependence of survival on both variables. It has been demonstrated that particle size of powder-like matrices can influence survival (Oni et al, 2015). Studies investigating storage temperature as a main predictor variable for survival of *Salmonella* in powder-like, low water activity matrices, or specifically, chocolate-related matrices, are very few, as most studies have focused on the influence of intrinsic properties such as a_w and moisture content, or the determination of inactivation profiles using higher temperatures as well as a combination of these factors (Archer et al, 1998; Jung and Beuchat, 1999; Laroche et al, 2005; Mattick et al 2001; Podolak et al, 2010; Doyle and Mazzotta, 2010). McDonough and Hargrave (1968) was one of the few studies to demonstrate effect of storage temperature on *Salmonella* survival in dried milk powder. Rate of destruction was found to increase with increasing storage temperature of up to 50°C for a duration of 15 weeks, but the authors expressed that this high storage temperatures may be ineffective in inactivating salmonellae from milk powder without adverse effects on quality. LiCari and Potter (1977) who performed similar studies reported that although storage at 45 and 55°C achieved substantial reductions in ~4 weeks, the adverse effects on quality of the milk powder were profound. It was recognized that storage at lower temperatures of 25°C and 35°C achieved some reduction but storage duration was limited to 8 weeks only. The current study has been able to further highlight the significance of taking storage temperature into account in a bid to optimize microbial inactivation in a dry powder-like matrix, especially when application of high temperatures are undesirable. A possible reason for a lack of focus research studies in this area as it specifically relates to chocolate and associated products may be because chocolate, as a product with rather sensitive sensory properties, is not considered a suitable candidate for temperature-manipulation studies. It is also important to mention that past attempts to hold cocoa powders at higher temperatures, a process known as "hot-boxing", were known to drive off volatile compounds and create off-flavor notes, thereby causing a decrease in the pleasurable properties of final chocolate product or other cocoa-based products. This is likely why hot-boxing has not been promoted in the chocolate industry and has hardly been investigated in recent literature. This study has demonstrated that there may be a way around this concern: strategically manipulating parameters such as temperature or water activity in order to find the optimal combination that may aid inactivation and reduce risk, yet maintain desired sensory properties. The two aforementioned studies were done on instant milk powders which are mostly ready for consumption, and as such, the concern regarding quality is important. The examination of milk crumb in this study is more related to its use as an intermediate product during milk chocolate processing, and it is possible that quality concerns are not as challenging as would be for instant or ready-to-eat products. Although, quality tests beyond visual inspection and monitoring of moisture levels were not conducted in this study, it is not farfetched to assume that small changes in quality, if observed, may be tolerable. Furthermore, the possibility of reducing contamination risk is encouraging.

5.6. Significance and Application

The major significance of this study to the chocolate manufacturing industry, as well as potentially other dry food producers, is the possibility of incorporating an additional risk mitigation step in processing. Traditionally, milk chocolate crumb is stored by manufacturers, usually under conditions no higher than ambient temperature. The temperatures chosen for our investigation were selected based on a number of factors including our curiosity as to what happens in a potential milk crumb contamination scenario, particularly given our knowledge of *Salmonella*'s behavior in dry food products, and notoriety of dairy products such as dried milk powder as a source of *Salmonella*. Furthermore, the current use of crumb in milk chocolate manufacturing is for convenience sake. It is often produced during slower production periods, allowing manufacturers to stockpile the major ingredients for milk chocolate. This way, the crumb can be readily converted into milk chocolate as demand requires. However, our research has now indicated that this crumb can also be used to reduce the risk of *Salmonella* contamination during processing. Specifically, it is demonstrated that the application of slightly elevated temperature to stored chocolate crumb can function as a pathogen mitigation strategy. Hence, storage parameters are no longer just for production convenience, but could now be manipulated to achieve *Salmonella* risk reduction and factored into a food safety risk assessment. This provides a way of maximizing the benefit of crumb storage while avoiding deleterious effects on the sensory properties of the final milk chocolate product.

5.7. Conclusion

There are no studies in the public domain addressing the use of milk crumb in chocolate processing and investigating its potential association with *Salmonella* risks. Thus, findings from our study introduces a new element which may be taken into consideration in risk assessment efforts in this regard. These results show that although *S. enterica*, particularly strains with increased resistance, may persist for extended periods in milk crumb, promising opportunities for risk reduction can be explored through further research into optimization of crumb storage parameters.

Chapter 6. Summary and Future Work

6.1. Summary

There is significant lack of information to help put together a good picture of major factors at play regarding *Salmonella* contamination of chocolate products. The absence of a comprehensive risk profile limits the ability to assess the effectiveness of existing control measures in a chocolate food safety plan. This work has taken the first few steps in formally assessing *Salmonella* risk within the farm-to-packaging continuum of a milk chocolate product and has gathered scientific information that can serve as a dossier for future reference.

In chapter 3, the food safety management systems of HACCP and HARPC were examined within the context of milk chocolate processing, and this helped define critical control points (CCPs) and preventive controls (PCs) in the conceptual model that was developed for this purpose. Sequel to this was the conduct of a qualitative risk assessment in Chapter 4, an important study that laid the foundation for risk assessment and helped characterize processes beginning from cocoa bean cultivation on the farm, up through conversion of beans to cocoa liquor and until final packaging. The use of a modular framework to assess risk in a stepwise manner, the creation of a set of criteria defining risk categories, and the assignment of risk rating to activities and ingredients throughout the continuum, were goals achieved by the qualitative assessment. We were however still interested in exploring some of the “blind spots” that may exist regarding *Salmonella* contamination during chocolate manufacture. In the course of our investigations and after visit to a chocolate manufacturing facility, our attention was drawn to milk chocolate crumb, particularly after learning about its current use in milk chocolate manufacturing. It became expedient to examine the crumb matrix especially after very little information was found in literature. Chapter 5 therefore covered the evaluation of milk crumb via experimental trials. Decline kinetics data of preadapted *S. enterica* in brown and white crumb was generated and predictive models for *Salmonella* survival under three isothermal conditions were subsequently developed. To our knowledge, our work is the first to identify that enhanced reduction at some elevated temperature can achieve greater inactivation of *Salmonella* in stored milk chocolate crumb and relate its applicability to risk assessment.

Of significance is the estimation that every 10-fold decrease in *Salmonella* leads to 10-fold decrease in risk; for example, a >5-log pathogen reduction represents a >100,000-fold reduction in the risk that a consumer would become ill from eating milk chocolate. Although, it is acknowledged that additional characterization is needed to optimize this potential control measure, findings from this study would be beneficial to global chocolate manufacturers as they seek ways to reduce risk and avoid incurring heavy financial losses, or worse still, a bad reputation. Other potential applications outside of chocolate production could be in the designing of decontamination efforts for low moisture ingredients that may carry *Salmonella* risk, including but not limited to soy lecithin, whey powder, infant formula, flour, dry milk powder, among others. Remedial treatment for lots or batches of these products or ingredients may be investigated, such that when applicable, rather than dumping a contaminated lot, optimized heat-storage treatment may provide a cost-effective way to salvage the product.

While it may considered impossible to create a risk-free farm-to-fork continuum, food manufacturers and distributors must be relentless in employing and seeking to improve control measures in order to drive risk toward zero and ultimately ensure the safety of a food product until it reaches the final consumer. Thus, any efforts in this regard – what this study has attempted to do - is considered an advancement for food safety optimization.

6.2. Future Work

Research in the following areas would provide a better understanding of risk factors for *Salmonella* contamination in chocolate, help fill some of the multiple data gaps identified and aid future risk analyses:

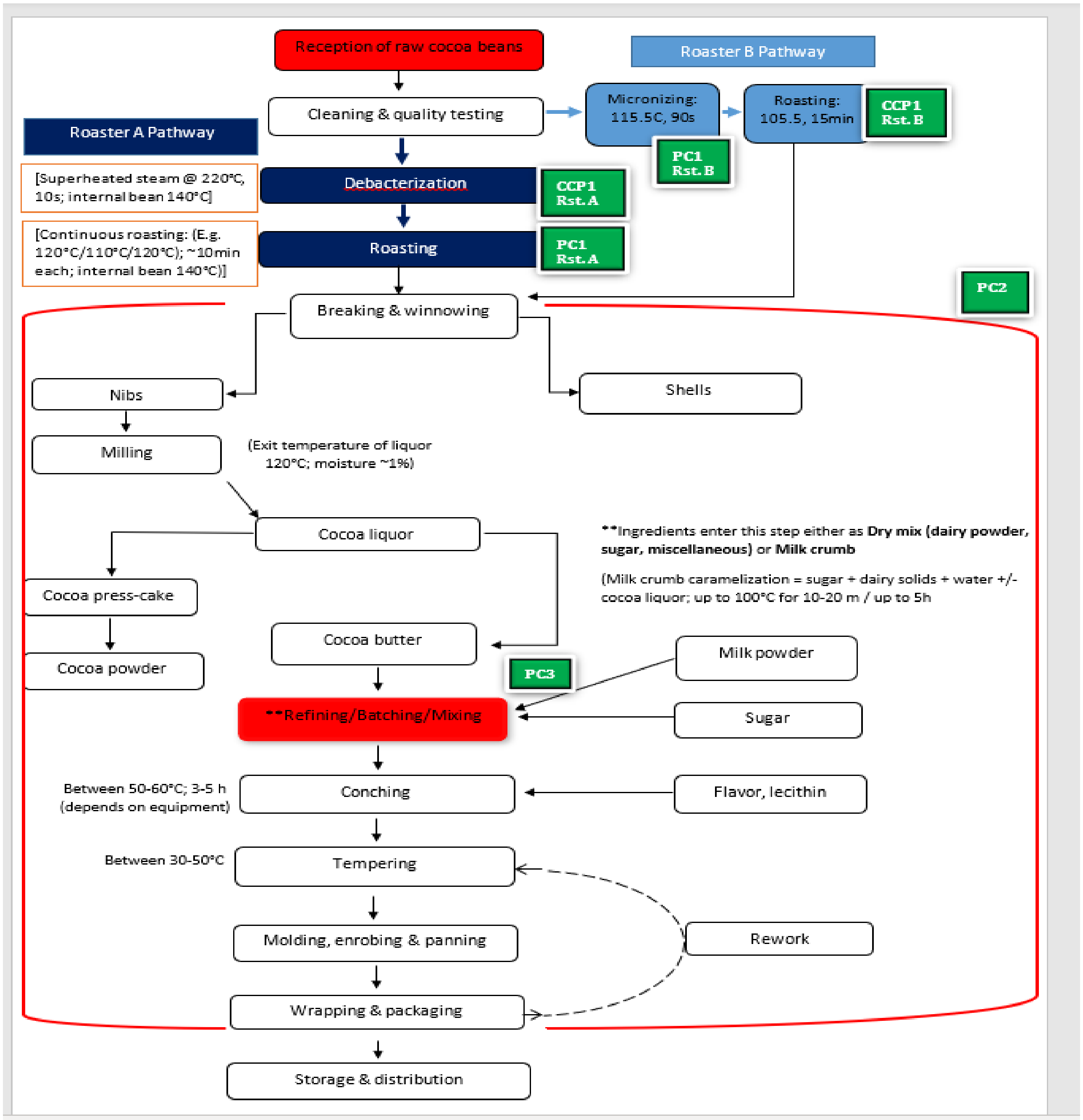
1. Conduct of an exposure assessment to evaluate thermal inactivation and resistance of *Salmonella* in various matrices associated with cocoa or chocolate - cocoa beans, cocoa liquor, cocoa butter, milk crumb and finished chocolate products.
2. Modeling of the thermal inactivation of *Salmonella* during milk chocolate processing, as well as an evaluation of the impact of integrated thermal treatments (roasting, debacterization, conching and refining) during processing. Validation studies to support an integrated inactivation model could also be helpful.
3. Regarding the potential risk reduction intervention identified, process optimization would be helpful in fine-tuning the application of this discovery and effectively tailor it to specific food ingredients and product; for example, water activity that will yield

maximal *Salmonella* inactivation. It would also be of interest to examine the effect of holding temperature on ingredient functionality and sensory characteristics.

Appendices

- Generic Conceptual Model of Milk Chocolate Processing –Appendix A
- Template for Expert Opinion Survey – Appendix B
- Snapshot of Risk Rating Tool in Excel - Appendix C

Appendix A - Generic Conceptual Model of Milk Chocolate Processing



Appendix B - Template for Expert Opinion Survey

UNIVERSITY OF MARYLAND

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES
DEPARTMENT OF NUTRITION AND FOOD SCIENCE

0112 Skinner Hall
College Park, Maryland 20742-7640
301.405.8977 TEL 301.314.3313 FAX

Expert Elicitation Survey

****Due to the nature of our ongoing research, kindly treat this survey and its contents as confidential.*

Instructions:
Please complete the following questions based on your knowledge of *Salmonella* and the products/ingredients in questions. When completed please e-mail the completed survey to: Ruth A. Oni at roni@umd.edu

Survey Questions

1. Please provide prevalence estimates of a) *Salmonella* b) Enterobacteriaceae (indicator organism) contamination of cocoa bean at the 5 stages of production listed below.

   Provide your estimates within a 90% confidence interval on the tables provided.

a. During fermentation

	Salmonella	Enterobacteriaceae
Minimum value		
Most likely value		
Maximum value		

b. During Drying

	Salmonella	Enterobacteriaceae
Minimum value		
Most likely value		
Maximum value		

c. During storage (post-drying)

	Salmonella	Enterobacteriaceae
Minimum value		

Most likely value		
Maximum value		

d. During shipping to manufacturing facilities

	Salmonella	Enterobacteriaceae
Minimum value		
Most likely value		
Maximum value		

e. Upon arrival at facility, if transportation takes an average of 30 days*

	Salmonella	Enterobacteriaceae
Minimum value		
Most likely value		
Maximum value		

*Estimate values can also be provided based on alternate transportation averages (e.g. 10 days, 60 days etc.)

2. With regards to thermal processes during milk chocolate production, should roasting be regarded as the only critical control point for *Salmonella*, or should considerations be given to:
    a. other heat-application activities such as *debacterization*; AND/OR:
    b. heat-generating activities such as *conching* and *refining*?

Roasting should be only CCP	Debacterization should be considered (in addition to roasting)	Conching should be considered (in addition to roasting)	Refining should be considered (in addition to roasting)	A combination should be considered (write down suggested combinations)

*Please indicate **Yes** or **No** in appropriate boxes on this table

3. In your opinion, should the COA (certificate of analysis) for the following milk chocolate ingredients be totally relied upon (rather than additional in-factory testing):

    a. cocoa butter/oil
    b. dairy solids (non-fat dry milk)
    c. lecithin

Please use table below to provide a brief rationale for your response:

Ingredient	**Should COA be relied upon (Y or N)**	**Rationale**
cocoa butter/oil		
dairy solids (non-fat dry milk)		
lecithin		

If needed, please use additional space below.

4. On the table below, which of the listed pathogens and/or indicator organisms should be tested for routinely?

Microorganism	**Tested Routinely (Y or N)**	**Testing Frequency (# hours or days)**
Total Aerobic Plate Count (TAP)		
Coliforms		
Enterobacteriaceae		
Salmonella spp.		
Listeria spp.		

5. During sampling, what is the suggested lower limit of detection for these microorganisms?

Microorganism	**Lower limit of detection***			
	1 CFU/g	1 CFU/10g	1 CFU/100g	1 CFU/125g
Total Aerobic Plate Count (TAP)				
Coliforms				
Enterobacteriaceae				
Salmonella spp.				
Listeria spp.				

*Put an "X" in the appropriate box

6. During the process of milk chocolate manufacture: if applicable, at what point(s) should testing for the aforementioned microorganisms (Question #4 above) be carried out?

    a) At point of receiving raw ingredients or COA
    b) In-line, during processing
    c) Finished products (just prior to packaging)
    d) Environmental testing from raw ingredient to finished, packaged product

*Please indicate your selection(s) from options (a – d) above by putting an "X" in the appropriate box on the Table below.

Microorganism	**Testing Point***			
	A	B	C	D
Total Aerobic Plate Count (TAP)				
Coliforms				
Enterobacteriaceae				
Salmonella spp.				
Listeria spp.				

7. What *Salmonella* strain would you suggest using as a standard for thermal resistance studies in a low-moisture food matrix such as molten milk chocolate (or milk crumb)?

[Please highlight your selection in red]

a. *S*. Senftenberg 775W
b. *S*. Eastbourne
c. *S.* Typhimurium
d. *S.* Enteritidis PT30
e. A combination of the above (please write down choices from (a–d)

………

f. Other. Please write down serotype(s) name:

………

Appendix C - Snapshot of Risk Rating Tool developed in Excel

	A	B	C	D	E	F
1	**Module**	**Risk Questions**	**Answers (risk rating options in comment box)**	**Risk Status for Base Model**	**Notes**	**References**
2	**Module I**	**FARM**				
3		**Harvesting**				
4		Can handling practices (harvesting, pod-opening) introduce *Enterobacteriacea* (indicator organism) and/or *Salmonella* into wet beans?	*See comment box + Notes*	↑	Step known to be first to potentially introduce contamination into sterile bean pods given handling and access to environment	Afoakwa 2010; Fowler 2009; Schwan and Wheals 2004
5		Prevalence of *Salmonella* in freshly harvested cocoa beans	*See Notes*	N/A	No data available, but possibility of contamination with *Salmonella* and *Enterobacteriacea* is acknowledged	Expert opinion via survey 2018
6		Prevalence of *Enterobacteriacea* in freshly harvested cocoa beans	*See Notes*	N/A	No data available, but possibility of contamination with *Enterobacteriacea* is acknowledged	Expert opinion via survey 2018
7		**Fermentation**				
8		Prevalence level of *Salmonella* during fermentation (estimates within a 90% confidence interval)	*Range Estimate: 0 - 10,000 CFU/g*	↑	Estimated guesses from experts	Expert opinion via survey 2018
9		Effect of fermentation and drying on *Salmonella* cells	*Possible reduction of bacterial load toward end of fermentation; increase during drying*	N/A	Limited scientific information available. Undetermined effect on risk	Camu et al. 2007; Derya Savran et al. 2018; Nascimento et al. 2013
10		Does the fermentation process act as an antimicrobial for *Salmonella*?	*See comment box + Notes*	N/A	Possibly; more data needed. Undetermined effect on risk	Nascimento et al, 2013
11		What happens in case of a fermentation failure?	*See Notes*	N/A	No data available. Undetermined effect on risk	—
12		**Drying**				
13		Drying method used: traditional sun-drying or mechanical?	*See comment box + Notes*	↑	Traditional drying methods has little environmental control and can increase risk of contamination from birds, rodents etc	Bell & Kyriakides 2002; Burndred 2009; Cordier 1994
14		Prevalence level of *Salmonella* during drying (estimates within a 90% confidence interval)	*Range estimate: 0- 100,000 CFU/g*	↑	Estimated guesses from experts	Expert opinion via survey 2018
15		Prevalence level of *Salmonella* during post-drying storage (estimates within a 90% confidence interval)	*Range estimate: 0- 10,000 CFU/g*	↑	Estimated guesses from experts	Expert opinion via survey 2018
16		Any controls to inactivate bacteria during or after farm processing (before transportation)?	*Decontamination efforts not usual*	—	No indication in literature of decontamination efforts	

Risk Rating Tool | Table A

Appendix D – Scenario Analysis Example of *Salmonella* estimates modeled in @Risk

Although the risk assessment conducted in this study was largely qualitative, scenario analysis was incorporated to provide a semi-quantitative illustration of risk using estimates provided by experts (Appendix C). This provides an example of how lack of data on pathogen prevalence can be treated.

Diagram A shows process steps for cocoa bean production on the farm until arrival at facility. *Salmonella* contamination estimates are in Log CFU/g. **Diagram B** is a screenshot of the model created in Excel using @Risk software. **Diagram C** is an output example showing result of simulation after model is run.

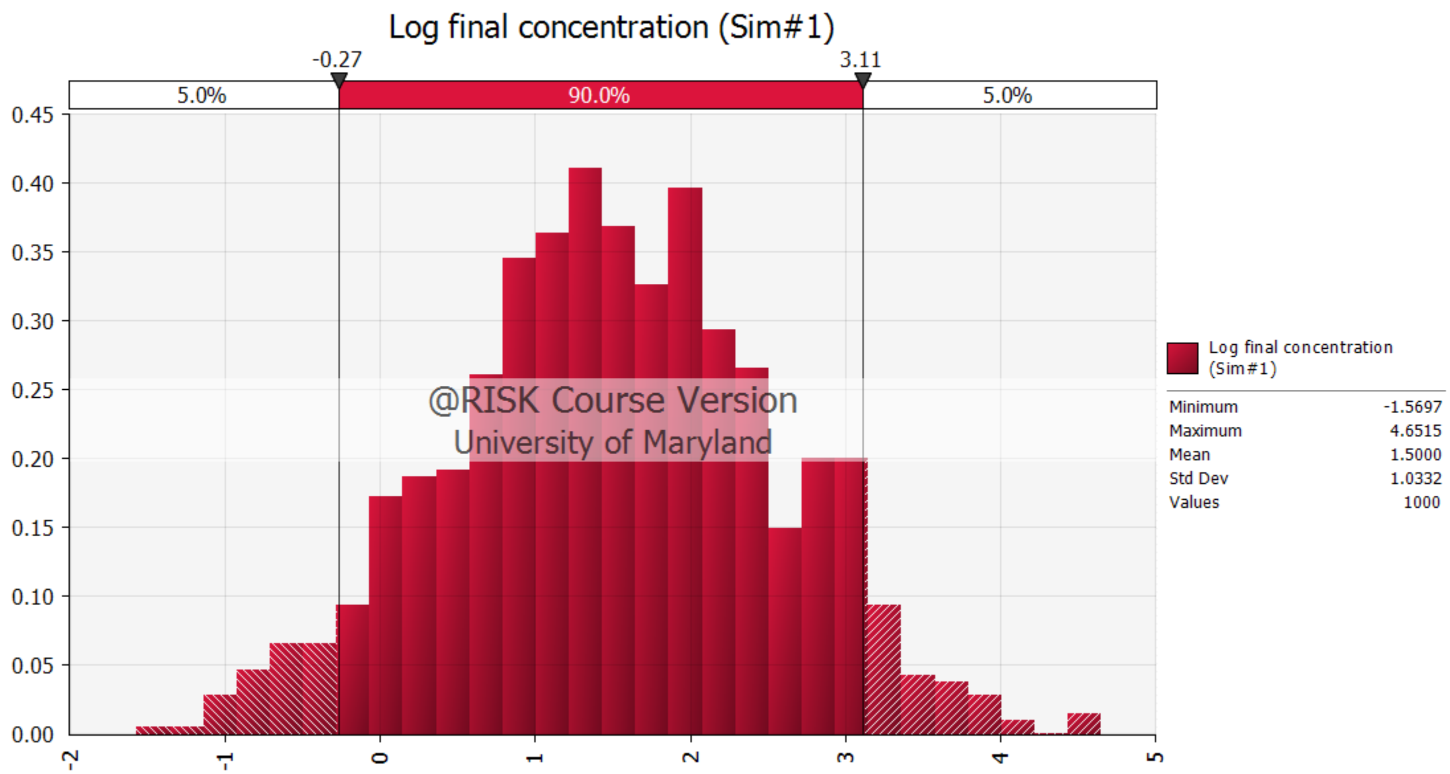
Diagram A

Process Step	*Salmonella* concentration (Log CFU/g)
Fermentation	
Min	1
Most likely	1
Max	4
Drying	
Min	1
Most likely	2
Max	5
Storage	
Min	1
Most likely	2
Max	4
Transportation	
Min	1
Most likely	2
Max	4

Diagram B

Model variable	Distribution	Units	Formula
Concentration of *Salmonella* during on-farm fermentation	3.186	Log CFU/g	RiskTriang(Q3,Q5)
Added contamination during drying stage	1.739	Log CFU/g	RiskTriang(Q7,Q9)
Reduction during storage	0.262	Log CFU/g	RiskTriang(Q11,Q13)
Reduction during transportation	2.120	Log CFU/g	RiskTriang(Q15,Q16,Q17)
Reduction due to roasting (Critical target is 5-log inactivation)	5.052	Log CFU/g	RiskTriang(4,5,6)
Amount of beans roasted	1000	g	
Final concentration in beans post-roasting	3.1 0.491191811	CFU per gram	

Diagram C



Log final concentration (Sim#1)
-0.27
3.11
5.0%
90.0%
5.0%
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-2
-1
0
1
2
3
4
5
@RISK Course Version
University of Maryland
Log final concentration (Sim#1)
Minimum -1.5697
Maximum 4.6515
Mean 1.5000
Std Dev 1.0332
Values 1000

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