POST HEAT TREATMENT MICROBIAL CONTAMINANTS AND QUANTITATIVE RISK ANALYSIS OF *S. AUREUS* IN SLICED VACUUM PACKED FRENCH POLONY

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DECLARATION

I **BONANG BEAULLAH MOYO** do hereby declare that I am the sole author of this thesis; I authorize **Midlands State University** to lend this thesis to other individuals and/or institutions for the purpose of academic research.

Signature.....Date.....

APPROVAL

This dissertation entitled **"Post heat treatment microbial contaminants and quantitative risk analysis of *S. aureus* in sliced vacuum packed French polony"** meets the regulations governing the award of the degree BSc Food Science and Nutrition at Midlands State University, and is approved for its contribution to knowledge and literal presentation.

Supervisor.....Date.....

DEDICATION

To Magreth Tsvara, my anchor.

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My humble gratitude goes to God, for holding me down throughout my studies and granting me strength to push forward when my spirit was down and lacked motivation. I'm grateful to Magreth Tsvara for sparing nothing to afford me a solid foundation. No words can thank Nigel Petre enough for his input throughout this research. To Debbie and Thabo Moyo, thank you for your unwavering love, support and encouragement. Bless.

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ABSTRACT

Contamination of luncheon meats by bacteria has resulted in premature product spoilage and food poisoning outbreaks as well as major product recalls. A quantitative microbial assessment of air, surfaces, equipment and personnel hands (swabs), and finished product was conducted on 3 batches during separate visits at various processing stages during production of sliced vacuum packed French polony at a large meat processing plant in Zimbabwe. The aim was to determine the routes of microbial cross contamination onto French polony during slicing and packing as well as the subsequent food safety threat posed to consumers. The post-cooking environment had relatively high levels of *S. aureus*, yeast and molds while equipment and surfaces proved to be unhygienic as indicated by high counts of TBC and coliforms. *S. aureus* was the only pathogen detected in the finished product (2.04log CFU/g) and personnel hands and the counts exceeded acceptable international standards. A Quantitative Risk Assessment Model process that described steps and behaviour of *S. aureus* along the sliced vacuum packed French polony chain was created and the exposure of *S. aureus* per serving was high (> 6log CFU/serving) in over 65% of each serving and consequently the probability of illness too. Overall, there is a relatively high level of risk of microbial contamination of French polony during slicing and packing from different sources at the meat processing plant investigated and it is paramount to consolidate the quality assurance programs so as to ensure the safety of consumers of products produced at this plant.

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ACRONYMS

HACCP	Hazard Analysis Critical Control Point
FSMS	Food Safety Management System
FSQMS	Food Safety and Quality Management System
GHPs	Good Hygiene Practices
GMPs	Good Manufacturing Practises
CFU	Colony Forming Unit
TBC	Total Bacterial Count
RTE	Ready-to-Eat
SE	Staphylococcal enterotoxin
SFP	Staphylococcal Food Poisoning
OTR	Oxygen Transmission Rate
CSLs	Critical Sampling Locations
QRAM	Quantitative Risk Assessment Model
ISO	International Standards Organisation
WHO	World Health Organisation
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration

CHAPTER ONE

INTRODUCTION

FAO (2016) indicated that meat consumption in developing countries has been continuously increasing from a modest average annual per capita consumption of 10kg in the 1960's to 26kg in 2000 and is estimated to reach 37kg around 2030. This rising demand is mainly a consequence of growing urbanization and the tendency among city dwellers to spend more on food. In general, as soon as consumers' income allow, they in-cooperate more animal protein, particularly meat, in the daily diet.

The great demand for meat output can only be met by making better use of meat resources available and reducing waste of edible livestock parts to a minimum through meat processing (FAO, 2016). Lean meat is a valuable source of animal protein, but costly and therefore not available to some population segments. Meat processing blends cheaper plant products thereby reducing the cost and allowing more consumers access to animal-protein products. Meat processing is the manufacture of meat products from muscle meat, animal fat and certain non-meat additives (Heinz and Hautzinger, 2007). The technology integrates certain animal tissues and internal organs which are otherwise not included on the fresh meat market (either because of their palatability or aesthetic value) into the food chain, fabricating nutrient rich products for human consumption (Heinz and Hautzinger, 2007). Unlike fresh meat, many processed meat products are made shelf stable, value added and display specific flavor, color, taste or texture components which are different from fresh meat, offering diversity (Heinz and Hautzinger 2007).

Meat processing accounts for a wide range of products on the market, ranging from cured, cooked, canned, chilled and frozen products. Cooked processed meats can either be eaten cold or warm, but both require no further preparation; they are ready to eat. Cold meats can be

bought pre-sliced in vacuum packs at a supermarket or grocery store or they can be bought at a deli counter where they can be sliced to order. AICR (2016), defined sliced cold meat, also called luncheon meat, cold cuts, sandwich meat or cooked meat as precooked cured meat (often in the form of meat loaves) that is sliced and served cold or warm on sandwiches or party trays. There is a strong mainstream market for pre-packed sliced cold meats; the main interests being speed, value and convenience. Consumers consider them to be more sanitary and of better bacteriological quality than those sold at the deli counter (FAO, 2016). Pre-packed sliced cold meats at company Z come in small portions of 150g or 200g, removing the monotony of buying one meat loaf of the same type allowing consumers to spread their money and buy a variety of products on the sliced cold meats range of products which covers products such as salami, polonies, pastarami, hams and kassler fleisch.

French polony requires no further heat processing, hence its bacteriological quality and consequently its shelf stability is a major concern. The presence of unfavorable microbes in high counts can cause premature deterioration in French polony. In general, as purported by Ray and Bhunia (2008), deterioration is a subjective judgment that can be influenced by economic and cultural aspects, knowledge and sensory acuity of the individual and intensity of deterioration. In the case of sliced cold meats, Ray and Bhunia (2008) reported that the main criteria for rejection are undesirable odour, slime formation, souring, changes in flavor, texture, color, pH level and gas production evidenced by blowing of vacuum packed products. Spoilage effects can be noticed from as early as a few days up to the end of shelf life, where the meat is dominated by spoilage. Organisms of interest in the sliced vacuum packed French polony at company Z are *S. aureus, E. coli,* coliforms, yeasts and molds because they are prevalent in the environment (*S. aureus* and *E. coli* constitute normal body flora, even of healthy individuals) and cause rapid deterioration of the products with food safety risks to the consumers (Leonard, 2011).

Food safety is a complex issue that has an impact on all segments of society, from the general public to government, industry, and academia. To ensure food safety, manufacturers should adopt strategies for maintaining and executing food safety programs by adhering to internal and external standards, regulations and requirements. Verran (2008) defined FSMSs as a series of specifications, procedures, processes, verifications, validations and documentation that comprises a processor's formal plan to ensure food safety and quality management. Following the system's procedures does not only produce safe food to reduce risk of foodborne illnesses but it also maintains the food at high quality especially if there is a quality criteria defined within the FSMS. FSMSs ultimately reduce loses to the processor that may be due to premature spoilage, product returns or recalls (Hoffman and Anekwe, 2013).

1.1 PROBLEM STATEMENT

Meat is the most valuable livestock product and for many people serves as their first choice source of animal protein. Meat is either consumed as a component of kitchen style food preparation or as processed meat products. Processed meat products are usually ready to eat, requiring little or no further heat treatment before consumption. It is therefore paramount that the manufacturing practices employed during production eliminate all hazards to ensure food safety (Heinz and Hautzinger, 2007). The current manufacturing practices in the sliced cold meat range of products at company Z have proved inefficient as some batches have failed to reach the end of their shelf life as shown in Figure 1 below. By not reaching the expected shelf life, this exposes consumers to risk of foodborne illnesses and threatens economic losses to the company due to product spoilage, returns and recalls.

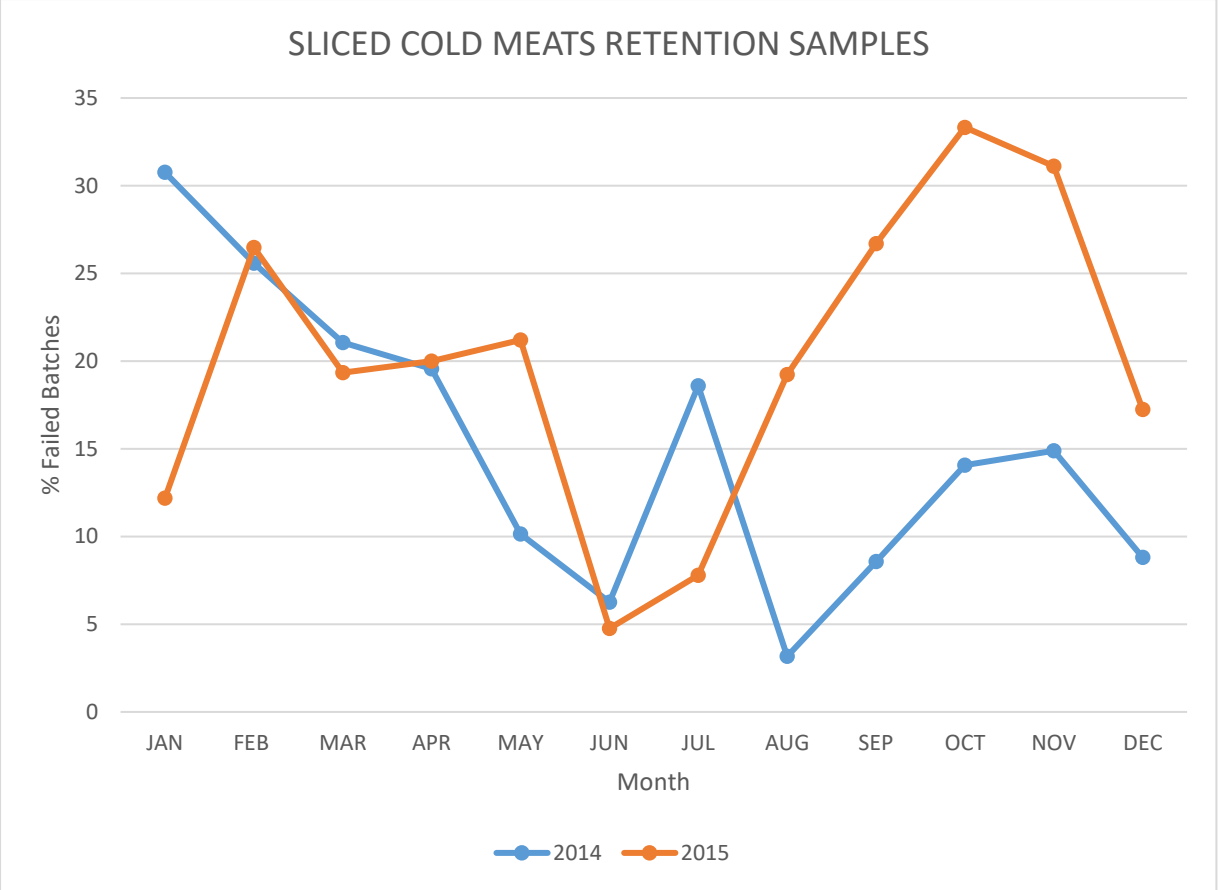

Figure 1 Internal performance tracking data at Company Z for 2014 and 2015 sliced cold meats retention samples.

According to Ray and Bhunia (2008), vacuum packaging has proven to be efficient in extending shelf life during refrigeration by reducing oxidation and the growth of aerobic micro-organisms hence preserving sensory characteristics inherent to the product for a period sufficiently long for its turnover. Some indicators of spoilage include surface discoloration of the meat or drip, evidence of gas production in the pack and off odors that may be detectable when the pack is opened. There are many factors that may contribute to the spoilage of vacuum packed meat e.g. temperature abuse, incomplete air evacuation, high meat pH (>6) and high initial microbial load (Hoffman and Anekwe, 2013)

At Company Z, the loaves of the French polony maintained shelf stability while the sliced vacuum packed French polony exhibited premature deterioration. The slicing process is

vulnerable to cross contamination (Chonnell, 2006), because it involves extensive handling and the products are exposed to air while being moved through different equipment, surfaces and personnel before vacuum packaging. Air is a natural habit for *S. aureus*, which is also prevalent on the skin, nose and hair even of healthy people (Chonell, 2006). According to FDA (2012), foods that require extensive handling, such as in the case in sliced vacuum packed French polony, are often involved in staphylococcal food poisoning. Soil, water, drains and meat processing equipment are suggested to be pivotal niches for the transmission of *L. monocytogenes*, *E. coli* and coliforms in the food chain (Mustapha, 2007), which can cause rapid deterioration if initial load is high in a product (Chonell, 2006). This research served to investigate the routes of microbiological cross contamination of French polony post heat treatment and the subsequent food safety risks to consumers of sliced vacuum packed French polony produced at the plant investigated.

1.2 OBJECTIVES

1.2.1 Main Objective

- To determine routes of microbial cross contamination onto French polony during slicing and packing and the subsequent food safety threats posed to consumers.

1.2.2 Specific Objectives

- To enumerate TBC, Coliform, *E. coli* and *S. aureus* on the hands of personnel and sliced vacuum packed French polony during storage up to the end of shelf life.
- To enumerate TBC and Coliform on equipment and surfaces.
- To enumerate yeast and molds in the environment and on the sliced vacuum packed French polony during storage up to the end of shelf life.
- To conduct quantitative risk analysis of *S. aureus* in sliced vacuum packed French polony.

1.3 HYPOTHESES

H_o1: There will be no significant difference in the microbial load (TBC, *E. coli*, coliform, yeast, molds and *S. aureus*) on CSLs across the French polony slicing and packing line.

H_o2: There will be no significant difference between the observed and the standard microbial limits (TBC and *S. aureus*) specified in SANS 885:2011 3rd Edition on sliced vacuum packed French polony.

1.4 ASSUMPTIONS

- Microorganisms are reducing the shelf life stability of sliced vacuum packed French polony.
- There was cross contamination from air, personnel hands, equipment and surfaces to the French polony during slicing and packing
- Consumers are at risk of SFP.

1.5 DELIMITATIONS

- This research was company based and focused on operations at company Z only.
- Primary research was only limited to microbial hazards.

1.6 SIGNIFICANCE OF THE STUDY

The meat industry

This study is of value to meat processors as it identified routes of microbial contamination during the processing of RTE meat which assists in the prevention and /or elimination of potential microbial hazards. Control of microbial hazards during processing reduces risk of foodborne illnesses posed to consumers, premature product spoilage, product recalls and customer complaints. Ultimately, this research helps the processor to realize more profits and to maintain or even enlarge their consumer base.

The customers

The study gives the processor an opportunity to control food safety risks which in turn protects the health of consumers. Consumers benefit through acquiring knowledge on proper refrigeration storage practices thereby taking charge of safeguarding their own health.

The University

The research adds to the university's library and opens up areas of further research in the field investigated.

1.7 OPERATIONAL DEFINITIONS

Critical Sampling Location – it is a potential source of microbial cross contamination where organisms are capable of surviving and/ or replicating.

CHAPTER TWO

LITERATURE REVIEW`

2.1 MEAT PROCESSING

The general increase in urbanisation in developing countries is characterised by increased inclusion of animal-based proteins in the diet. This has raised a demand for meat output which can only be met by making better use of the meat resources already available and reducing waste of edible livestock parts to a minimal (Heinz and Hautzinger, 2007). Table 1 below summarises the different categories of processed meat.

Table 1: Categories of processed meat products

CATEGORY	DESCRIPTION
Fresh processed meat products	Meat mixes composed of comminuted muscle meat with varying quantities of animal fat. They are only salted but not cured. Heat treatment such as frying or cooking is applied immediately before eating to make them palatable. Examples are hamburgers, kebabs, chicken nuggets etc .
Cured meat pieces	Entire pieces of muscle meat that are either cured-raw (only curing, fermentation and ripening under controlled climatized conditions for palatability e.g. raw ham) or cured-cooked (after curing undergoes heat treatment to achieve desired palatabilitye.g. bacon).
Raw-cooked products	Muscle meat, fat and non-meat ingredients are comminuted and mixed then the viscous batter is portioned into casings and cooked. Heat treatment induces protein coagulation resulting in typical firm-elastic texture, desired palatability and a certain degree of bacterial stability. Examples are frankfurters, Kessler fleisch and French polony.
Precooked-cooked products	Mixes of lower grade muscle trimmings, fatty tissues, head meat, animal skin, blood, liver and other edible slaughter by-products e.g. corned beef. Precooking of raw materials prior to grinding or chopping as well as utilization of a variety of animal by-products is the main distinction of this category from other categories.
Raw (dry) fermented sausages	Uncooked meat products consisting more or less course mixtures of lean meats and fatty tissues combined with salts, nitrite, spices and other non-meat ingredients filled into casings. Their characteristic properties (flavour, firm texture and red cured colour) are achieved through fermentation. These products are not subjected to any heat treatment but are distributed and consumed raw e.g. salami.
Dried meat	Result of simple dehydration or drying of lean meat in natural conditions or artificially created environments. Many of the nutritional properties such as protein content remain unchanged e.g biltong

Adapted from Heinz and Hautzinger (2007)

Processed meat is classified into different categories as shown in Table 1 above. Meat processing is a technology that comprises different steps and procedures in the manufacture of meat products from muscle meat and other tissues which would otherwise not be sold on the fresh meat market such as skin, animal fat, internal organs, and blood as well as certain non-meat additives to enhance product flavour and appearance and in some cases increase volume (Leo, Nollet and Toldra, 2009). The technology is highly mechanised involving a wide range of physical and chemical treatment methods such as cutting, tumbling, salting/curing, fermentation, drying, heat treatment etc.

2.1.1 Preservation Techniques

The primary purpose of food preservation is to prevent food spoilage; the chief cause of food spoilage is the action of microorganisms (bacteria, molds, or yeasts) aided by enzymes. Shaltout (2007) found that the principle of all preservation methods is the creation of conditions unfavorable to the growth or survival of spoilage microorganisms. Under sub-optimal conditions as shown in Figure 2 below, the lag phase is prolonged and growth rate is significantly decreased delaying spoilage thereby extending the shelf life of products. The main inhibitory factors employed in preservation of meat are oxygen reduction (vacuum packaging), freezing, heat treatment (cooking), dehydration, chemical, fermentation and irradiation. The combined use of several of these inhibitory factors to make a product shelf-stable, improve quality and provide additional safety is known as “combined method technology” or hurdle technology (Shaltout, 2007).

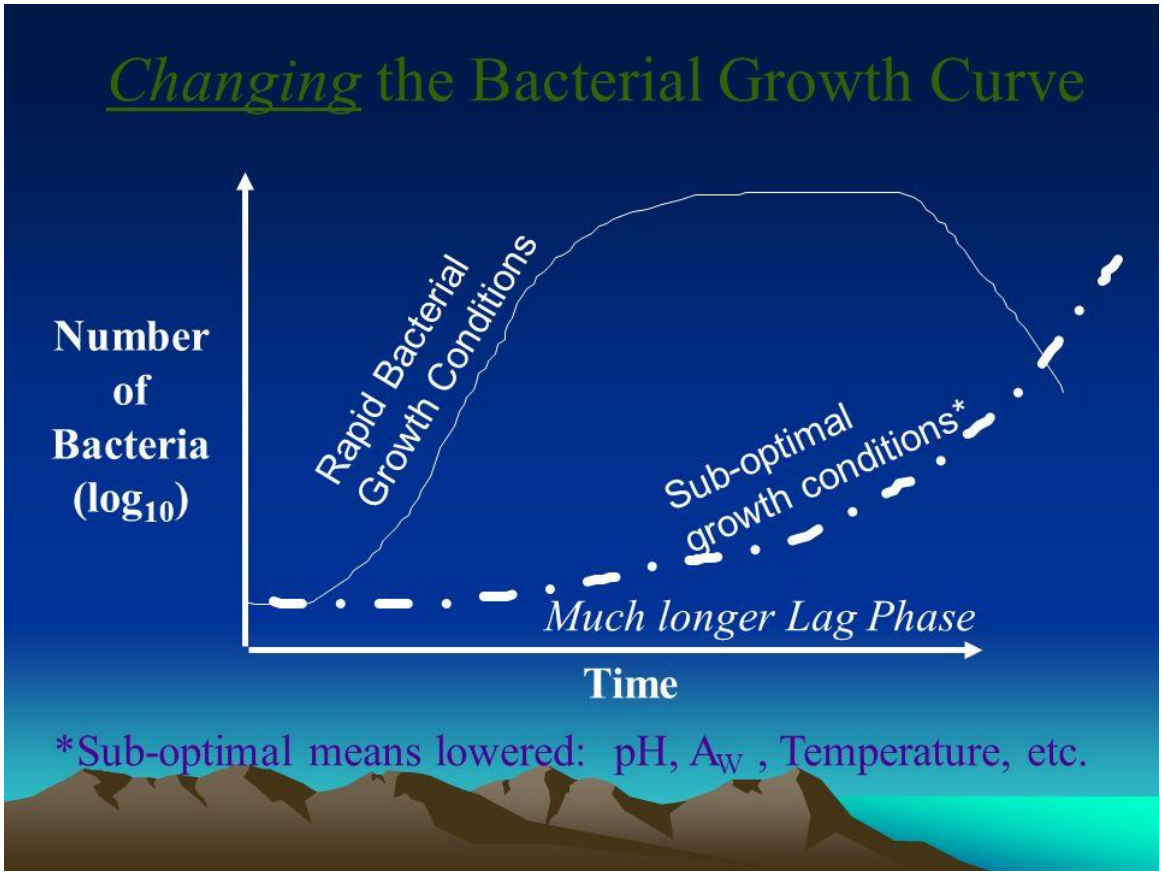

Figure 2: Effect of inhibitory factors on growth and activity of bacteria

Adapted from Shaltout (2007).

Hurdle Technology

While one hurdle (inhibitory factor) may not be sufficient to inhibit microbial growth on its own, combined hurdles may be more effective (Heinz and Hautzinger, 2007). In modern meat processing the effect of heat treatment can be supported by the application of additional "hurdles" which have the potential to slow down microbial growth. These allow keeping heat treatment of commercially sterile products at lower temperature levels such that the product quality is less affected. Alternatively this technology can be used to produce shelf stable products that are of non-sterile type through heat treatments below 100°C (Heinz and Hautzinger, 2007). Heat treatment alone at such temperatures would not be enough to stop microbial growth but additional "hurdles" complete the effect (Heinz and Hautzinger, 2007).

Thermal Destruction of Microorganisms

Heat is lethal to microorganisms but each species has its own particular heat tolerance. Goff (2008) found that microorganisms are not killed instantly when exposed to a lethal agent. Theoretically, as illustrated in Figure 3 below, if a homogenous suspension of microorganisms is heated at a constant temperature, the microorganism's destruction commonly follows logarithmic order of death i.e. they decrease by a constant fraction at constant intervals.

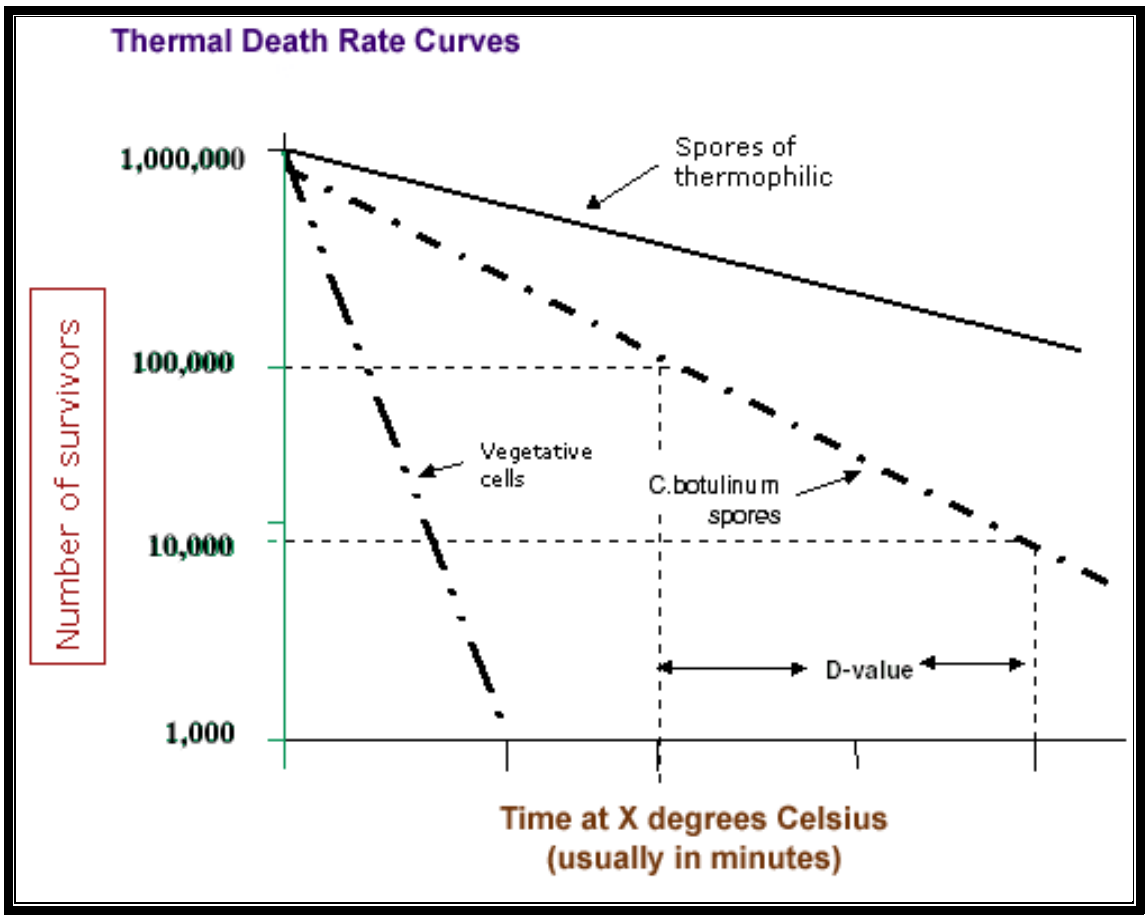

Figure 3: Thermal death rate curves of microorganisms during heat treatment

Adapted from Goff (2008)

Thermal death time (TDT) according to Goff (2008) is the shortest time necessary to kill all organisms in a suspension at a specific temperature and under defined conditions. The decimal reduction time (D value) as indicated in Figure 3 above is the time required to kill 90% (1 log reduction) of the organisms in a sample at a specific temperature while the Z value is the

increase in temperature required to reduce the D value to a tenth of its previous value (Goff, 2008). Figure 3 above, shows that the type of species in a sample determines the amount of time it will take to destroy them, vegetative cells take less time to destroy at a given temperature while spores of thermophilic bacteria take longer. The thermal stability of the enterotoxins produced by *S. aureus* greatly exceeds that of its vegetative cells. As a comparison, the D values for vegetative cells at 60°C are reported to be in the range of 0.43-8.0 minutes; however to gain a reduction in toxin activity of similar scale, a heat treatment of 121°C for 3-8 minutes would be required. For this reason, it is advisable that heat-treated foods be examined for the presence of residual toxin in addition to, or instead of, viable cells of S. *aureus* (Juffs and Deeth, 2007), because absence or low numbers of *S. aureus* in a heat treated food product does not guarantee its safety; absence of the enterotoxin must also be demonstrated.

Vacuum Packaging

It is the process of removing air around a food product in an impermeable package and then sealing it. Different factors affect the effectiveness of vacuum packaging as shown below in Table 2. The aim of this technology is to extend shelf life of perishable products by creating an unfavourable environment for microbes found in air at the surface of the product (Leo et al., 2009). After removal of air, oxygen levels continue to drop while CO_2 levels increase due to tissue respiration and activity of microbes. The low O_2 and high CO_2 environments significantly reduce growth of normal spoilage organisms such as bacteria, mold, and yeast, allowing longer shelf life.

Table 2: Factors affecting effectiveness of vacuum packaging.

FACTOR	EFFECT	COMMENT
Gas Atmosphere	Required gas atmosphere for increased shelf life is partly achieved when pack is evacuated and sealed leaving about 18% residual O_2 which is further reduced to 1% by meat tissue and bacteria respiration releasing CO_2. This combination supresses the growth of spoilage bacteria.	If required gas atmosphere is created slowly or if the sufficiently low level of O_2 in the pack is not achieved, the meat may develop brown patches of discolouration and spoilage bacteria may grow on the meat.
Packaging Film (OTR)	After gas atmosphere has been created it must be maintained and this is achieved by use of a packaging film with a low permeability to O_2. O_2 can pass through the material but if it can permeate at a slow rate continued muscle meat respiration and bacteria can consume the O_2 passing through.	If the OTR is less than 100 at 25°C and 98% relative humidity, it is suitable for vacuum packaging film for meat. If it is higher, O_2 can pass through at a faster rate than it can be consumed by meat tissue and bacterial respiration and it could accumulate in the pack.
Meat Temperature	Keeping quality of vacuum packed meat is affected by temperature of meat. Packaging meat below 10°C while fat surface is cool and firm, contributes to efficient evacuation and sealing of packs. As a guide vacuum packed meat should reach 2°C in 24hrs after packing and 0°C in 48hrs regardless of boning or packing temperature.	Failure to comply with these temperatures will result in increased bacterial growth on meat and early spoilage.
Meat pH	pH of meat affects keeping quality because at pH > 6.0 growth of several types of spoilage bacteria is favoured.	These spoilage microbes produce H_2S (g) which causes meat to turn green and develop off odour and flavour.
Initial Bacterial Contamination	If there is a large number of spoilage bacteria on the meat at the time of packing, even limited growth of the bacteria could contribute to early spoilage of the meat	N/A
Storage and Handling	Vacuum packaged meat should reach 0°C before distribution and should be stored at temps <7°C	Above this temperature it is possible that food poisoning bacteria could grow.

Adapted from Feiner (2006); Ray and Bhunia (2013); Brightwell et al., (2007).

Because some organisms are anaerobic, their growth is retarded at lower temperatures -1– 0 °C, hence vacuum packed products should still be refrigerated. Leo et al., (2009) suggested that some of the advantages of vacuum packaging are reduced product shrinkage, enhanced product quality (at chilled temperatures aging and tenderising are not hindered), more efficient use of time and more hygienic distribution. However vacuum packaging has its own

disadvantages. Retailers and wholesalers who buy packaged meat do not have the opportunity to judge the quality of the meat meaning they may not be confident about the product they are selling. Other elements such as appearance, odour and display characteristics which differ from conventionally packed meat but not necessarily making it inferior pose a great disadvantage too.

2.2 READY-TO-EAT MEAT

RTE foods are those that do not require further preparation by heat treatment prior to consumption. Ray and Bhunia (2013) reported that hygienic failing during slaughter allow contamination of meat and industrial facilities with animal faeces. In addition, failures in compliance with good manufacturing and hygiene practices allow the bacterium to be carried into the industry by shoes, clothing, transport equipment and people. These become mediums of contamination in RTE meat being further processed after the lethality heat treatment.

2.2.1 Routes of Contamination in RTE Meat Products

Further processing after heat treatment is vulnerable and exposes products to cross contamination. FDA Food Code (2007) defined cross contamination as the unintentional transfer of microorganisms, chemical contaminants (including allergens) or any foreign matter from food, person or object to another food product. It usually occurs from raw foods to RTE or between products that contain allergens and those that do not. Cross contamination can cause food spoilage or food borne illnesses when viable microorganisms and/or toxins are transferred to products. There are 3 major routes through which cross contamination can occur: food to food, equipment and food contact surfaces to food and people to food (FDA Food Code, 2007).

Food to food

Raw meat like all raw agricultural products can be contaminated with microorganisms some of which may be pathogenic. Faecal coliform e.g. *E coli* exist as natural flora in the intestines and faeces of people and animals; its presence in a sample indicates recent faecal contamination

pointing to an even greater threat of presence of pathogenic microbes (FDA Food Code, 2007). Whole muscle parts may be contaminated with *E coli* during slaughter; if they come into contact with RTE processed meat such as luncheon meats cross contamination might occur. Raw meat can contaminate cooked meat products during preparation, processing, cooking, cooling or storage. Careful handling of cooked meat products and avoiding contact of cooked and raw meat can prevent this (Ray and Bhunia, 2008).

People to food

According to Rodrigues, Cordeiro and Barros (2016) food handlers that do not follow GMP and GHP can transfer microorganisms and allergens to food through soiled uniforms, gloves or dirty boots, handling food without washing hands properly after using ablution services, wiping hands on protective clothing then handling food etc. A major concern is the contamination of RTE products with pathogenic bacteria (Heinz and Hautzinger, 2007). The chief source of *S. aureus* contamination is the hands of food handlers, however it is also found in the nose and skin of warm-blooded animals including humans. *S. aureus* is versatile hence it can grow (in different types of food, producing SE which causes SFP), over a wide range of temperature, salt concentration, a_w and pH. Though the organism is destroyed by heat treatment, SE is more heat resistant and may not be destroyed during cooking processes (Heinz and Hautzinger, 2007).

Equipment and food contact surfaces to food

Residues on equipment and other contact surfaces may provide cross contamination opportunities. RTE products may become contaminated with bacteria and or allergens due to improper washing and sanitation of equipment and utensils, use of dirty cloths to clean surfaces and equipment as well as use of contaminated packaging material. Lin et al., (2006) conducted a research that showed that *Listeria* can survive on processing equipment such as meat slicers and can be transferred from a contaminated slicer onto meats where it will grow and survive.

Mustapha (2007) supported their findings through a similar research which indicated that *E.coli* and *Listeria* remained viable on air-dried stainless steel surfaces for considerable periods of time. To prevent this type of contamination, use separate equipment for different product categories, clean and sanitize all surfaces and equipment after each task/batch using proper sanitization concentrations and prepare allergen free products separate from allergen containing foods. All primary packaging materials should be of sterile type (Lin et al., 2006).

Biofilms

Microorganisms attach and grow universally on a variety of surfaces and may form biofilms. Rodrigues, Cordeiro and Barros (2016) defined a biofilm as surface-associated microbial cells that are irreversibly assembled (not removed by gentle rinsing) and enclosed in a polysaccharide extracellular polymeric substance (EPS). Because the EPS is highly hydrated, it protects cells in some natural biofilms against drying out and conduces to their antimicrobial resistance by hindering antibiotic mass transport through the biofilm by directly binding to the antibiotics (Rodrigues, Cordeiro and Barros, 2016). Cells can migrate to other sites and resume the biofilm formation process formation. Biofilms can persist for a long time, even years and they form within a few hours or days. Pathogens such as *S aureus, Vibrio Cholrea, L. monocytogenes, Campylobacter spp., E. coli O157:H7, H. pylori* etc. are capable of attaching to surfaces and already formed biofilms; their ability to attach to surfaces and existing biofilms as well as their associations and metabolic interactions with indigenous microbes consolidates their survival in biofilms. Biofilms are a concern in food processing because they are a potential source of microbial spoilage and pathogenic contaminants and also have greater resistance to disinfectants and sanitizers. Once ingested they are resistant to host organism immune system clearance and may result in infections in the blood stream or urinary tract (Rodrigues, Cordeiro and Barros, 2016).

2.3 THE MICROBIOLOGY OF MEAT SPOILAGE

Meat can be spoiled extremely quickly by certain species of bacteria owing to its chemical composition, favourable water activity (a_w) value and pH value. Their numbers soon reach levels that cause sensory deviations and ultimately lead to spoilage of the meat (Doulgeraki, Ercolini, Villani and Nychas, 2012). The selection, growth and metabolic activity of food spoilage microbes is influenced by a large number of factors, which can be divided into 4 groups as; intrinsic factors (physical and chemical properties of the matrix itself), extrinsic factors (storage conditions that affect both the food and their microbes), processing factors (physical or chemical methods of treatment during processing) and implicit factors (synergistic or antagonistic effects between bacteria) (Doulgeraki et al., 2012).

2.3.1 Factors Affecting Microbial Spoilage of Meat

Intrinsic Factors

1. **Water Activity (a_w)**

Growth of microbes in a food matrix relies on the availability of water in a free form. a_w ranges from 1.00 for pure water to 0.00 in a completely dehydrated food. a_w is the extent to which water is "bound" in a food as well as its availability for chemical/biochemical reactions and growth of microorganisms. Optimum growth level for most microbes is 0.97 - 0.99, such is found in most fresh foods such as fruit, vegetables and fresh meat. The a_w in foods can be altered by solute (salt or sugar) addition which binds free water causing the food to dehydrate or draws water from microbial cells via osmosis (Belitz, Grosch and Schieberle, 2009). Higher sensitivity to low a_w is exhibited in Gram (-) bacteria more than in Gram (+) bacteria. Many pathogens can be curbed at a_w levels around 0.86, however below 0.90, *S. aureus* can grow and produce toxins (Feiner 2006). Table 3 shows general a_w needs for different classes of microorganisms**.**

Table 3: General a_w needs for different classes of microorganisms

Group of Organisms	Minimal a_w Value
Most spoilage bacteria	0.91
Most spoilage yeasts	0.88
Most spoilage molds	0.8

Adapted from Feiner (2006)

2. **pH (Hydrogen Concentration)**

Every microbe has a defined pH range within which it can be active. Increasing food acidity by fermentation or addition of weak acids has been used for preservation for centuries. Fruits and vegetables are relatively more acidic than fresh meat and fish. According to Hui (2006) interaction of pH with other inhibitory factors has a bactericidal effect. The pH of a food modifies the lethality of heat treatment on the food; as pH drops less heat is needed to destroy bacterial cells. Yeast and molds have a higher tolerance for acid thus they degrade and spoil foods with a pH level <4.5 more readily than do bacteria. Cerveny, Meyer and Hall (2009) reported that many pathogens are capable of survival at pH levels below their optima. Molds can proliferate over a wider pH range compared to most yeast and bacteria. Spoilage is more rapid in meat because of high pH and presence of adipose tissue; the combination allows for greater nutrient consumption by microbes facilitating faster growth rates (Ray and Bhunia, 2013).

3. **Reduction-oxidation potential (Eh)**

The redox potential is a result of the gaseous atmosphere, pH and reductants. It measures in millivolts (mV), a system's potential difference generated by a coupled reaction wherein one substance is oxidized while the other is simultaneously reduced (Cenci-Goga, Rossitto, Sechi, Parmegiani, Cambioti and Cullor, 2012). Aerobic microbes require positive Eh values

(oxidised, +500 - +300 mV) for growth while anaerobes require negative Eh values (reduced, e.g. in meat due to presence of the SH groups, +100 - -250 mV and below) while facultative anaerobes require +300 - -100 mV. Raw meat has been reported to have an Eh of -200 mV, +225mV for ground raw meat and +90 - -50mV for cooked meat (Doulgeraki et al., 2012). Presence of salt and other food constituents, pH, packaging material and the storage environment's O_2 partial pressure impact Eh and consequently microbial growth (Ray and Bhunia, 2013).

4. Nutrient content

Meat is a natural ecosystem rich in proteins, lipids, minerals and vitamins, but poor in carbohydrates. Microorganisms require certain basic nutrients (vitamins and minerals), water and a source of energy (carbohydrates, alcohols, fats and amino acids) for their metabolism. Cell constituents and other essential substances that they need for effective survival but cannot synthesise are obtained from the surrounding environment during the lag phase. (Cenci-Goga, 2012). Because of their fastidious nutrient requirements, Gram (+) bacteria such as *S. aureus* are generally less competitive (Barbosa, Fernandes, Ushimaru, Probst and Fernandes, 2009), thus Gram (-) bacteria which are generally able to derive all their nutrient requirements from a wide range of food sources tend to dominate in food matrices with mixed bacterial populations (Ray and Bhunia, 2013).

5. Naturally occurring and added antimicrobials

Certain foods have naturally occurring antimicrobial substances that confer microbiological stability to them. Plant-based antimicrobial substances include essential oils, tannins, glycosides, resins etc. (Fratianni, Martino, Melone, Feo, Coppola and Nazzaro, 2010) while lactoferrin, conglutinin, lactoperoxidase, lysozyme etc are animal-based (Fratianni et al., 2010). Some types of food processing result in the formation of antimicrobial compounds such as phenol production on the surface of fish and meat during smoking which lowers the surface

pH or maillard compounds which are a result of condensation reactions between sugars and amino acids or peptides upon heating of certain foods (Fratianni et al., 2010). Bacteriocins, antibiotics, and other related inhibitors are antimicrobial substances that are a result of natural fermentation (Barbosa et al., 2009). Based on their interactive and synergistic effects, various additives and chemical preservatives have been used singly or in combination to stabilise food products by inhibiting pathogens and/or extend shelf life (Cenci-Goga et al., 2012).

6. Initial microbial load and competitive micro-flora

Leornard (2011) reported that initial microbial load and substrate availability determine the time for microbial proliferation in meat and meat products and consequently shelf life. High initial total viable counts are an indication of poor hygiene and considerably shorten shelf life even in ideal storage conditions. Meat spoils at TVCs of about 10^6 CFU/cm^2 with off odour production while slime formation and discoloration appear at 10^8 CFU/cm^2 (Barbosa et al, 2009). The species that dominates in a mixed bacterial population is determined by individual growth rates and mutual interactions of species.

Association and Succession of Microorganisms in Food Matrices

When metabolically active organisms are present in a food matrix they continue to interact and thus flora dominance over time is dynamic (Fratianni et al., 2010). The interactions are either synergestic or antagonistic (competing for nutrients and/or adhesion sites or unfavourable environmental alterations). While metabolic products' accumulation may limit the growth of certain species, if other species can utilise the limiting metabolic product these may take over partly or wholly thereby creating an association or succession. This phenomenon can be observed in raw ground beef where *S. aureus* is often found in low numbers but SE is not produced because the *Pseudomonas-Acinetobacter-Moraxella* association that is always present in this food grows at a higher rate, outgrowing the staphylococci (Cenci-Goga et al., 2012). Staphylococci are poor competitors sensitive to nutrient exhaustion in both fresh and

frozen foods (Nycha, 2008). In some associations, optimum growth and normal metabolic activity will not developed unless both organisms are present and such information has been used to control microbes in temperature-sensitive foods through the hurdle technology.

Extrinsic Factors

1. **Storage Temperature**

According to Doulgeraki et al., (2012), all microbes operate within a defined temperature range. Temperature impacts an organism's generation time, lag period, maximum growth rate and the final cell count. Low temperatures slow reaction rates for individual enzymes in the organism (Nychas, Skandamis, Tassou and Koutsoumanis, 2008), and transport mechanisms are interrupted by reduced fluidity of the cytoplasmic. Structural cell components of heat sensitive enzymes are denatured and inactivated at high temperatures. Four major groups of microorganisms are listed in Table 4 based on their temperature ranges for growth. Psychrotrophic organisms are of major interest during chilled storage and these constitute spoilage bacteria, yeast molds as well as certain foodborne pathogens. The expression of virulence genes and an organism's thermal sensitivity in certain foodborne pathogens is regulated by growth temperature (Doulgeraki et al., (2012).

Table 4: Temperature ranges for prokaryotic microorganisms

GROUP	MINIMUM (°C)	OPTIMUM (°C)	MAXIMUM (°C)
Thermophiles	40 – 45	55 -75	60 – 90
Mesophiles	5 – 15	30 – 45	35 – 47
Psychrophiles	-10	12 – 15	15 – 20
Psychrotrophs	-10	25 – 30	30 – 35

Adapted from Doulgeraki et al., (2012)

2. **Relative humidity of storage or holding conditions**

The effect of storage environment's relative humidity on food safety is vague (Audenaert et al., 2010). The a_w of a food may or may not be altered by relative humidity of the environment since its product dependent. In generally foods whose microbiological quality and shelf life stability depend on a_w should be stored in environments that do not significantly change such characteristics because foods will eventually attain moisture equilibrium with their surroundings. In essence, storage should be such that environmental moisture does not have an opportunity to unfavourably alter the a_w of the product (Audenaert et al.,2010).

3. **Packaging and gaseous atmosphere**

Composition of spoilage bacteria is influenced by packaging conditions and surrounding gaseous atmosphere's composition. Audenaert et al., (2010) reported that growth of Pseudomonads, a major spoilage organism, is favoured in aerobic storage conditions at -1 - 25°C. The lag phase of aerobic microbes is extended by CO_2 and N_2 gas usage in MAP, however growth of facultative and strict anaerobes such as LAB, which constitute the majority of spoilage microorganisms, is promoted under these conditions (Nychas et al., 2008).

2.4 SPOILAGE DETECTION

Meat is considered spoiled when it is rendered unfit for human consumption. Spoilage can be caused by a wide array of factors such as improper handling, exposure to air and high temperatures or conditions that trigger chemical reactions or microbial contamination. However, the most common cause is the presence of microbes together with their metabolite production (Nychas et al., 2008). The detectable effects and major reasons for inedible spoiled meat are off odour and flavour but consumer rejection is also due to discolouration, blown packages, souring, visible growth (slime, colonies) and other alterations of meat quality such as textural changes due to degradation of polymers (Nychas et al., 2008).

2.4.1 Detectable Sensory Signs of Spoilage

1. Off odors and off flavors

Ketones, aldehydes, fatty acids, ethyl ester, organic acids, ammonia, alcohol, sulphur compounds etc., constitute the volatilome portion of microbial metabolites. Casaburi, Piombino, Nychas, Villani and Ercolini (2015) suggested that these molecules affect the sensory quality of meat depending on the interaction of volatile and non-volatile compounds as well as their olfactory thresholds (first concentration at which all panel members can detect the odour). Putrid, sulphuric, cheesy, fruity and sweet odours are common in meat stored under aerobic conditions (Baborsa, 2009) however perception of these is notable at TBC counts from 10^7 - $10^{7.5}$ CFU/g. Metabolic activities of *Pseudomonas* spp. *and B. thermosphacta* are the major contributors of foul odours (Nychas et al., 2008). At 10^8 CFU/g contamination levels carbohydrates are depleted; Pseudomonads along with psychrotrophic Gram (-) microbes like *Moraxella* spp. start utilising amino acids for energy. Free amino acids and nitrogen compounds like ammonia are associated with nauseating odours (Koutsoumanis, Stamatiou, Drosinos and Nychas 2008) while sulphuric odours are due to hydrogen sulphide formed from sulphur-containing compounds by enterobacteriaceae (Casaburi et al., 2015). Anaerobic metabolism produces less intense odours than aerobic metabolism, so the use of low O_2 concentration in MAP is better for maintaining acceptable qualities (Casaburi et al., 2015)

2. Colour alteration

When microbial counts reach $10^{7.5}$-10^8CFU/g, bacterial patina (green coloured layer due to oxidation) is observed on the surface of meat products (Barbosa, 2009). *L. sakei, H.alvei,* and *S. putrefaciens* produce hydrogen sulphide which changes muscle colour to sulphomyoglobin which is green, however the green colour is not produced under anaerobic conditions. Hydrogen peroxide which is produced by *Leuconostoc* spp. and *Weissella viridescens*, can also cause meat products to turn green when exposed to O_2 (Barbosa, 2009).

3. Gas production

A large amount of H_2 and CO_2 gas are produced by *Clostridium* spp. especially in canned foods (Yang et al., 2011). Blown pack spoilage characterised by deformed pack due to large amounts of gas accumulating has been observed in chilled vacuum packed meat due to the activity of psychrophiles and psychrotrophs. According to (Yang et al., 2011), yeast and LAB also contribute to the production of volatile organic compounds constituted in the package headspace of meat that has spoiled.

4. Filaments and ropy slime

The stretchy ropy slime (long-chain, high-molecular mass gelling or viscosifying exocellular polysaccharides) is a secretion of *Lactobacillus* spp. and *Leuconostoc* spp. and it is common in cooked vacuum packed meat (Barbosa, 2009). Slime production is advantageous for some bacteria as it forms a protective layer around them.

Table 5: Common alterations associated with microbial spoilage of meat

Alteration	Product	Aetiology
H_2S production	Cured meat	Enterobacteriaceae
Sulphide odour	Vacuum packaged meat	*Clodtridium* spp, *Hafnia* spp.
H_2O_2 greening	Meats	*Weisella* spp, *Leuconostoc* spp, *enterococcus* spp, *Lactobacillus* spp.
Slime production	Meats	*Pseudomonas* spp., *Lactobacillus* spp., *Enterococcus* spp, *Brochothrix* spp.
Blown Pack	Vacuum packaged meat	*Clostridium* spp., LAB
Putrefaction	Ham	Enterpbacteriaceae, *Proteus* spp.
Souring	Ham	LAB, *Enterococcus* spp.

Adapted from Nychas et al., (2008) and Yang et al., (2011).

2.5 MICROBIOLOGICAL GUIDELINES FOR RTE MEAT

Food safety control aims to safeguard public health and provide assurance on food safety and quality. It is an offence to sell food that is unfit for human consumption. To this end, microbiological analyses are useful ways to assess the safety and quality of food involved. Microbiological guidelines are criteria indicating the microbiological condition of the food items which reflects the safety and hygienic quality of the food. Table 6 below lists microbiological criteria in RTE meat used as guidelines in different countries.

Table 6: Microbiological guidelines for RTE meat in different countries.

ORGANISM	Limit CFU per ml or gram				
	SANS 885:2011 South Africa	**Woolworth Foods Group South Africa 2006**	**Hong Kong Centre for Food Safety 2014**	**FDA Philippines 2013**	**Food Administration Manual 1995**
Total Bacterial Count	$<10^6$	$<10^5$	$<10^6$	**N/A**	**N/A**
Enterobacteriaceae	**N/A**	$<10^3$	$<10^2$	**N/A**	**N/A**
Coliforms	**N/A**	$<10^2$		**N/A**	**N/A**
E.coli	<10	<20	<20	<20	<20
S. aureus	<20	<20	<20	$<10^2$	$<10^2$
Yeast and Moulds	**N/A**	$<10^3$	**N/A**	**N/A**	**N/A**
Lactic Acid Bacteria	**N/A**	$<10^5$	**N/A**	**N/A**	**N/A**
Escheria coli O157	not detected	not detected	not detected	**N/A**	**N/A**
Clostridium perfringens	$<10^4$	<20	<10	**N/A**	$<10^2$
Bacillu cereus		$<10^3$	$<10^3$		$<10^3$
Salmonella spp	not detected in 25g	not detected in 25g	not detected in 25g	not detected in 25g	not detected in 25g
Listeria monocytogenes	<100	<20	not detected in 25g	**N/A**	0

The role of microbiological guidelines is to provide assistance in the interpretation of microbiological analyses of foods and give recommendations on the appropriate follow-up

action to monitor and control food safety. They also serve to facilitate producers in devising measures to improve their food safety practices. Food samples failing any of the microbiological criteria stipulated will be considered as "Unsatisfactory" meaning the food is potentially injurious to health and/or unfit for human consumption. In other words, the affected products should be prevented from being released for human consumption. In such cases, appropriate actions should be taken i.e. immediate investigation and parties concerned (e.g. vendors) should be instructed to stop sale of food item in question. Investigate immediately and find out the causes and adopt measures to improve the situation. Take investigative samples and in addition, warning letters, source tracing and other enforcement actions should be considered.

2.6 FOOD SAFETY DURING MEAT PROCESSING

Food safety is a scientific discipline describing handling, preparation and storage of food in a way that prevents foodborne illnesses (Narmanno et al., 2006), hence it must be fully implemented at all stages during food production to provide safe and wholesome products. Food safety hazards can be classified as biological, chemical or physical.

Biological Hazards

These hazards can come from raw materials or from food-processing steps used to make the final product. The main cause is food that is contaminated with microorganisms such as molds, bacteria and viruses. According to Hoffmann and Anekwe (2013), the input levels of microbes can be minimized by good lay out, hygienic operations and rapid chilling. On the contrary, uncontrolled storage conditions and poor hygiene result in contamination leading to proliferation of contaminants. In order to produce toxins in meat products or raw materials and to cause harm pathogens need to grow sufficiently to 10^6 CFU/g. Under warm semi-dry conditions, *S. aureus* may become established on equipment and produce toxin hence sliced cold meat is at risk of contamination posing risk of foodborne illnesses (Narmanno et al., 2006).

Table 7 highlights different optimum conditions for staphylococcal enterotoxin production. Some infectious pathogens e.g. *E. coli* are hazardous at such low levels that their mere survival is sufficient to be hazardous (Hoffmann and Anekwe, 2013).

Table 7: Factors affecting *S. aureus* growth and SE production

Factor	Optimal Growth	Growth Limits	Optimal SE Production	SE Production Limit	Effect(s) on SEnproduction	Food Source
Temperature °C	35─41	6–48	34–40	10–46	Temperature affects SE production more than growth	Milk, ham, egg products
pH	6─7	4─10	7─8	5─9.6	More tolerant to aerobic than anerobic growth conditions. Lactic acid inhibits toxin formation	Ham, Sausage
O_2	Aerobic	Anaerobic-aerobic	Aerobic	Anaerobic-aerobic	increases yield of SE upto 10-fold, 10% dissolved oxygen is optimal for SE production	Ham, prawn, Sausage
a_w	0.99	0.83≥0.99	0.99	0.86≥0.99	**N/A**	Curred beef slurry, bacon, shrimp slurry, sausage
Salt %	0%	0─2	0%	<12	raises temperature limit for SE production and low osmotality increases SE production	Ham, sausage
Eh	>+200mV	≥ 200 ─ >+200mV	>+200mV	≥100mV ─ > +200mV	**N/A**	**N/A**

Adapted from Schelin et al., (2011)

Chemical Hazards

Chemical contamination can happen at any stage in food production and processing and may lead to acute poisoning or long term diseases such as cancers. Narmanno et al., (2006) found that the presence of a chemical may not always indicate a hazard but the amount determines whether or not it is a hazard. Some require prolonged exposure to be toxic. Allergens e.g. those found in seafood or nuts are naturally occurring chemicals which produce an allergic reaction in sensitive people. Additives are safe when used within permitted limits but when these are exceeded, they become toxic e.g. Vitamin A can be toxic in high concentrations (Narmanno et al., 2006). Primary packaging materials can be sources of incidental contaminants such as ink.

Lubricants and cleaning agents should be approved because their residues on food contact surfaces and equipment pose a health risk (Hoffmann and Anekwe, 2013).

Physical Hazards

Physical hazards include any potentially harmful extraneous matter not normally found in food. When a consumer mistakenly eats the foreign material or object, it is likely to cause choking, injury or other adverse health effects. Physical hazards are the most commonly reported consumer complaints because the injury occurs immediately or soon after eating, and the source of the hazard is often easy to identify. Glass and metal can be physically hazardous when present in foods because they can cause cuts, broken teeth and bleeding and these may require surgery to find or remove (Hoffmann and Anekwe, 2013).

2.6.1 Food Safety Management Systems

As the world's population grows, the intensification and industrialization of agriculture and animal production to meet increasing demand for food creates both opportunities and challenges for food safety putting greater responsibility on food producers and handlers to ensure food safety. FSMS means the adoption of GMP, GHP, HACCP, ISO 22000 and several other such procedures (Verran et al., 2008). If well designed with appropriate control measures, FSMS can help food establishments comply with guidelines and ensure that food prepared for sale is hygienic and safe for human consumption. FSMS provide a preventative approach to identify, prevent and reduce food-borne hazards (Verran et al., 2008).

Serious foodborne disease outbreaks have occurred on every continent in the past decade, often amplified by globalized trade (Hoffmann and Anekwe, 2013). Examples include the contamination of infant formula with melamine in 2008, affecting 300 000 infants and young children, six of whom died, in China alone, and the 2011 enterohaemorrhagic *Escherichia coli* outbreak in Germany linked to contaminated fenugreek sprouts which caused US$ 1.3 billion in losses for farmers and industries and US$236 million in emergency aid payments to 22

European Union Member States. Other incidences include *E. coli* outbreak in the United States, bovine spongiform encephalopathy (BSE) on British beef and dioxin contamination of animal feed in Belgium (Hoffmann and Anekwe, 2013).These scenarios generated the need to cope with foodborne hazards from farm-to-fork by strengthening integrated management along the food supply chain.

Prerequisite Programs

Prerequisite programs are procedures, including GMP and GHP that address operational conditions providing the foundation for HACCP. Certain programs and activities are required and must be in place if a HACCP program is to be effective. Examples of the most common PRPs are waste disposal, cleaning and sanitation, personal hygiene, pest control and traceability and recall. HACCP is only one component of an aquaculture food safety program. Without PRPs a HACCP plan cannot be effective (Hoffmann, 2010).

Hazard Analysis Critical Control Point

HACCP is a certificate that ensures safety of consumer food products. According to Narmanno et al., (2006), HACCP certifies food safety via a systematic preventive process, which includes guidelines to protect food from physical, biological and chemical risks. It also controls the supply chain to manage ample supply of products throughout the year. HACCP is generally applicable on every stage of the food chain, from farm to fork, such as food production, processing, packaging, sorting or distribution. The basic objective of HACCP is to create a model for manufacturers and producers that they can follow to ensure product safety and reduce the loss incurred on spoilage, defective products, recalls and returns. Following HACCP not only improves food safety, but it also systematizes the entire food chain in our economy, which spreads awareness about food safety, promotes internal safety reviews and increase food and material traceability (Verran et al., 2008).

ISO 22000:2005

ISO 22000:2005 is an internationally recognised standard that incorporates HACCP principles and specifies requirements for a food safety management system where an organization in the food chain needs to demonstrate its ability to control food safety hazards in order to ensure that food is safe at the time of human consumption (Narmanno et al., 2006). The standard covers the key components for ensuring food safety including interactive communication, system management, implementation of pre-requisite programmes and the continual review and improvement of the system. Management involvement and commitment through time and other resources is key for ISO 22000:2005 to be effective in ensuring food safety (Narmanno et al., 2006).

2.7 FOODBORNE ILLNESSES

According to Schelin et al., (2011), foodborne illness is any illness involving a combination of intestinal symptoms such as nausea, vomiting and diarrhoea caused by eating food that is contaminated by microorganisms or chemicals. Other symptoms include headaches, muscle aches, chills and fever. Food infection is a type of foodborne illness caused by eating food that contains certain types of live bacteria. Once the food is consumed, the bacterial cells continue to grow and their growth and activity inside the host lead to illness. Salmonellosis is a good example of foodborne infection (Narmanno et al., 2006). Food intoxication is when an individual consumes food containing preformed bacterial toxins; *S. aureus* and *Clostridium botulinum* are examples of species of bacteria that cause food intoxication. The toxicity of biotoxins depends of a variety of factors such as exposure, dose (actual amount of the toxin that enters the body) and the relationship between exposure and health effects (dose response) (Schelin et al., 2011). Symptoms can start in a few minutes or take as long as six weeks after eating the contaminated food (Schelin et al., 2011). Onset times depend on the type and amount of bacteria in the food. The severity and length of illness can also vary. Greatest risk from

consumption of harmful bacteria is posed to the elderly and people with weakened immune systems as well as pregnant women and young children (Schelin et al., 2011).

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 RESEARCH DESIGN

This research was carried out using the exploratory research design. 10 sampling locations were assessed to determine their microbiological quality. The method was employed based on its capacity to clarify and define the nature of the problem i.e. determining whether one variable is a more consistent indicator of microbiological quality than the other. The method was useful in determining the best approach to achieve the researcher's objectives. Scientific methods were used to enumerate microbial load during French polony slicing and packing at the selected CSLs. Enumeration was carried out for TBC, coliforms, *S. aureus, E. coli,* yeast and molds. The method was quantitative with the aim of determining sources of microbial contamination and the subsequent growth and activity of microbes in the finished product over its intended shelf life period.

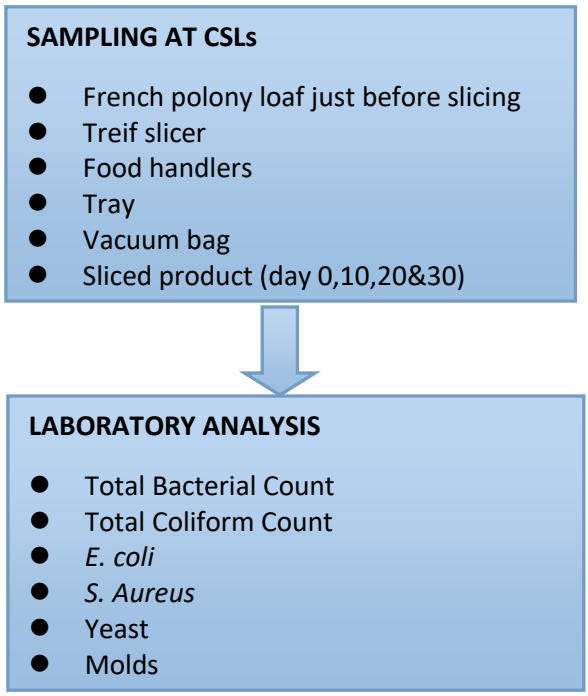

Figure 4 Research Design

3.2 RESEARCH POPULATION

The research population was sub-divided into 3 groups:

- A- Slicing and packing environment i.e. air, Treif slicer, trays and vacuum bag.
- B- Food handlers
- C- 4kg French polony loaf and 200g sliced vacuum packed French polony

3.3 SAMPLING TECHNIQUES

- **Random sampling-** On a produced batch of 4kg French polony and on the sliced vacuum packed French polony. 4 loaves were randomly selected from a batch and 4 sliced packs were randomly picked from the sliced loaf.
- **Systematic sampling-** The technique was employed on the 4kg French polony loaves and the 200g packed sliced vacuum packed French polony. A 4kg loaf was sampled every 10 days for slicing and a finished sliced vacuum packed product was selected every 10 days to check for growth and activities of microbes.
- **Non-probability sampling-** On equipment, surfaces and personnel.

3.4 SAMPLE SIZE

- Total of 12 4kg French polony loaves were selected from 3 batches, (4 from each batch)
- Total of 3 meat handlers were selected for all batches, (same handlers on all batches and loaves)
- Equipment and contact surfaces used during slicing and packing of French polony.

3.5 SELECTED SAMPLING METHOD

Sampling, sample preparation and microbiological analyses followed ISO guidelines. ISO 6887-1:1999(E) and ISO 6887-2:2003 (ISO, 2003b) were used for destructive samples (final product) while ISO 18593:2004 (ISO, 2004a) was employed for environmental samples (surfaces).

3.5.1 DETECTION METHODS

Table 8: Methods for isolation and enumeration of microorganisms

Organism	Media	Incubation Temperature (°C)	Incubation Time (Hrs)	Guideline
E. coli	Eosin Methylene Blue	37	18-24	ISO 16654:2001(E)
S. aureus	Mannitol Salt Agar	37	18-24	ISO 6888-3:2003(E)
TBC	Plate Count Agar	37	18-24	ISO 4833-1:2013(E)
Total Coliform Count	Violet Red Bile Agar	37	18-24	ISO 4832:2006(E)
Yeasts	Malt Extract Agar	37	18-24	SAZ ISO 7954:2005
Molds	Malt Extract Agar	37	18-24	SAZ ISO 7954:2005

3.6 QUANTITATIVE RISK ANALYSIS FOR *S.AUREUS*

A QRAM process was constructed in a Microsoft Excel spread sheet for sliced vacuum packed French polony from despatch to consumption (Appendix 16) and was simulated in @Risk a spreadsheet add-in program. The despatch-to-table pathway was modelled (Node 1-6) as a series of unit operations and *S. aureus* events that started with initial contamination at despatch. Two scenarios were crafted; the actual sequence of events and a hypothetical situation that assumed optimum storage temperatures at the retailer and consumer. *S. aureus* growth was calculated using predictive microbiology. Using minimum time, minimum temperature and minimum pH the best and worst case growth was calculated using predictive microbiology hence the growth followed a uniform distribution.

3.7 RESEARCH INSTRUMENTS AND MATERIALS

Table 9: Equipment and materials used for microbial analysis

EQUIPMENT	MATERIALS
Lasany colony counter LI-37	Petri dishes
Nicolas scale 4.1kg WT41002CFE	Wash bottles
Autoclave 80L	Cotton wool
SMC 36L Econo Water Bath WBA 36	70% ethanol
Incubator (walk-in) 37°C	1000µl pipette filler
Labcon 25°C incubator	Mac-Cutney bottles
Distiller	Beakers
Gas torch PT2000	Tongs
Micro-pipette gun	Spatula
Bunsen burner	Media
Tripod stand and wire gauze	
Gas lighter	

3.8 DATA PRESENTATION AND ANALYSIS

3.8.1 DATA PRESENTATION

All data collected was presented in the form of tables, line and bar graphs and word narratives.

3.8.2 DATA ANALYSIS

GraphPad Prism 4 software was used for statistical analysis to test and draw conclusions on hypothesis. ONE WAY ANOVA was used to compare microbial loads across CSLs while t-test was used to compare observed microbial loads in the finished product against set guidelines at ($P < 0.05$) significance level. ComBase software was used to predict growth of *S. aureus* and @Risk software was used to calculate exposure of *S. aureus* and probability of illness per serving.

3.9 VALIDITY AND RELIABILITY

In quantitative research, the extent to which results are consistent over time and are an accurate representation of the total population under study is referred to as reliability. If the results of a study can be reproduced under a similar methodology, then the research instrument is considered to be reliable. Validity determines whether the research truly measures that which it was intended to measure or how truthful/genuine the research results are. In this research, data was collected and manipulated from samples that were a true representative of the intended population and results were obtained with minimal or no bias. Approved standard methods, i.e. ISO techniques, were used for detection and enumeration. All experiments were conducted in the laboratory at the plant investigated.

To ensure validity and reliability internally:

- Instrument calibration was routinely done by reputable companies (SAZ and SIRDC)
- Duplication of analysis, carried out on 3 batches
- Sampling twice on the same CSL instead of once for comparison purposes
- All analysis were carried out under the direct supervision of the company's qualified and experienced microbiologist following standard laboratory protocols to minimize errors and to ensure proper implementation of all protocols and standard procedures.
- Sterilization of all lab equipment and surfaces including hands, before and during analyses
- Aseptic techniques were employed throughout the experiments
- A control was used for each microbiological analysis.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 MICROBIAL ANALYSIS

Key	
CLS 1: Food handler 1	CSL 6: Tray
CSL 2: Food handler 2	CSL 7: Vacuum bag
CSL 3: Food handler 3	CSL 8: Sliced vacuum packed French polony
CSL 4: Treif slicer	CSL 9: Slicing area air
CSL 5: French polony loaf before slicing	CSL10: Packing area air

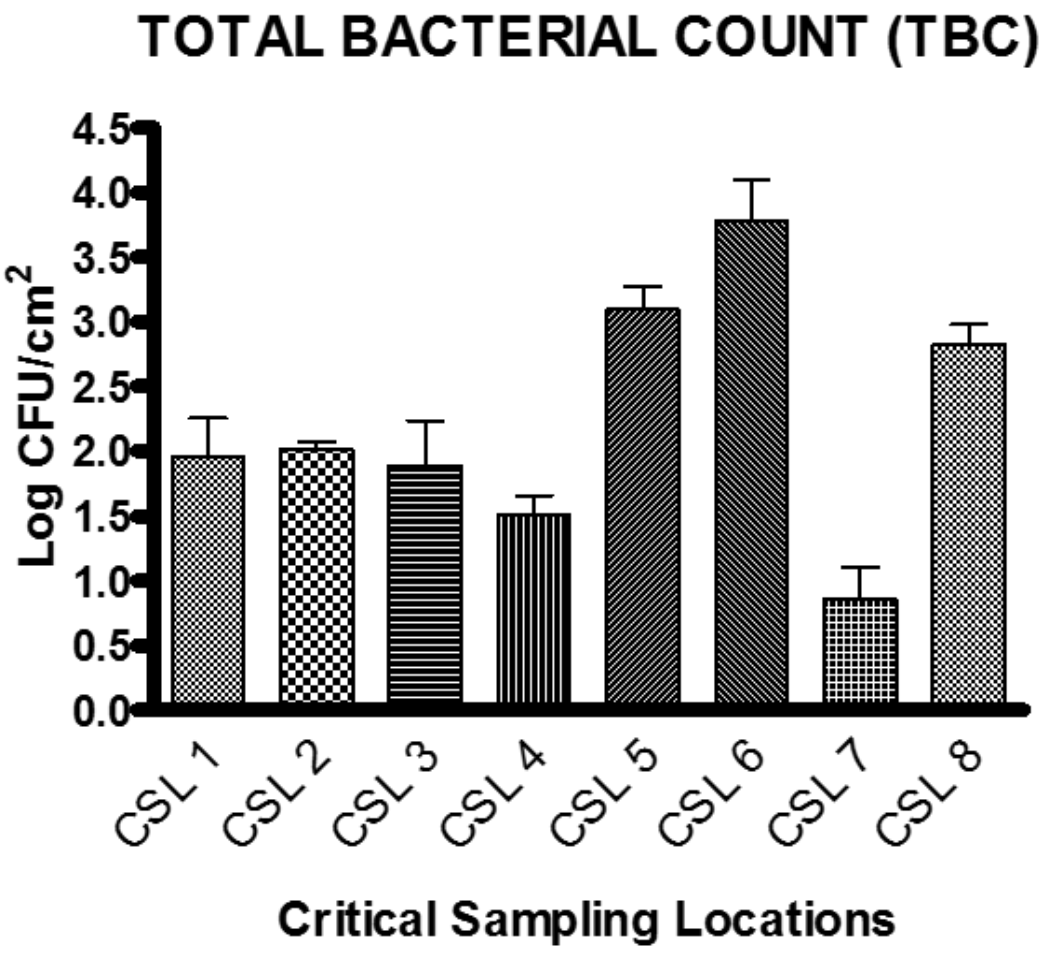

Figure 5: TBC across the cold meats slicing line

The observed TBC across the cold meats slicing line varied on different sampling locations. The highest count was observed on CSL 6 which had 3.77log CFU/cm^2 followed by CSL 5 which had 3.09log CFU/cm^2. The lowest count was recorded on CSL 7 which had 0.860log CFU/cm^2.

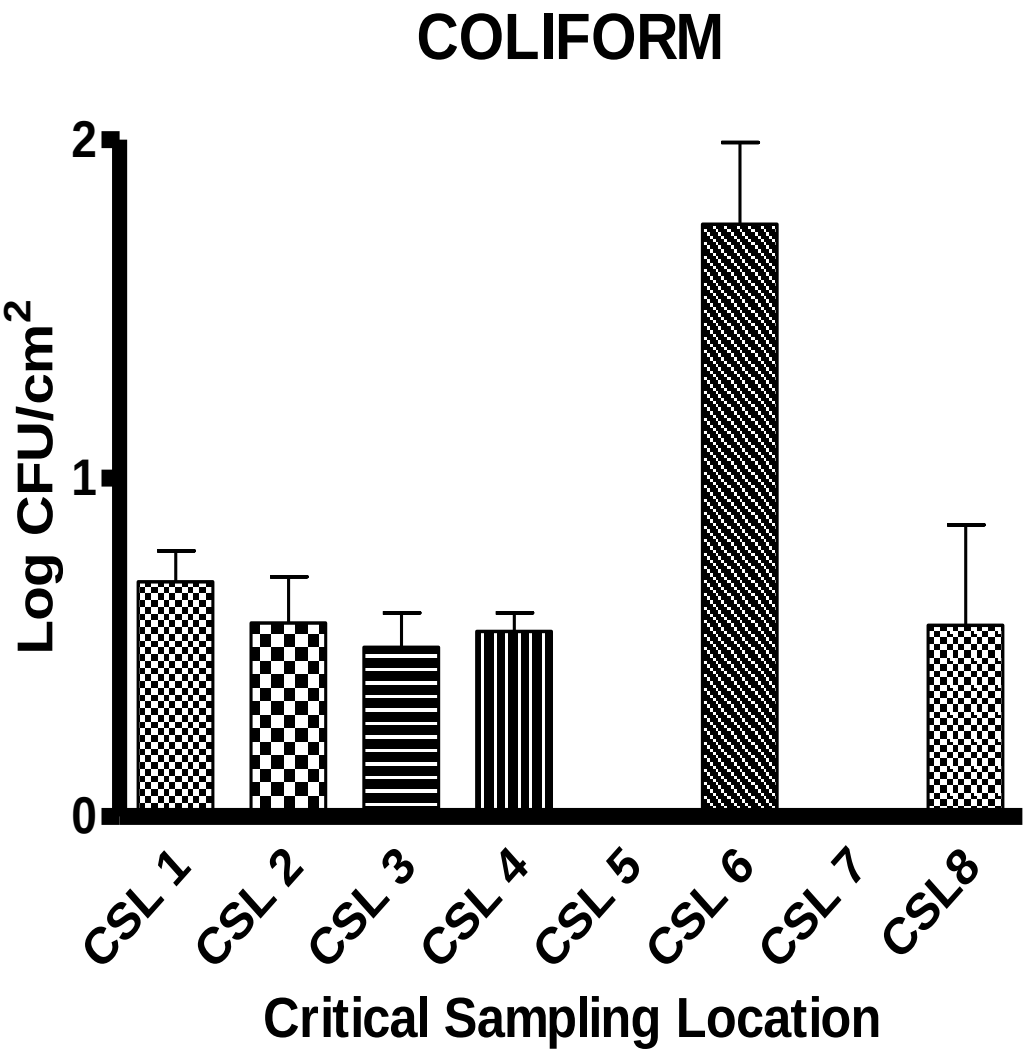

Figure 6: Total coliform count across the cold meats slicing line

The observed Total coliform count was generally low on all critical sampling locations. The highest count was found on CSL 6 which had 1.65log CFU/cm^2 while CSL 5 and 7 were found with no coliforms.

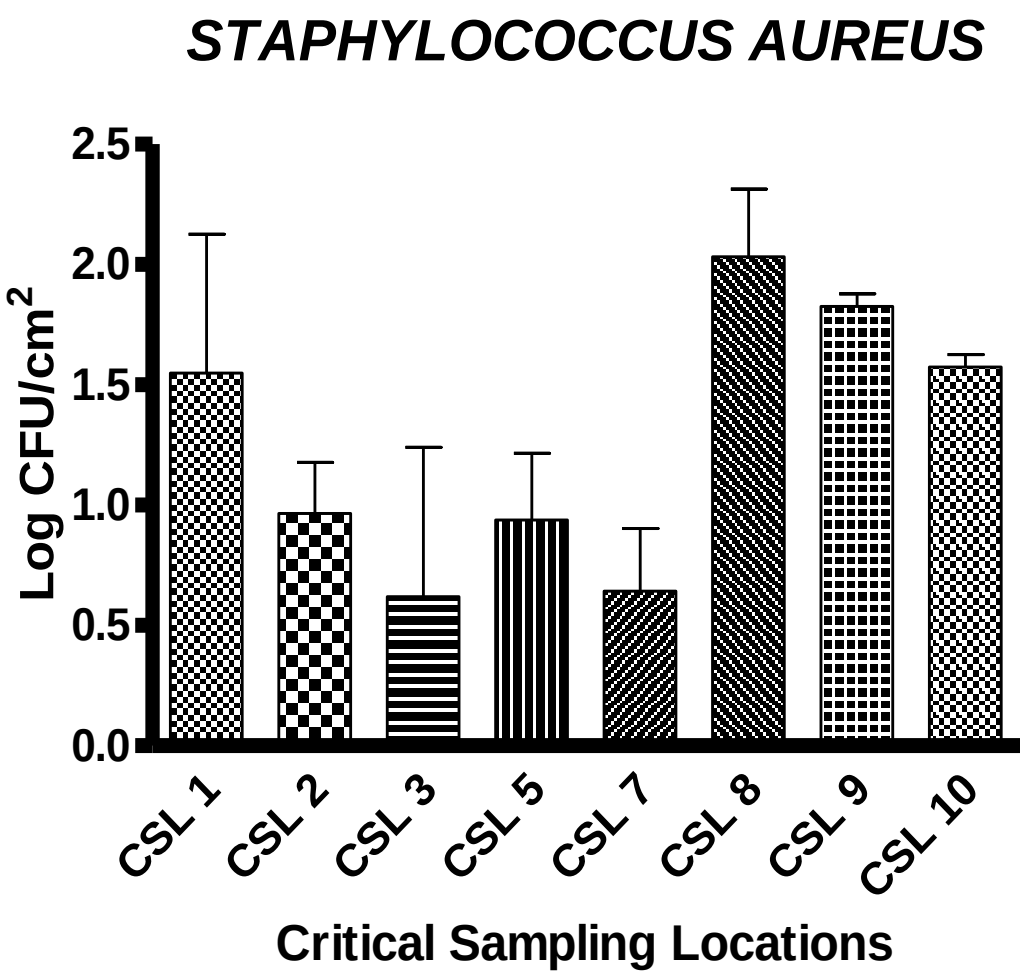

Figure 7: *S. aureus* counts across the cold meats slicing line

CSL 8 exhibited the highest counts of *S. aureus* with a high of 2.04log CFU/cm^2. Highest standard deviations were recorded on CSL 1 and CSL 3.

E. coli

No *E.coli* was observed on all critical sampling locations.

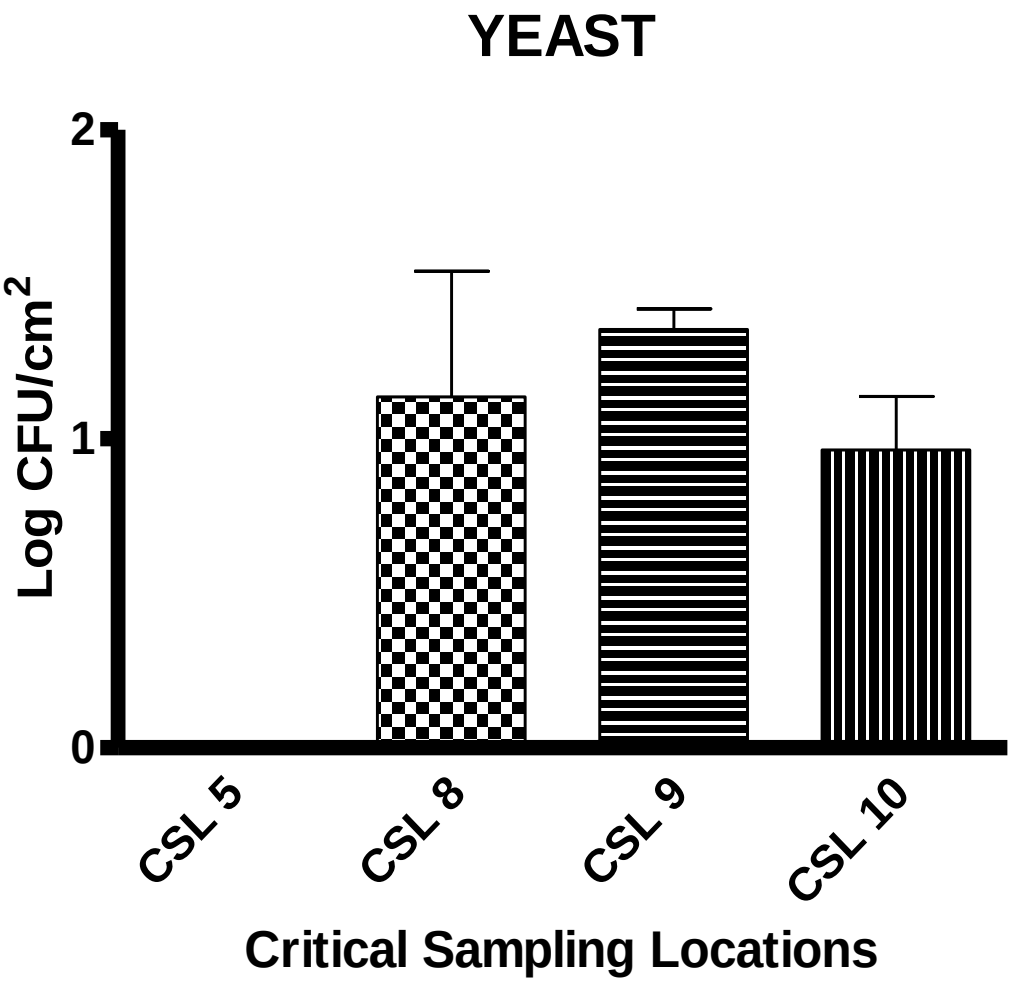

Figure 8: Yeast across the cold meats slicing line

No yeast was observed on CSL 5. CSLs 8, 9 and 10 all had yeast but in varying counts, with the highest being observed on CSL 9.

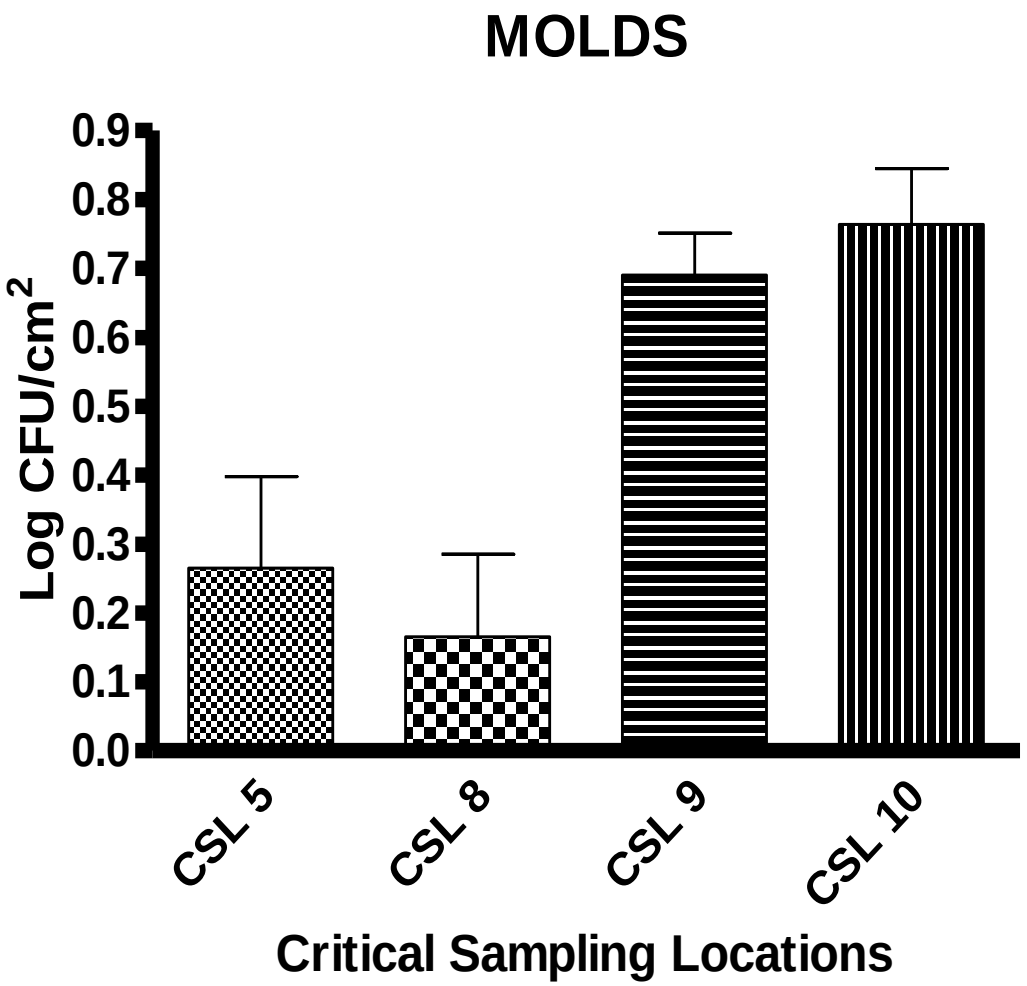

Figure 9: Molds across the cold meats slicing line

Air circulating around both the slicing and packing area was found to contain molds with the packing area having more molds, 0.764log CFU/cm^2. CSLs 5 and 6 both had very low counts less than 0.3logCFU/cm^2**.**

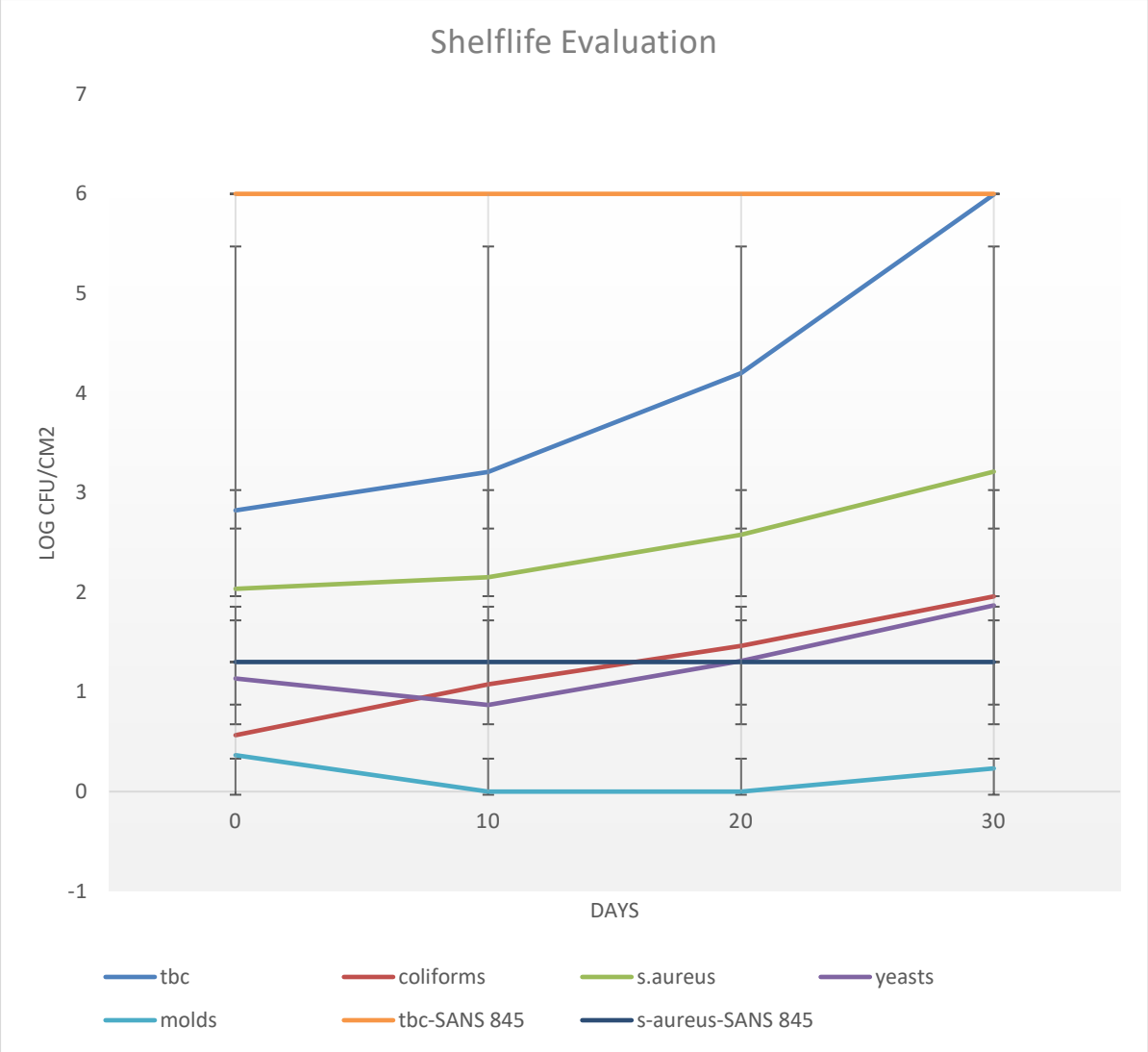

Figure 10: Growth of microorganisms in sliced vacuum packed French polony throughout its 30-day shelf life.

The number of microorganisms in the sliced French polony generally increased over the shelf life of the product with the exception of molds that decreased in the first 10days, then started increasing in the last 10days during shelf life. High standard deviations such as in the case of TBC indicate inconsistences in the manufacturing practises employed at company Z. No *E.coli* was observed throughout the shelf life of the product.

4.2 QUANTITATIVE RISK ANALYSIS

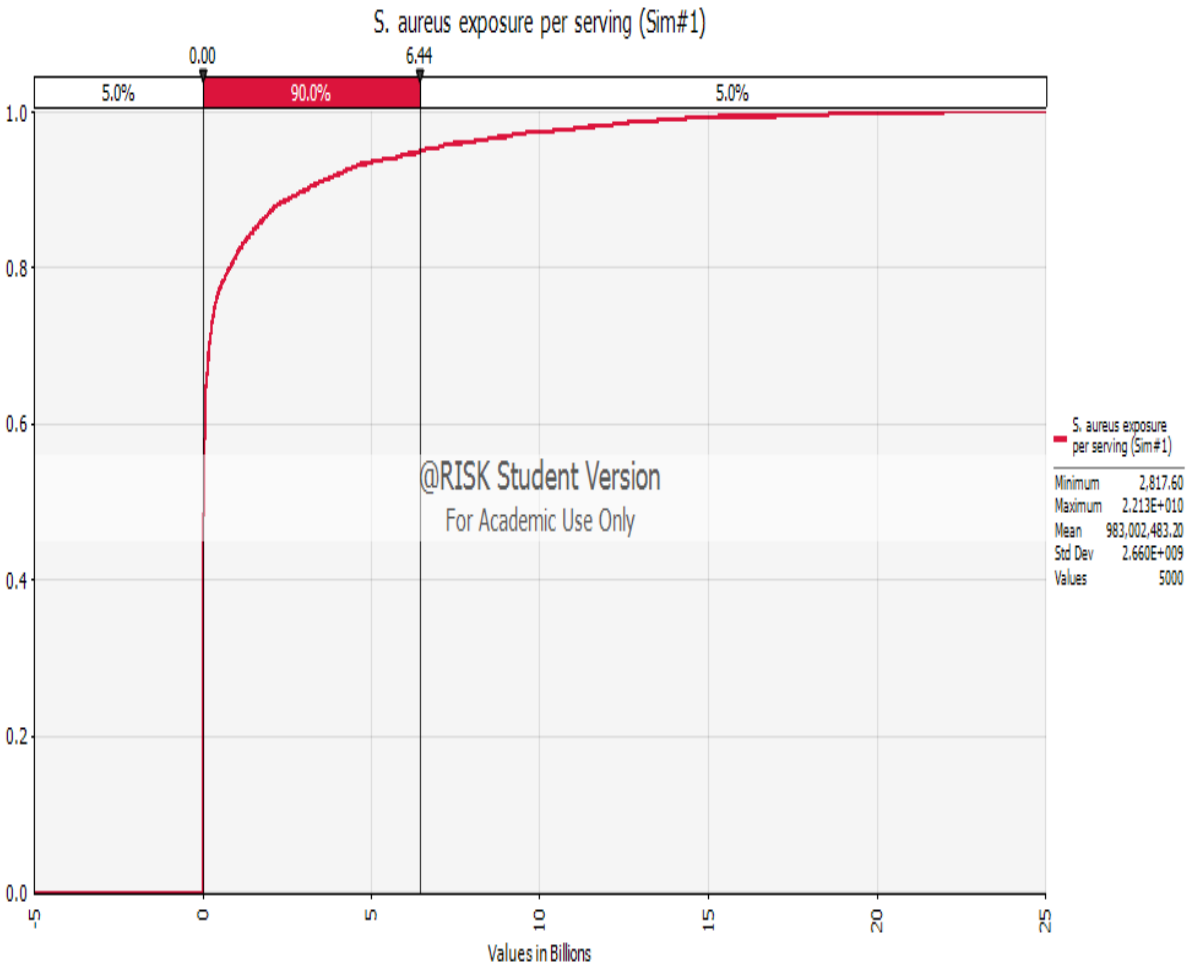

Figure 11: Actual Scenario of *S. aureus* exposure to consumers per serving

For each serving consumed, 5% of the serving had *S. aureus* below detectable levels while 90% ranged from undetectable levels to 6.44 X 10^9 CFUs and the remaining 5% had counts higher than 6.44 X 10^9 CFUs

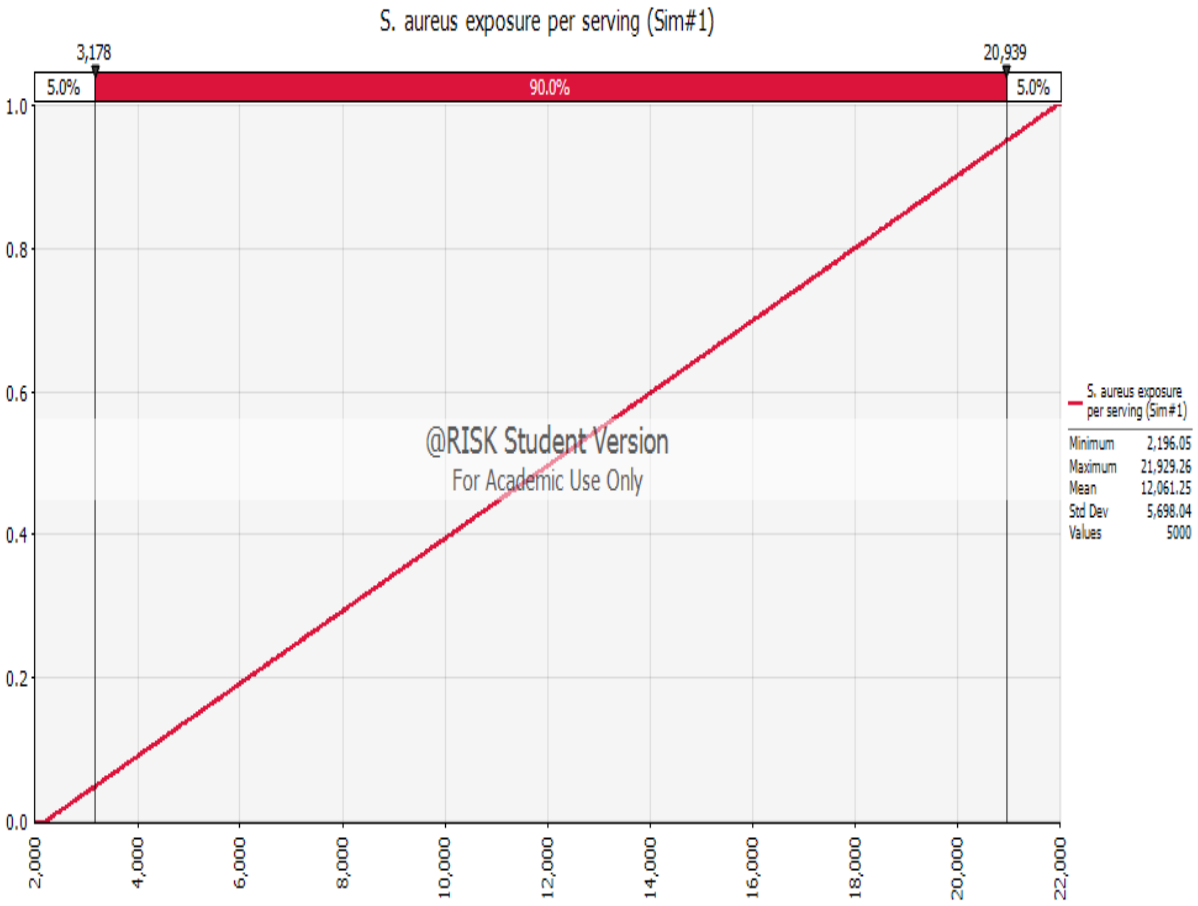

Figure 12: Hypothetical Scenario of *S. aureus* exposure to consumers per serving

For each serving to be consumed, 5% of the serving would have *S. aureus* counts of 3.178 X 10^3 CFUs and below while 90% would have range from 3.178 X 10^3 to 20.939 X 10^3 CFUs and the remaining 5% would be higher than 20.939 X 10^3 CFUs.

Table 10: Comparison of actual and hypothetical scenarios of *S. aureus* exposure to consumers per serving

Description	*S. aureus* exposure (CFUs/serving)	
	Actual Scenario	Hypothetical Scenario
Minimum	2817.603	2194.977
Maximum	22133670000	21926.84
Mean	983002500	12061.25
5% Perc	20946.28	3176.63
50% Perc	10917170	12060.6
75% Perc	337437200	16992.23
90% Perc	3024351000	19953.31
95% Perc	6440751000	20941.02

Maximum *S. aureus* exposure per serving consumed is more than 10^6 times higher in the actual scenario compared to the hypothetical scenario.

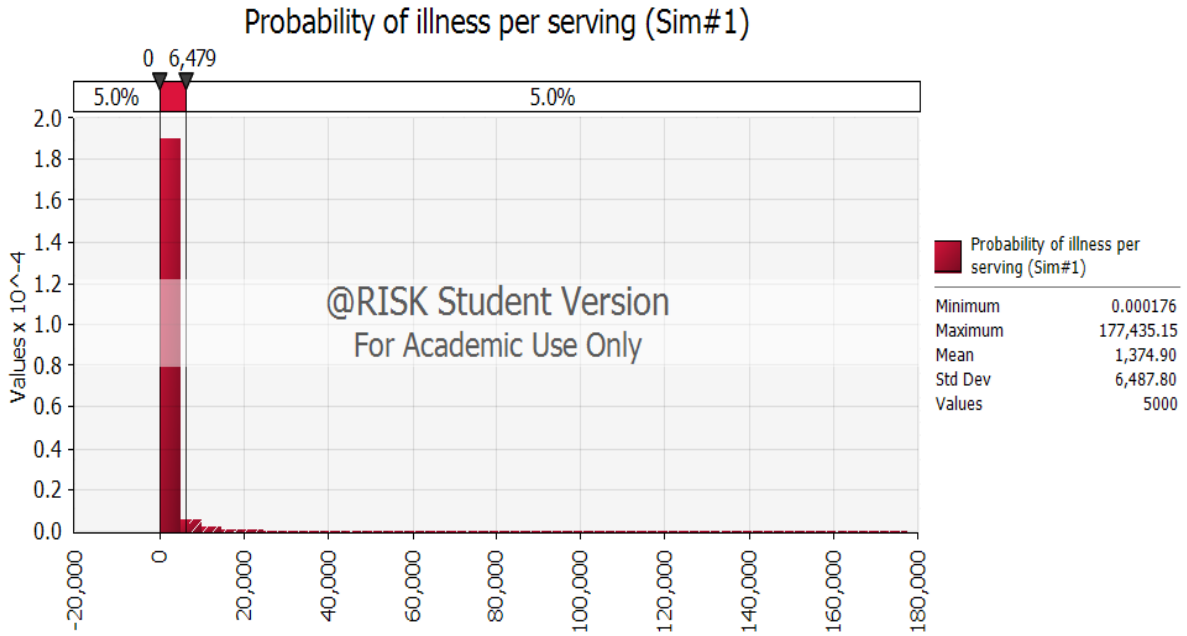

Figure 13: Probability of consumer illness after exposure to *S. aureus* in the actual scenario.

For each serving, only 5% of the portion has no risk of causing illness while the remaining 95% has a high risk of causing SFP to the consumer.

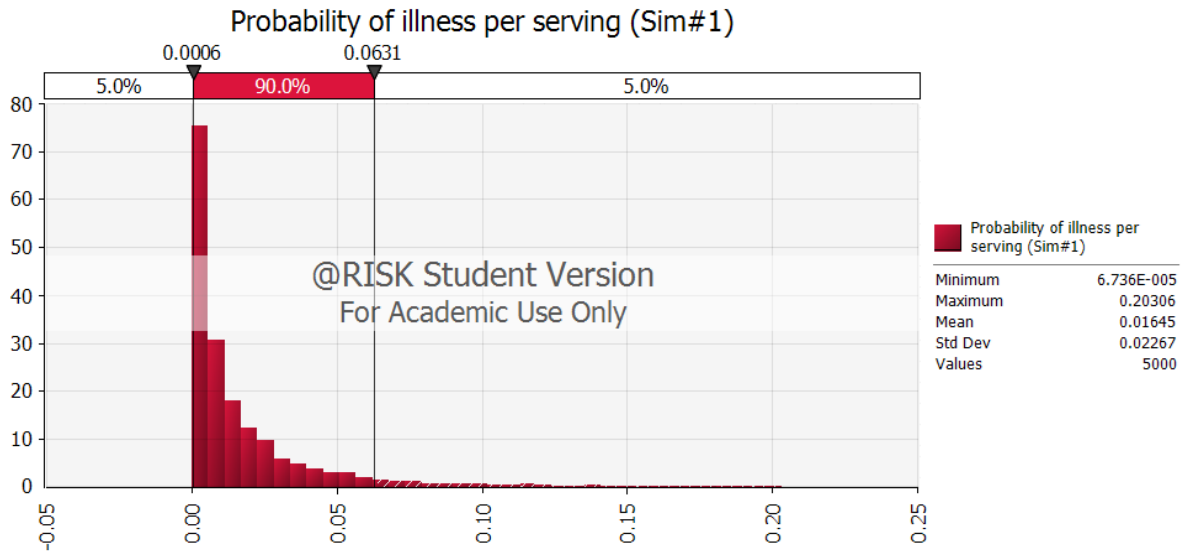

Figure 14: Hypothetical scenario probability of consumer illness after exposure to *S. aureus*

The maximum probability of causing illness to the consumer is less than 0.25, hence this scenario has very low risk of causing SFP.

Table 11: Comparison of probability of illness of consumers per serving under actual and hypothetical scenarios after exposure to *S. aureus*

Description	Probability of illness per serving	
	Actual Scenario	Hypothetical Scenario
Minimum	0.000192652	5.61688E-05
Maximum	113094	0.1717719
Mean	1240.504	0.01603782
5% Perc	0.008237399	0.000609035
35% Perc	0.9632069	0.004378221
40% Perc	2.027072	0.005244165
50% Perc	7.940082	0.007591653
75% Perc	242.0921	0.02028355
90% Perc	2269.665	0.0423305
95% Perc	6004.645	0.06090503

There is low risk of SFP under the hypothetical scenario because maximum probability of illness is less than 1. On the other hand over 60% of a serving portion has a probability of illness greater than 1, threatening SFP to consumers.

4.3 HYPOTHESES TESTING

H_o1: There is no significant difference in the microbial load (TBC, *E. coli*, coliform, yeast, molds and *S. aureus*) on CSLs across the French polony slicing and packing line.

S. aureus

$F_{tabulated} > F_{calulated}$ (Appendix 9)

Decision: Do not reject H_o1

Conclusion: There is no significant difference in the microbial load (*S. aureus*) across the French polony slicing and packing line

TBC, Coliform, Yeast and Molds

$F_{tablulated} < F_{calculated}$ (Appendix 10, 11,12,13)

Decision: Reject H_o1

Conclusion: There is a significant difference in the microbial load (TBC, Coliform, Yeast and molds) across the French polony slicing and packing line

H_o2: There is no significant difference between the observed and the standard microbial limits (TBC and *S. aureus*) specified in SANS 885:2011 3rd Edition on sliced French polony.

S. aureus

$P_{value} > P_{\alpha}$ (Appendix 15)

Decision: Reject H_o2

Conclusion: There is a significant difference between the observed and standard *S. aureus* load on sliced French polony.

TBC

$P_{value} < P_{\alpha}$ (Appendix 14)

Decision: Do not reject H_o2

Conclusion: There is no significant difference between the observed and standard TBC load on sliced French polony.

4.4 DISCUSSION

S. aureus

There is risk of microbial contamination of French polony during slicing and packing. The most important risk factor that was flagged in this study was the presence of *S. aureus* on the hands of personnel and consequently in the sliced vacuum packed French polony. *S. aureus* can be found as part of the human skin and hair's natural microbiota and personnel hands have been reported to be the chief source of *S. aureus* contamination in foods processed post heat treatment (Rodrigues et al., 2016; Schelin, 2011; Heinz and Hautzinger, 2007). There was an increase in counts of *S. aureus* in the French polony after slicing and packing. The concurrent presence of high counts of *S. aureus* on the hands of personnel and in the processing environment is indication that cross contamination may have occurred from these sources to the polony during slicing and packing. This observation follows a report by Aycicek, Cakiroglu and Stevenson, (2005) that processed foods requiring more handling during preparation are prone to *S. aureus* contamination. Syne, Ramsubhag and Adesiyun (2013) detected *S. aureus* in environmental samples and consequently in food processed in that environment post heat treatment and advised that cross-contamination can adversely affect the microbiological quality of meat with impact on people's health hence implementation of GHPs by employees should be strictly monitored and food handlers need to be aware of activities with high risk of causing food hazards. Air samples showed relatively low counts of *S. aureus* in the manufacturing room

compared to the raw meat environment. This indicates that the air filtration model (installing air filters in the air conditioning system and directing air flow from slicing and packing room towards the raw meat area) in use was reducing the bacteria entering the room but was not entirely effective. It is therefore important to closely monitor and regulate the equipment that manage the aerosol particles in a food processing plant (Lutgring, Linton, Zimmerman, Peugh and Heber, 1997).

TBC

Highest TBC counts were observed on the trays which are used for layering sliced French polony before vacuum packaging. The tray sanitization procedure employed at this plant was not effectively reducing the bacterial load on the trays. The trays were rinsed with warm water (40°C) in-between loads throughout the 8 hour shift. According to the FDA Food Code (2009), effective sanitization of equipment using heat can be accomplished by either pumping the water at 77°C for at least 5 minutes through assembled equipment or immersing equipment at 77°C for 30 seconds. According to Toyofuku, Inaba, Kiyokawa, Obana, Yawata and Nomura (2015), when elevated numbers of bacteria are present in an area, the concentration of auto-inducers in the region will be higher facilitating quorum sensing between the cells leading to biofilm formation. Once the microbes grow into a well-developed biofilm, cleaning and sanitization become more difficult since biofilms have a shielding effect on the bacterial cells within them and increase biocidal resistance of the cells due to the film. Kostakioti, Hadjifrangisko and Hultgren (2013), found that biofilms can be a continual source of pathogenic and spoilage organisms if not completely removed.

Coliforms

Coliforms were present on the trays and personnel hands which is indicative of unhygienic practices. According to the NSW Food Authority (2009), enterobacteriaceae represent bacteriological quality and are used as a hygiene indicator. Coliform bacteria are easily killed by heat and for an effective kill you require temperatures above 72°C for at least two and a half minutes (Ishaku, Ajamobi and Olayinka, 2013). The presence of coliforms in cooked processed food products points to recontamination and threatens presence of other pathogenic bacteria, (Ishaku, Ajamobi and Olayinka, 2013). Coliforms were isolated from the French polony after slicing and packing which was attributed to post heat treatment contamination because the loaves had none. These findings were in line with a research conducted by Ishaku, Ajamobi and Olayinka (2013), where coliforms, *E coli, S. aureus*, psychrotrophic and psychrophilic microbes were isolated from different RTE foods but these microbes had tested negative prior to post heat treatment handling which was indicative of contamination after heat treatment.

Yeast and Molds

Molds were isolated from the air around the processing area and from the finished product. USDA (2012) proposed that molds' presence in a sample is a risk factor of spoilage of the product before the end of its shelf life and some pose health risks to consumers. Molds such as *Aspergillus, Rhizopus and Penicillium* are responsible for the spoilage of cured meats while some molds, under right conditions produce mycotoxins such as aflatoxin which can cause allergic reactions and respiratory problems. According to USDA (2012), presence and growth of yeast in canned food may result in spoilage, generally in the form of alcohol production and large amounts of CO_2 gas which swells the container. In Figure 8 no yeast were isolated from the French polony loaf suggesting that the heat treatment applied during the cooking process was effective in eliminating the yeast. The effectiveness of cooking could also be concluded

from the fact that *E. coli* and coliforms were also not detected in the loaves after cooking. USDA (2012), noted that growth of yeast in processed foods does not pose a health risk, however its can cause spoilage of products before end of shelf life costing the company both financially and in consumer confidence.

Shelf life Evaluation

Overall, as shown in Figure 10 the microorganisms in the sliced vacuum packed French polony grew normally following the normal growth pattern. The only exception to this observation were yeast and molds whose numbers decreased in the early days then later increased until the end of shelf life. Molds are more sensitive to CO_2 than yeast. By definition molds are strict aerobes and hence sufficient residual O_2 must be present in the package headspace after vacuuming to allow mold growth (Gynot, Marin, Sanchis and Ramos, 2003). When the pack is evacuated about 18% residual oxygen remains (Ray and Bhunia, 2013) but it continues to drop to about 1% (Feiner, 2006) due to tissue and microbe respiration releasing CO_2. This combination suppresses growth of molds and the sensitive species may die (Brightwell, 2007). This phenomenon explains the drop in molds following vacuum packaging. According to Ray and Bhunia (2013), CO_2 has an important fungistatic effect (inhibits fungal growth without destroying them). Presence of CO_2 has been attributed to longer lag phases which corresponds to lower growth rates. This theory was supported in a study by Gynot et al., (2003) where no fungal growth occurred up to day 28 in their samples packed with 100% CO_2 incubated at 25°C, regardless of the water activity. In this research, molds resumed growth but at relatively slower rates after day 20 up to the end of the product's shelf life. Reduced O_2 tension might have destroyed sensitive molds while presence of CO_2 increased the lag phases, however some molds e.g. *A. niger* and *Penicillium spp* can tolerate and even grow in O_2 concentrations as low as 0.02 to 0.03%. In general, molds can grow in the presence of elevated CO_2 levels if O_2 is present (Gynot et al., 2003). Yeast can grow both aerobically and anaerobically. The air

evacuation process during vacuum packaging may have induced physical damage or shock to the cell population and some may have died leading to a reduction in the number of cells. When residual O_2 is exhausted, yeast follow the anaerobic pathway utilizing CO_2 that would have accumulated in the package headspace, (Gynot et al., 2003). Anaerobic growth is not as fast as aerobic growth because the sugars in the meat matrix are used for alcohol synthesis instead of benefiting the cells (Gynot et al., 2003). TBC remained within limit (SANS 885, 2011; Woolworth Foods Group, 2006; Hong Kong Centre For Food Safety, 2014; FDA Philippines, 2013; Food Administration Manual, 1995) throughout the 30day shelf life of the sliced vacuum packed French polony, however *S. aureus* was out of specification (SANS 885, 2011; Woolworth Foods Group, 2006; Hong Kong Centre For Food Safety, 2014; FDA Philippines, 2013; Food Administration Manual, 1995) throughout the entire shelf life of the product which points to increased risk of product spoilage through the organism's fermentation activities which produce lactic acid, (USDA, 2012) as well as increased risk of SE production.

Quantitative Risk Assessment

The sliced vacuum packed French polony in this study posed risk of SFP to its consumers because *S. aureus* colonies isolated and enumerated from the samples were almost double the permitted limit (SANS 885, 2011; Woolworth Foods Group, 2006). SANS 885 (2011) defined the threat posed as the presence of enterotoxin and not the mere presence of *S. aureus* in a sample and recommended a limit of 1.30log CFU/g because the organism grows well, either aerobically or under anaerobic conditions and produces good growth within 24 hours. However, enterotoxin production only begins at > 5log CFU/g, (Baeza, et al., 2007; Schelin et al., 2011). Baeza, et al., (2007) conducted a research in Mexican cities where they used predictive microbiology to estimate the growth of *S. aureus* in cooked meat products exposed to changing environmental temperatures in warm climates and their results showed that it took 6-8 hour for *S. aureus* to reach 6log CFU/g in the samples. In 2009 they conducted a similar

study in different cities in Argentina at ambient temperatures 25-40 °C and it took 6.8-8.1 hours for *S. aureus* to reach 6log CFU/g in the cooked meat samples. Such findings are useful to estimate the time needed for *S. aureus* to grow to toxic levels when a contaminated food is kept at ambient temperature in warm climates such as in the case of street vendors. In this study the QRAM simulated for the actual dispatch-to-table pathway showed that in each serving, consumers were exposed to *S. aureus* counts above 6log CFU/serving which is suggestive that enterotoxins may be present. The subsequent high risk of illness (probability >1) per serving calculated under the same pathway indicated the graveness of the health risk and the need for prompt intervention. A hypothetical scenario forged in the same pathway reduced exposure per serving by ensuring no growth occurred and reduced the risk of illness (probability <1) by manipulating storage temperatures during refrigeration to an optimum of 0-5 °C throughout the pathway. According to Schelin et al, (2011), SE production occurs at 10-46 °C and no *S. aureus* growth occurs below 6°C. It is important to note that the risk reported in this study is overstated because although home and retailer refrigeration temperatures might fluctuate between 6.5-12 °C (Ntuli 2016; Kamana 2015), when fridge door is opened, they may not remain high, hence growth might occur to numbers supporting SE production, but when the temperature is below 10°C no SE will be produced. The overstating was also caused by unavailability of an *S. aureus* predictive growth model that factors in competition from other organisms in a RTE meat product.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY

The goal of this study was to determine the routes of post heat treatment microbial cross contamination onto French polony during slicing and packing and the subsequent food safety threats posed to consumers. TBC, coliforms, *E. coli*, *S. aureus*, yeast and molds were isolated and enumerated from food handlers, equipment, surfaces, air and the product. All tests conducted on the product were used as an overall indicator of the microbiological quality of the product, while coliforms and *E coli* were used as hygiene indicators. *S aureus* was used both as a personal hygiene and pathogenic indicator. Coliforms, *S. aureus*, yeast and molds were detected but *E. coli* was not detected on all CSLs. A quantitative risk analysis was conducted for *S. aureus* in the sliced vacuum packed French polony on the despatch-to-table pathway to determine the exposure per serving and the resultant risk of SFP illness to consumers.

5.2 CONCLUSIONS

The research gave an indication that the current manufacturing practises, particularly the hygiene procedures employed are ineffective to curb microbial cross contamination. This was confirmed by the relatively high total viable counts and coliforms on trays as well as presence of *S. aureus* and coliforms on the hands of personnel. The presence of viable cells including *S. aureus* on the vacuum bag which is of sterile type is a clear indication that cross contamination occurred from the environment during processing. The hands of personnel harboured *S. aureus* and coliforms which have a zero tolerance policy at the company investigated suggesting that the handwashing protocols and monitoring procedures are ineffective. The heat treatment was effective in destroying yeast but the organism was reintroduced into the product from the

environment during slicing and packing. On the other hand *S. aureus* and molds were not completely destroyed during cooking and their relatively high initial counts in the sliced vacuum packed French polony indicates poor microbiological quality and considerably shorten the shelf life of the product. *E coli* was not detected on any CSL which indicates that procedures put in place to curb faecal contamination were effective. Exposure of *S. aureus* per serving was high and the consumers are at high risk of illness from consuming the sliced vacuum packed French polony produced at the company researched. In conclusion, there is need for improving the quality control and quality assurance programs such as handwashing techniques, equipment and surface sanitization protocols and fumigation schedules to safeguard the health of consumers of this product.

5.3 RECOMMENDATIONS

To Processor

- Implement FSQMSs such as HACCP. By implementing such a programme all hazards, which could affect human safety, will be identified, monitored when considered critical and eliminated, reduced or prevented.
- Conduct random personnel hand swabs online instead of scheduled swabs offline which do not give a clear indication of how well the employees follow the prescribed hand washing procedure when unmonitored.
- Provide hot water outlets at appropriate temperatures (77°C), in the processing area to wash equipment and surfaces. Warm water rinsing does not curb microbial growth.
- Provide antibacterial soap and hand sanitisers on hand washing basins in the processing area for food handlers to have more thorough handwashing instead of washing with warm water only.

To Retailer and Consumer

- Do not store sliced vacuum packed French polony in the fridge for long and maintain optimum refrigeration temperatures during storage.
- Consume cold meats immediately after taking them from fridge, do not hold at ambient temperatures for hours e.g. preparing as snacks to consume later in the day at work or school.

5.4 SUGGESTIONS FOR FURTHER RESEARCH

- Research on presence of staphylococcal enterotoxins in the French polony loaves after cooking and in the sliced vacuum packed French polony at predetermined intervals during shelf life.
- Conduct a survey on consumer practices such as portion sizes per serving to facilitate more accurate risk calculations.

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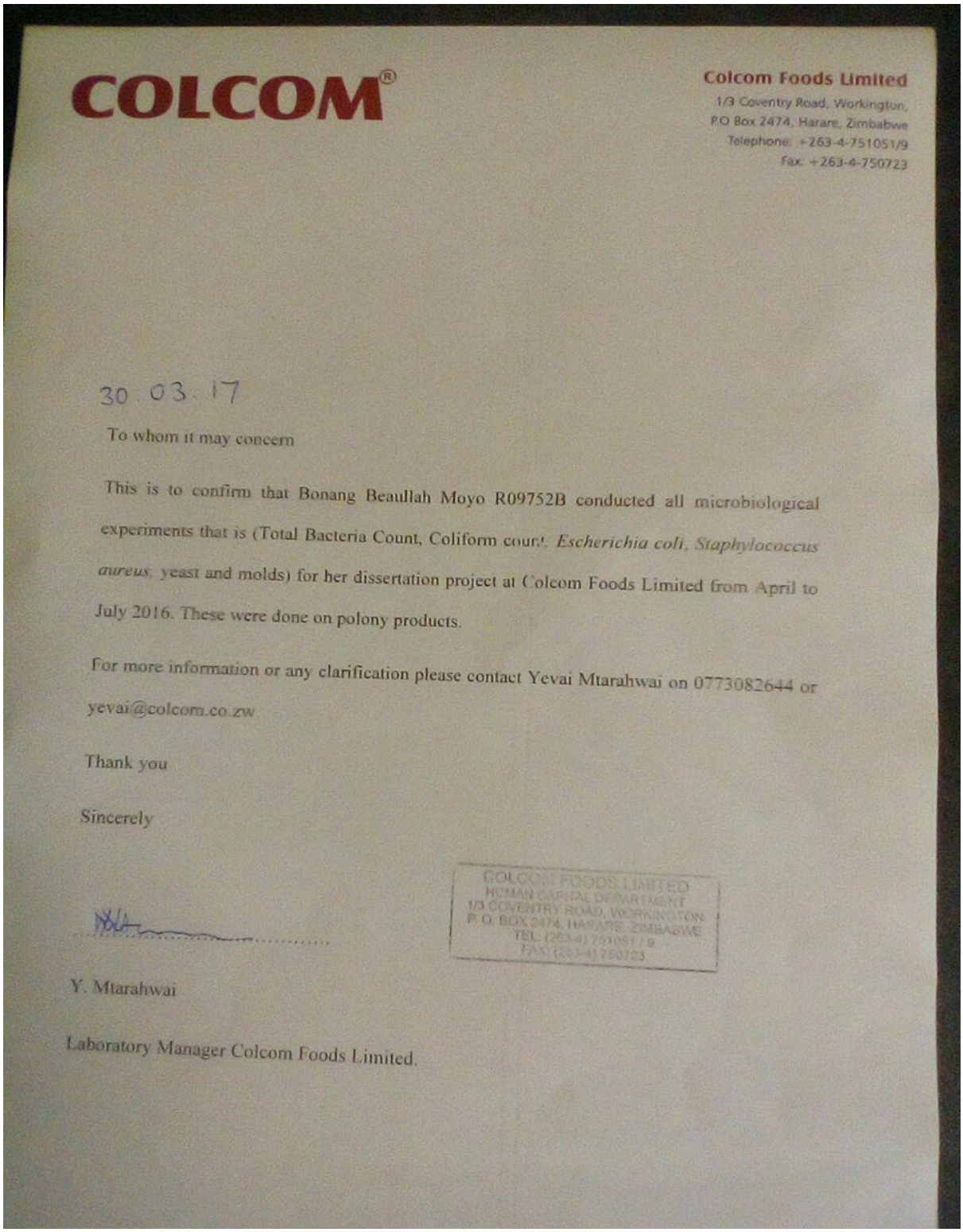
APPENDICES

APPENDIX 1: Confirmation letter



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30.03.17

To whom it may concern

This is to confirm that Bonang Beaullah Moyo R09752B conducted all microbiological experiments that is (Total Bacteria Count, Coliform count, *Escherichia coli*, *Staphylococcus aureus*, yeast and molds) for her dissertation project at Colcom Foods Limited from April to July 2016. These were done on polony products.

For more information or any clarification please contact Yevai Mtarahwai on 0773082644 or yevai@colcom.co.zw

Thank you

Sincerely

COLCOM FOODS LIMITED
HUMAN CAPITAL DEPARTMENT
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Y. Mtarahwai

Laboratory Manager Colcom Foods Limited.

APPENDIX 2: Microbial Results – TBC

CSLs	Batch 1	Batch 2	Batch 3	Average
Food Handler 1	1.36173	2.25588	2.23742	1.95167
Food Handler 2	1.97313	1.95061	2.12953	2.01775
Food Handler 3	1.54407	1.52827	2.58659	1.88631
Slicer Equipment	1.64345	1.6744	1.19728	1.50505
French polony loaf before slicing	3.41363	3.08279	2.78533	3.09392
Tray	3.20952	3.77869	4.32593	3.77138
Vacuum Bag	1.11394	1.11394	0.35218	0.86002
Packed Sliced French polony	2.8654	3.05881	2.54407	2.82276
Plate Count Agar	0	0	0	0
Ringers Solution	0	0	0	0

APPENDIX 3: Microbial Results – *S. aureus*

CSLs	Batch 1	Batch 2	Batch 3	Average
Food Handler 1	0.39794	2.123852	2.128722	1.550171
Food Handler 2	0.544068	1.10551	1.243038	0.964205
Food Handler 3	0	0	1.861833	0.620611
French polony loaf before slicing	0.39794	1.30103	1.122216	0.940395
Slicing Area Air	1.841985	1.911158	1.730378	1.82784
Packing Area Air	1.469822	1.653213	1.599337	1.574124
Vacuum Bag	0.90309	0.90309	-0.12494	0.560414
Packed Sliced French polony	1.942008	2.562293	1.60206	2.035454
Mannitol Salt Agar	0	0	0	0
Ringers Solution	0	0	0	0

APPENDIX 4: Microbial Results – Coliform

CSLs	Batch 1	Batch 2	Batch 3	Average
Food Handler 1	0.54407	0.67669	0.86034	0.6937
Food Handler 2	-0.301	0.72016	0.69897	0.3727
Food Handler 3	-0.301	-0.6021	-0.6021	-0.5017
Slicer Equipment	0.43933	-0.6021	-0.6021	-0.2549
French polony loaf before slicing	0	0	0	0
Tray	2.22531	1.57403	1.14613	1.64849
Vacuum Bag	0	0	0	0
Packed Sliced French polony	1	0.69897	0	0.56632
Violet Red Bile Agar	0	0	0	0
Ringers Solution	0	0	0	0

APPENDIX 5: Microbial Results – Yeast

CSLs	Batch 1	Batch 2	Batch 3	Average
French polony loaf before slicing	0	0	0	0
Slicing Area Air	1.43136	1.21748	1.41497	1.35461
Packing Area Air	1.29003	0.69897	0.90309	0.96403
Packed Sliced French polony	0.39794	1.79588	1.21085	1.13489
Malt Extract Agar	0	0	0	0
Ringers Solution	0	0	0	0

APPENDIX 6: Microbial Results – Molds

CSLs	Batch 1	Batch 2	Batch 3	Average
French polony loaf before slicing	0.39794	0	0.39794	0.26529
Slicing Area Air	0.77815	0.72016	0.57403	0.69078
Packing Area Air	0.60206	0.86034	0.8293	0.7639
Packed Sliced French polony	0.39794	0	0.69897	0.36564
Malt Extract Agar	0	0	0	0
Ringers Solution	0	0	0	0

APPENDIX 7: Microbial Results – *E. coli*

CSLs	Batch 1	Batch 2	Batch 3	Average
Food Handler 1	0	0	0	0
Food Handler 2	0	0	0	0
Food Handler 3	0	0	0	0
Slicer Equipment	0	0	0	0
French polony loaf before slicing	0	0	0	0
Tray	0	0	0	0
Vacuum Bag	0	0	0	0
Packed Sliced French polony	0	0	0	0
Media	0	0	0	0
Ringers Solution	0	0	0	0

APPENDIX 8: Microbial Results – Shelf Life Evaluation

Organism	Day 0	Day 10	Day 20	Day 30
TBC	2.82276	3.20923	4.19922	5.99379
Coliform	0.56632	1.07682	1.46187	1.95936
S. aureus	2.03545	2.15062	2.57808	3.21177
Yeast	1.13489	0.8696	1.31034	1.86908
Molds	0.36564	0	0	0.23299
E. coli	0	0	0	0

APPENDIX 9: Hypothesis Testing H_o1– TBC

Parameter	Value		
Table Analyzed			
TOTAL BACTERIAL COUNT 1 WAY ANOVA			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	8		
F	15.17		
R squared	0.8691		
ANOVA Table	SS	df	MS
Treatment (between columns)	18.4	7	2.628
Residual (within columns)	2.772	16	0.1732
Total	21.17	23	

$F_{calculated}$ =15.17

$F_{tabulated}$ = 2.66

Decision: Reject H_o1

APPENDIX 10: Hypothesis Testing H_o1– *S. aureus*

Table Analyzed			
STAPHYLOCOCCUS AUREUS			
One-way analysis of variance			
P value	0.0694		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	8		
F	2.403		
R squared	0.5125		
ANOVA Table	SS	df	MS
Treatment (between columns)	6.24	7	0.8914
Residual (within columns)	5.936	16	0.371
Total	12.18	23	

$F_{calculated}$ =2.403

$F_{tabulated}$ = 2.66

Decision: Do not reject H_o1

APPENDIX 11: Hypothesis Testing H_o1– Coliform

Table Analyzed			
COLIFORMS			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	8		
F	12.63		
R squared	0.8468		
ANOVA Table	SS	df	MS
Treatment (between columns)	6.186	7	0.8837
Residual (within columns)	1.119	16	0.06995
Total	7.305	23	

$F_{calculated}$ =12.63

$F_{tabulated}$ = 2.66

Decision: Reject H_o1

APPENDIX 12: Hypothesis Testing H_o1– Yeast

Table Analyzed			
YEASTS			
One-way analysis of variance			
P value	0.0119		
P value summary	*		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	7.144		
R squared	0.7282		
ANOVA Table	SS	df	MS
Treatment (between columns)	3.21	3	1.07
Residual (within columns)	1.198	8	0.1498
Total	4.408	11	

$F_{calculated}$ =7.144

$F_{tabulated}$ = 2.66

Decision: Reject H_o1

APPENDIX 13: Hypothesis Testing H_o1– Molds

Table Analyzed			
MOLD			
One-way analysis of variance			
P value	0.0072		
P value summary	**		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	8.511		
R squared	0.7614		
ANOVA Table	SS	df	MS
Treatment (between columns)	0.8083	3	0.2694
Residual (within columns)	0.2533	8	0.03166
Total	1.062	11	

$F_{calculated}$ =7.144

$F_{tabulated}$ = 2.66

Decision: Reject H_o1

APPENDIX 14: Hypothesis Testing H_o2– Finished Product TBC

TBC FINISHED PRODUCT	OBSERVED
Number of values	3
Minimum	2.54
25% Percentile	
Median	2.87
75% Percentile	
Maximum	3.06
Mean	2.823
Std. Deviation	0.2631
Std. Error	0.1519
Lower 95% CI of mean	2.17
Upper 95% CI of mean	3.477
One sample t test	
Theoretical mean	6
Actual mean	2.823
Discrepancy	3.177
95% CI of discrepancy	-3.830 to -2.523
t, df	t=20.91 df=2
P value (two tailed)	0.0023
Significant (alpha=0.05)?	Yes
Sum	8.47

$P_{value} = 0.0023$

$P_{\alpha} = 0.05$

Decision: Do not reject H_o2

APPENDIX 15: Hypothesis Testing H_o2– Finished Product *S. aureus*

Number of values	3
FINISHED PRODUCT *S. aureus*	
Minimum	1.6
25% Percentile	
Median	1.94
75% Percentile	
Maximum	2.56
Mean	2.033
Std. Deviation	0.4868
Std. Error	0.281
Lower 95% CI of mean	0.8242
Upper 95% CI of mean	3.243
One sample t test	
Theoretical mean	1.3
Actual mean	2.033
Discrepancy	-0.7333
95% CI of discrepancy	-0.4759 to 1.943
t, df	t=2.609 df=2
P value (two tailed)	0.1208
Significant (alpha=0.05)?	No

$P_{value} = 0.1208$

$P_{\alpha} = 0.05$

Decision: Reject H_o2

APPENDIX 16: Quantitative Risk Analysis Model for *S. aureus*

Cells	Variables	Descriptions	Units	Distribution/ Model/Value Actual Scenario	Distribution/ Model/Value Hypothetical Scenario	Sources
Node 1: Initial Contamination						
E3	N	Number of samples	No units	48	48	Experimental
E4	P	Number of positive samples	No units	48	48	Experimental
E5	PS	Prevalance of *S. aureus* in sliced french polony	No units	E4/E3	F4/F3	Experimental
E6	IC	Initial concentration of *S. aureus* in sliced french polony	log CFU/g	2.04	2.04	Experimental
E7	ICo	Initial contamination output	log CFU/g	E6	F6	Experimental
Node 2: Distribution To Retailer						
E9	T	Temperature	°C	3-7	3-7	Experimental
E10	Hr	Time	Hours	1-8	1-8	Experimental
E11	pH	pH	No units	5.4-5.8	5.4-5.8	Experimental
E12	GS	Growth concentration of *S. aureus* in sliced french polony	log CFU/g	0	0	ComBase
E13	DRo	Distribution to retailer output	log CFU/g	E7+E12	F7+F12	Calculated
Node 3: Storage At Retailer						
E15	T	Temperature	°C	5 -12	6	Literature
E16	Hr	Time	Hours	24 -720	24 -720	Experimental
E17	pH	pH	No units	5.4 - 5.8	5.4 - 5.8	Experimental
E18	GS	Growth of *S. aureus* in sliced french polony	log CFU/g	RiskUniform(0;6.05)	0	ComBase
E19	SRo	Storage at retailer output	log CFU/g	E13+E18	F13+F18	
Node 4: Distribution To Consumer						
E21	T	Temperature	°C	16-25	16-25	Literature
E22	Hr	Time	Hours	1/2 - 2	1/2 - 2	Experimental
E23	pH	pH	No units	5.4 - 5.8	5.4-5.8	Experimental
E24	GS	Growth of *S. aureus* in sliced french polony	log CFU/g	0	0	ComBase
E25	DCo	Distribution to consumer output	log CFU/g	E19+E24	F19+F24	Calculated
Node 5: Storage At Consumer						
E27	T	Temperature	°C	7 -12	6	Literature
E28	Hr	Time	Hours	24-168	24-168	Experimental
E29	pH	pH	No units	5.4 - 5.8	5.4-58	Experimental
E30	GS	Growth of *S. aureus* in sliced french polony	log CFU/g	0	0	ComBase
E31	SCo	Storage at consumer output	log CFU/g	E25+E30	F25+F30	Calculated
Node 6:Consumption						
E33	CC	Concentration of *S.aureus* at consumption	log CFU/g	E31	F31	Calculated
E34	CCg	Concentration of *S.aureus* at consumption	CFU/g	10^E33	10^F33	Calculated
E35	SVD	Serving portion distribution	g	RiskUniform(20;200)	RiskUniform(20;200)	Estimated
E36	EPS	*S. aureus* exposure per serving	CFU/serving	E34*E35	F34*F35	Calculated
E37	DRD	Dose Response Distribution	CFU	10^RiskPert(5;6;8)	10^RiskPert(5;6;8)	Literature
E38	Pi	Probability of illness per serving	No units	E36/E37	F36/F37	Calculated

APPENDIX 17: *S. aureus* exposure per serving of vacuum packed French polony

Description	*S. aureus* exposure (CFUs/serving)	
	Actual Scenario	Hypothetical Scenario
Minimum	2817.603	2194.977
Maximum	22133670000	21926.84
Mean	983002500	12061.25
Std Deviation	2659699000	5698.041
Variance	7.074E+18	32467670
Skewness	3.978808	1.04032E-05
Kurtosis	21.07379	1.799987
Errors	0	0
Mode	22158.5	10778.12
5% Perc	20946.28	3176.63
10% Perc	43446.17	4165.194
15% Perc	81759.01	5151.747
20% Perc	171208.8	6138.426
25% Perc	329563.8	7125.139
30% Perc	679343.1	8113.628
35% Perc	1355746	9099.426
40% Perc	2771206	10086.92
45% Perc	5463212	11073.05
50% Perc	10917170	12060.6
55% Perc	21898410	13047.05
60% Perc	44422420	14033.42
65% Perc	89171750	15019.75
70% Perc	182124600	16006.89
75% Perc	337437200	16992.23
80% Perc	781438700	17978.63
85% Perc	1521143000	18965.62
90% Perc	3024351000	19953.31
95% Perc	6440751000	20941.02

APPENDIX 18: Probability of illness per serving of sliced vacuum packed French polony

Description	Probability of illness per serving	
	Actual Scenario	Hypothetical Scenario
Minimum	0.000192652	5.61688E-05
Maximum	113094	0.1717719
Mean	1240.504	0.01603782
Std Deviation	5230.817	0.02156312
Variance	27361440	0.000464968
Skewness	8.866579	2.61207
Kurtosis	113.0119	11.53034
Errors	0	0
Mode	0.000838368	0.000723285
5% Perc	0.008237399	0.000609035
10% Perc	0.02581247	0.0010084
15% Perc	0.0554505	0.001546168
20% Perc	0.1102432	0.00218569
25% Perc	0.2249914	0.002821373
30% Perc	0.476481	0.003540087
35% Perc	0.9632069	0.004378221
40% Perc	2.027072	0.005244165
45% Perc	4.004717	0.006359527
50% Perc	7.940082	0.007591653
55% Perc	15.80459	0.009125561
60% Perc	31.65476	0.01098791
65% Perc	60.85793	0.01351622
70% Perc	125.3777	0.01650303
75% Perc	242.0921	0.02028355
80% Perc	490.1855	0.024864
85% Perc	1010.798	0.03229659
90% Perc	2269.665	0.0423305
95% Perc	6004.645	0.06090503

APPENDIX 19: Research in Pictures


AMR2
AMR1