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The Safety of Ready-to-Eat Meals Under Different Consumer Handling Conditions

**A thesis presented in partial fulfilment of the
requirements for the degree of**

Master

in

Food Technology

at Massey University, Manawatū,

New Zealand

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2016

Abstract

Microbial count is an important index to measure the safety status of a food. This trial aimed to determine the safety of eight meals (four meats and four vegetarians) by using the agar plate counting method to measure the populations of total bacteria and specific pathogenic microorganisms during four day' abusing. The results showed that chicken & lemon sauce, pork & cranberry loaf and lasagne veg can be considered as acceptable after a series of handling steps including heating and holding in different environments. BBQ beef, quiche golden and pie rice & vegetable were all marginal for the microbial load before heating, but afterwards all of them were acceptable. Casserole chickpea and hot pot sausage were in marginal for the microbial load by the end of trial.

Keywords: microbial count; eight meals; pathogenic microorganisms

Acknowledgements

I am like to acknowledge my chief supervisor Prof. Steve Flint for his consistent support and guidance. Without his insightful advice, this project would not go smoothly. I learnt a lot from him how to deal with the challenges faced in the research.

I am also grateful to Mrs Julia Good at the Microbiology Laboratory for providing me with her expert guidance on the experimental operation.

I would like to acknowledge Massey University Institute of Food, Science and Technology for providing a wonderful laboratory. Many thanks to Ann-Marie Jackson, Julia Good, Kylie Evans for providing me technical advice and resources to complete my study.

I owe many thanks to all the students in the microbiology research team, who were always keen to discuss my work and answer lots of doubts.

I am indebted to Compass Group (NZ) Limited for supporting this study.

Finally, I would like to thank my family and friends for always supporting and encouraging me.

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Chapter One: Introduction

Ready-to-eat meals that require minimal or no processing before consumption are growing in popularity for busy working people and the elderly. For a ready-to-eat meal, shelf life is an important factor for the quality and safety of the food. Shelf life is the period of time, established under intended conditions of distribution, storage, retail sale and use, that the food remains safe (MPI, 2014). There are many ways to determine the shelf life of a food, depending on whether the primary interest is in the overall acceptability or the safety of the food. Shelf life can be determined by the chemical changes or microbiological spoilage in foods. Measuring the population of different pathogenic microorganisms is important to ensure the food is safe. Some of the most important foodborne pathogens of concern in ready to eat meals are discussed below.

The *Enterobacteriaceae* refer to coliforms, fecal coliforms, *E. coli* and other bacteria in the family. The *Enterobacteriaceae* are used as an indicator of food quality and an index of food safety in Europe. In the United States, coliforms are considered as indicators of poor food quality or inadequate sanitation in food processes (Kornacki & Johnson, 2001). In the family of *Enterobacteriaceae*, the common foodborne genera include *Enterobacter*, *Escherichia* and *Salmonella*. *Escherichia coli* (*E. coli*) is a member of the family of *Enterobacteriaceae*. As *E. coli* is a part of normal flora of the intestinal tract, most of *E. coli* would not cause illness, but some groups can result in serious diarrhoea causing death (Doyle & Padhye, 1989). The detection of *E. coli* in foods also shows that there is a potential for other pathogens such as *Salmonella* spp.

Salmonella spp. are facultative intracellular pathogens (Jantsch et al., 2011). There are two types of *Salmonella* serovars: typhoidal and nontyphoidal. Typhoidal serovars are adapted to humans and do not occur in animals while nontyphoidal serovars can infect animals and be transferred between humans and animals. The infection of *Salmonella* will usually cause self-limiting gastrointestinal disease.

The presence of *Staphylococcus aureus* (*S. aureus*) in foods shows a potential health hazard as many strains of *S. aureus* will produce enterotoxins to cause food poisoning. When people get infected by pathogenic strains of *S. aureus*, it may cause many illnesses from minor skin infections like pimples to life-threatening diseases such as meningitis and toxic shock syndrome (Lancette & Bennett, 2001).

Clostridium perfringens (*C. perfringens*) is also a common cause of foodborne illness. People usually get infected from contaminated food such as poorly prepared meat products or the same products prepared well but left for a long time (Benz et al., 2003). The *C. perfringens* enterotoxin is heat labile and inactivated when the temperature exceeds 75°C. Thus to reduce the risk of *C. perfringens* food poisoning, the food should be heated above 75°C for minutes before consuming (Duncan, 1973).

Bacillus cereus (*B. cereus*) can be isolated from many raw and processed foods. Consuming $> 10^5$ *B. cereus* cells/g will cause foodborne illness (Hatheway & McCroskey, 1987). There are two types of illness can be caused by the consumption of contaminated food with *B. cereus*: diarrhoeal syndrome and emetic syndrome. The diarrheal toxin appears in all kinds of food that have been contaminated while emetic toxin will be only produced in food containing starch. Another difference between those two is diarrhoeal toxin is heat-labile but the emetic toxin is not. In addition, if the food is improperly refrigerated, the refrigeration temperature will allow the endospores to germinate (McKillip, 2000).

These microorganisms are the common causes of foodborne illness. The detection of those microorganisms above the specification limit (Food Standard Australia New Zealand, 2001) represents a potential public health hazard.

The purpose of this study is to determine the safety of eight meals (four of meat and four of vegetarian) after a series handling steps by measuring the microbial count.

Chapter Two: Literature Review

2.1 Ready to eat food

Ready to eat (RTE) foods are foods that can be consumed without any further process like heating as they have already been through processes to make them safe and ready for consumption. These kinds of foods do not need any further preparation before consuming except thawing, washing, or moderate reheating (Farber & Harwig, 1996).

RTE foods include a large range of products, such as seafood (cold smoked salmon and sushi), fruits and vegetables (salads, leafy greens, and salsa), dairy products (cheese, yogurt and sour cream), meats (deli meats, sausages and hot dogs) (Jaroni et al., 2010). Some frozen foods can be also considered as RTE foods, such as frozen meals, ice cream, and frozen fruit.

RTE foods are convenient for busy consumers, thus the demand for such products is increasing. Surveys of consumer purchase behaviours by Anonymous (2001) confirm the increase in the consumption of RTE foods. The market for RTE products is large and growing.

Because RTE food products require no further processing before consumption, the microbiological risks of these products for customers is a concern. Many RTE products such as sandwiches or meats, involve cutting or slicing, and handling which provides the opportunity for contamination with pathogenic bacteria (Jaroni et al., 2010).

The common pathogenic bacteria found in refrigerated RTE foods are *Clostridium perfringens*, *Salmonella enterica*, and *Escherichia coli* (Jaroni et al., 2010).

Because *E.coli* and *Salmonella* are considered as part of the natural intestinal flora of animals, the faecal contamination of RTE foods may result in these pathogens being found in foods. *E. coli* and *Salmonella* have good resistance to pH and low moisture, enabling them to survive in food products (Jaroni et al., 2010).

Clostridium perfringens is often found in meat and poultry products. This microorganism will cause foodborne illness. Its presence in foods at levels that can cause food poisoning often results from inadequate refrigeration or reheating of RTE meat or poultry products. If people consume a food that has been contaminated with $>10^6$ viable vegetative cells of *C. perfringens*, the pathogen will survive through

the stomach and sporulate and release enterotoxin in the intestines (Labbe & Juneja, 2002).

2.2 Shelf life test

The definition of shelf life is the time during which a food product remains safe, complies with label declarations for nutritional data and retains desired sensory, chemical, physical and microbiological characteristics when stored under the recommended conditions (IFST, 1993). Shelf life is a function of time, environmental factors and susceptibility of product to change in quality. There are two main shelf life testing methods: real-time and accelerated shelf life testing. Challenge testing is another method to determine the effect of the intrinsic and extrinsic properties of a food on the growth of pathogens or spoilage microorganisms.

2.2.1 Real-time shelf life testing (Magari, 2003)

Real-time shelf life testing is used to test those products where microbial stability is the limiting factor. This is particularly important for some perishable foods with high water activity, including many ready-to-eat meals, salads, and refrigerated foods. Spoilage microorganisms growing in the food will result in changes to the sensory characteristics of food products such as smell, taste and appearance. Through analysing the changes in the sensory characteristics of a particular food, the shelf life of a food product can be predicted.

2.2.2 Accelerated shelf life testing (Magari, 2003)

Accelerated shelf life testing (ASLT) is a method used to test the safety and quality of foods through subjecting them to a range of controlled conditions, such as temperature, humidity and light. These conditions exceed normal storage conditions and therefore accelerate the changes that food products would undergo in normal circumstances. This method can be used to assess microbiologically stable products for deterioration in sensory characteristics caused by chemical and physical changes and can provide results more quickly than real time testing.

2.2.3 Challenge testing (Ragnarsson & Labuza, 1977)

Challenge testing enables the assessment of the properties of the food (intrinsic factors) and the storage environment (extrinsic factors) in controlling specific pathogenic or spoilage microorganisms. During the challenge testing, through monitoring the growth

of microorganisms, it is able to establish critical factors that may enable the elimination of pathogens or slow the growth of spoilage microorganisms (Campelos, 2015). For such trials, it is important that a broad selection of microbial isolates, from the food sector concerned, are used as the challenge cultures and that the conditions used in the trial are representative of the storage and handling of the food.

2.2.4 Quality indices for testing the shelf life of food (Singh & Cadwallader, 2004)

Using an appropriate index to model the quality change of a food is a good way to test the shelf life of food. Common indices used include physical, chemical and microbiological changes as well as sensory evaluation.

Physical changes are usually caused by the mishandling during the harvesting, processing and distribution of foods. Those physical changes will result in the reduction of shelf life of foods. For example, freeze damage in fruits can cause emission of volatiles like methyl hexanoate and ethanol (Obenland et al., 2003). In addition, textural or flavour changes caused by thawing or refreezing, melting or solidifying of fat in bakery products, those changes will all influence the shelf life of foods.

The chemical changes occur during food processing or storage and may result in food deterioration and reduced shelf life. The chemical changes can be caused by external environmental factors (extrinsic factors) or internal food constituents (intrinsic factors). Enzymatic reactions and oxidative reactions, especially non-enzymatic browning and lipid oxidation, are the most important chemical changes related with the shelf life of foods. Lipids are sensitive to oxidation, which leads to oxidative rancidity. To determine the oxidation of lipids, many methods have been used, such as the 2-thiobarbituric acid method and gas chromatography to monitor volatiles (St Angelo & Spanier, 1993). Non-enzymatic browning will also lead to the change in food quality, which is generally caused by the interaction between amino acids and a reducing sugar. Enzymatic reactions also change the quality of foods. For example, lipoxygenase can change the quality of a food at sub-freezing temperature if it is not denatured during the blanching process (Robinson, 2001). Other undesirable changes include the development of free radicals, which result in alteration in color, loss of vitamins, and degradation of proteins (Singh, 2000).

In a food product, the growth of microorganisms can result in the change in pH, formation of toxins and slime, production of enzymes and the production of gas and off-

flavor (Singh & Cadwallader, 2004). Microbial enumeration using agar plating techniques is commonly used to measure the total numbers of bacteria. However, new techniques are available for enumerating bacteria. These include electrical impedance used to evaluate the shelf life of milk and milk products (Bishop & White, 1986). A rapid method used to determine the number of bacteria in milk is to measure adenosine triphosphate through using luciferase and cofactor to produce light (Bossuyt, 1982).

2.2.5 Techniques used to detect specific food poisoning bacteria

The traditional method used is agar plating technique. The basic step involved in this method is pre-enrichment, selective enrichment, selective plating, biochemical screening and confirmation (Vunrcrzant & Pllustoesser, 1987). This method requires a few days to present the result as it is based on the ability of the bacteria tested to multiply to visible colonies.

Fourier transform infrared spectroscopy (FT-IR) is a method used to generate bacterial spectral scans based on the molecular composition of a sample. FT-IR is usually used to classify or identify foodborne pathogens. The method involves such as flow cytometry, the electronic nose (Gupta et al., 2005). In flow cytometry, the cells are carried by laminar flow of water through a focus of light, whose wavelength matches the absorption spectrum of the dye with which the cells have been stained (Mandal et al., 2011). This technique is effective and suitable on the rapid analysis of individual cells. The electronic nose is a system used to identify bacteria, which includes many components such as sensors, flow controllers, and a statistical analyser. In microbiology, a bacterium can be identified by the smell of itself as a clue. The gaseous components released by the microorganisms can be caught by the sensor and analysed. This electronic nose can also be applied to monitor the factors that influence spoilage, such as some volatile components released during the spoilage (Mandal et al., 2011).

Based on the presence or absence of particular genes, bacteria can also be identified by DNA probe hybridization. The polymerase chain reaction (PCR) has been used frequently in research for food microbiology due to its high sensitivity (Olsen et al., 1995). Even though PCR is a highly sensitive and powerful technique, it can still be influenced by the presence of inhibitory compounds in foods (Biswas et al., 2008).

Even there are so many techniques can be used to enumerate or detect bacteria, the agar plating technique is still the most common method used in the assessment of shelf life.

As the assessment of shelf life is more used in commercial areas rather than research, the cost and fitness is the main concern. Plating counting is cheaper and more universal than other novel techniques, thus it is most commonly used in the food industry.

2.2.6 Steps of shelf life testing (Nicoli et al., 2009)

Figure 1 outlines a series of steps for shelf life testing. Firstly, the most critical chemical, physical, and biological events that can cause the deterioration of the food products are identified. Indicators, such as temperature, humidity, or light conditions are defined. Next, shelf life testing under actual or accelerated storage conditions is used to evaluate the changes of the selected quality indicators as a function of time. The final step is to apply the classic kinetic approach and calculate the shelf life at actual storage conditions. If the food products undergo accelerated shelf life testing, the effect of the accelerated factor on the quality decay rate needs to be determined so the accelerated data can be related to the actual storage conditions for the product. Although many articles have produced data on the rate of quality deterioration of many food products under different conditions and supplied in-depth knowledge on food deterioration kinetics, these kinetics are not equivalent to shelf life data. Eventually, some trials need to be done on the actual storage conditions.

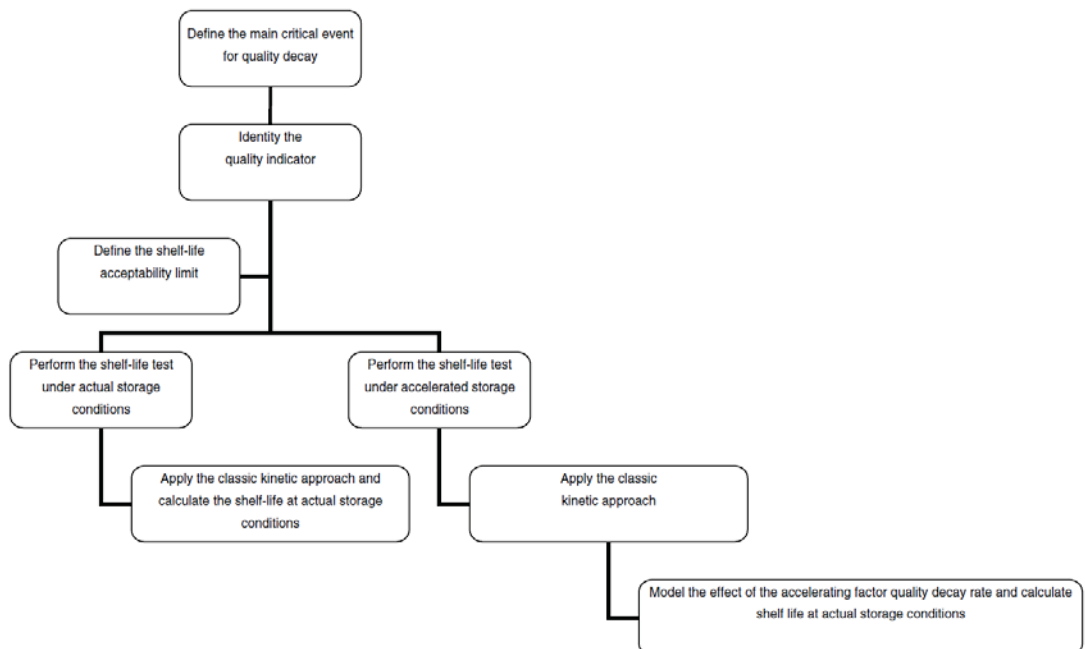


Figure 1. Steps of shelf life testing (Nicoli et al. , 2009)

2.3 Pathogenic bacteria in ready to eat foods.

Among all the pathogenic bacteria that may exist in foods, there some specific bacteria that are of most concern. Some of these are of more concern in specific food products. For example, *Vibrio* species are more often associated with seafood. Table 1 is a list of the bacteria if most concern in ready to eat foods, along with a general indicator of quality, the Standard Plate Count (SPC).

Table 1. Guideline levels for determining the microbiological quality of ready-to-eat foods (Food Standard Australia New Zealand, 2001)

Test	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
SPC	$<10^4$	$<10^5$	$\geq 10^5$	-
<i>Enterobacteriaceae</i>	$<10^2$	10^2-10^4	$\geq 10^4$	-
<i>E. coli</i>	<3	3-100	≥ 100	-
Coagulase +ve <i>staphylococci</i>	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$ set +ve
<i>C. perfringens</i>	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$
<i>B. cereus</i> and other pathogenic <i>Bacillus</i> spp	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$
<i>Vibrio parahaemolyticus</i>	<3	$<3-10^2$	10^2-10^4	$\geq 10^4$
<i>Campylobacter</i> spp	Not detected in 25g	-		≥ 10
<i>Salmonella</i> spp	Not detected in 25g	-	-	$\geq 10^2$
<i>Listeria monocytogenes</i>	Not detected in 25g	Detected but $<10^2$		$\geq 10^2$

* Microbiological Quality = CFU per gram

2.3.1 *Enterobacteriaceae*

The *Enterobacteriaceae* are a large family of Gram-negative bacteria including many harmless symbionts and pathogens like *Escherichia coli*, *Salmonella*, and *Yersinia*.

Members of the *Enterobacteriaceae* family are rod-shaped and usually 1-5µm in length. Most members of this family are a part of the gut microflora of humans' intestines.

In Europe, the *Enterobacteriaceae* have been used for a long time as an indicator of food quality and an index of food safety. In the United States, coliforms have been tested as an indicator of food quality and poor hygiene in food processing environments (Kornacki & Johnson, 2001).

There are several ways to detect *Enterobacteraceae*. One method is culturing the suspect sample on violet red bile glucose (VRBG) agar. After incubation, *Enterobacteraceae* present as purple-red colonies surrounded by a zone of precipitated bile acids.

Another way to count the *Enterobacteraceae* is Petrifilm. Petrifilm is a rapid method for the direct enumeration of *Enterobacteraceae*. Petrifilm used to detect *Enterobacteraceae* is a film containing selective and/or differential agents together with a cold-water soluble gelling agent. *Enterobacteraceae* colonies in the plates are presented as red coloured colonies that are associated with gas bubbles and surrounded by a yellow zone indicative of acid production (Kornacki & Johnson, 2001).

2.3.1.1 *Escherichia coli*

Escherichia coli (*E. coli*) are Gram-negative, facultatively anaerobic, rod-shaped bacteria of the genus *Escherichia* that are commonly found in the lower intestine of warm-blooded animals (Singleton, 2004). *E. coli* is considered as a part of the normal intestinal flora and generally non-pathogenic. Some special serotypes of *E. coli* are pathogenic to both animals and humans, especially for infants and young animals. There are seven groups of *E. coli* that are pathogenic: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EaggEC), diffusely adherent *E. coli* (DAEC), and adherent-invasive *E. coli* (AIEC). Those serotypes of *E. coli* can cause life-threatening diarrhoea and severe sequelae or disability (Croxen et al., 2013).

The general method used to detect *E. coli* is LST-MUG MPN method. Through sub-culturing microorganisms in LST broth, the tubes with *E. coli* will have air bubbles produced within two days' incubation. To confirm *E. coli*, the suspect colonies are sub-cultured on L-EMB plates. Typical *E. coli* colonies are nucleated, dark-centered colonies with or without a metallic sheen.

2.3.1.2 *Salmonella*

Salmonella is a rod-shaped bacillus with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, which is non-spore-forming, predominantly motile enterobacteria and facultative anaerobes (Fàbrega and Vila, 2013). There are only two species of *Salmonella*, *Salmonella bongori* and *Salmonella enterica*. The latter one includes six subtypes: *S. enterica*, *S. salamae*, *S. arizonae*, *S. diarizonae*, *S. houtenae* and *S. indica* (Brenner et al., 2000). Salmonellae are found all around the world in both warm-blooded and cold-blooded animals as well as in the environment. When humans consume food that infected by *Salmonella*, they may get food poisoning leading to uncomplicated enterocolitis or even worse, enteric (typhoid) fever. Enteric (typhoid) fever is a serious disease, resulting in diarrhoea, fever, sleepiness, abdominal pain and headache. *Salmonella* will also cause systemic infections, leading to chronic reactive arthritis (Echeita et al., 1999).

To detect *Salmonella*, samples are pre-enriched to revive injured cells and to provide the best conditions for microbial growth. The culture should then be enriched in a selective medium such as Rappaport-Vassiliadis (RV) medium followed by agar plating (Vassiliadis, 1983). Several different agar can be used to detect *Salmonella*. Bismuth sulphite (BS), Hektoen enteric (HE), and Xylose lysine desoxycholate (XLD) agars are all capable of detecting *Salmonella*. Typical *Salmonella* colonies on BS agar are green colonies with or without a metallic sheen. In addition, the colour of medium surrounding *Salmonella* colonies will change to black from the original brown colour. In terms of HE agar, typical *Salmonella* colonies occur as blue-green to blue colonies with or without black centres. On XLD agar, *Salmonella* occur as pink colonies with or without black centres.

2.3.2 *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*) is a Gram-positive coccobacillus bacterium which belongs to the family of Firmicutes. It is also known as “golden staph” and generally found in the nose, respiratory tract and skin. The presence of *S. aureus* in food products shows a potential health risk to humans as many strains of *S. aureus* can produce enterotoxins that cause food poisoning. When consuming foods that contaminated by *S. aureus*, people may have following symptoms such as nausea, vomiting, abdominal

cramps and diarrhoea. In some severe cases, patients may also have headaches, sweating and fever (Ash, 1997).

The common selective agar used to detect *S. aureus* is Baird-Parker. After incubating the plates of Baird-Parker agar, *S. aureus* colonies are typical circular, smooth, convex, moist, 2-3mm in diameter on uncrowded plates with the colour of gray-black to jet-black (Bennett & McClure, 1993). Generally the colonies have a light-coloured margin and are surrounded by an opaque zone with a clear outer zone. In addition, a coagulase test is used to identify *S. aureus*. Through sub-culturing cultures with coagulase plasma with EDTA, the formation of a clot indicates whether *S. aureus* is coagulase positive or not. There is also another direct enumeration method used specifically for dairy products (Jürgens et al., 1987). This method detects *S. aureus* through melted rabbit plasma fibrinogen agar. Typical *S. aureus* on rabbit plasma fibrinogen agar is grey to black colour and surrounded by an opaque or cloudy zone, which indicates the coagulase activity of *S.aureus*.

2.3.3 *Clostridium perfringens*

Clostridium perfringens (*C. perfringens*) is a rod-shaped, Gram-positive bacterium which is anaerobic, spore-forming and pathogenic (Sherris, 1984). *C. perfringens* can be categorized into five types (A, B, C, D, E) based on the type of toxin. In developed countries, most food poisoning cases of *C. perfringens* reported are caused by type A strains of *C. perfringens* (Bates & Bodnaruk, 2003).

C. perfringens are the third most common cause of foodborne illness in the United States (Scallan et al., 2011). When people are infected by *C. perfringens*, patients will have profuse watery diarrhoea as well as severe abdominal pain. The illness caused by *C. perfringens* is because of the toxin produced in the intestine of human (McClane, 2007).

According to Labbe (2001), the common method used for the enumeration of *C. perfringens* is Tryptose-sulphite-cycloserine (TSC) or egg yolk (EY)-free TSC agar. However, not all strains of *C. perfringens* will produce distinguishable opaque halos on the plates of TSC agar Thus *C. perfringens* requires further confirmation. After incubating anaerobically, *C. perfringens* on TSC agar appear as black colonies, which may be surrounded by a zone of precipitate. To confirm the black colonies on TSC agar are *C. perfringens*, the suspect colonies are transferred into motility-nitrate and lactose

gelatin media. Because *C. perfringens* is non-motile, thus the growth of bacteria will only occur along the line of inoculum and not spread from the stab. In addition, *C. perfringens* can liquefy the gelatin (Hauschild & Hilsheimer, 1974). Thus the lactose gelatin medium will change from red to yellow and also have air bubble produced due to lactose fermentation and gas production.

2.3.4 *Bacillus cereus*

Bacillus cereus (*B. cereus*) is a rod-shaped, Gram-positive bacterium that is usually found in food and soil. *B. cereus* is a spore-forming bacterium, with the spores resistant to dehydration and heating during cooking or storage. When food is contaminated by *B. cereus*, an appropriate temperature between 10 and 50°C can support the bacteria to grow and produce toxin, which can make people sick (Roberts et al., 1996). If people consume any food contaminated by *B. cereus*, it may cause vomiting or diarrhoea based on the type of toxin that *B. cereus* produces. There are two toxins that *B. cereus* can produce. Enterotoxins, also called diarrhoeal toxin, produce diarrhoea. Enterotoxins are produced in the intestines of the host after people consume contaminated foods (Berthold-Pluta et al., 2015). The second toxin, emetic toxin, is formed in the food before consuming, and results in vomiting (Rajkovic, 2014). The formation of emetic toxin requires the presence of starch, therefore foods containing starch are more hazardous if they have been contaminated by *B. cereus*.

There are two agars that can be used for the enumeration of *B. cereus*. In Europe and United States, Mannitol yolk polymyxin (MYP) agar has been used widely. After incubation, *B. cereus* on MYP agar appear as pink to violet colonies. Kim-Goepfert (KG) agar is another medium that can be used for the enumeration of *B. cereus* (Bennett & Belay, 2001). KG agar is used less frequently compared with MYP agar, but both agars are similar in terms of sensitivity and selectivity. *B. cereus* on KG agar appears as translucent or creamy white colonies, which are surrounded by a wide precipitate zone of lecithinase activity (Da Silva et al., 2012). *B. cereus* also requires confirmation using motility tests. *B. cereus* confirmation by testing for rhizoid growth and haemolytic activity is also used (Bennett & Belay, 2001).

2.3.5 *Vibrio parahaemolyticus*

Vibrio parahaemolyticus (*V. parahaemolyticus*) is a Gram-negative and rod-shaped bacterium, which is usually found in seawater, sediments, shellfishes and other different marine species (Pavia et al., 1989, Fishbein *et. al.*, 1974). *V. parahaemolyticus* was firstly identified as a cause of foodborne gastroenteritis after a big outbreak in Japan in 1951, which had caused 272 illnesses and 20 deaths (Fujino et al., 1953).

When people consume raw fish or shellfish that is contaminated by *V. parahaemolyticus*, it can cause vomiting, diarrhoea and abdominal cramps (Rippey, 1994). *V. parahaemolyticus* can produce a haemolysin called thermostable direct haemolysin (TDH), which is enterotoxic and also cardiotoxic. TDH may results in watery diarrhoea, cardiac arrhythmia and involvement and other symptoms (Nishibuchi et al., 1992). Watery diarrhoea usually accompanies nausea, vomiting, fever and abdominal cramps. Generally, those symptoms will resolve in three days, however, they may persist for more than ten days if the patients are immunocompromised (Ryan & Ray, 2004).

To detect *V. parahaemolyticus*, the common way is using thiosulfate citrate-bile salts-sucrose (TCBS) agar. After incubation, a typical *V. parahaemolyticus* colony on TCBS agar is bluish green colour (Karunasagar et al., 1986). As *V. parahaemolyticus* can produce TDH, the presence of TDH can also be an index to enumerate *V. parahaemolyticus*. Chang et al. (1994) identified *V. parahaemolyticus* by using a commercial latex agglutination kit to detect TDH. Honda et al. (1985) developed enzyme linked immunomagnetic sorbent assay to detect TDH.

2.3.6 *Campylobacter* spp.

Campylobacter is a genus of curved bacteria, which is typically spiral-shaped, Gram-negative, oxidase-positive, microaerophilic and non-fermentative. Most of *Campylobacter* species are pathogenic and able to infect both humans and animals. Dozens of species of *Campylobacter* have been involved in human disease. *C. jejuni* is considered as one of the primary sources of bacterial foodborne diseases all around the world, followed by *C. coli* (Ryan & Ray, 2004). Those two species, *C. jejuni* and *C. coli*, together account for more than 95% of all human cases (Lastovica & Allos, 2008).

Campylobacteriosis is the foodborne disease caused by *Campylobacter*. The symptoms of campylobacteriosis include headache, fever and myalgia. In addition, there may be diarrhoea, dysentery, abdominal pain, cramps and fever (may as high as 40°C) (Silva et al., 2011). This is classified as invasive/inflammatory diarrhoea or dysentery. The diarrhoea may be bloody and can be accompanied by nausea and vomiting (Humphrey et al., 2007).

Many methods have been developed for the detection or enumeration of *Campylobacter*. Most methods require a pre-enrichment before plating on agar. Preston, charcoal cefoperazone deoxycholate (CCDA) and Butzler agars are equally effective in detecting *Campylobacter* (Silva et al., 2011). Some rapid methods have also been developed to detect or confirm *Campylobacter*, such as fluorescence *in situ* hybridization (Lehtola et al., 2006), and latex agglutination (Wilma et al., 1992). However, PCR is the most effective way to confirm the bacterium (Silva et al., 2011).

2.3.7 *Listeria monocytogenes*

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive, facultatively anaerobic and rod-shaped bacterium, which exists in many environments includes soil, plants, and meat. *L. monocytogenes* is one of the most virulent food borne pathogens, which can cause the infection listeriosis (Ramaswamy et al., 2007).

Listeriosis is a food borne infection that can take many forms. Ingestion of live microorganisms can lead to temporary intestinal infection with or without diarrhoea. In an immunocompromised host, the pathogen can go on to invade the bloodstream causing septicemia and move on to the central nervous system causing encephalitis or meningitis (Armstrong & Fung, 1993).

For the detection or enumeration of *L. monocytogenes*, Oxford agar and *Listeria* chromogenic agar are effective ways can be used. Pre-enrichment with Fraser broth is required before the plating. On Oxford agar, the colonies of *L. monocytogenes* appear as small, greyish colonies surrounded by black halos. With the incubation, the colonies will become darker and sometimes with a greenish sheen. On *Listeria* chromogenic agar, typical colonies of *L. monocytogenes* appear as blue to blue-green colonies surrounded by an opaque halo after 24 hours' incubation (Speck, 1992).

2.4 Conclusions

RTE foods, also known as convenience foods, are usually ready to consume without any further preparation. Because of their convenience, the demand of RTE foods is increasing and the market is large and growing. The safety of products is critical as they do not require further processing and can be consumed directly. Microbiological risk is a major concern for the RTE foods. The common pathogenic bacteria may found in RTE foods include *Enterobacteriaceae*, *E. coli*, *Salmonella*, *S. aureus*, *C. perfringens*, *B. cereus*, *Campylobacter spp.*, and *L. monocytogenes*. Enrichment and agar plating or selective enumeration of pathogenic bacteria are the most common methods used to ensure the safety of foods as they are cheap and reliable.

2.5 Aim of this study

The aim of this study is to determine the safety of different RTE meals through enumerating several pathogenic bacteria using traditional enrichment and identification or selective enumeration on agar plates.

Chapter Three: Materials and Methods

3.1 Materials:

Eight ready-to-eat meals including four meat and four vegetarian (Compass Group (NZ) Limited, New Zealand);

Buffered peptone water (Merck, New Zealand);

Infrared detector (Rolson, United Kingdom)

Microwave (MGA)

Oven (Kelvinator, United States)

Stomacher (BioMérieux, France)

Coagulase Plasma, Rabbit with EDTA (BD BBL™, France)

3.2 Media used:

Nutrient agar (Merck, New Zealand)

Violet Red Bile Glucose (VRBG) Agar (BD Difco™, France)

Lauryl Sulfate Tryptose Broth with MUG (LST-MUG) (BD BBL™, France)

Baird-Parker Agar (Fort Richard Laboratories Ltd, New Zealand)

Tryptose-sulphite-cycloserine (TSC) Agar (Fort Richard Laboratories Ltd, New Zealand)

Mannitol yolk polymyxin (MYP) Agar (Fort Richard Laboratories Ltd, New Zealand)

Selenite Cysteine (SC) Broth (BD Difco™, France)

Tetrathionate Broth (TB) Base (BD Difco™, France)

Xylose lysine desoxycholate (XLD) Agar (BD Difco™, France)

Hektoen Enteric (HE) Agar (BD Difco™, France)

Brain Heart Infusion (BHI) Broth (Oxoid, New Zealand)

Nitrate Motility Medium Supplemented (Fort Richard Laboratories Ltd, New Zealand)

Lactose Gelatin Medium (Fort Richard Laboratories Ltd, New Zealand)

3.3 Methods:

3.3.1 Sample preparation:

All the samples (eight meals) were received frozen from Compass Group (NZ) Limited and kept at -20°C until required for testing.

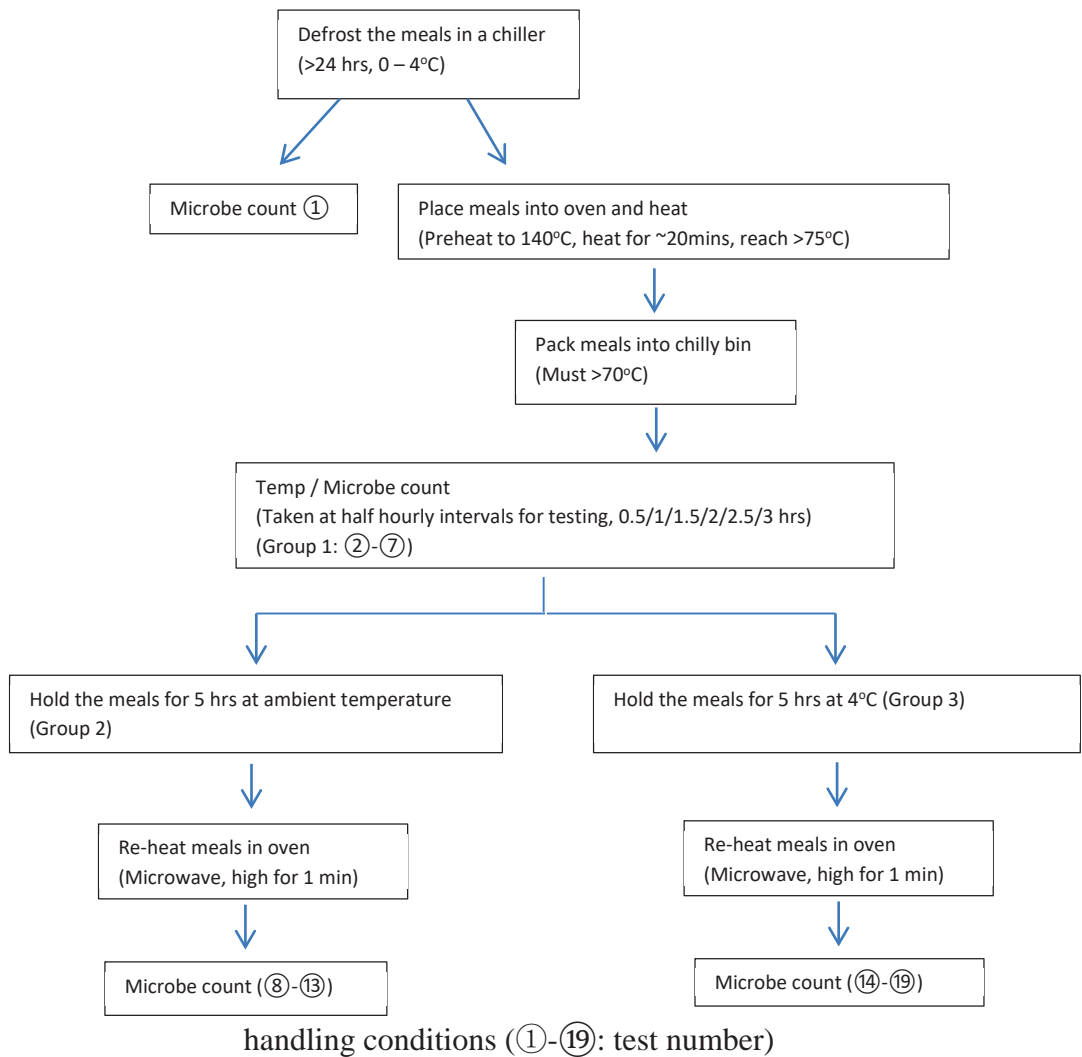
3.3.2 Initial test

This test was designed to explore the effect of different typical consumer handling conditions on the safety of RTE foods at the point of consumption. The process of initial test is shown as Figure 2. Meals were defrosted in a chiller (0-4°C) over 24 hours, and then a 25g sample was aseptically removed from the meal and placed in a stomacher bag with 225 ml sterile peptone water. This 25g sample was collected from each part of the meal. This was homogenised in a peristaltic blender (stomacher) for 30 seconds. Serial 10-fold dilutions (10^{-1} – 10^{-4}) were prepared in 9 ml peptone water and 1 ml volumes were used to inoculate petri dishes in duplicate to be filled with the appropriate agar for microbiological testing (See below for test details). This was to determine the initial microbial count of the meal.

The rest of sample was heated in an oven, which had been pre-heated to 140°C. After reaching above 75°C, which was measured by an infrared detector, the sample was divided into 3 lots and treated in different ways. To measure the temperature, the sample was mixed after removed from the oven, and the surface of sample was measured by the infrared detector. Each lot contained 6 packs of sample. The first group was stored in a chilly bin to keep warm and the samples were tested at half hourly intervals. The second group also stored in a chilly bin at the beginning. Samples were taken out at half hourly intervals. After being taken out from the chilly bin, the samples were left on a bench for another 5 hours before microbiological testing. The treatment of the third group was similar to the second group. The only difference was after taking out from the chilly bin, the samples of third group were put into a cold room of 4°C for another 5 hours. For the first group, the six samples underwent microbial testing as soon as they were removed from the chilly bin. For the second and third group, the samples were re-heated in a microwave to above 60°C after the 5 hours' storage in bench or cold room, and then underwent a microbial test.

3.3.3 Abuse test

This test was designed to determine the effect of gross abuse of RTE foods as may occasionally happen under consumer handling. The process for an abuse test is shown as Figure 3. The preparation of samples used in abuse tests was the same as in the first experiment – defrosting and heating. After defrosting, 25g of sample was tested for the initial microbial count. The rest of sample was heated in the oven. The meal was divided into six packages and held on the bench (abuse). Over the next three days of the abuse test, two packs of samples were tested each day. One pack was tested for the microbial count directly. Another one was heated in a microwave for 1 minute before microbiological testing. The treatments of samples on the second and third day were the same as the first day. **Figure 2.** Process for initial testing of the meals under simulated



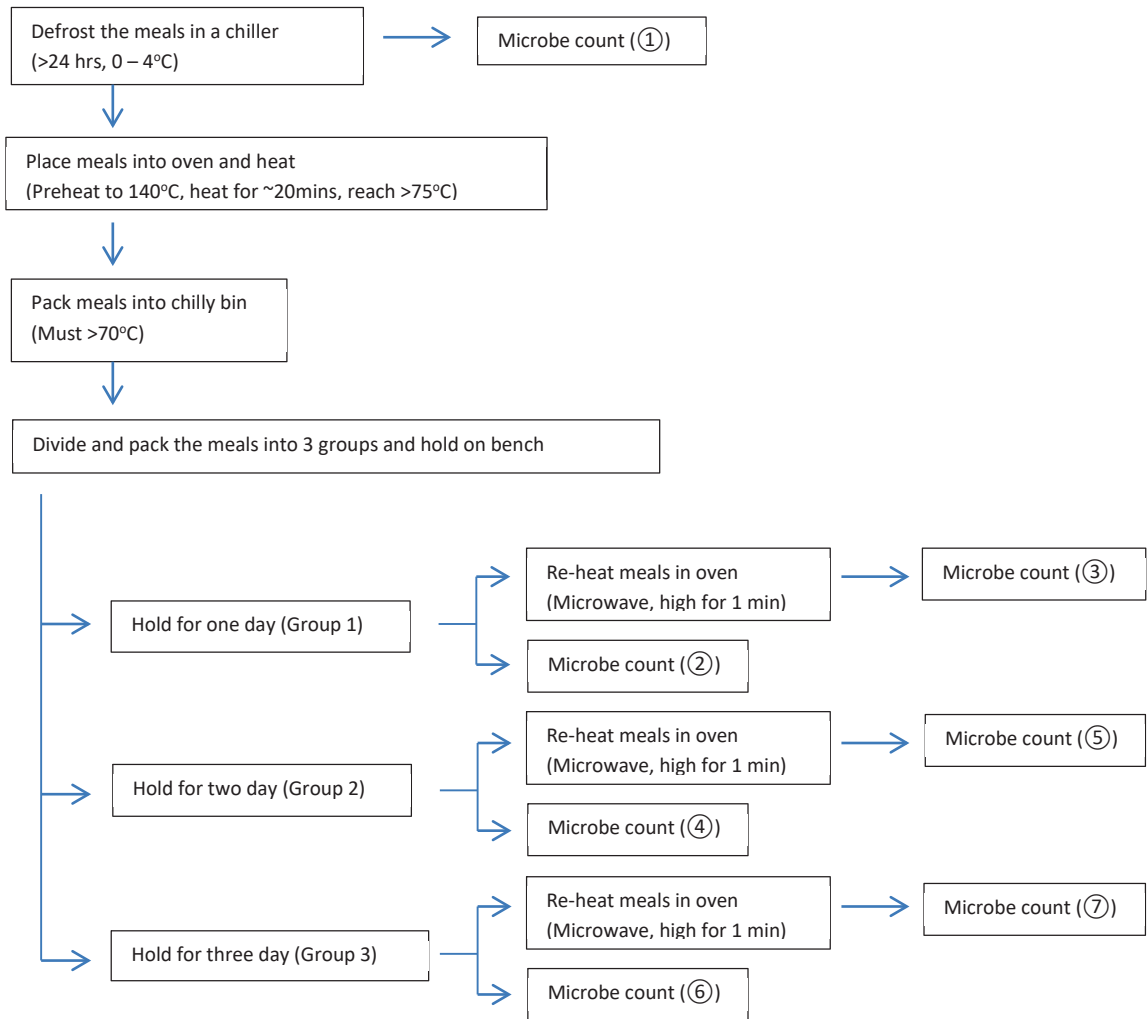


Figure 3. Process of abuse test (①-⑦): test number

3.3.4 Microbial tests:

All the samples were tested for a standard plate count and the population of *Enterobacteriaceae*, *E. coli*, *S. aureus*, *C. perfringens*, *B. cereus* and *Salmonella*. All the microbial tests were based on the APHA methods (Downes & Ito, 2001). The selection of the bacteria was chosen by the client sponsoring this trial and was constrained by costs. Other pathogens that would have been worthwhile testing were *Campylobacter* (in particular for the chicken product) and *Listeria*.

The following are the details for each method used in this trial:

SPC: 1ml of sample was added to petri dishes and then about 15ml of nutrient agar was poured into the plate and allowed to set. The plates were inverted and incubated at 30°C for 1-2 days.

Enterobacteriaceae: 1ml of sample was added to petri dishes and then about 15ml of VRBG was poured into the plate and allowed to set. The plates were inverted and incubated at 35°C for 18-24 hours. The suspect positive colonies should be purple-red and surrounded by a zone of precipitated acids.

E. coli: Most probable number (MPN) method was applied to test *E. coli*. Each dilution of sample was tested in triplicate. 1ml of sample was added to each of three tubes with 10ml of LST-MUG. All the tubes were incubated at 35°C for 1-2 days. After the incubation, all the tubes were checked under UV light. The positive results should be those tubes with pale blue fluorescence showed as well as air bubbles appeared inside the small glass in the tubes.

Salmonella: 1.0 ml of sample was transferred replicate to 10 ml of SC broth and 10 ml of TBG broth. Both of SC and TBG broths were incubated at 35°C for 24 hours. A 3 mm loop of SC culture was streaked on XLD and HE agar. Repeat plate inoculations with TBG enrichment culture. All the plates were incubated at 35°C for 24 hours. In XLD agar, the suspect positive *Salmonella* strains should be red colonies with or without black centres. In HE agar, the suspect positive colonies should be green to blue colonies with or without black centres.

S. aureus: 0.1 ml of sample was spread over a pre-made plate of Baird-Parker agar. The plates were incubated at 35°C for 45-48 hours. After incubation, *S. aureus* showed a precipitate and clearing of the medium. The suspect positive colonies should be black colonies surrounded by clear zones normally.

C. perfringens: 0.1 ml of sample was spread over a pre-made plate of TSC agar. The plates were incubated anaerobically at 35°C for 18-24 hours. The suspect positive colonies should be black colonies surrounded by a zone of precipitate.

B. cereus: 0.1 ml of sample was spread over on a pre-made plate of MYP. The plates were incubated at 30°C for 20-24 hours. The suspect positive colonies should be pink to violet colour and surrounded by a zone of precipitate.

3.3.5 Confirmation test:

S. aureus: Suspect colonies were transferred to tubes containing 0.2 ml BHI broth. A 0.5 ml volume of coagulase plasma with EDTA was added to the tubes. Those tubes were incubated at 37°C and examine periodically during a six hour interval for clot formation. (Downes & Ito, 2001)

C. perfringens: ①Motility nitrate reduction test: Suspect colonies were stabbed into motility nitrate medium and then incubated at 37°C for 24 hours. ②Lactose gelatin medium: Suspect colonies were stabbed into lactose gelatin medium and then incubated at 37°C for 22-24 hours. (Downes & Ito, 2001)

B. cereus: Suspect colonies were collected and transferred to a sterile 0.2ml PCR tube with 20ul RNase free water. Then the following reagents were added into a 0.2ml PCR tube sequentially: 25µl master mix, 20µl RNase free water, 1µl primer BCFomp1 (5'-ATCGCCTCGTTGGATGACGA-3', forward, 24 to 43), 1µl primer BCRomp1 (5'-CTGCATATCCTACCGCAGCTA-3', reverse, 598 to 577), and 3µl sample from last step, to make up a final reaction volume of 50µl. After mixing well, those tubes were placed in the PCR machine and the programme corresponding was selected to make the primers being used. The next step is interpreting the results. E gel unit was inserted into E gel iBase power system. Then 10µl of diluted DNA ladder was loaded into lane M of E gel and 10µl of sample was loaded into each well of the top row. After running the system, the result could be checked with the UV light (Oliwa-Stasiak et al., 2010).

3.5.6 Statistical analysis

To ensure reproducible results, all counts were done in triplicate on agar plates and the results reported as mean plus standard deviation. Each test on a particular meal under specific conditions was repeated. Each standard deviation was calculated by Microsoft Excel worksheet.

Chapter Four: Results

All the results are summarised in Tables 2, 3 and 4. Table 2 shows the initial population of bacteria existed in eight meals after those meals defrosted and before the heating process. Table 3 shows the population of different bacteria detected after heating in the initial trial. The results of abuse test were listed in Table 4, showing how the population of bacteria changed during the four days' testing period.

4.1 Initial test

The initial tests of each meal, the populations microorganisms in four meals (chicken & lemon sauce, pork & cranberry loaf, casserole chickpea, and vegetable lasagne) were less than the limits in the specification as shown in Table 1 (Food Standard Australia New Zealand, 2001), which means that those four meals were considered safe at the start of the trial. After heating the microorganisms were less than the detectable limits of the tests (<1 cfu/mL) in three of the meals (chicken & lemon sauce, pork & cranberry loaf, and vegetable lasagne).

For the chickpea meal, *S. aureus* and *C. perfringens* were detected in the end of the test. They appeared in the food after heating and three hours in a chilly bin plus 5 hours in a cold room at 4°C. The population of *S. aureus* was $1.67 \pm 1.15 \times 10^2$ cfu/g, which is marginal according to the specifications. In addition, the population of *C. perfringens* was $1.17 \pm 0.35 \times 10^2$ cfu/g, which exceeds the marginal safe level, according to the specifications. It was also shown that a few bacteria were detected in the standard plate count in other test point. A count of $2.07 \pm 0.68 \times 10^3$ cfu/g of bacteria was detected after 2 hours in the chilly bin plus 5 hours at 4°C.

For the other four meals (hot pot sausage, BBQ beef, quiche golden, and pie rice & vegetable), the results exceeded the acceptable specification limits for one or more tests.

For the hot pot sausage, *S. aureus*, *C. perfringens* and *B. cereus* were detected in the initial test before heating process. The tests showed that the population of *S. aureus* ($1.33 \pm 0.57 \times 10^2$ cfu/g) was marginal while the population of *C. perfringens* ($4.17 \pm 1.91 \times 10^3$ cfu/g) was unsatisfactory and *B. cereus* ($2.73 \pm 1.8 \times 10^4$ cfu/g) was potentially hazardous. The result of the standard plate count was $5.77 \pm 2.63 \times 10^4$ cfu/g, which is marginal. After heating in the oven, no microorganisms were detected until the last two tests. After storing at the chilly bin for 2.5 or 3 hours plus 5 hours at

4°C, *C. perfringens* was detected at $1.67 \pm 0.11 \times 10^2$ cfu/g and 1.33 ± 0.58 cfu/g, respectively. Both these results were considered “marginal”.

The result for BBQ beef showed *C. perfringens* and *B. cereus* were detected in the initial test before heating ($5.50 \pm 1.57 \times 10^4$ cfu/g of *C. perfringens* and $4.67 \pm 0.67 \times 10^4$ cfu/g of *B. cereus*), both of which were “unsatisfactory”. After heating, no microorganisms were detected in the sample.

S. aureus, *C. perfringens* and *B. cereus* were detected in the initial tests of quiche golden. The population of *S. aureus*, *C. perfringens* and *B. cereus* were $1.67 \pm 1.15 \times 10^2$ cfu/g, $2.07 \pm 0.96 \times 10^3$ cfu/g, and $4.67 \pm 0.58 \times 10^2$ cfu/g, respectively. Compared with the standard, the population of *S. aureus* and *B. cereus* were “marginal” while *C. perfringens* were “unsatisfactory”. The result of standard plate count was $4.01 \pm 1.64 \times 10^3$ cfu/g, which is “satisfactory” but close to the “marginal” level. After heating, no microorganisms were detected during the test period.

For the last meal, pie rice & vegetable, *C. perfringens* and *B. cereus* were detected in the initial test. Standard plate counts were $4.77 \pm 1.08 \times 10^3$ cfu/g which is below the marginal level. The population of *C. perfringens* was “marginal” at $1.33 \pm 0.58 \times 10^2$ cfu/g. *B. cereus* was at the marginal level. Its population was $1.03 \pm 0.25 \times 10^2$ cfu/g. After heating, both tests showed no microorganisms.

Table 2. Initial test results of bacterial count

Meal type	SPC	<i>S.aureus</i>	<i>C.perfringens</i>	<i>B.cereus</i>
1	$1.33 \pm 0.58 \times 10^1$	<100	<100	<100
2	$1.67 \pm 1.15 \times 10^1$	<100	<100	<100
3	$5.77 \pm 2.63 \times 10^4$	$1.33 \pm 0.58 \times 10^2$	$4.17 \pm 1.91 \times 10^3$	$2.73 \pm 1.8 \times 10^4$
4	$5.17 \pm 1.35 \times 10^4$	<100	$5.50 \pm 1.57 \times 10^4$	$4.67 \pm 0.67 \times 10^4$
5	$6.33 \pm 3.78 \times 10^1$	<100	<100	<100
6	$3.33 \pm 2.08 \times 10^1$	<100	<100	<100
7	$4.01 \pm 1.64 \times 10^3$	$1.67 \pm 1.15 \times 10^2$	$2.07 \pm 0.96 \times 10^3$	$4.67 \pm 0.58 \times 10^2$
8	$4.77 \pm 1.08 \times 10^3$	<100	$1.33 \pm 0.58 \times 10^2$	$1.03 \pm 0.25 \times 10^2$

* The unit of each data is cfu/g

* Yellow-Marginal; Turquoise-Unsatisfactory; Pink-Potentially Hazardous

* Meal type: 1: Chicken & Lemon Sauce; 2: Pork & Cranberry Loaf; 3: Hot Pot Sausage; 4: BBQ Beef; 5: Casserole Chickpea (Vege); 6: Lasagne Vege; 7: Quiche Golden; 8: Pie Rice & Vegetable.

Table 3. The bacterial count after heating in different meals in initial test

Meal type	Test Number	SPC	<i>S.aureus</i>	<i>C.perfringens</i>
1		<10	<100	<100
2		<10	<100	<100
3	T18	1.33±0.58x10 ²	<100	1.67±1.15x10 ²
	T19	1.70±1.13x10 ²	<100	1.33±0.58x10 ²
4		<10	<100	<100
5	T19	2.07±0.68x10 ³	1.67±1.15x10 ²	1.17±0.35x10 ³
6		<10	<100	<100
7		<10	<100	<100
8		<10	<100	<100

* The unit of each data is cfu/g.

* Yellow-Marginal; Turquoise-Unsatisfactory

* Meal type: 1: Chicken & Lemon Sauce; 2: Pork & Cranberry Loaf; 3: Hot Pot Sausage; 4: BBQ Beef; 5: Casserole Chickpea (Vege); 6: Lasagne Vege; 7: Quiche Golden; 8: Pie Rice & Vegetable.

* T18: Stored at a chilly bin for 2.5 hours plus 5 hours at 4°C; T19: Stored at a chilly bin for 3 hours plus 5 hours at 4°C

4.2 Abuse test

The abuse testing result of BBQ beef meal is shown in Table 4. *S. aureus*, *C. perfringens* and *B. cereus* were all detected in the sample. The microbial count of initial test after defrosting showed that both *C. perfringens* and *B. cereus* were at a “potentially hazardous” level, at greater than 10⁴ cfu/g. The populations of *S. aureus* and SPC were in “marginal” level, at 1.00 ± 0.56 x 10² cfu/g and 2.97 ± 0.55 x 10⁴ cfu/g, respectively. After heating in the oven and abusing for one day, the population of those pathogenic bacteria were lower than the initial result. No *S. aureus* was detected and the result of SPC was 1.17 ± 0.21 x 10⁴ cfu/g, which was still in “marginal” level. For *C. perfringens* and *B. cereus*, both of them were still “potentially hazardous”, with the population of

1.05 ± 0.13 x 10⁴ cfu/g and 1.90 ± 0.56 x 10⁴ cfu/g, respectively. However, after reheating in microwave, no bacterium was detected.

In the third day of abuse, all the bacteria detected reached a “potentially hazardous” level before the sample was heated. The population of *C. perfringens* and *B. cereus* had reached to above 10⁷ cfu/g, while the population of *S. aureus* was 3.67 ± 1.05 x 10⁵ cfu/g. The standard plate count was 1.15 ± 0.12 x 10⁸ cfu/g. After heating, *S. aureus* and *C. perfringens* were still detected. The populations were at the “marginal” level with the population of 1.0 ± 0.58 x 10² cfu/g. In addition, the stand plate count was 5.67 ± 3.06 x 10¹ cfu/g, which is regarded as “satisfactory”.

On the fourth day, the sample before heating showed a similar result to the third day with the bacteria detected at levels that were “potentially hazardous”. The populations of *S. aureus*, *C. perfringens*, and *B. cereus* were 1.40 ± 0.10 x 10⁶, 7.67 ± 2.08 x 10⁷, and 6.33 ± 2.31 x 10⁷ cfu/g, respectively. A population of 1.83 ± 0.15 x 10⁸ cfu/g of bacteria was detected in the stand plate count. After heating in the microwave, no *S. aureus* was detected. The result of SPC was at a “satisfactory” level with the population of 8.33 ± 3.51 x 10¹ cfu/g. However, *C. perfringens* and *B. cereus* were detected. Both of them were “marginal”, with populations of 1.00 ± 0.58 x 10² and 2.00 ± 1.15 x 10² cfu/g, respectively.

Table 4. The bacterial count of abuse test of BBQ beef meal

Day	Test number	SPC	<i>S. aureus</i>	<i>C. perfringens</i>	<i>B. cereus</i>
1	T1	2.97±0.55x10 ⁴	1.00±0.56x10 ²	3.67±0.42x10 ⁴	2.87±0.38x10 ⁴
2	T2	1.17±0.21x10 ⁴	<100	1.05±0.13x10 ⁴	1.90±0.56x10 ⁴
	T3	<10	<100	<100	<100
3	T4	1.15±0.12x10 ⁸	3.67±1.05x10 ⁵	5.43±2.71x10 ⁷	2.83±0.50x10 ⁷
	T5	5.67±3.06x10 ¹	1.00±0.58x10 ²	1.00±0.58x10 ²	<100
4	T6	1.83±0.15x10 ⁸	1.40±0.10x10 ⁶	7.67±2.08x10 ⁷	6.33±2.31x10 ⁷
	T7	8.33±3.51x10 ¹	<100	1.00±0.58x10 ²	2.00±1.15x10 ²

* The unit of each data is cfu/g.

* Yellow-Marginal; Pink-Potentially Hazardous

*T1: Initial test after defrosting; T2, T4, T6: Direct test on each day; T3, T5, T7: Test after re-heating

Chapter Five: Discussion

As the results show that the microorganisms were less than the detectable limit of the tests in three of the meals (chicken & lemon sauce, pork & cranberry loaf, and vegetable lasagne), those three meals appear to be low risk. In the chicken & lemon sauce meal, the addition of lemon sauce would reduce the pH of the meal, which could possibly inhibit the growth of bacteria. Chicken is a high risk food (Santacruz, 2016), thus this meal may undergo an especially strict process to ensure the safety.

BBQ beef produced the highest initial population of microorganisms. *C. perfringens* and *B. cereus* were both detected in the initial test and their populations were unsatisfactory. After heating, in terms of bacterial population, this meal could be considered as acceptable as the level of each microorganism was satisfactory. However, *B. cereus* and *C. perfringens* can produce enterotoxin. Thus if the meal has not been heated thoroughly before consuming, it may still cause foodborne illness. The meal should be heated above 56°C for 5 minutes to inactivate the enterotoxin (Watson, 1998).

The result of abuse testing of the BBQ beef meal showed that heating is an effective process to kill microorganisms in the meal. Comparing the population detected before and after heating the microwave in the same day showed that the treatment of heating almost killed all the microorganisms in the sample. After heating, no microorganisms or only a few microorganisms could be detected. Thus in terms of the population of microorganisms, the meal can be considered as acceptable after heating, even after the meal had been held for three days at room temperature following heating. However, if toxins were produced, as would occur with contaminants *B. cereus*, *C. perfringens*, and *S. aureus*, the meal may still be hazardous. The numbers of these toxin producing bacteria reached levels that would be a concern for toxin production after the third day of the trial, although few bacteria were detected after re-heating. The enterotoxin of *B. cereus* can be inactivated by heating above 56°C for at least 5 minutes, but the emetic toxin is extremely heat tolerant. According to Watson (1998), the emetic toxin produced by *B. cereus* can survive in 121°C for 90 minutes.

In the hot pot sausage meal, *C. perfringens* appeared after 2.5 hours in a chilly bin and 5 hours when held at 4°C. The populations of *C. perfringens* in the meal after 2.5 hours in a chilly bin plus 5 hours at 4°C and after 3 hours in a chilly bin plus 5 hours at 4°C were both marginal, which means that this meal becomes a food safety risk after the 8 hours

storage. *S. aureus* and *C. perfringens* were detected in the sample of casserole chickpea in the end of the test, after the sample had been stored in a chilly bin for 3 hours and then held at 4°C for another 5 hours. *S. aureus* reached a marginal level and the *C. perfringens* was graded unsatisfactory. The detection of these two microorganisms shows that the food under these handling conditions becomes a food safety risk. When bacteria were detected at the end of the trial, this showed that the handling conditions enabled low levels of bacteria in the food to grow after heating. The sausage and chickpea meals may provide a suitable environment for *S. aureus* and *C. perfringens* growth as these bacteria appeared to multiply quickly. For other meals, the nature of the ingredients (intrinsic properties of the food) such as the lemon sauce in the chicken meal, might limit the growth of bacteria.

Chapter Six: Conclusions

Three meals (chicken & lemon sauce, pork & cranberry loaf, and lasagne veg) are considered as acceptable during the handling and storage conditions tested in this trial. The populations of each microorganism tested were determined to be satisfactory at each test point.

The meal of casserole chickpea was satisfactory at the beginning of the trial. *C. perfringens* and *S. aureus* were detected at the end of testing causing these meals, resulting in this meal grading as marginal for food safety, based on the reference criteria. Thus casserole chickpea should be handled with caution after eight hours storage following heating and storing in a chili bin for 3 hours plus another 5 hours at 4°C.

The other four meals (hot pot sausage, BBQ beef, quiche golden, and pie rice & vegetable) were considered unsatisfactory with microbial levels reaching marginal or unsatisfactory levels on initial testing.

In hot pot sausage and quiche golden meal, marginal levels of *S. aureus* and *B. cereus* were detected and *C. perfringens* was unsatisfactory at the start of the trial. After heating in an oven and storing for several hours, quiche golden was free of microorganisms, which shows that quiche golden is acceptable after heating. However, *C. perfringens* was detected in the sausage meal at the end of the trial. A marginal level of *C. perfringens* was detected after 2.5 hours in a chilly bin plus 5 hours at 4°C.

In BBQ beef meal, the initial test showed that this meal had the highest population of microorganisms. After heating, no microorganisms were detected. However, there is concern about the initial population of *C. perfringens* and *B. cereus*, as both these bacteria produce enterotoxin. Thus the meal should be heated before consuming to inactive the enterotoxin. As heating can inactive the enterotoxin and kill the pathogenic bacteria as well, therefore, the meal should be safe after heating.

The pie rice & vegetable meal was marginal in the beginning of the trial with 2.0×10^2 cfu/g of *C. perfringens* and 1.0×10^3 cfu/g of *B. cereus* detected. Because no microorganisms were detected in the meals for the rest of the trial, it can be considered that the pie rice & vegetable meal was acceptable after heating. However, the toxins produced by *B. cereus* and *C. perfringens* may still be a risk. As mentioned above, the enterotoxin would cause foodborne illness if the meal has not been heated thoroughly

before consuming. Also this meal contains rice, which means the starch would enable *B. cereus* emetic toxin production. The emetic toxin produced is heat-stable and can withstand 121°C for 90 minutes (Watson, 1998). Considering the marginal level of *C. perfringens* and *B. cereus* detected in the initial test, the toxin may be a low risk for consumers.

When the meals had been abused for more than one day, even if the re-heating treatment could kill the pathogenic bacteria and inactivate some toxins in the meal, there is still the possibility of the survival of some toxins such as emetic toxin of *B. cereus*.

The meals chosen to be tested include most common meat people may consume, such as pork, beef and chicken, and vegetables like potato, broccoli, and carrot. The ingredients involved are all common foods that most people may have. Among all those ingredients in eight meals, chicken and rice may be more risky than other ingredients. *B. cereus* was found in the rice meal and it can produce emetic toxin in the presence of starch. The emetic toxin produced is heat-stable and hard to inactivate. Chicken is a high risk food causing food poisoning as it is commonly contaminated by *Campylobacter* and *Salmonella* (Santacruz, 2016). *Salmonella* and *Campylobacter* were not detected in the chicken meal as these were not required by the sponsor of this trial. However, it is recommended that these be included in the assessment of any chicken product for safety.

Reproduction of the trial is ensured by testing meals in triplicate. The result of triplicate tests showed good agreement. In this trial, the meals provided by the manufacturer were assumed to be typical. Variations between batches of product are possible and have not been considered in this trial.

In summary, with the meals that we tested, the overall recommendation would be to insist that customers heat the meals as we were instructed to do for these trials and consume the meals immediately after defrosting and heating to ensure safety. If the meals had been abused for few days, the recommendation is not to consume those meals even if reheating is an effective way to control the pathogenic bacteria and enterotoxin.

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