MODELLING THE EFFECTS OF LOCAL FOOD SPICES ON THE GROWTH OF *Escherichia coli* 0157:H7 AND *Campylobacter* sp.

BY

ADELEYE, SAMUEL ADENIYI

(B.TECH MICROBIOLOGY)

REG. NO: 20154990278

A THESIS SUBMITTED TO THE POST GRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI, IMO STATE, NIGERIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE DEGREE (M.Sc) IN FOOD AND INDUSTRIAL MICROBIOLOGY

NOVEMBER, 2018

CERTIFICATION

This is to certify that this research work titled "**Modelling the effects of Local Food spices on the growth of *Escherichia coli* 0157:H7 and *Campylobacter* sp**" was carried out by **Adeleye, Samuel Adeniyi** (**20154990278**) of the Department of Microbiology, Federal University of Technology, Owerri, Nigeria under the supervision of Dr. W. Braide and Dr. E.C. Chinakwe in partial fulfillment of the award of a Master of Science (M.Sc.) degree in Food and Industrial Microbiology.

..... 2-11-18

Dr. W. Braide — Date

(Supervisor)

..... 2/11/2018

Dr. E.C. Chinakwe — Date

(Co-Supervisor)

..... 2/11/2018

Dr. C.O. Nweke — Date

(HOD, Microbiology)

....

Prof. J.N. Ogbulie — Date

(Dean, School of Biological Science)

....

Prof. (Mrs) Nnenna. N. Oti — Date

(Dean, Post-Graduate School)

..... 5/11/18

Prof. D.J. Aiyegoro — Date

(External Examiner)

DEDICATION

This research work is dedicated to God who has always remained a father and provider of unlimited grace to accomplish every academic race.

ACKNOWLEGEMENTS

I give gratitude to God for giving me the privilege and grace to continue my education to this Level. He has been the invisible force behind my excellence.

I wish to use this opportunity to appreciate my supervisors Dr. Wesley Braide and Dr. E.C. Chinakwe for their tireless efforts devoted to the accomplishment of this task. I also appreciate the Dean of School of Biological Sciences, Prof. J.N. Ogbulie for his mentorship and Fatherly advice all through my program. In addition, My Postgraduate coordinator (Dr. Wesley Braide), and the Head of Department (Dr. C.O Nweke) have been awesome in making sure this program is accomplished with good speed.

My profound gratitude also go to Ogwumike Stephanie, who has assisted me continually in the laboratory, and for being an unending source of encouragement towards the pursuit of my academic goals.

Special thanks goes to My family, My Friend and all well-wishers who has played special roles in one way or the other to accomplish this work. To others too numerous to count, I sincerely appreciate you.

TABLE OF CONTENT

LIST OF FIGURE

LIST OF TABLES

ABSTRACT

The activity of food spices (pepper, ginger and salt) were investigated for their ability of inhibit or stimulate the growth of *Campylobacter jejuni* and *Escherichia coli* 0157:H7, isolated from meat and coleslaw samples, in singles and in combination. In addition, the isolates were subjected to temperature dependent inactivation for the development of primary models using the GinaFit Software. A 2^k Factorial plot was carried out to determine the single and combined effect of the spices on the test isolates. Results shows that at a concentration of 800 µg/ml, ginger extracts had stationary/inhibitory effect and pepper had inhibitory effects while salt also had inhibitory effects at 4% concentration. At 8% concentration, results indicated a bactericidal effect as the mean growth declined rapidly below the initial bacterial population. Similar results was also recorded for ginger at 1200 µg/ml. However, pepper had stimulatory effect on *Campylobacter jejuni*. On the other hand, results on *Escherichia coli* 0157:H7 shows that the pepper extracts had mean stimulatory effect at 400 µg/ml, while the salt extracts had similar results at 4% salt concentration. Mean inhibitory effect was obtained for ginger extracts at 400 µg/ml and 800 µg/ml but, stimulatory at 1200 µg/ml. Also, at a concentration of 800 µg/ml, pepper extracts had inhibitory effects, while salt solution also had stimulatory effects at 8% concentration. At 12% salt concentration, the effect on mean growth was bactericidal demonstrated by rapid decline below the initial bacterial population. GINAFIT Models were used to determine values of "A, δ_1, P, Log N, K_{max}, R^2, MSE" which were adopted for model development. Findings had it that at -4 and 4 degrees, the isolates was inactivated. In addition, *Campylobacter jejuni* tested grew at 25 and 35 degrees while *Escherichia coli* grew at 25, 35 and 45 degrees but inactivated at -4 degrees.

Keywords: Inactivation, Modelling, Spices, Factorials, *Campylobacter* sp, *Escherichia coli*.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

God has blessed us with so much in Nature that the secret to all healthy living has been embedded in Nature. Indeed, the use of plants as sources of remedy to different ailments, food and shelter are dated as the existence of Man (The KJV Holy Bible, 1979). Among others, the burden of foodborne infections and illnesses is globally ravaging. Africa has numerous food spices of plant origin with promising features, and diverse applications (Okorondu *et al*., 2015). Many of these plant spices have been explored for antimicrobial properties (Adeleye *et al*., 2016; Adeleye *et al*., 2018; Mike-Anosike *et al*., 2018). However, fewer of the plants have been modeled for industrial applications.

Pepper (*Piper nigrum*), ginger (*Zingiber officinale* roscoe) and Salt are common spices used in Nigerian delicacies which have also found a wide application in medicine. Ginger, the underground rhizome of the ginger plant that belongs to family Zingiberaceae, is an important plant with several medicinal, ethnomedicinal and nutritional values (Kumar *et al*., 2011; Subash and Anand, 2014). Among other potentials; it has been demonstrated to have great antimicrobial potentials as it has been used in folk medicine for curing heart problems, treat stomach upset, diarrhea, and nausea (Shukla and Singh, 2007). Besides, it is used as curry powder, hot drinks like ginger beer, ginger wine, ginger tea (Kumar *et al*., 2011). Ginger has been identified as an herbal medicinal product with pharmacological effect (Subash and Anand, 2014).

Pepper, a member of Piperaceae family, contains some of the antimicrobial components such as terpinene, α-pinene, β-pinene, linalool and terpineol (Dean *et*

al., 1992; Sweetman and Martindale, 2002; Khani *et al*., 2011). Capsaicin is the major active chemical compound of peppers (8-methyl-N-vanillyl-6-nonenamide) (Sweetman and Martindale, 2002). Capsaicin and several related compounds are called capsaicinoids and are produced as secondary metabolites by chili peppers, which are used as deterrents against herbivores. Pure capsaicin is a hydrophobic, colourless, odourless, and crystalline to waxy compound (Khani *et al*., 2011). Khan and Siddiqui (2007) evaluated the antibacterial potential of aqueous decoction of a member of the Piper genus, "*Piper nigrum* (black pepper)", *Laurus nobilis* (bay leaf), *Pimpinella anisum* (aniseed), and *Coriandum sativum* (coriander) against different bacterial isolates from oral cavity of two hundred individual volunteers. Black pepper extracts (aqueous decoction) showed the strongest antibacterial activity comparable to an aqueous decoction of *Laurus nobilis* and *Pimpinella anisum* at the concentration of 10µL/disc.

Meat and vegetables are good media for the growth and proliferation of pathogenic microorganisms (Line *et al*., 2013; Chai *et al*., 2017; Braide *et al*., 2017). This is because they contain most of the vital minerals, organic carbon and nitrogen sources and water activity. Microorganisms, ambient or transient, find these food materials interesting owing to the fact that their properties support microbial proliferation. As a result, the need to inhibit the microbial activity by food processing industries that use them as raw materials is highly needed (Chai *et al*., 2017).

This burden and cost of food preservation, and the increased rate of incidence of foodborne infections, locally set a challenge to food microbiologist and places the need for methods that could substitute conventional food processing preservatives and preservation methods. This drives the research into the use of locally available spices to inactivate microbial activities in a procedure known as Predictive

Microbiology. In the first book on the subject, published just over 20 years ago, McMeekin *et al*. (1993) defined it as a quantitative science that enables users to evaluate objectively the effect of processing, distribution and storage operations on the microbiological safety and quality of foods. The goal of Predictive Microbiology is to develop mathematical equations that describe the behaviour of microorganisms under different environmental factors (physical, chemical, competitive). Predictive modeling of bacterial growth and inactivation is an important research topic among Food Microbiologists (Buchanan, 1993, Skinner and Larkin, 1994, McMeekin *et al*. 1997). Predictive models allow estimating the shelf-life of foods, isolate critical points in the production and distribution process and can give insight on how environmental variables affect the behaviour of pathogenic or spoilage bacteria. Predictive Microbiology provides us with an estimate of the potential growth of particular microorganisms under a variety of conditions. The models used in are developed from experimental work and derives immediate and futuristic inference.

1.1 Statement of problem

Foodborne infection and spoilage is a global concern and annually, millions of tons of food are lost to spoilage. Among others is the predominant infection and spoilage caused by bacteria such as the infections/spoilage caused by *Campylobacter* spp and *Escherichia coli* 0157: H7. Interestingly, Nigerian spices (Usually used for preparing local delicacies) have been identified to possess antimicrobial and medicinal properties. However, inhibitory thresholds of these spices are commonly investigated in singles and do not conform to their application in traditional methods of food preparation but, rather its potentials for medical applications. These spices have been poorly harnessed locally for industrial applications owing to the absence of models that can predict the action of

these spices on microorganisms in food in the presence and absence of temperature. This work tends to investigate the effect of selected food spices in singles and in combinations on selected microbial activities. Primary models will be developed for informed application of these spices in the homes locally/domestically and in industries.

1.2 Aim and Objectives of the project

1.2.1 Aim of the project

This project studies the modeling of the effects of Local Food spices on the growth of *Escherichia coli* 0157: H7 and *Campylobacter* sp.

1.2.2 Objectives of the project

i. To Isolate and characterize of *Campylobacter* sp and *Escherichia coli* from meat and Coleslaw samples using standard microbiological procedures and ABIS Online identification system.
ii. To investigate the effect of single and mixed spices of three food spices on the growth of *Escherichia coli* and *Campylobacter* sp using factorial design executed with MINITAB 17 Statistical Software.
iii. To Model, the temperature dependent inactivation of *Escherichia coli* and *Campylobacter* sp in the absence of different spices mix using GinaFiT modeling software.
iv. To develop primary models for application in food industries using the GinaFiT Modelling Software.

CHAPTER TWO

LITERATURE REVIEW

2.1 Modelling of bacterial survival in foods and water

Microbial models are mathematical expressions that quantify populations of microorganisms in a given food matrix or system as a function of relevant intrinsic or extrinsic variables (Whiting and Buchanan, 1993). Hence, modeling of bacterial growth or survival is described as a function of environmental factors such as temperature, pH, and water activity (McMeekin *et al*., 1993; Olumide and Florence, 2016). In predictive microbiology, there are several derived mathematical equations that describe the bacterial behaviour under different external conditions. The different conditions can be modeled into primary, secondary and tertiary models. There are several ways to classify models. Models can be classified, by the microbiological event into kinetic and probability models (Roberts, 1989); the modeling approach that adopts Empirical and Mechanistic ways (Roels and Kossen, 1978); or by the variables considered into primary, secondary and tertiary (Whiting and Buchanan, 1993).

2.1.1 Kinetic and probability models

Kinetic models are considered with the rates of response (Growth or death). Examples include the Gompertz and square root models which, describe the rates of response, like lag time, specific growth rate and maximum population density (McMeekin *et al.,* 1993; Whiting and Buchanan, 1994) or inactivation/ survival models that describe destruction or survival over time (Xiong *et al*., 1999).

Probability models, originally used for predicting the likelihood that organisms grow and produce toxin within a given period of time (Hauschild, 1982; Stumbo *et al.,* 1983), have been more recently extended to define the absolute limits for

growth of microorganisms in specified environments, for example, in the presence of a number of stresses which individually would not be growth limiting, but collectively prevent growth (Baker and Genigeorgis, 1990). Probability models indicate only the probability of growth and/or toxin production and do not indicate the speed at which they occur (Roberts, 1989).

2.1.2 Empirical and mechanistic models

Empirical models usually take the form of first or second-degree polynomials and are essentially pragmatic describing the data in a convenient mathematical relationship (curve fitting). An example is the quadratic response surface used by Gibson *et al.* (1988). Mechanistic or deterministic models are built up from theoretical bases and allow interpretation of the response in terms of known phenomena and processes. Attempts, like those of McMeekin *et al.* (1993), to find a fundamental basis for the square root model are important steps towards more mechanistic approaches. Draper (1988) considers the mechanistic models to be more preferable than the empirical ones, as they usually contain fewer parameters, fit the data better and extrapolate more sensibly. Whiting and Buchanan (1993) have proposed a three-level classification method described as primary, secondary and tertiary.

2.1.3 The Weibull model

Nonlinearities in inactivation data have been addressed by several mathematical models (Anderson *et al*., 1996; Augustin *et al*., 1998; Peleg and Cole, 1998; Baranyi and Pin, 2001; Geeraerd *et al*., 2005). Among those models, considered to be the most important has probably been the use of the Weibull model. The Weibull distribution is considered to be the most convenient and flexible among the various distribution functions that describe monotonic survival curves. This

distribution is named after Waloddi Weibull (1887-1979), a Swedish engineer and scientist, who was well-known for his work on the strength of materials and fatigue analysis (Weibull, 1939). The Weibull model is applicable to materials, structures and also to biological systems because it has an increasing failure rate and can describe wearing out processes. Nonthermal treatment studies are based on the hypothesis that the resistance to stress of a population follows a Weibull distribution (Peleg and Cole, 1998; Corradini and Peleg, 2003; Hajmeer *et al*., 2006). The Weibull model, when applied to describe microbial inactivation, is the cumulative form of the asymmetric Weibull probability density function for the heat resistance of individual microbial cells. The cumulative distribution of the Weibull model can be applied in a variety of forms. For example in the logarithmic form.

$$\log_{10} N = \log_{10} N_0 - (\frac{t}{\delta})^p$$

Where p is a shape parameter, δ [time unit] is a scale parameter and can be explained as the time for the first decimal reduction when p = 1, in which case the Weibull model is capable of describing a wide range of inactivation phenomena, for which the log-linear is (p = 1). Convex curves are obtained for p > 1, whereas concave curves are described for p < 1.

2.1.3 The log-linear model

Traditionally microbial inactivation has been described to be analogous to chemical kinetics as a first-order decay reaction of the microbial population N (CFU/mL) during time t (Chick, 1908). In the linear model, it is assumed that all cells in a population have equal sensitivity to external factors and that the death of an individual cell is dependent upon the random chance that a key molecule within it receives sufficient heat (Cole *et al*., 1993).

$$\frac{dN}{dt} = -kN \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq\ 2.1$$

Full integration of the equation gives

$$\int_{N_0}^{N} \frac{dN}{N} = \int_{t_0}^{t} -K = kdt \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq\ 2.2$$

This becomes

$$\ln \frac{N}{N_0} = -kt \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq\ 2.3$$

Recall that

$$\ln x = \ln 10\ (\log_{10} x)$$

Therefore

$$\log_{10} \frac{N}{N_0} = \frac{-k_{max} t}{\ln 10} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq\ 2.4$$

$$\log_{10} N = \log_{10} N_0 - \frac{k_{max} t}{\ln 10}$$

$$\log_{10} N = \log_{10} N_0 - \frac{t}{D} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq\ 2.5$$

Where N represents the microbial cell density, expressed in, [CFU/ml], for example, N_0, the initial microbial cell density [CFU/ml], K_{max} [1/time unit] the first order inactivation constant and D [time unit] the decimal reduction time (the time required to achieve a 1-log reduction in the population) can be computed as $\ln(10)/k_{max}$. The log-linear model (Eq. 2.5) is a single parameter model, which has the advantage of computational simplicity, in that it only requires the regression of survival data.

a. Primary model

A primary model describes the microbial behaviour (growth or survival) as a function of time under specific conditions. Quantities and parameters include colony forming units (CFUs), biomass, absorbance measurements, in addition to substrate levels or metabolic products depending on the model (Whiting, 1995). The most frequently used primary inactivation model is a log-linear model. It is favoured due to its simplicity. Nowadays there is strong evidence that the curves for bacterial cell survival are not logged linear as the first order kinetic model entails (van Boekel, 2002). Among the various distribution functions that can describe monotonic survival curves, the Weibull distribution is probably the most convenient and flexible. It can be assumed that the inactivation patterns are due to biological response. There is no reason to accept that one model form would be universally valid for all microorganisms, substrates and physical conditions (Whiting, 1995).

b. Secondary models

Secondary models deal with the response of parameters that appear in primary modeling approaches as a function of one or more environmental conditions such as temperature or pH. The quality of the original data set is extremely important in generating the estimates. McDonald and Sun (1999) and Vereecken *et al*. (2000) presented a general overview of secondary model types. Nowadays, approaches that receive considerable attention for new developments are: (i) Bělehrádek type models (also referred to as Ratkowsky-type or square root models) (Ratkowsky *et al*., 1982), (ii) polynomial models (Gibson *et al*., 1988), (iii) cardinal values models (Rosso *et al*., 1995), and (iv) artificial neural network models (Hajmeer *et*

al., 1997). Great caution should be exercised to avoid extrapolation when using purely empirical secondary models because the model could yield nonsensical results when applied outside the domain of the data from which the parameters were estimated. Most of these secondary models have little or no microbiological basis, which makes interpretation of some model parameters difficult and sometimes their performances are not stable (Hajmeer *et al*., 1997).

2.2 Model validation

To assess the reliability of models before they are used to aid decisions, they (models) have to be validated. Two steps must be taken to validate a model once it has been built. The first is to test its accuracy with new data and new combinations of variables to determine if the model can describe the experimental data sufficiently(Whiting, 1995). This is called internal validation, also termed 'Curve fitting'. This will allow an estimation of the goodness of fit and will show if and where additional data is needed. Complex models tend to be very specific, which can be a limitation when testing new data.

The second step is to compare model predictions with microbial responses in actual foods. This is called external validation. This will show the model's limitations and may show if additional factors must be tested and included in the model. Errors in growth or survival should always tend towards faster growth rates or better survival, respectively, to make a conservative prediction (Whiting, 1995).

Models cannot be used with confidence until this validation is done. Growth rates or statistical measures like root mean-square error (RMSE) and regression coefficient or coefficient of determination (r^2) values were used by Duh and Schaffner (1993) to assess the reliability of predictive equations developed based on measurements in brain heart infusion broth and those of literature values in

food. These terms have been used to mathematically compare data derived from the literature (Giffel and Zwietering, 1999). McClure *et al*. (1993) compared their models on the basis of the sum of the squares of the differences of the natural logarithm of observed and predicted values and suggested that a smaller value indicates a model, which, on average, better predicts the observed response. Two important factors for validation of predictive models are accuracy factor and bias factor introduced by Ross (1996). Accuracy and bias factors are the mean square differences between predictions and observations (Baranyi *et al.*,1999).

2.3 Limitations of the model

There are some limitations of predictive microbiology that need to be considered. The models cannot be extrapolated outside the ranges (for example, T°C, a_w) in which they were derived. This is because the models are derived from fitting the observed data and therefore do not model microbial behaviour. Predictions outside the experimental ranges are usually not accurate and in some cases are nonsensical. Secondly, the models usually predict faster growth rates comparable to rates that are actually observed. This makes them failsafe but they may be overly conservative. The reason for this is the models are usually conducted in laboratory media and while they are validated in foods, they may not have widespread application in the food industry. In addition, several workers have also pointed out that models derived in static conditions may not be applicable to fluctuating conditions *i.e.* those in which environmental conditions like temperature, pH, gaseous atmosphere and water activity change during the life of the product (Mackey and Kerridge, 1988; Gibbs and Williams, 1990). Also, previous incubation conditions of the test organisms can affect the subsequent rate of growth of organisms (Walker *et al*, 1990). Fu *et al.* (1991) termed this a

"Temperature history effect" and other environmental conditions like pH have also been investigated under this "history effect".

Therefore, great caution is required in the use of microbial models as scepticism exists that models derived in an experimental system can reliably predict the growth of the modeled organism in a food and It is very important that the model is accompanied by a description of its limitations; specific microorganisms, factors tested and considered in the model, ranges for each of these factors, and combinations of factors. The model user must be aware that using the model outside its limitations may not give valid answers.

2.4 Challenges in predictive microbiology

Considerable progress has been made in defining philosophic approaches and experimental protocols for growth model development and many models have been developed and published, as a result, more validation studies are required, particularly involving independent and industry-based trials. More emphasis should be placed on modeling the death kinetics of foodborne pathogens with low infective doses. Measurement of environmental factors (for example, temperature) can be achieved with precision, but in some situations, (for example, in chilling of meat carcasses), it is more difficult (McMeekin *et al*., 1997). Location of the sensor can be an important consideration (Gill *et al.*, 1991a and b). Furthermore, development of techniques to measure constraints such as water activity, pH, or redox potential on a microscale might provide useful information for a complex food such as salami. This would allow the definition of the role of the microenvironment in determining microbial behaviour (McMeekin *et al.*, 1997).

The inherent variability of response times (generation time and lag phase duration) is an issue in Predictive Microbiology (Ratkowsky *et al*., 1996). The variance was

shown to be proportional to the square or cube of the response time (Ratkowsky *et al.*, 1996). The practical implication of these findings for the application of kinetic models is that inherent biologic variability increases markedly with increasing response times, and thus, the confidence limits associated with predictions also increase markedly. However, if the probability distribution of the response time is known, one can determine the probability that an organism will grow more quickly than a predicted response time (Ratkowsky *et al*., 1996). Thus, kinetic models are appropriate to describe consistent microbial growth responses, but under extreme conditions, a probability approach may be required (McMeekin *et al.*, 1997).

Models must be validated in foods under conditions that mimic situations encountered in normal practice, for example,, decreasing temperature and water activity during active chilling of meat carcasses or fluctuating temperatures during the distribution and storage of many food commodities (McMeekin *et al*., 1997).

Modeling lag phase duration is also a problem (Baranyi *et al.*, 1995). Predicting the lag phase duration in foods is very difficult not due to the lack of a suitable model, rather the difficulty comes from the lack of knowledge of the physiologic status of the microorganisms contaminating the food. The organisms may include cells that are actively growing, exhibiting a physiologic lag phase, damaged and under repair, exhibiting physiologic (endospores) or exogenous dormancy (VNC cells), damaged but unable to reproduce because of ineffective repair mechanisms, and dead (McMeekin *et al.*, 1997).

Methods to define the physiologic status of food-borne contaminants under various conditions need to be developed. This will require observations on individual cells or small populations of cells either directly by microscopy or an indicator of single-cell metabolic activity (Baranyi and Roberts, 1995). Luminescent *Salmonella* strains have been used as real-time reporters of growth and recovery

from sub-lethal injury (Chen and Griffiths, 1996). Alternatively, a parameter to describe the suitability of cells to grow in a new environment may be incorporated in the model (Baranyi and Roberts, 1995).

2.5 Introduction to food-borne pathogens

According to the U.S Food and Drug and Administration (FDA), there are several food-borne pathogens that are of concern and harmful to the general public and are particularly harmful to pregnant women. These organisms are listed in Table 2.1 as culled from FDA (2013). Microorganisms frequently encountered in food disease cases include *Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens,* Pathogenic *Escherichia coli (E. coli), Listeria monocytogenes, Norovirus(Norwalk-like Virus), Salmonella enteritidis, Salmonella typhimurium, Shigella, Staphylococcus aureus, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus and Yersinia enterocolitica*. These organisms have varied symptoms, gestation period and also route of infection.

Aside from these 14 organisms listed, there are other well-known pathogens some of which are foodborne, including *Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Pseudomonas aeruginosa* (Brito-Argáez *et al*., 2009).

2.6. *Escherichia coli* 0157: H7 and its associated diseases

2.6.1 Overview

The genus *Escherichia coli* comprised Gram-negative, facultative anaerobic bacilli, a common inhabitant of the gastrointestinal tract of mammals, and belong to the Enterobacteriaceae family. They are bile-tolerant, non-fastidious organisms that are easily cultured on routine laboratory media(Bell, 2002). They ferment lactose and grow best under mesophilic temperatures with an optimum at 37^0C. Most *E. coli* have the b-glucuronidase (enzyme) that breaks down complex carbohydrates. This

enzyme is used in a fluorogenic assay that takes advantage of the breakdown of 4-methyl umbelliferone glucuronide (MUG) by b-glucoronidase yielding a fluorescent compound. However, *E. coli* 0157: H7 does not have b-glucuronidase. Further, *E. coli* 0157: H7 cannot ferment sorbitol within 24 h, while 90% of *E. coli* can.

Enterohemorrhagic *Escherichia coli* (EHEC) are the cause of serious illness and mortality in outbreaks of foodborne illness linked variety of foods (Armstrong *et al*., 1996). There are a number of different enteropathogenic groups of *E. coli* that have been shown to cause various types of gastrointestinal infections. Six main pathotypes of *E. coli* can be distinguished: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), and EHEC. All these pathotypes of *E. coli* use multistep systems of pathogenesis, comprised in general of colonization of the mucosal site, evasion of the host defenses, and multiplication and host damage (Kaper, 2005). After the first outbreak in 1982, *E. coli* 0157: H7 has become the most widely known EHEC strain. Reports have shown that an *E. coli* 0157: H7 strain that was involved in an outbreak of hemorrhagic colitis in the United States produced shiga toxins. Shiga toxin *E. coli* (STEC) was epidemiologically associated with hemolytic uremic syndrome (Bell, 2002).

Generally, *E. coli* can be a member of the normal microflora in animals including humans. However, virulence genes acquired in various ways in the ecosystem enabled *E. coli* to acquired different forms of genes that lead to increased pathogenicity. In general, many pathogenic strains behave biochemically and ecologically like any other nonpathogenic *E. coli*, making their detection among commensal *E. coli* an important problem, especially among EHEC (Bettelheim, 2007). Serotype 0157 has been found to be incapable to ferment the carbohydrate sorbitol. Compared to other pathogenic *E. coli*, this serotype would cause

hemorrhagic colitis (HC) and other severe symptoms. Other serotypes, such as 026, 0111, and sorbitol-fermenting 0157: NM, have also been related with HC and subsequently classified as EHEC (Armstrong *et al*., 1996). The ability to produce shiga toxins is the common characteristics of all EHEC that are often referred to as shiga toxin-producing *E. coli* (STEC).

2.6.2 Pathogenesis and virulence factors

The production of shiga toxin is fundamental to the pathogenesis of bloody diarrhea and haemolytic-uraemic syndrome. *Escherichia coli* O157 strains have the locus of enterocyte effacement genes but other serogroup strains without these genes have also caused haemolytic-uraemic syndrome). The pathogenicity of STEC is determined by several virulence factors that are encoded by chromosomal pathogenicity islands, phage chromosomes integrated into the bacterial genome as well as plasmids. Shiga toxins are members of a toxin family that share many common features. The shiga toxins identified in EHEC are classified into two distinct subgroups: Stx1 and Stx2 (Persson *et al*, 2007). The toxins are produced by the pathogen in the colon and cause local damage. The ability to pass through the bloodstream to the kidney plays a role in causing HC and HUS. *E coli* O157 can produce two different Shiga toxins encoded by bacteriophage. The shiga toxin, Stx1, is very similar to the type 1 toxin of *Shigella dysenteriae*; while Stx2 is genetically and immunologically distinct with 55–60% similarity in genetic and amino acid sequences. The possession and expression of the Stx2 gene and the variant Stx2c (which often occurs with Stx2) correlate strongly with the causation of bloody diarrhea and haemolytic-uraemic syndrome (Persson *et al*, 2007). Shiga toxins bind to glycosphingolipid globotriaosylceramide (Gb3), a cell surface receptor. They are then internalized by clathrin-dependent endocytosis, and go on to specifically depurinate 28S eukaryotic rRNA, inhibiting protein synthesis (Persson *et al*, 2007). This step induces a ribotoxic stress response that can lead to

cytokine release and apoptotic cell death. In the human kidney, Gb3 is present on glomerular endothelial cells, podocytes, and various tubular epithelial cell types. Shiga toxin binds to these cells in renal sections from patients with haemolytic-uraemic syndrome, and damage markers from these cells can be detected in their urine; biopsy samples from these patients show apoptosis of glomerular and tubular cell types and fibrin-rich glomerular microangiopathy (Tarr *et al.*, 2005). Blood from patients with haemolytic-uraemic syndrome showed an increase in microparticles with surface-bound tissue factor and in functional tissue factor. Tissue factor can contribute to a prothrombotic state (Stahl *et al*, 2009).

Other structures that help EHEC in adhering to host cells are fimbriae and fimbrial adhesins, thread-like structures that extend out from the bacterial surface. Type 1 fimbriae are the first adhesins described in *E. coli* and are the most common adhesins produced. These adhesins mediate the adherence of the pathogen to mannose-containing glycoproteins found on the surfaces of eukaryotic cells. Enterohemorrhagic *Escherichia coli* (EHEC) clinical isolates from HUS patients have been found to have a distinct virulence profile. Strains capable of producing both shiga toxins have been found to be highly associated with bloody diarrhea or HUS, while strains with only Stx1 are rarely found in HUS patients. In addition, clinical strains associated with HUS have also been found to be more enterohemolytic and are more likely to possess intimin (Law, 2000). The high virulence of STEC strains like *E. coli* 0157:H7 is not only dependent on virulence factors but partially also on the ability to survive environmental unfavourable conditions, such as resistance to low pH levels found in the gastrointestinal tract which contributes to very low infectious dose of 50–100 cells or lower (Armstrong *et al*., 1996).

2.6.3 Prevalence and Epidemiology

Shiga toxin *E. coli* (STEC) and specifically *E. coli* 0157: H7 are considered as emerging foodborne pathogens that occur globally. In the United States, *E. coli* 0157: H7 is estimated to cause 73,480 illnesses annually, with approximately 2168 hospitalizations and 61 deaths. In Europe, 14,000 cases in over 24countries have occurred from 2000 to 2005, of which 62% belong to the 0157 serogroups (Fisher and Meakins, 2006). A review of 90 outbreaks in Britain, Ireland, Scandinavia, Canada, USA and Japan indicated that about 20% of outbreak cases resulted from the secondary spread (Snedeker *et al*., 2009). However, the duration of outbreaks shows that continued transmission thereafter in the affected communities is very rare (Snedeker *et al*., 2009).

The primary habitat of *E. coli* is the intestinal tract of warm-blooded animals as well as humans. *Escherichia coli* infections in humans are transmitted directly from animals mainly through contaminated foods. Enteric pathogens are distributed from livestock to food crops and can occur in various ways such as the application of manures, irrigation with contaminated water, dispersal by air, and dispersal via biological vectors, such as wildlife and insects (Janisiewicz *et al*., 1999). Many studies have measured the prevalence of *E coli* 0157 in cattle. Comparisons of reported data have shown big differences between studies. For dairy cattle, the prevalence estimated by testing faeces ranged from 0.2% to 48.8%. In the USA (prevalence in calves 0.4–40%) and Canada, Italy, Japan, and the UK (prevalence in calves 1.7–48.8%) (Janisiewicz *et al*., 1999).The highest figure was for carriage by calves with a functioning rumen rather than cows or heifers. Prevalence was higher in warmer months than in cooler months (Bell, 2002).

Escherichia coli 0157 can also be present in sheep and pigs; in a study conducted in Great Britain in 2003, intestinal contents of 4.7% of cattle, 0.7% of sheep, and 0.3% of pigs tested positive for *E coli* 0157 at slaughter. The type of cattle (female breeding cattle) and cattle stress (movement and weaning) were identified as risk

factors. Results from a study of 474 Scottish cattle farms have identified a robust pattern in which about 80% of transmission arises from the 20% of animals that are most infectious (Chase-Tapping *et al.*, 2007). Bovine super-shedding is associated with the colonization of a lymphoid follicle-dense mucosal region at a short distance proximal to the recto-anal junction (Chase-Tapping *et al.*, 2007). Cattle colonized at this site shed higher numbers of organisms for a longer period than those colonized at other sites. The presence of these animals on a farm is associated with a high prevalence of low-level shedders, and they are likely to infect another animal in the same pen. Risk factors for the presence of super-shedders on farms have been studied in Scotland (Chase-Tapping *et al.*, 2007).

Ground beef is still the most frequently implicated source of *E. coli* 0157: H7 outbreaks, accounting for 75% of *E. coli* 0157: H7 outbreaks (Vugia *et al*., 2006). Dairy products and undercooked minced beef can be directly contaminated by cattle faeces during either milking or slaughtering processes. Results from a study of 90 outbreaks confirmed microbiologically in the UK, Ireland, Denmark, Norway, Finland, USA, Canada, and Japan, occurring between 1982 and 2006, showed that the source of transmission was food in 42.2% of the outbreaks, dairy products in 12.2%, animal contact in 7.8%, water in 6.7%, environmental in 2.2%, and unknown in 28.9% (Snedeker *et al*, 2009). Many foods and dairy products have acted as vectors—ground beef hamburgers, ready-to-eat cold meats including poultry, pork and beef products, cheese, milk; butter; yogurt; ice cream, apple juice, coleslaw; lettuce, spinach, sprouts, and melons (Rangel *et al*, 2005). The list continues to expand—for example, consumption of prepackaged raw cookie dough was strongly associated with a multistate outbreak in the USA in 2009, with 72 cases of *E coli* 0157 infection, ten with haemolytic-uraemic syndrome (CDC, 2009a). Waterborne outbreaks have been associated with recreational waters (lakes, ponds, and paddling and swimming pools), drinking water (municipal and

local, from springs and wells), and ice. Outbreaks attributable to direct and indirect contact with ruminant animals have occurred on farms, agricultural shows (UK), county fairs (USA), open farms and camps (CDC, 2009a).

The dominance of ground beef as a vector in the USA has been striking; it was the transmission route in 41% of foodborne outbreaks between 1982 and 2002. Such outbreaks are rare in the UK, where butcher-associated outbreaks have occurred much more often than in any other country; 30 outbreaks were recorded between 1995 and 2004 (EFSA, 2011). In the USA, 24 multistate outbreaks were recorded between1992 and 2002, with at least one occurring per annum. In 2006, the incidence of infection per 100 000 in European countries was 2.1 in England and Wales, 2.87 in Ireland, 4.7 in Scotland (from 1998 to 2007 the mean yearly rate was 4.28), 0.43 in Germany, and 0.08 in France in the same year, the incidence was about 1.3 and in Canada. (EFSA, 2011; CDC, 2009b). The incidence of all shiga-toxin-producing organisms (including *E coli* O157) in Japan from April 1999, to October 2004, was 2.74 (Sakuma *et al*, 2006).

Major national differences exist also in the proportion of isolates of verocytotoxin-producing organisms accounted for by *E coli* 0157, ranging from 99.6% in the UK, to 93.7% in Canada (2004), 74.27% in the USA (2005), to 30.5% in Germany, where serogroups O103, O26, O91, O145, and a sorbitol-fermenting strain of serogroup 0157are common (EFSA, 2011). Such strains of *E coli* O157 have also been found in the Czech Republic, Austria, Finland, Scotland, and Australia. The serogroup O111 has had an important public health effect in Australia, whereas O157 has predominated in New Zealand (Leotta *et al.*, 2008). Substantial regional variations exist within countries. Studies in Sweden and Canada using geographical information systems have shown that the incidence of the human disease is greater in rural areas, which have high densities of cattle and sheep, than in urban areas (Snedeker *et al.,* 2009).

The review of secondary spread in the 90 outbreaks that arose between 1982 and 2006 (Snedeker *et al.*, 2009) ranked the route of secondary transmission as person to person in the home (45.6%); person to person in nurseries(11.1%); recreational water (ie, swimming and paddling pools, 10%); person to person in institutions (4.5%); and others and unknown (5.5%). The highest mean proportion of secondary cases was recorded in outbreaks in which patients had a median age of fewer than 6 years. The lowest was in outbreaks in which the median age of patients was 17–59 years (Snedeker *et al.*, 2009). A recent study on the persistence of *E. coli* 0157 in irrigation waters that could potentially be transmitted to fresh produce was conducted in Kubanni River in Nigeria (Chigor *et al*., 2010). The prevalence of the pathogen in the river was studied over a 10-month period. The detection rate for *E. coli* 0157 was 2.1% and faecal coliform counts exceeded acceptable limits. The researchers concluded that the Kubanni River represented a public health risk and unfit for fresh produce irrigation. The factors responsible for the emergence of the problem are changes in the produce industry which include intensification and centralization of production, wider distribution of produce over longer distances, introduction of minimally processed produce, increased importation of fresh produce; Changes in consumer habits such as increased consumption of meals outside the home, the increased popularity of salad bars, increased consumption of fresh fruits and vegetables, and fresh fruit juices and increased size of the at-risk population; others are enhanced epidemiological surveillance, improved methods to identify and track pathogens, and emerging pathogens with low infectious dose. (Brandl, 2006)

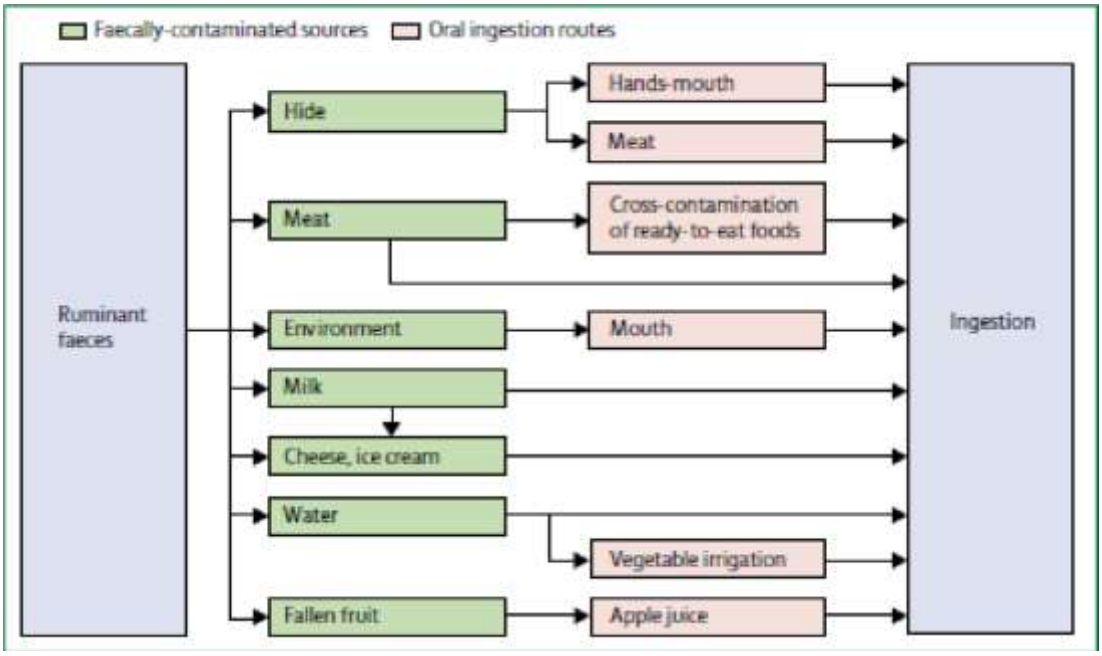


Figure 2.1: Routes of transmission

Source: Brandl, 2006

2.6.4 Disease Caused by *E coli* 0157

One of the main characteristics of EHEC that are required to cause disease in humans is their ability to attach to intestinal cells and to colonize the human gut.The infectious dose of EHEC is very low (1-100 cfu) which is lower compared to that of other pathogens (Paton and Paton, 1998). Since the 1980s, EHEC strains have been established as foodborne pathogens associated with an array of human infections including Hemolytic Colitis, milder forms of diarrheal illness, and as the major etiologic agent responsible for the fatal infection, haemolytic uraemic syndromme (HUS). In general, infection with EHEC 0157: H7 is self-limiting, which depends on the virulence of the infecting strain. Hemolytic Colitis is the principal disease associated with EHEC and is characterized by severe abdominal cramping and bloody diarrhea. haemolytic uraemic syndromme (HUS) may eventually develop as a sequel to EHEC infection and HC. Approximately 8% of those infected with EHEC 0157: H7 will develop HUS (Manning *et al*, 2008). The

endothelial cell damage leads to swollen detached endothelial cells, which in turn exposes the basement membrane. Hemolytic anemia is an abnormal breakdown of erythrocytes. This results from clots and possible side effects from leukocytes on the erythrocyte cell membranes(Manning *et al*, 2008).

Outbreaks vary in the severity of illness and the frequency of the most serious complication, the haemolytic-uraemic syndrome; because of differences in the virulence of the causative *E coli* 0157 strains (Manning *et al*, 2008). The effects of an *E. coli* 0157 infection range from asymptomatic to lethal. Children less than 5 years of age have a higher incidence of HUS. They express higher levels of the Gb3 receptor present on the renal endothelial cells and form an attachment to Shiga toxin that may be circulating due to infection. Renal injury occurs from leukocyte infiltrates and clots that may lead to acute renal failure and azotemia. Azotemia is characterized by the increase of nitrogenous compounds due to poor filtering by the kidneys (Tarr *et al*., 2005).

2.6.5 Isolation and Diagnosis of *E coli* 0157

According to the USDA's Food Safety and Inspection Service (FSIS), ground beef is considered adulterated if as little as 1 cfu of EHEC O157: H7 is detected in 25 g of ground beef of which rapid diagnosis is essential (Pallock *et al*, 2009). Early separation of infected individuals from their siblings will substantially reduce secondary transmission, and the development of oligoanuric renal failure is associated with delays in the start of intravenous volume expansion (Pallock *et al*, 2009). The earlier epidemiological investigations of outbreaks start, the sooner control measures can be implemented.

Enteric bacteria have similar physiological characteristics and therefore enrichment may cause the outgrowth of competitive microflora as well). *Escherichia coli* 0157 is identified by culture on selective indicator media (Sorbitol MacConkey or the

same agar containing cefixime and tellurite) (CDC, 2009a). To improve selective growth of *E. coli* 0157: H7, selective media have been developed through the addition of antibiotics. There are three types of enrichment media that are often used when recovering *E. coli* 0:H7: buffered peptone water supplemented with 8 mg/L Vancomycin, 10 mg/L cefsulodin, and 0.05 mg/L cefixime, modified EC broth (mEC with novobiocin), or mTSB with 20 mg/L novobiocin or 10 mg/L acriflavine (OIE, 2006). Strains of *E. coli* 0157 are relatively easy to isolate because of their unique biochemical characteristics. Shiga toxin producing *Escherichia coli* (STEC) 0157 is unable to ferment the carbohydrate Sorbitol, which led to the development of the SMAC agar used for its isolation. More specific media have also been developed, such as Rainbow Agar, CHROM agar, and 0157: H7 ID agar, that is able to recover STEC 0157 along with sorbitol-fermenting 0157 and in-0157 strains (Bettelheim, 2007). Sorbitol MacConkey agar (SMAC) containing Cefixime and Tellurite (CT-SMAC) provides highly selective recovery of *E. coli* 0157: H7 from other *E. coli* and enteric bacteria. Currently, CT-SMAC is widely used to isolate *E. coli* 0157: H7 followed by PCR or latex agglutination confirmation. However, the use of CT-SMAC is not recommended for detection of non-0157 EHEC because most non-0157 EHECs that produce Shiga toxins behave physiologically the same as other commensal *E. coli* strains (Arthur *et al*., 2002). Overnight colonies are colourless and have a diameter of 2–3 mm.

Their identity is confirmed by agglutination with specific antiserum. Immunomagnetic beads have been designed for the capture of the O antigen of O157 and some have been developed for the most commonly reported non-O157 strains such as O111 and O26 (Oxoid, Inc.). Enrichment broth culture and immunomagnetic separation with antibody-coated beads are used to increase the sensitivity of culture methods in outbreak investigations and food testing.

Retrospective diagnoses are sometimes made by measurement of antibodies to lipopolysaccharide (Arthur *et al*., 2002). Non-O157 serotypes can be differentiated from commensal *E. coli* by using specialized molecular techniques such as multiplex PCR. In the UK, strain differentiation by phage typing and pulsed-field gel electrophoresis is done at reference laboratories in London and Edinburgh. A few phage types dominate; pulsed-field gel electrophoresis is much more discriminatory than is phage typing and has been widely used in outbreak investigations (Arthur *et al*., 2002).

2.6.6 Antimicrobial Resistance

Multidrug-resistant strains of *E. coli* from food, animal, and humans are increasingly being encountered. The most frequently reported resistance phenotype of *E. coli* 0157: H7 and non-0157 isolates are found to resist streptomycin, sulfisoxazole and tetracycline. Increasing resistance of Fosfomycin the drug of choice for pediatric gastrointestinal infections due to Shiga toxin *E. coli* infection in Japan has been documented (White *et al*., 2002). Resistance level in *E. coli* have been reported to be high for broad-spectrum penicillin and trimethoprim, and low for third-generation cephalosporins and nitrofurantoin (White *et al*., 2002). It is hypothesized that adaptation to antimicrobials by bacteria is an essential survival strategy particularly for microbes having their environment within the host (White *et al*., 2002).

2.6.7 Control

i) Temperature

Thermal processing is one of the most common interventions applied to foods to inactivate EHEC. The heat sensitivity of the pathogen has been extensively studied and reviewed. In recent years, mild heat treatments and high-temperature short

time treatments have been evaluated to inactivate STEC on raw produce and meat. However, a new study raised an important question. Pasteurization temperatures have been validated for STEC, but not for free Shiga toxin (White *et al*., 2002).

ii) High pressure

The application of high-pressure processing (HHP) to enhance the safety of seeds or sprouts has been studied in the past (Penas *et al.*, 2008) and various degrees of efficacy has been shown.

iii) Ionizing irradiation

Food irradiation uses high-energy gamma rays, electron beams, or X-rays; all are penetrating processes and are used commercially to eliminate pathogens from meat products. Irradiation may be better than most technologies in penetrating fresh produce and it could be a powerful tool if used correctly in different produce items and among different varieties. Irradiation is able to effectively eliminate *E. coli* 0157: H7 from lettuce (Niemira *et al*., 2002).

iv) Ozone

Ozone destroys microorganisms through progressive oxidation of critical cellular components, with the cell surface suggested as the primary target of the process. Chlorine, one of the most commonly used disinfecting agents, destroys certain intracellular enzyme systems, while ozone causes widespread oxidation of internal cellular proteins ultimately leading to rapid cell death (Komanapalli and Lau, 1996).

v) Cinnamaldehyde

There has been an increased interest in the development and application of new effective and nontoxic antimicrobial compounds. Plant essential oils (EOs) have been found to have antimicrobial activity against a multitude of pathogens and show promise as an alternative to the currently used sanitizers. Plant-derived EOs

can be used as flavoring agents in foods and beverages and have potential as natural agents for food preservation due to their content of antimicrobial compounds. Cinnamon oil is commonly used in the food industry because of its special aroma. Amalaradjou *et al*. (2010) treated polystyrene plates and urinary catheters inoculated with uropathogenic *E. coli* (5–6.0 log CFU) with different concentrations of trans-cinnamaldehyde at 37^0C. They found that all concentrations of the antimicrobial resulted in effectively preventing the pathogen from forming a biofilm on plates and catheters while producing no cytotoxic effects on human bladder epithelial cells.

vi) Electrochemically activated water

Electrochemically activated water (EAW) has been reported to have strong bactericidal effects on most pathogenic bacteria that are important to food safety (Huang *et al*., 2008). EAW is produced by passing a diluted salt solution through an electrolytic cell that contains an anode and cathode separated by a membrane. By subjecting the electrodes to direct current voltages, negatively charged ions such as chloride and hydroxide in the diluted salt solution move to the anode and become oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid, and hydrochloric acid, while positively charged ions move to the cathode to take up electrons becoming hydrogen gas and sodium hydroxide of which the main advantage of EAW is its safety.

2.6.8 Vaccination

Vaccination is used to prevent pathogen colonization and faecal excretion in ruminants, and it is based on inducing the animal's immune system to protect itself from antigens expressed by *E. coli* 0157: H7. In a recent clinical vaccine trial, commercially fed cattle were used to test the effect of a two-dose regimen of a vaccine against type III secreted proteins of *E. coli* 0157: H7 (Smith *et al*., 2008).

The study found that pens of vaccinated cattle were less likely to test positive for *E. coli* 0157: H7.

2.6.9 Feed management

Feed management has been suggested as a viable method to affect conditions within ruminant gastrointestinal tracts and ultimately modify the survival of *E. coli* 0157: H7. Corn silage, barley, and beet pulp have been found to increase the prevalence of 0157 in cattle (Berg *et al*., 2004). A *Lactobacillus acidophilus* culture has demonstrated effectiveness in reducing *E. coli* 0157: H7 in feedlot cattle by up to 50%. This particular product is currently available commercially in the United States and is being used in many large U.S. feedlots.

Pathogenic property of *E. coli* 0157: H7 coupled with its ability to survive environmental stress make it a powerful threat to public health efforts. Commonly used methods for controlling bacteria such as boiling cannot eliminate the effect of the Shiga toxin produced by the *E. coli*. Preventive measures applied to arrest the spread or transmission of the pathogen remained the best alternative. There is a need for food handlers to be educated on good hygienic practices and all necessary measures to be taken to control waste disposal especially agricultural waste to reduce the health burden that can arise from food and water sources (Berg *et al*., 2004).

2.7 *Campylobacter* and Campylobacteriosis

2.7.1. Overview

Recent years have witnessed the emergence of several foodborne pathogens such as *Aeromonas hydrophila, Yersinia enterocolitica, Campylobacter jejuni*, *Listeria monocytogenes, Vibrio parahaemolyticus*, *Escherichia coli* 0157: H7, *Arcobacter butzleri, Cyclospora cayetanensis, Cryptosporidium parvum*, etc. (Pal, 2013; Pal and Mahendra, 2015). Among these agents, *Campylobacter jejuni* is one of the

most significant cause of bacterial diarrhea in humans throughout the world including Nigeria (Allos, 2001; Mukherjee *et al*., 2013; Pal, 2014). The association of *C. jejuni* with human enteric illness was first elucidated by Butzler and co-workers in 1973. Subsequently, Skirrow in 1981 reported the etiologic role of this bacterium in enteritis of dogs and cats and considered campylobacteriosis as a new zoonotic disease.

As Campylobacteriosis is not a notifiable disease, the exact data on its incidence is grossly inadequate. In the United States of America, 2.5 million people suffer annually from *Campylobacter* infections, of which 80% of the cases are related to ingestion of contaminated foods. Among the several species of *Campylobacter*, *C. jejuni* is most commonly isolated from diarrheal disease in humans and animals. Other species such as *C. jejuni* subsp. doylei, *C. coli*, and *C. lari* are occasionally found to be associated with diarrhea in human beings (Pal, 2014). *Campylobacter jejuni* occurs as a commensal in the intestinal tract of many warm-blooded animals including poultry. Consumption of raw chicken, undercooked red meat, unpasteurized milk and untreated water is implicated in several outbreaks of disease (Hadush and Pal, 2013). The intestines of warm-blooded animals including birds act as the amplification vessel for the organisms.

Manure from animals may contaminate surface water through runoff from pasture, and thus presents a risk for humans when untreated water is consumed. Furthermore, humans can be exposed to surface water through direct contact during swimming or by indirect contact through the ingestion of raw products irrigated with surface water (Wagenaar *et al*., 2013). Organisms are present in faeces, vaginal discharges, and the products of abortions and can be spread by direct contact, on fomites and by arthropods acting as mechanical vectors. Contaminated food and water is often the source of infections (Allos, 2001).

The disease is characterized by enteritis, abdominal cramps, nausea, and profuse diarrhea (Pal, 2007). Guillain-Barre syndrome (GBS), reactive arthritis and irritable bowel syndrome are major complications of *C. jejuni* infections (WHO, 2012). Guillain Barré syndrome (GBS), a neurological disease, can be fatal in immunocompromised populations such as AIDS patients. Globally, approximately one-third of GBS cases are attributed to *Campylobacter* infection (WHO, 2012). Laboratory techniques such as microbiological, immunological and molecular are employed to establish an unequivocal diagnosis of *C. jejuni* infection. Treatment is advised in long-lasting infections with antimicrobial agents such as erythromycin, chloramphenicol, gentamicin, azithromycin, or clarithromycin (Pal, 2013). As a farm to fork approach is of pivotal importance in food safety, it must be observed in all food establishments to protect the health of consumers against the foodborne diseases (Pal and Mahendra, 2015).

In developed countries, including Australia, France, Japan, Netherlands, New Zealand, Sweden U.K. and the US, *Campylobacter* is one of the most frequently reported causes of acute infectious diarrhea. An estimated 2.5 million cases of Campylobacteriosis are recorded every year in the USA (Bhaduri and Cottrell, 2004). *Campylobacter*s are responsible for approximately 17% of hospitalizations resulting from food-borne infections. Annual economic cost due to *Campylobacter* associated illnesses is estimated up to the US $8 billion in the United States. In 2004, 183,961 cases of *Campylobacter* infections were reported from 25 European Union countries (Pal, 2014).

In developing countries, the direct animal-to-man transmission may be common as people live in close association with food animals; and waterborne transmission can occur due to poor sanitation (Suzuki and Yamamoto, 2009). The failure of routine water treatment in developed nations can cause sporadic and large outbreaks (Suzuki and Yamamoto, 2009). The present communication delineates

the growing role of *C. jejuni i*s an emerging foodborne pathogen of global public health importance.

2.7.2 Etiology

The name *Campylobacter* derived from the Greek word "Kampylos," which means curved. Presently, the genus *Campylobacter* consists of 17 species and 6 subspecies of which *C. jejuni* is most commonly reported as a cause of gastroenteritis in humans (WHO, 2016). The organism is Gram-negative, non-spore-forming, microaerophilic, motile, catalase positive, oxidase positive, nitrate positive, hippurate positive, indole production negative and glucose utilization negative (Pal, 2014). The bacterium can be curved, spiral, or occasionally straight rods, with size ranging from 0.2 to 0.9 μm wide and 0.5 to 5 μm long, and optimum growth temperature range from 35 to 45°C.

2.7.3 Chicken food chain and *Campylobacter*

Poultry encompasses chicken, turkey, duck and laying hens, of which chicken (Gallus gallus) is the predominant species used for meat production (70%–80%) (OECD, 2015). Global poultry meat production has increased from 58.5 million tonnes in 2000 to 95.5 million tonnes in 2014. Production is not equally distributed; the Americas accounted for 43% of the total production, Asia (mainly China) for 34%, Europe for 17% and Africa and Oceania for 5% and 1 % of the whole production in 2012 (93 million tonnes), respectively. In 2023, poultry meat is expected to be the largest meat sector with around 130.7 million tonnes (OECD, 2015).

An expert opinion assessment by the European Food Safety Authority has estimated that chicken meat consumption accounts for 20%–30% of Campylobacteriosis in the EU in which 50%–80% may be attributed to the chicken reservoir as a whole. This indicate that broiler meat production accounts for

variable numbers of campylobacteriosis cases in different countries (EFSA Panel on Biological Hazards (BIOHAZ, 2011). This also means that the approximate doubling of the chicken meat production from 58.5 million tonnes in 2000 to 95.5 million tonnes in 2014 has clearly affected the global burden of Campylobacteriosis and the continuing growth of poultry meat production will put further pressure on the poultry industry and public health authorities to reduce poultry/ chicken-associated human *Campylobacter* infections (BIOHAZ, 2011).

The stages in the chicken meat production and processing chain consist of primary production at rearing farms, transport to slaughter, the slaughter process and subsequent processing of chicken meat products, selling products at the retail level, and handling and consumption of chicken meat products at home and in public places such as restaurants (BIOHAZ, 2011). All of these stages have a role in the transmission of *Campylobacter* from farm to fork. Production chain conditions vary between countries, and this is also reflected in the annual number of *Campylobacter*-positive chicken flocks. In the EU, the variation in *Campylobacter* prevalence has been from 0.6% to 13.1% in the Nordic countries Finland, Norway, and Sweden, up to 74.2%–80% in several other countries (EFSA Panel on Biological Hazards (BIOHAZ), 2013). Moreover, *Campylobacter* prevalence on farms subsequently reflects the presence of *Campylobacter* found on carcasses and meat.

The most important factors for slaughter batches to become *Campylobacter* positive have been shown to be partial depopulation of the flock (thinning), slaughter in the summer (June, July, and August), increasing bird age at slaughter (from 36 days to >40 days), common health status of the flock (measured as mortality) and increasing number of rearing houses at the farm (Agunos *et al*., 2014). This indicates that the major contamination site in the chicken meat

production is at the rearing farm (Lawes *et al*., 2012; Agunos *et al*., 2014). Vertical transmission from parent to young chicks is uncommon and a flock is usually identified as *Campylobacter* positive at the age of approximately 2 weeks. Flocks at commercial production systems consist of approximately 10 000–30 000 birds per house, with several houses present at a farm, potentially facilitating high levels of *Campylobacter* amplification and rapid spread within the flock. A flock is either colonized by one strain only or, at farms with less stringent biosecurity, multiple strains can colonize the same flock simultaneously (Jorgensen *et al*., 2011).

Transport has only a limited effect on the contamination of carcasses, whereas during the slaughter process, plucking and evisceration lead to contamination of carcasses. At the end of the processing line, various types of products are on sale in different countries, starting from fresh or frozen whole carcasses to pieces of cuts and portions which accounts for the divergent quantities of infection risk. Generally, skinless portions such as breast fillets and slices contain lower *Campylobacter* counts than portions with skin (EFSA Panel on Biological Hazards (BIOHAZ), 2011).

2.7.4 Epidemiology

The great majority of *Campylobacter* infections are sporadic, and a wide variety of animal species can carry the organisms in high numbers and act as a reservoir, which complicates tracking and attribution of the original source of infection. Human exposure can come through direct contact with animals, food (for example, raw or undercooked meat and unpasteurized milk) or environmental reservoirs (for example, natural bodies of water) (Kaakoush *et al*., 2015). Pulsed-field gel electrophoresis, multilocus sequence typing (MLST) and Fla-typing have all been methods commonly used to study the distribution of different genotypes in various

reservoirs and sources (Kittl *et al*., 2013). As a result of the weakly clonal population structure, especially of *C. jejuni*, MLST based on sequencing of seven housekeeping genes has been particularly suitable to study the long-term changes in sequence type (ST) distribution at both local and global levels (de Haan *et al*., 2010; Kittl *et al*., 2013). Subsequently, mathematical models using MLST data have been employed to assign patient isolates to potential sources. In studies employing MLST and mathematical modeling, it has been found that chicken is the most common reservoir/source of *Campylobacter* infection, with attributions varying from 38% to 77%, whereas cattle have been named as the second most common source, with attribution rates varying between 16% and 54%. Generally, source attribution studies have greatly improved our understanding of the relative contributions by different sources to human infection. However, the assignment of one source to one genotype by the source attribution model may subsequently result in over-attribution of particular sources to human infections. This has been particularly seen for generalist genotypes, such as ST21 and ST45, which are commonly found from a large number of sources and reservoirs, but often bovines or chickens are assigned as their sources (Lévesque *et al*., 2013). This issue can partially be resolved by studying the allelic variation at genome level in a whole genome MLST approach, which allows for a more refined way to resolve the association of possibly epidemiologically linked isolates (Kovanen *et al*., 2014; Dearlove *et al*., 2015). In addition, when MLST data are only available for a limited number of potential sources apart from poultry, this can lead to over-estimation of the role of poultry. Ultimately, epidemiological studies using case-control data combined with robust typing of the isolates improve the sensitivity of the source attribution (Dearlove *et al*., 2016).

Seasonal peaks in human *Campylobacter* cases, mostly in July–August, are commonly observed in western countries with temperate climates (EFSA Panel on Biological Hazards (BIOHAZ), 2013; Strachan *et al*., 2013), whereas this is less marked in Australia, New Zealand and countries with tropical climates (Spencer *et al*., 2012; Strachan *et al*., 2013). In line with the summer peak in human infections, higher isolation rates of *Campylobacter* from chickens in the summer time, compared with the winter season, have been observed (Jorgensen *et al*., 2011; Ozbey *et al*., 2014; Williams *et al*., 2015). However, the human infection peak often precedes the prevalence peak of chicken slaughter batches, suggesting that both may have acquired *Campylobacter* from the same source (EFSA Panel on Biological Hazards (BIOHAZ), 2011; Bahrndorff *et al*., 2013; Williams *et al*., 2015).

Furthermore, MLST typing has shown that the same sequence types (ST45, ST230, and ST677) occur during the summer peak in both human patients and chickens, which raises the question of common environmental sources for these types. Although the reasons for *Campylobacter* seasonality are not well understood, increase in potential reservoirs, human behaviour and climate may all play a role in the shedding and subsequent transmission of the bacteria. In addition, variation in the risk of acquiring Campylobacteriosis between rural and urban regions has been documented (Spencer *et al*., 2011; Lévesque *et al*., 2013; Deckert *et al*., 2014). These studies suggest that chicken may play a more prominent role in the transmission of *Campylobacter* to humans residing in urban regions, whereas ruminant-associated genotypes have often been more commonly detected from people living in rural areas (Spencer *et al*., 2011; Lévesque *et al*., 2013; Deckert *et al*., 2014).

Despite the recognition of poultry as a substantial source and reservoir for *Campylobacter* more risk factors have been described. A substantial proportion of

human *Campylobacter* infections are travel-related and the genotypes from travel associated isolates are often divergent compared with domestically acquired *Campylobacter* isolates (Strachan *et al*., 2013).

Workers at poultry abattoirs are an interesting group in which to study the effect of occupational exposure to potentially contaminated poultry on *Campylobacter* infection. Recently, two studies, one conducted in Sweden (Ellström *et al*., 2014) and the other conducted in the USA (de Perio *et al*., 2013), showed divergent results on Campylobacteriosis in poultry abattoir workers. In the US-based study, the great majority (83%) of the symptomatic workers with laboratory-verified *Campylobacter* infection had been working at the slaughterhouse for less than a month (de Perio *et al*., 2013). In the Swedish prospective study, workers who became stool culture positive for *Campylobacter* did not exhibit symptoms, although more than half (57%) had been employed for less than a year (Ellström *et al*., 2014). Both studies included a small number of workers and it will be of great interest to have more studies conducted on the role of occupational exposure to better understand protective immunity in humans and virulence of poultry-associated *Campylobacter*.

2.7.5 Detection of *C. jejuni* from food and Clinical samples

Campylobacter jejuni is one of the most common causes of bacterial diarrhoeal disease worldwide. This significant zoonotic pathogen is reported to have a low infective dose with high pathogenicity (Angesomand and Mahendra, 2013). Poultry and poultry products have long been associated with *Campylobacter* infection, through a variety of food materials and other vectors have been implicated in the transmission such as unpasteurized milk, and water (Bang *et al*., 2001). Evaluation of food and samples for the presence of *Campylobacter* can be challenging. Isolation of the organism from highly-contaminated samples may require different

media depending on the food/sample type and with incubation under microaerobic conditions. They are not the fastest growing organisms in which it can take up to a week to obtain a final test result (Bang *et al*., 2001).

2.7.6 Detection Techniques adopted in Literature

The pathogen can be isolated from stool, rectal swab, bile, blood and other clinical specimens on several media such as Butzler's agar, Preston medium, Skirrow medium, *Campylobacter* thioglycollate medium, *Campylobacter* cefoperazone desoxycholate agar, semi-solid *Campylobacter* medium, *Campylobacter* Blaser agar and Campy-Brucella agar plate (Jeffrey *et al*., 2000, Pal, 2007;2014). Immunological tests such as passive haemagglutination, complement fixation, and ELISA are also useful to demonstrate antibody titer in sera samples. Currently, real-time PCR is employed to screen *C. jejuni* in faecal samples (Mukherjee *et al*., 2013). Multiplex-PCR can be applied for confirmatory identification of *C. jejuni* and *C. coli* isolates, both at genus and species level (Behringer *et al*., 2011). Pulsed-field gel electrophoresis (PFGE) is considered as the reference typing method for *Campylobacter* species (Behringer *et al*., 2011).

In food and feedstuffs, the sample is added to selective enrichment broths which can be obtained as a base powder to which supplements may be added or ready-to-use formats. These are incubated at 37°C for 4 h and then at 41.5°C for 44 h. Selective agars either in powder format or ready-to-use are inoculated from this enrichment or incubated for a further 48 h. Clinical samples are sub-cultured directly to selective agars. Incubation atmosphere is critical for recovery of *Campylobacter*, microaerophilic conditions must be provided. Several proprietary atmosphere systems are available for this purpose. During the broth enrichment, a 10 to 15% aerobic headspace is sufficient (Martin *et al*., 2002). As an alternative to growth on agar, there are a 360 variety of technologies which may provide rapid results such as antibody/antigen interactions using immunoassay methods;

molecular methods such as PCR/nucleic acid techniques which reduce the time to result such as concentration using cell separation. Sometimes combinations of these techniques are used to further enhance the speed to result. Polymerase Chain Reaction (PCR) can eliminate the need for identification in the event of a positive result and may also provide quantitative information. Quality control organisms are available to ensure that method performance is within standard criteria (Martin *et al*., 2002).

2.7.7 Sample preparation and processing

Samples are collected using sterile instruments, under aseptic condition. Twenty five grams (25 g) sample of food is put into a sterile stomacher bag, mixed with Bolton broth nine times the weight or volume. This is homogenized for 2 min to get a homogenized sample (NSM, 2007).

a. **Surface rinse technique**

This is done by rinsing the surface of the sample then shaking or massaging it with 250 ml of nutrient broth (without agar) in a sterile plastic bag and filtering through two layers of cheesecloth and centrifuging the filtrate at 16,000 rpm for 20 min. Finally, supernatant fluid is discarded and the pellet is suspended in a minimum (2 to 5 ml) cubic volume of pre-enrichment broth (NSM, 2007).

b. **Swab technique**

This is done by dipping a sterile swab into an enrichment broth and pressing the swab against the container wall to remove excess moisture. The carcass is then swabbed with the moist swab and the swab is put in pre-enrichment broth to incubate at 37°C for 4 to 6 h. There is no statistically significant difference in the isolation rates of *Campylobacter* species on a carcass in different swabbing sites (Woldemariam *et al*., 2009).

c. **Recognition of colonies**

The plates should be examined as quickly as possible after removal from the microaerobic environment for characterization. *Campylobacter jejuni* has gray/moist flat, glossy, effuse colony with a tendency to spread along the inoculation track having well-spaced colonies resembling droplets of fluid and on moist agar a thin, spreading film and with continued incubation colonies become convex often with a dull surface (NSM, 2007).

d. **Confirmatory tests**

For oxidase test, immerse a swab in freshly prepared oxidase reagent and touch lightly the surface of the colony to be tested, the immediate appearance (in 10 s) of a dark purple colour at the point of contact denotes a positive reaction which confirms *C. jejuni*. For microaerobic growth test, subculture suspected colonies from *Campylobacter* selective agar into two blood agar plates, then incubate one plate in microaerobic condition and the other aerobically at 41.5 ± 1°C to 22 ± 1 h. The growth in micro-aerobically incubated plates and no growth in aerobic conditions in line with other tests confirm the test (Chaban *et al*., 2010). As optional, cell morphology and motility tests can also be used by preparing a wet preparation and using a phase contrast microscope. If *Campylobacter* species are present, there will be highly motile, slender rods with curved morphology and a characteristic darting or corkscrew-like movement. Agglutination under normal lighting conditions indicates that the test organism is *C. jejuni* (Chaban *et al*., 2010).

2.7.8 Treatment

In many patients, therapy with antimicrobial drugs is usually not attempted due to mild and self-limited nature of *Campylobacter* infections. Nevertheless, treatment is recommended in patients who are immunocompromised or show high fever, bloody stools, and prolonged illness. Erythromycin and azithromycin are

considered as the drugs of choice (Pal, 2014). Some isolates of *Campylobacter jejuni* are susceptible to azithromycin, clarithromycin, erythromycin, chloramphenicol and gentamicin and some showed resistance to ciprofloxacin, norfloxacin, ofloxacin and nalidixic acid. Multiple resistances to two or more antibacterial antibiotics are also observed (Mukherjee *et al*., 2013). It is advised that electrolyte balance should be restored to prevent dehydration in *Campylobacter* enteritis.

2.7.9 Antimicrobial resistance

Fluoroquinolones, such as ciprofloxacin, and macrolides, such as erythromycin, have been the primary antimicrobials used for the treatment of human *Campylobacter* infections. Resistance to fluoroquinolones requires only one point mutation in the gyrA gene and resistance has increased rapidly among chicken and human *Campylobacter* isolates since the early 1990s (Wieczorek and Osek, 2013). Studies have shown a clear positive association between the use of fluoroquinolones in poultry production and increased resistance among chicken and human *Campylobacter* isolates (Wieczorek and Osek, 2013; Garcia-Migura *et al*., 2014), whereas in countries not permitting the use of fluoroquinolones in poultry production, such as Australia and the Nordic European countries, few resistant *Campylobacter* isolates are found from chickens and humans with domestically acquired infections (Garcia-Migura *et al*., 2014). The USA banned the use of the fluoroquinolone enrofloxacin in chickens in 2005. Despite this, resistance to ciprofloxacin in C. jejuni from chicken slaughter batches has remained stable at 22% between 2005 and 2013. Although at the retail level, ciprofloxacin resistance decreased from 17% in 2005 to 11% in 2013. Moreover, ciprofloxacin resistance in human *C. jejuni* isolates in 2013 remained at the same level as in 2005 (22%). The reasons for the persistence of resistance are not well understood.

2.7.10 Public Health Significance

Thermophilic *Campylobacter* species have received considerable attention in recent years as a major cause of bacterial enteritis in man. *Campylobacter* enteritis is recognized as an important source of diarrheal illness worldwide (CDC, 2008). The pathogen is also an important causative agent of 'traveler diarrhea' accompanied by predisposing debilitating factors such as pregnancy, premature birth, chronic alcoholism, neoplasia and cardiovascular disease (Mandrell *et al*., 2006). Campylobacteriosis affects all age groups; however, infections are recognized with increasing frequencies in infants, children, aged individuals, and immune-compromised persons. According to the Centre for Disease Control (CDC) report, *Campylobacter* infections accounted for approximately one-third of laboratory-confirmed foodborne illness that occurred globally in food net surveillance areas (CDC, 2008). A serious consequence of diarrheal diseases in human is called Guillain-Barrè syndrome (GBS) which is characterized by polyneuritis of the peripheral nerves that may lead to either short-term or lengthy paralysis. Guillain-Barrè syndrome (GBS), a demyelinating disorder resulting in acute neuromuscular paralysis, is serious sequelae of *Campylobacter* infection (Shane, 2000).

2.7.11 Economic Significance

As reviewed by Angesomand and Mahendra (2013), Campylobacteriosis cause severe economic loses both in the public health and food industry sector. Campylobacteriosis has an enormous economic impact in terms of treatment costs, loss of production, and human welfare. In livestock, particularly sheep and cattle, *Campylobacter* species are the cause of important economic losses associated with infertility problems and abortion (Beatriz and Ana, 2011).

2.7.12. Prevention and Control

Currently, no prophylactic vaccine is available to protect the susceptible population against *C. jejuni* infection. However, measures such as avoiding the use of untreated water, unpasteurized milk, undercooked red meat, raw poultry, hygienic handling of foods, sanitary disposal of faeces and other faeces soiled articles, biosecurity at poultry farm, good hygienic practices at meat processing plants, application of hazard analysis critical control point, training of abattoir workers, keeping children away from sick pets, active surveillance, personal hygiene and health education on basic principles of food hygiene will certainly reduce the incidence of campylobacteriosis, which has emerged as an important foodborne zoonosis of global importance (Pal, 2007; WHO, 2016).

2.8 A Review of *Zingiber officinale* Roscoe

Ginger scientifically known as *Zingiber officinale* Roscoe, belonging to family Zingiberaceae is one of the most important plants with several medicinal, nutritional and ethnomedical values, therefore, used extensively worldwide as a spice, flavoring agent, and herbal remedy (Grzanna *et al*., 2005). Traditionally, *Z. officinale* is used in Ayurveda, Siddha, Chinese, Arabian, Africans, Caribbean and many other medicinal systems to cure a variety of diseases viz, nausea, vomiting, asthma, cough, palpitation, inflammation, dyspepsia, loss of appetite, constipation, indigestion and pain (Grzanna *et al*., 2005).

2.8.1 Description

Ginger is herbaceous rhizomatous perennial, reaching up to 90 cm in height under cultivation. Rhizomes are aromatic, thick lobed, pale yellowish, bearing simple alternate distichous narrow oblong-lanceolate leaves (Kawai, 1994). The herb develops several lateral shoots in clumps, which begin to dry when the plant matures. Leaves are long and 2 - 3 cm broad with sheathing bases, the blade gradually tapering to a point. Inflorescence solitary, lateral radical pedunculate

oblong-cylindrical spikes. Flowers are rare, rather small, calyx superior, gamosepalous, three toothed, open splitting on one side, corolla of three subequal oblongs to lanceolate connate greenish segments (Kawai, 1994).

2.8.2 Antioxidant activity

Antioxidants are compounds or systems that can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. They can use several mechanisms such as scavenging species that initiate peroxidation, chelating metal ions so that they are unable to generate reactive species or decompose peroxides, quenching O_2^- preventing formation of peroxides, breaking the auto-oxidative chain reaction, and/or reducing localized O_2 concentrations (Asimi *et al*., 2013). Ginger can be regarded as the storehouse of antioxidants. It has an extraordinary property of scavenging reactive oxygen species (ROS), free radicals, peroxides, and various other damaging oxidants. The active ingredients like gingerols, shogaols, zingerone, and so forth present in ginger exhibit antioxidant activity. It inhibits an enzyme, namely, xanthine oxidase, which is mainly involved in the generation of reactive oxygen species. Zingerone has been reported to protect *in vitro* DNA against stannous chloride induced ROS oxidative damage (Rajan *et al*., 2013). Zingerone provides a direct adaptogenic effect by preventing oxidative stress on smooth muscles of the intestine (Banji *et al*., 2014). These findings lead to a conclusion that zingerone is a potent antioxidant.

Geraniol is an important chemopreventive agent and various studies have demonstrated that geraniol has a potent antioxidant effect by scavenging oxygen-free radicals and increasing the level of total glutathione content (GSH) in the murine skin (Dugasani *et al*., 2010).

Eleazu and Eleazu (2012) studied the antioxidant potentials of six varieties of ginger. All the varieties were observed to possess strong antioxidant activities and

had high quantities of phenols, which may be responsible for their antioxidant activities. Correlation analysis in the study revealed that the total phenolic contents of the ginger varieties correlated negatively with their total oleoresin contents. This finding suggested that the oleoresin contents might not have come from their phenolics constituents and that the oleoresins present could have little contribution to the antioxidant activities of the ginger varieties.

2.8.3 Antimicrobial activity

Foodborne illnesses are a major concern for consumers, the food industry, and food safety authorities. In recent years, considerable effort has been made to find natural antimicrobials that can inhibit bacterial and fungal growth in foods in order to improve quality and shelf-life. Natural extracts of plants have been used for many years for different purposes and recently they have been screened for their potential use as alternative remedies and food preservatives (Jones, 1996). The antibacterial activities of plant extracts and oils can be useful for the preservation of raw and processed food, in the pharmaceutical industry and as alternative medicines and natural therapies (Balchin and Deans, 1997).

Ginger has strong antibacterial and to some extent antifungal properties. Studies have revealed that a methanol extract of *Z. officinale* rhizomes possesses significant antibacterial activity against *Escherichia coli, Salmonella enteriditis,* and *Staphylococcus aureus* (Sunilson *et al*., 2009). *Escherichia coli* induced diarrhea is the leading cause of death in developing countries and recently it was documented that zingerone exerted a protective effect on *E. coli* induced diarrhea (Chen *et al*., 2008). Zingerone also showed a protective effect in hypermotility mediated diarrhea that was linked to inhibition of gastrointestinal motility. A recent study also indicated that zingerone supplemented Pacific white shrimp (*Litopenaeus vannamei*) juveniles showed a strengthening of immunity and protection against *V. alginolyticus* challenge(Chang *et al*., 2012).

The essential oil from ginger was studied for antimicrobial activity against *Aspergillus niger, Saccharomyces cerevisiae, Mycoderma* sp., *L. acidophilus* and *Bacillus cereus*, as determined by paper agar diffusion method (Guptha and Ravishankar, 2005). Another study reports on the bioassay-guided isolation of antifungal compounds from an African landrace of ginger, *Zingiber officinale* Roscoe, and the identification of 6, 8 and 10-gingerols and 6-gingerdiol as the main antifungal variables.

2.9 A review of Chili Pepper

Human use of chili peppers dates back to prehistoric times. Preserved peppers have provided evidence that South Americans ate and grew *aji*, (chili in English), in 2500 B.C (Mortensen and Mortensen, 2009). The peppers became increasingly common and integrated into the diet of particular cultures. However, chili peppers and similar spices remained isolated in these cultures until the 13th century, when they became available to civilizations throughout the world (Mortensen and Mortensen, 2009). The pungency of chili peppers is due to the accumulation of capsaicinoids (also known as capsinoids, a group of naturally produced compounds that are unique to the *Capsicum* genus (Jones *et al*., 1997). The chili pepper is a member of the *Solanaceae* family. It is a diploid, facultative, self-pollinating crop, and closely related to potato, tomato, eggplant, tobacco, and petunia. It is one of the oldest domesticated crops in the Western hemisphere, the most widely grown spice in the world, and is a major ingredient in most global cuisines (Mortensen and Mortensen, 2009). *Capsicum* species are commonly grown in warm humid regions such as the tropics and subtropics and their fruits are mainly used in local cuisine.

Chili peppers are widely used as spices in traditional Mexican foods. The flavor and pungent power of these peppers vary widely and so do their contents of

capsaicin and its capsaicinoid analogs (Jones *et al*., 1997). When eaten, many chili peppers evoke a sensation of heat and/or pain to the neurological systems in mammals, and these adverse effects can be overcome through the consumption of foods containing casein such as milk, cheese, or yogurt (Jones *et al*., 1997). Studies of the botanical pharmacopeia of the indigenous Mayan inhabitants of Mesoamerica have shown that chili peppers (*Capsicum* species) are incorporated into a number of medicinal preparations. These preparations were applied for a variety of ailments including respiratory problems, bowel complaints, earaches, and sores. Early European observers noted the omnipresent nature of chili peppers in the Mayan diet, reporting that nothing was eaten without them. While typically regarded as a spice, the substantial role that chili peppers occupy in this culture's diet may have important nutritional consequences for these people (Brito-Argáez *et al*., 2009).

Chili peppers have a wide range of uses, including pharmaceutical, natural colouring agents, and cosmetics, as an ornamental plant, and as the active ingredient in most defense repellants (that is, pepper sprays). Capsaicin, a well-studied chemical component of the *Capsicum* species and one of the pungent capsaicinoids found in chili peppers, has already demonstrated a high degree of biological activity affecting the nervous, cardiovascular, and digestive systems (Brito-Argáez *et al*., 2009). Chemical analysis has demonstrated that *Capsicum* fruits contain relatively high concentrations of several essential nutrients, including vitamin C (up to 6 times the concentration of an orange) (Brito-Argáez *et al*., 2009).

Strong consumer demand for safe and high-quality foods can be attributed in part to the widespread availability and accessibility of quality health data and information. There are also new concerns about food safety due to increasing occurrences of new food-borne disease outbreaks caused by pathogenic

microorganisms. This raises considerable challenges, particularly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit the growth of spoilage and pathogenic microorganisms (Cichewicz and Thorpe, 1996). In addition, currently available treatment options for food-borne pathogen infections have drug-related side effects, bacterial resistance to antimicrobials, and in some cases, no medical treatment exists for organisms such as *Escherichia coli* 0157: H7 (Cichewicz and Thorpe, 1996). Therefore, newer treatments which are safe, cost-effective, and simple to administer are urgently needed. In light of this, the use of nutritional agents is an attractive alternative to conventional therapeutics and warrants further investigation. Consequently, natural antimicrobials, such as chili peppers, are receiving a good deal of attention for a number of microorganism-control issues (Cichewicz and Thorpe, 1996). Recent reports state that the *Capsicum* genus, among other plant genera, is a good source of antimicrobial and antifungal compounds (Tajkarimi *et al*., 2010).

2.9.1 Studies on Antimicrobial Effects of Chili Pepper extracts on Some Foodborne and/or Human Pathogens

Bacillus subtilis (not typically associated with foodborne illness)

According to Molina-Torres *et al*. (1999), capsaicin (pure, purchased from Sigma Aldrich), had a strong inhibitory effect towards *B. subtilis* starting from 25 µg/ml (minimum concentration assayed).

Escherichia coli

Molina-Torres *et al*. (1999) determined that capsaicin (pure, purchased from Sigma Aldrich), at concentrations up to 200 or 300 µg/ ml only retarded the growth of *E. coli*.

Figure 2.2: different variants of Pepper

Source: Molina-Torres *et al.*, 1999

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection

Meat samples (Beef and Chicken meat) and Coleslaw ingredients/Vegetables (cabbage, Carrots, Green peas and Mayonnaise) were purchased from four different markets in Owerri, Nigeria. These were: Relief Market, Orji Market, Ihiagwa Market and Obinze Market. Twenty (20) samples were collected from each market at random at different tables, making a total of 80 samples. Each sample was properly labeled in sterile polythene bags, placed in an ice pack and transported to the Microbiology laboratory for sample preparation and analysis.

3.2 Sample preparation

Samples were prepared on a bench previously swabbed with 70% ethanol and allowed to dry. The meat samples were chopped into pieces and 20 g of each meat sample was minced in a sterile blender with 180 ml of distilled water. The meat was placed in sterile containers for further analysis. Vegetables for the Coleslaw was prepared according to methods described by FCJ refugee center community kitchen (2008). Approximately 20 g of the Coleslaw sample was placed into a sterile blender and blended with 180 ml of water. Each blended sample was placed in sterile Polythene bags for further analysis.

3.3 Isolation and characterization of *Campylobacter jejuni* and *Escherichia coli.*

3.3.1 Sample analysis for *Campylobacter jejuni* and *Escherichia coli.*

A ten-fold serial dilution was adopted as described by Cheesbrough (2009). Ten (10) ml of each blended sample was placed into 90 ml of sterile water. Dilution

was done up to the 6^{th} dilution factor. From the 6^{th} dilution factor, 0.1 ml was plated on a surface dried Sorbitol MacConkey agar (CT-SMAC) (Titan Biotech, India), Eosin Methylene Blue (EMB) (Titan Biotech, India) agar and *Campylobacter* Agar base (Oxoid, England). Eosin Methylene Blue (EMB) and CT-SMAC inoculated media was cultured aerobically at 37^0C for 24 hours while *Campylobacter* agar was cultured in a microaerophilic environment for 48 hours.

3.3.2 Pure culture technique

Discrete colonies formed after incubation was subcultured onto the surface of a surface dried prospective medium as described in 3.2.1 above. Pure cultures obtained was inoculated into 10 ml of Tryptic Soy Broth and on the surface of agar slants and stored in the refridgerator for further use.

3.3.3 Bacterial identification

The identification of organisms used in this study was based on selected biochemical tests and data obtained was analyzed using the international web-based software (ABIS Online) built with standard Bergy's Manual identification procedures. The software is available at http://www.tgw1916.net/bacteria_logare_desktop.html. To identify the organisms, the selected biochemical tests, also available at http://www.tgw1916.net/bacteria_logare_desktop.html for a group of bacteria, were conducted and the results were fed into the software which in return provide related microbial identities. Below are the set of tests required for the identification of the test isolates.

a) Selected target 1: *Escherichia coli*

Recommended tests (best for identification): pigment, catalase, oxidase, lactose, indole, citrate, h_2s, urease, voges proskauer (VP), phenylalanine, motility,

gelatin hydrolysis, potassium cyanide (KCN), malonate, glucose, gas, mannitol, inositol, sorbitol, arabinose, nitrates, cellobiose, maltose, mannose, amdg, trehalose, xylose, mucate, tartrate, acetate, deoxyribonuclease, lipase, methyl red and adonitol.

b) Selected target 2: *Campylobacter jejuni*

Recommended tests (best for identification): urease, indoxyl, hippurate, nitrates, selenite, H_2S, growth at 25°c and 42°c, oxidase, 2%nacl, 2%bile, minimal medium, nalidixic acid, Sodium Fluoride (NaF), safranin, alkaline phosphatase activity (pal), cefoperazone, cephalotin, and macconkey.

3.4 Standardization of isolates

The McFarland's (MF) Standard was prepared following the reaction between 1% barium chloride and 1% Sulfuric acid standardized into MF standard 0.5, 1, 2, 3 and 4. The MF plot was obtained by plotting values obtained from the A_{500} readings against the predefined standard number of cells. The cells used in this work were harvested and washed; re-suspended and the absorbance was read at A_{500}. The Number of cells used was therefore calculated by interpolation.

3.5 Experimental design

A General full factorial design (2^k) was carried out using three different food additives (Ginger, Pepper, and Salt) so as to ascertain the effect of each factor in singles and in synergy. The effect of four concentration levels of each spice was tested against the standardized inoculum. The spices concentrations include Salt (0%, 2%, 6% and10%), Ginger (0 µg/ml, 400 µg/ml, 800 µg/ml and 1200 µg/ml) and pepper (0 µg/ml, 400 µg/ml, 800 µg/ml and 1200 µg/ml). All Percentage were expressed in weight percent.

3.6 Experimental set up for Microbial inactivation

The growth of the test isolates on the meat sampled was studied at different temperatures so as to obtain specific temperatures that could inactivate the microorganisms over a selected and graded incubation period. In this study, a 24-hour inoculum of the test isolates was diluted using tenfold serial dilution and 1 ml of the 10^6 diluent was inoculated in minced chicken meat sample (10 g portions). The inoculated test isolates were mixed with the meat and the samples stored in polyethylene bags at different temperatures (-4°C, 4°C, 25°C, 35°C and 45°C).

3.6.1 Sampling and cultivation

To determine the temperature dependent inactivation of the selected test isolates, samples of each storage temperature treatment at the time of inoculation, after 4h, then 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 hours were taken for microbial analysis using the spread plate technique. Experiments that evaluated survival at each storage temperature was replicated. At each sampling interval, frozen samples were thawed at room temperature for 20 min and 10 g quantities were subsequently diluted 1:10 in 0.9% peptone saline water. These were subsequently mixed for 20 s and plated selective agar media plates using the spread plate method. Incubation of the inoculated plates was done at the appropriate growth conditions for each organism. These include microaerophilic conditions (for *Campylobacter* sp) at 37°C and in aerobic conditions (for *E. coli*) at 37°C. The microbial Load was determined in colony forming units (cfu/ml).

3.7 Data analysis

3.7.1 Data analysis for model prediction

Data was transferred to Microsoft® Excel 2016 for the primary analysis. GInaFiT software described by Geeraerd *et al*. (2005) was used to identify appropriate survival models that fit the dataset by least squares regression and a logarithmic form of the Weibull model was selected in order to build predictive models.

3.7.2 Statistical analysis

Statistical analyses were performed using MINITAB 17 software. Analysis of variance and covariance (ANOVA) was carried out to determine the differences that occurred around the mean of bacterial counts. The F-test was used to compare the goodness of fitting between the log-linear model and the Weibull model at $P<0.05$. Mean and standard deviations were calculated for all data sets and the results were indicated as Mean ± Standard Deviation. The Mean Sum of Square Error (MSE) in conjunction with The R^2 Values was used to determine the Goodness of Fit at $P<0.05$.

CHAPTER FOUR

RESULTS

4.1 Isolation and identification of isolates

The isolates were identified based on morphological and biochemical characterization in line with specifications available at ABIS online. *Escherichia coli* 0157: H7 (A) was purified on sorbitol MacConkey agar and its colonial morphology is shown in Plate 4.1. Pure cultures of *Campylobacter* sp (B) was obtained on *Campylobacter* agar base (Oxoid) and its colonial features are shown in Plate 4.2 as a white mucoid, moist and shiny colonies growing over the black charcoal background. The biochemical tests result is shown in Table 4.1. Data obtained was feed into the ABIS online software and results obtained are displayed in Plate 4.3 and 4.4 for *Escherichia coli* 0157: H7 and *Campylobacter jejuni* respectively. Plate 4.3 showed a degree of relatedness of the isolates identified by the software to have similar biochemical tests. Results indicated that the isolates (A) were closely related to *Escherichia coli* 0157: H7 by 99% compared *to Citrobacter koseri* (90%), *Citrobacter farmeri* (88%) and *Citrobacter sediakii* (88%). On the other hand, Plate 4.4 showed that *Campylobacter jejuni* subsp. doylei was identified using the ABIS online with a 99% degree of relatedness compared to the other isolate (B) with close relatednesses such as *Helicobacter fennelliae* (92%), *Helicobacter canis* (81%) and *Campylobacter helveticus* (76%).

Plate 4.1: Colonial morphology of pure cultures for *Escherichia coli* 0157: H7 identification on CT-SMAC (Titan Biotech, India).

Plate 4.2: Colonial morphology of pure cultures for *Campylobacter* sp identification on *Campylobacter* agar base (Oxoid).

Table 4.1. Biochemical tests for the identification of *Escherichia coli* and *Campylobacter* spp.

Identification using Biochemical tests			
Test	**Result**	**Test**	**Result**
Escherichia coli			
Catalase	+	Glucose	+
Oxidase	-	Gas	+
Indole	+	Mannittol	+
Lactose	+	Sorbitol	+
Citrate	-	Nitrates	+
Voges Proskaur	-	Maltose	+
Motility	+	Mannose	+
KCN	-	Tartrate+	+
Methyl red	+	Acetate	+
***Campylobacter* sp**	**Result**	**Test**	**Result**
Indoxyl hydrolysis	-	Nalidixic acid	-
Hippurate hydrolysis	+	NaF	-
Nitrates	-	Safranin	-
Selenite	-	PAL	+
H_2S	-	Cefoperazone	-
Growth at 42°C	Nd	Cephalotin	-
Urease	Nd	Oxidase	+
MacConkey	Nd	2%NaCl	-

+ = Positive, - = Negative, nd= not done

Plate 4.3: ABIS Online results for *Escherichia coli* identification

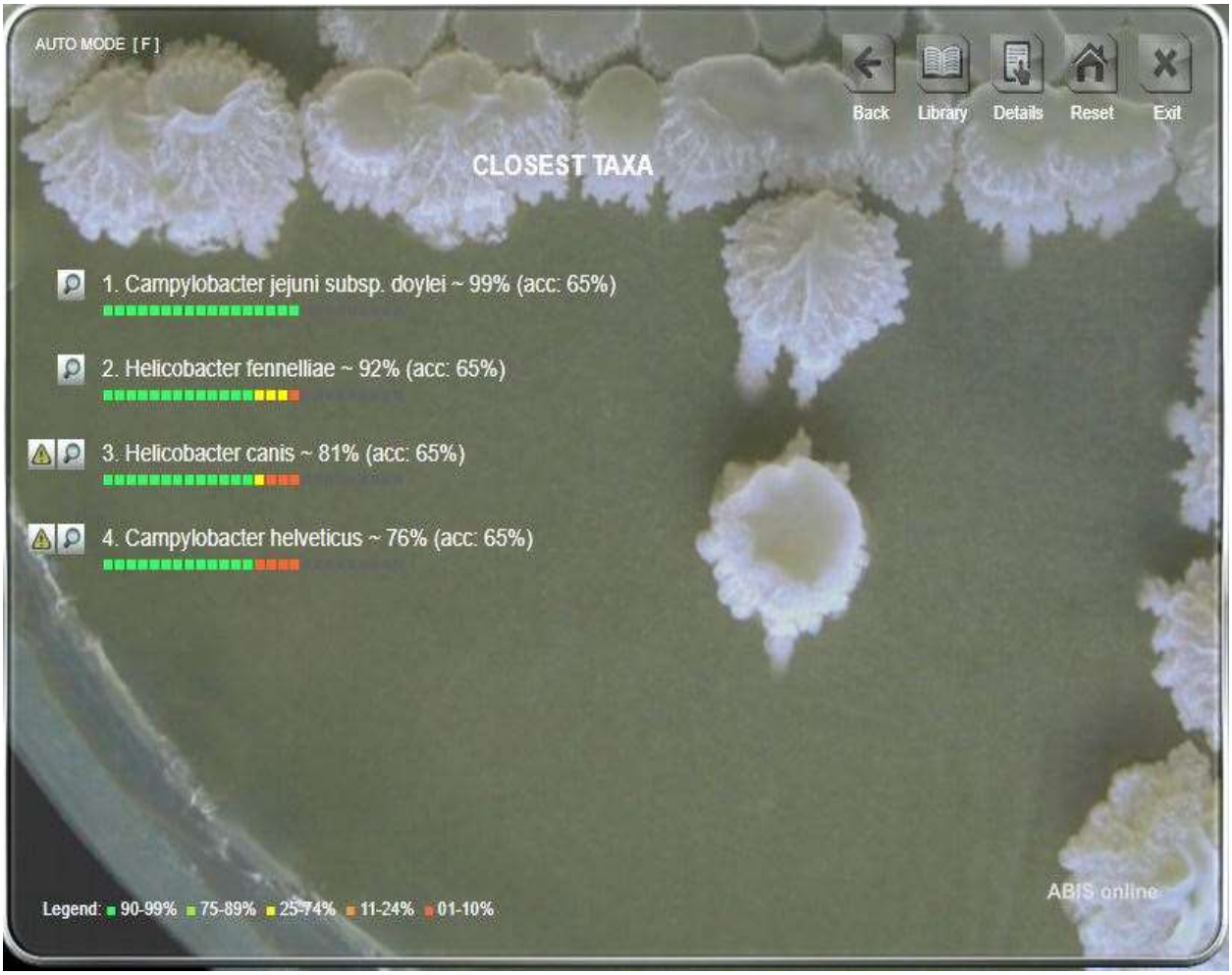


Plate 4.4: ABIS Online results for *Campylobacter jejuni* subsp. doylei identification

4.2 Microbial load of samples obtained from four Local Markets

Three sample varieties were assessed for their microbial loads using Standard plate counts and their results are shown in Figure 4.1. Considering the meat samples, higher counts of *Campylobacter* spp was obtained from the Chicken meat samples than the beef (Red meat) samples except meat samples obtained from relief and Ihiagwa market. The counts obtained ranged from 5.0 $x10^3$-1.18 $x10^4$ Cfu/g for Chicken meat; 6.4 $x10^3$-8.8 x 10^3Cfu/g for red meat samples and 5.0 x 10^3-1.18 x 10^4 Cfu/g for both samples. Lower counts were obtained from Salad samples with a range of 2.32 x 10^3-3.2 x 10^3 Cfu/g. Counts were significantly different from each other by comparing their means using ANOVA at $P < 0.05$.

Similarly, the counts on CT-SMAC were higher from Chicken meat than red meat samples obtained from Obinze market and Relief Market. Also, the red meat samples had higher counts than Chicken meat samples obtained from Orji market. The same trend was obtained from samples obtained from Ihiagwa market. The counts obtained from Chicken meat ranged from 8.4 x 10^3-1.06 x 10^4 Cfu/g for Chicken meat samples and 6.6 x 10^3-1.28 x 10^4 Cfu/g for the red meat samples. The Salad vegetables also had generally lower counts than the chicken and red meat samples with a range of 4.0 x 10^2-2.55 x 10^3 Cfu/g. Counts were also significantly different from each other by comparing their means using ANOVA at $P<0.05$.

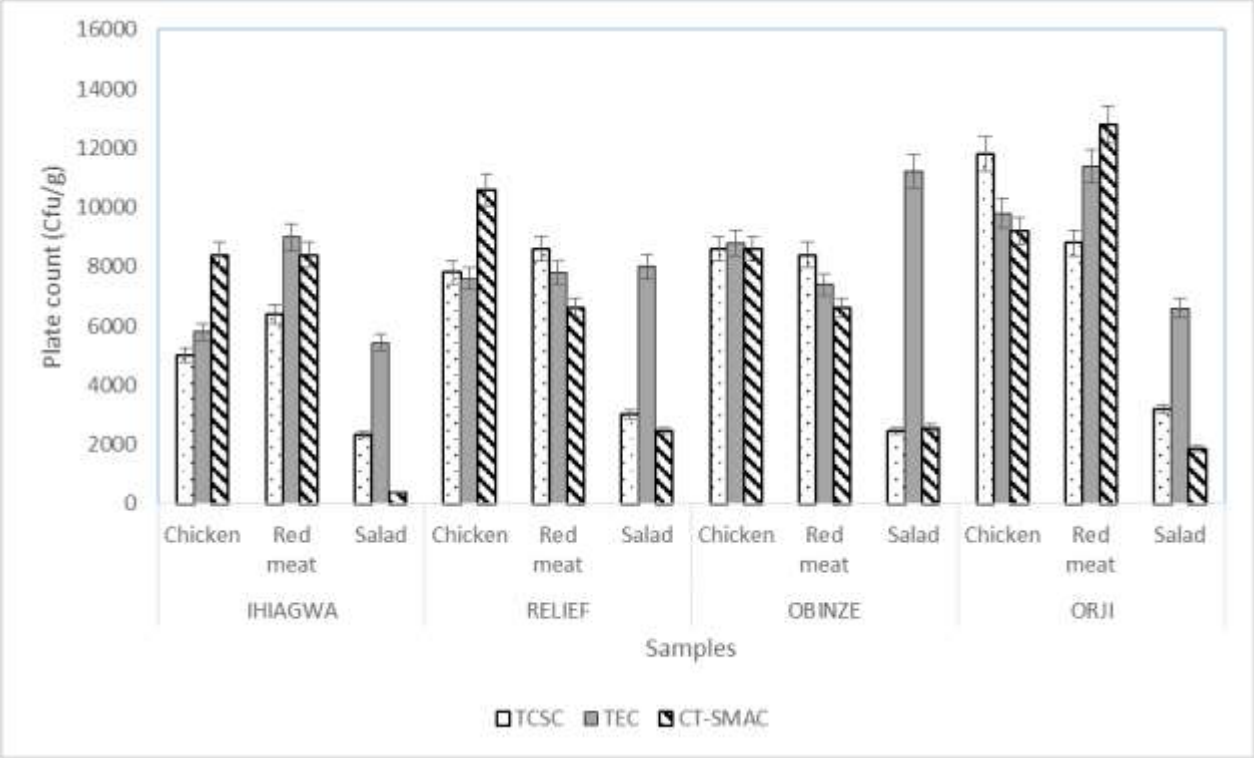


Figure 4.1: Total plate counts of bacterial isolates from chicken meat red meat and salad, sample isolated from samples

TEC=Total Enterobacteriaceae Count

TCSC= Total Campylobacter sp Count

CT-SMAC= Counts recovered on CT-SMAC

4.3. Standardization of isolates

McFarland's (MF) Standard was obtained following the reaction between barium chloride and sulfuric acid standardized into MF standard 0.5, 1, 2, 3 and 4. The MF plot was obtained by plotting values obtained from the A_{500} readings against the predefined standard number of cells whose results are displayed in Table 4.2 and the standard plot shown in Figure 4.2 with a linear/interpolatory equation of

$$y = 6 \times 10^{-10}x + 0.0297$$

With R^2 Values of 0.9972. Where y = Optical density and x=cell concentration.

The OD readings obtained for the 24-hour cultures were standardized to McFarland's standard 0.8 which corresponds to 1.28 x 10^9 cells.

Table4.2. McFarland's standard table for determination of McFarland's Standard plot

MF STd	Abs	Log N	No of Cells
0.5	0.108	8.176	1.50E+08
1	0.212	8.477	3.00E+08
2	0.352	8.778	6.00E+08
3	0.549	8.954	9.00E+08
4	0.700	9.079	1.20E+09

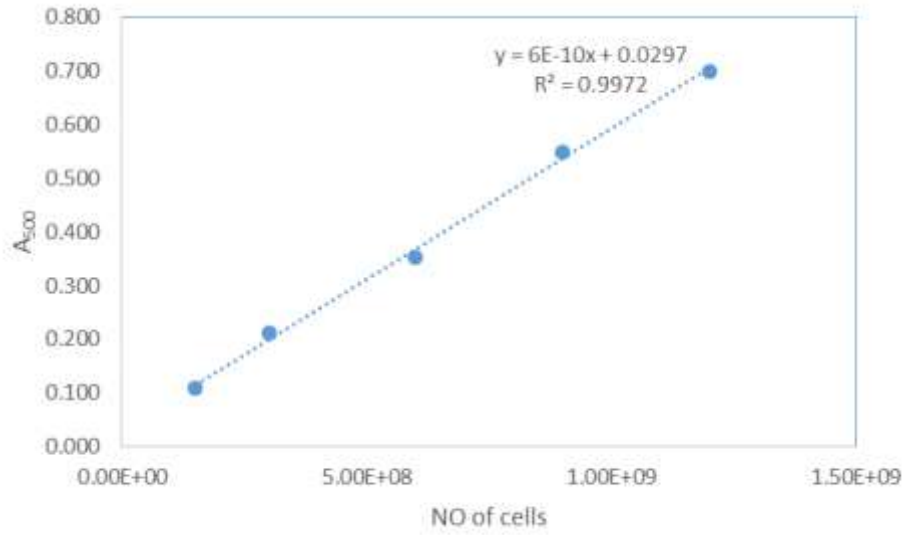


Figure 4.2. McFarland's standard plot for the determination of cell number of broth cultures used as standard inoculum.

4.4. The single effect and combined effects of extracts of ginger, pepper, and salts on *Campylobacter jejuni* and *Escherichia coli* 0157: H7

4.4.1 The single effect and combined effects of extracts of ginger, pepper, and salts on *Campylobacter jejuni*

The main effect and combined effects of extracts of ginger, pepper, and salts on *Campylobacter jejuni* and *Escherichia coli* 0157: H7 was determined using a 2^k factorial plot are displayed in Figures 4.3- 4.6. Figure 4.3 shows the single treatment of spices on *Campylobacter jejuni*. Results show that ginger and pepper extracts had mean stimulatory effect at 400 µg/ml while the salt extracts had similar results at 4% salt concentration. Also, at a concentration of 800 µg/ml, ginger extracts had a stationary/inhibitory effect and pepper had inhibitory effects while salt also had inhibitory effects at 4% concentration. At 8% salt concentration, the effect on Mean Growth was bactericidal demonstrated by a rapid decline below the initial bacterial population. Similar results were also recorded for ginger at 1200 µg/ml. However, pepper had a stimulatory effect on *Campylobacter jejuni* at that concentration.

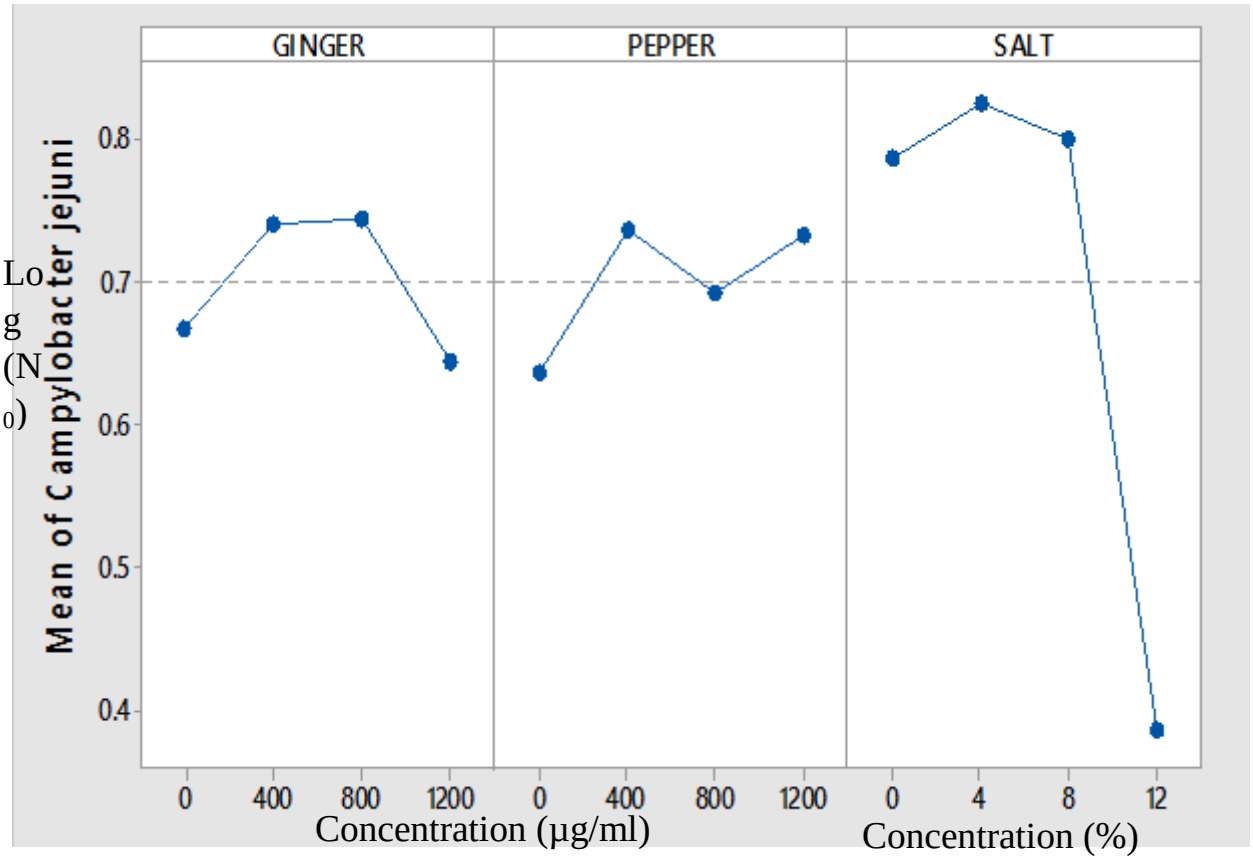
Figure 4.4 shows the interaction plot between the mean effects of extracts of the selected spices extracts against *Campylobacter jejuni*. The interaction of salt x ginger and salt x pepper had significant interactions causing inhibitory effects against *Campylobacter jejuni* with an increase in concentration. The maximum interaction was observed at a concentration of 800µg/ml of pepper and ginger and 8% Salt concentration resulting in the death of the isolate above that concentration. More so, similar results were obtained for salt x pepper at 1200 µg/ml and 12% salt. The mean inhibition was observed in ginger x pepper than pepper x ginger indicated that ginger had better inhibitory activity than pepper in mixed concentrations. Despite the interactions observed in other combinations, the

interactions were synergistic in ginger x pepper, ginger x salt, salt x ginger, salt x pepper while they were antagonistic in pepper x salt and pepper x ginger respectively.

4.4.2 The Single effect and combined effects of extracts of ginger, pepper, and salts on *Escherichia coli* 0157: H7

Figure 4.5 shows the main effect plots for the effects plot for the effect of the spices against *Escherichia coli* 0157: H7. Results show that the pepper extracts had mean stimulatory effect at 400 µg/ml while the salt extracts had similar results at 4% salt concentration. However, the mean inhibitory effect was obtained for ginger extracts at 400 µg/ml and 800 µg/ml but stimulatory at 1200 µg/ml. Also, at a concentration of 800 µg/ml, pepper extracts had inhibitory effects while salt solution also had stimulatory effects at 8% concentration. At 12% salt concentration, the effect on mean growth was bactericidal demonstrated by a rapid decline below the initial bacterial population.

Figure 4.6 shows the interaction plot for the mean effects of extracts of the selected spices against *Escherichia coli* 0157: H7. As observed in the effects of the extracts on *Campylobacter jejuni*, the interaction of salt x ginger and salt x pepper had significant interactions causing inhibitory effects against *Escherichia coli* 0157: H7 as the concentration increases. The maximum interaction was observed at a concentration of 800 µg/ml of pepper and ginger and 8% salt concentration resulting in the death of the isolate above 800 µg/ml of pepper and ginger and 8% salt concentration. Combinations of pepper x salt and ginger x salt had weak interaction and effect resulted in a combined antagonistic effect using inhibition as a synergistic measure. Combinations of pepper x ginger and ginger x pepper had a mean stationary effect on the growth rate of *Escherichia coli* 0157: H7.



Log (N_0)

Figure 4.3: The activity of the selected food spices against *Campylobacter jejuni*

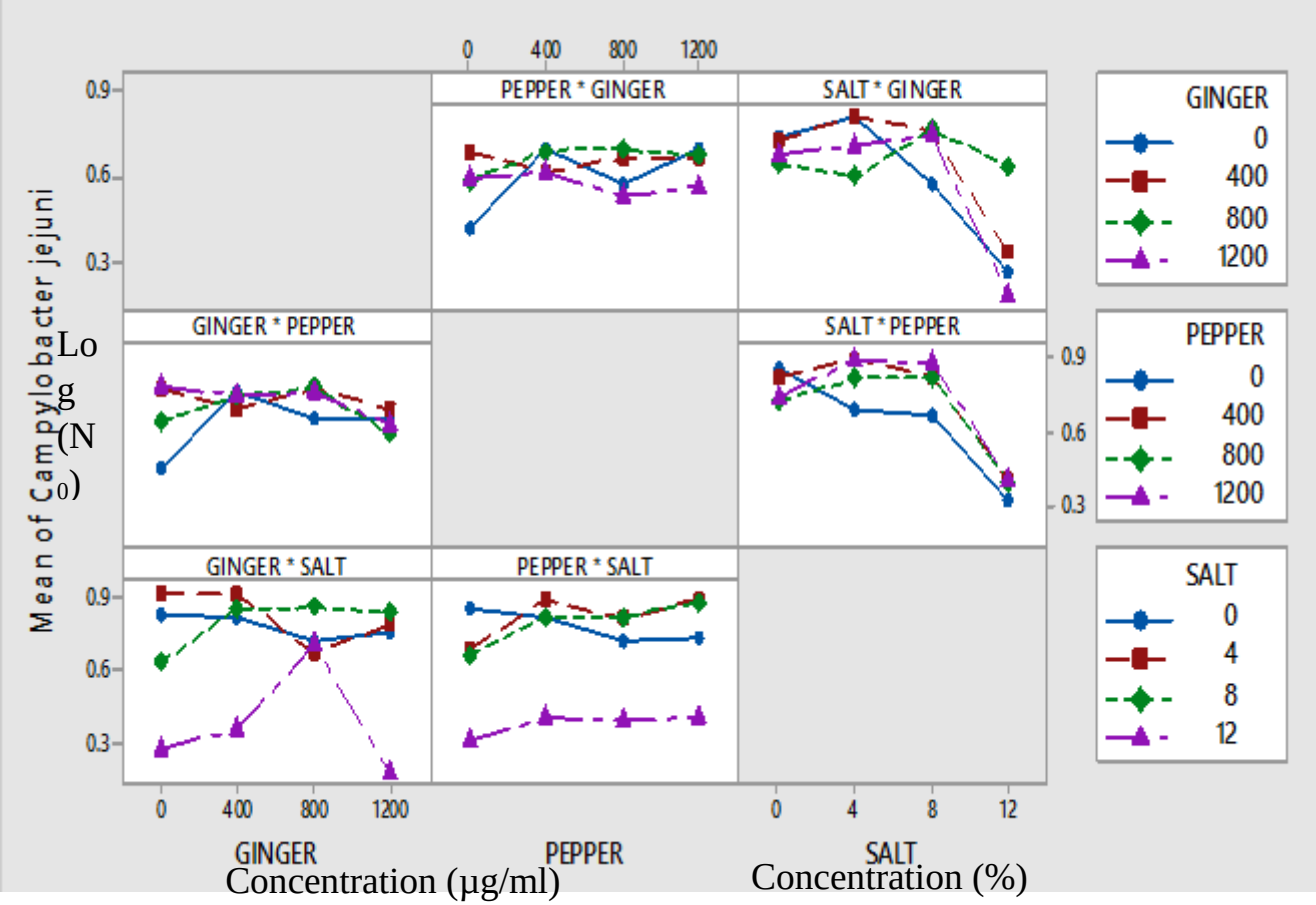


Figure 4.4: Combined effects of the selected food spices against *Campylobacter jejuni*

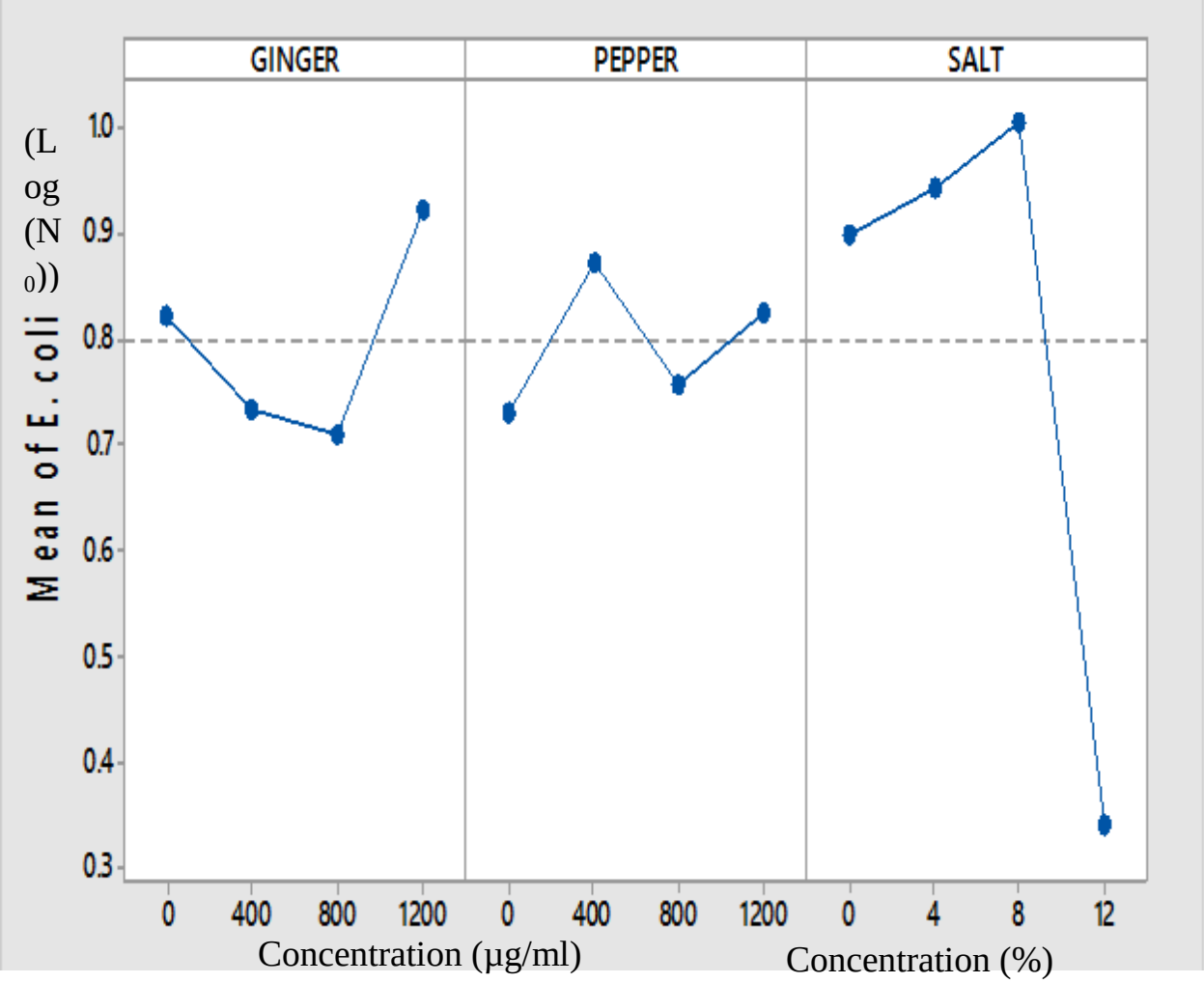


Figure 4.5: The activity of the selected food spices against *Escherichia coli 0157: H7*

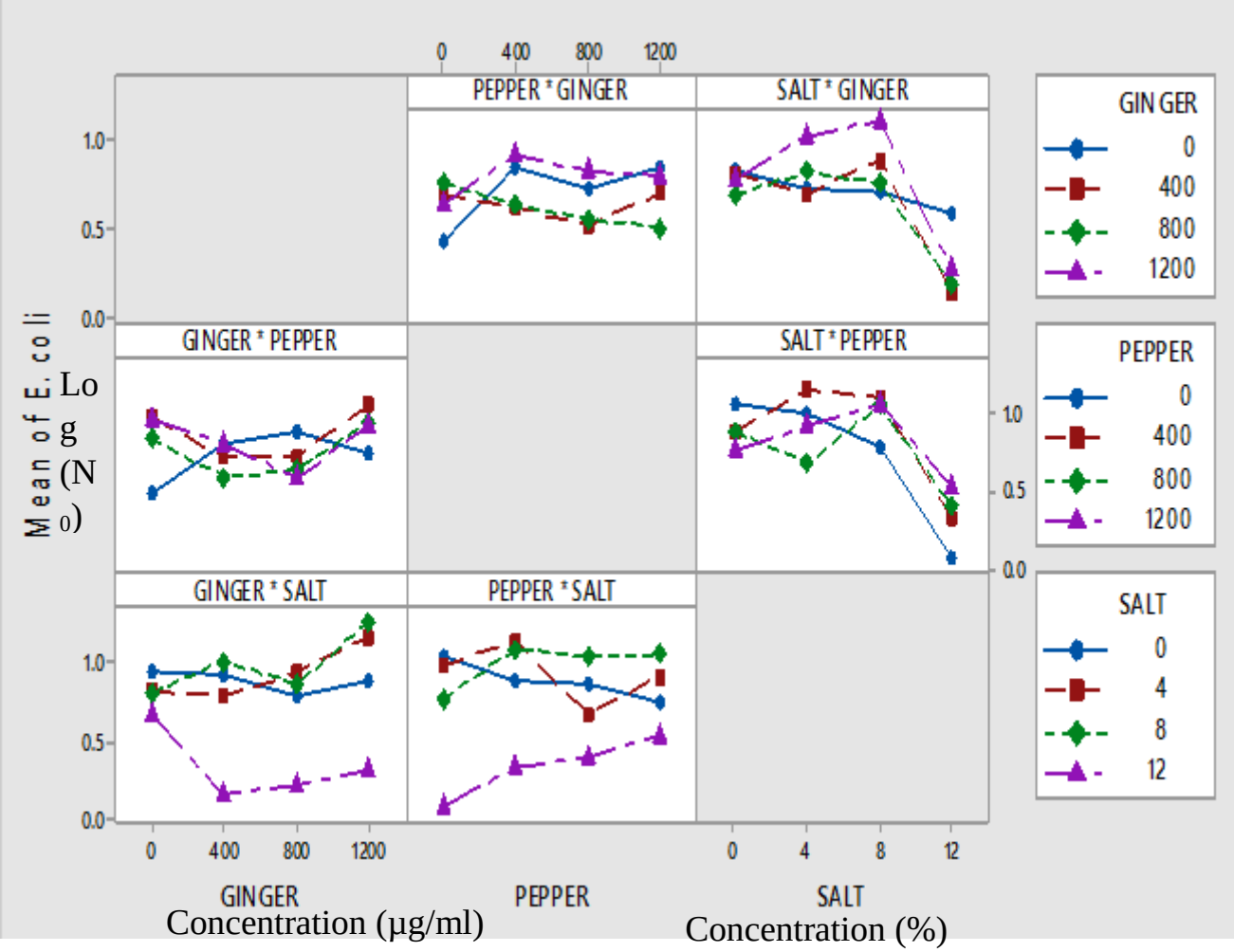


Figure 4.6: Combined effects of the selected food spices against *Escherichia coli* 0157: H7

4.5. Maximum and minimum effects of Spices' extracts on microbial growth

Figure 4.7 and 4.8 showed the maximum and minimum effects of the extracts on *Campylobacter jejuni* and *Escherichia coli* 0.57: H7 respectively. The maximum effects represent the highest non-inhibitory effect obtained from the factorial plots and interactions while the minimum effects represent the inhibitory effects obtained at given optimal predicted conditions. Results of these plots are summarized in Figure 4.7. The results recorded a highest inhibitory growth of 88.85% and 98.5% growth at 12% salt concentration; and a maximum non-inhibitory effect of 12.38% and 10.8% at 1200 µg/ml Ginger, 400 µg/ml Pepper and 4% Salt for both *Campylobacter jejuni* and *Escherichia coli* 0.57: H7 respectively.

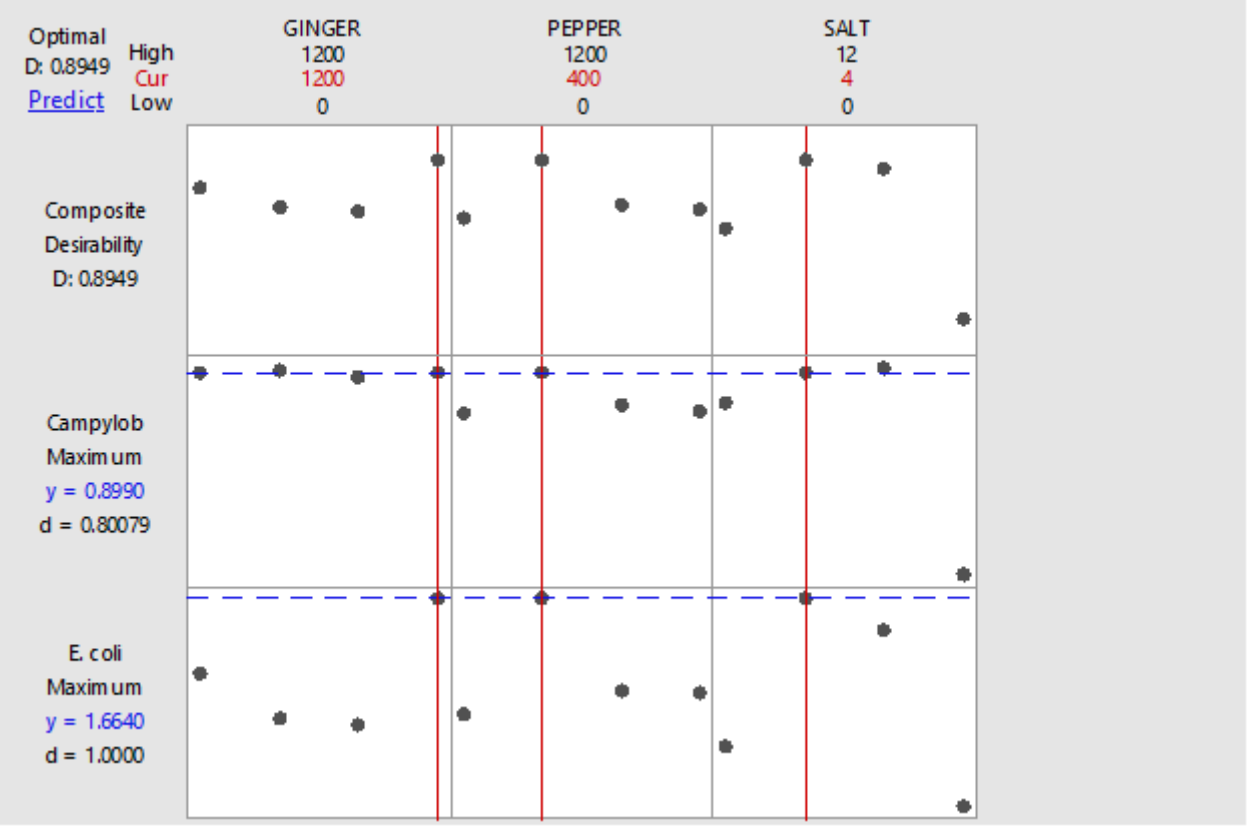


Figure 4.7: Optimization plot (Maximum) for the activity of the spices on the test isolates

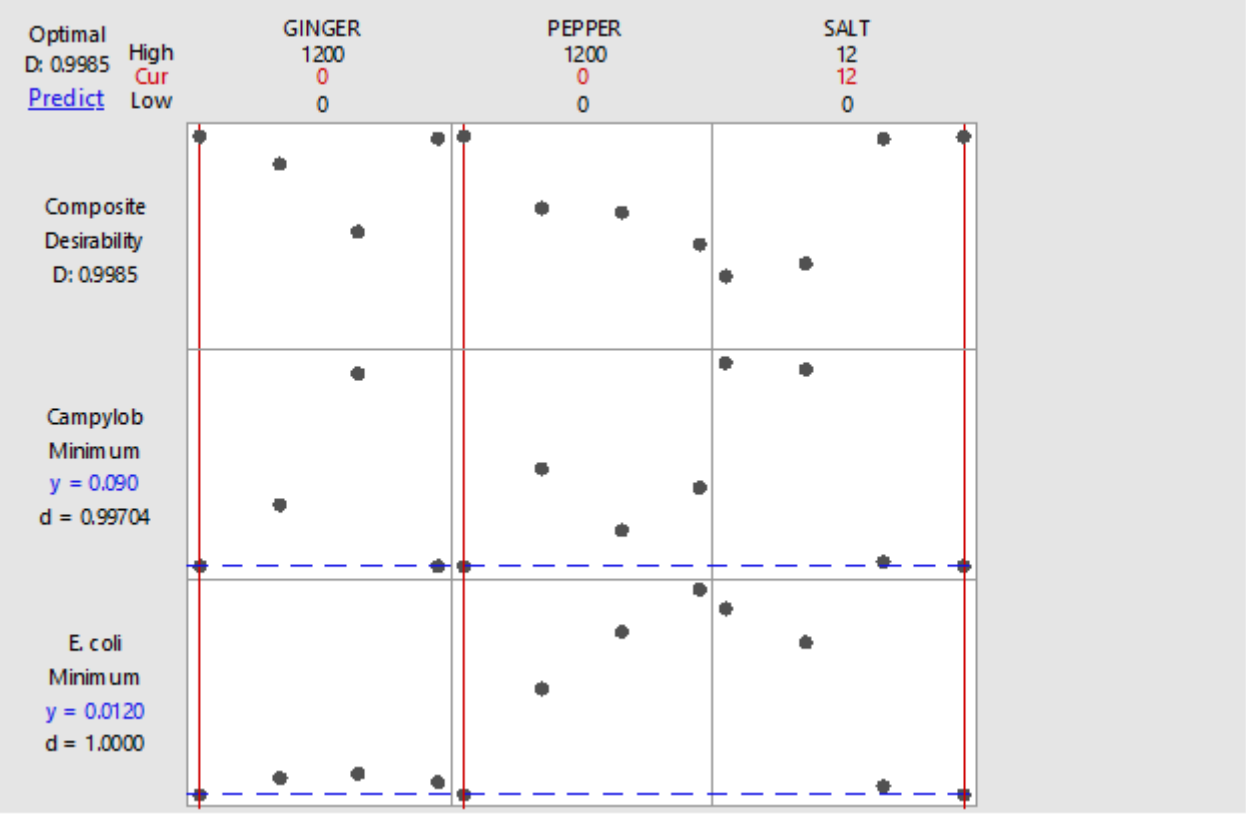


Figure 4.8: Optimization plot (Minimum) for the activity of the spices on the test isolates

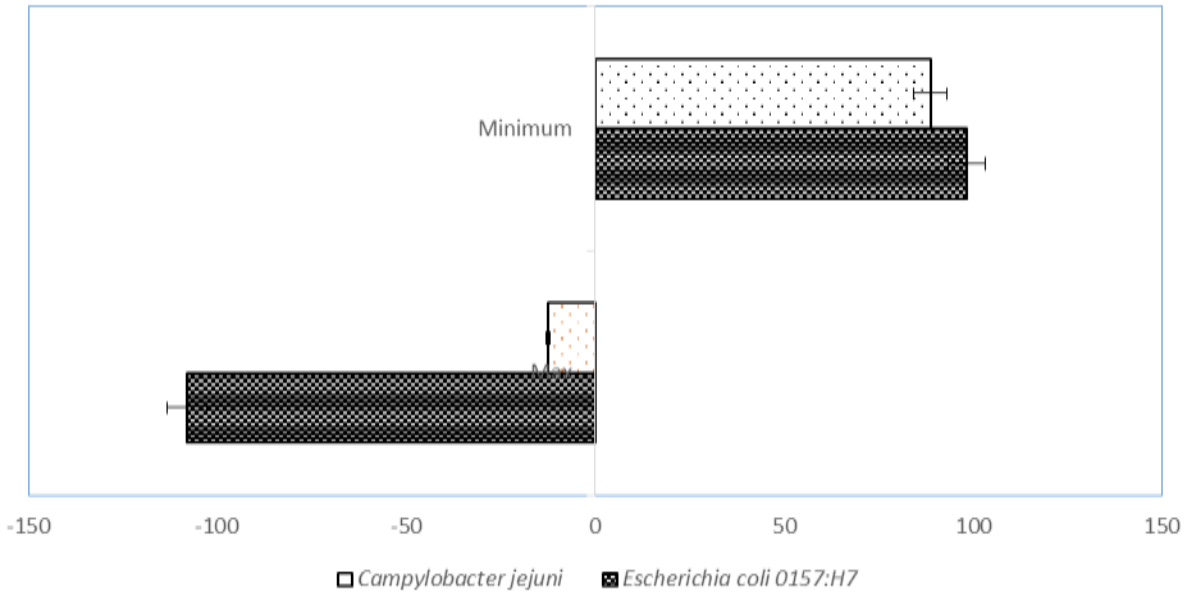


Figure 4.9. Summarized maximum and minimum effects of extracts on selected test isolates.

4.6 Modelling temperature dependent inactivation of *Campylobacter jejuni*

At -4^{0}C the data fitted into the Two Mixed Weibull Distributions with an R^2 value of 0.9680. The fitted model is described in Figure 4.10. The model identified conformed to:

$$N = \frac{N_0}{(1+10^{\alpha})} \times \left[10^{-\left(\left(\frac{t^{-1}}{\partial_1}\right)^p + \alpha\right)} + 10^{\left(-\left(\frac{t}{\partial_2}\right)^p\right)}\right] \ldots\ldots\ldots\ldots \quad \text{Equation 1}$$

Where the values of each parameter were calculated and outlined in Table 4.3 with values of alpha, delta1, p, and $Log_{10}(N_0)$ as 0.00, 39.98, 1.60 and 9.03 respectively.

At 4^{0}C the data also fitted into the Two Mixed Weibull Distributions. The fitted model is described in figure 4.11. The model identified conformed to the model displayed in Equation 1.

With an R^2 Value of 0.9394 and Where the values of each parameter were calculated and outlined in Table 4.4 with values of alpha, delta1, p and $LOG_{10}(N_0)$ values of 0.00, 192.46, 1.45 and 9.05.

Considering the modeling of the growth of the microorganisms at -4^{0}C and 4^{0}C, it is important to note that values of alpha, delta and ρ are only derivable for inactivation Models with a negative slope. Where positive slopes exist, the Bigelow and Esty's Model will be adopted and values of K_{max} and $Log_{10}(N_0)$ will be obtained.

At 25^{0}C and 35^{0}C, the data fitted into the thermal death Model with an R^2 Value of 0.9706 and 0.9573 as shown in Figure 4.12 and 4.13 respectively. The Model conformed to:

$$N = N_0 \times e^{-K_{max} \times t}$$

Which when the Logarithmic values of both sides were taken, it transformed to:

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{\ln 10}$$

Which can be further simplified as:

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{2.303} \ldots\ldots\ldots\ldots \quad \text{Equation 2.}$$

As stated earlier, this model signifies a positive slopped model with k_{max} and LOG_{10} (N_0) values of -0.01 and 9.06; and -0.01 and 9.16 for 25^0C and 35^0C respectively. This signifies that the inactivation of the isolate is not possible at near room temperature and indicates the risk associated with samples displayed openly at market temperatures.

At 45^0C, the predicted data also fitted into the Two Mixed Weibull Distributions. The fitted model is described in figure 4.14. The model identified conformed to the model displayed in Equation 1.

With an R^2 Value of 0.9816 and where the values of each parameter were calculated and outlined in Table 4.4 with values of Alpha, delta1, ρ and $LOG_{10}(N_0)$ values of 0.00, 82.16, 1.06 and 9.05.

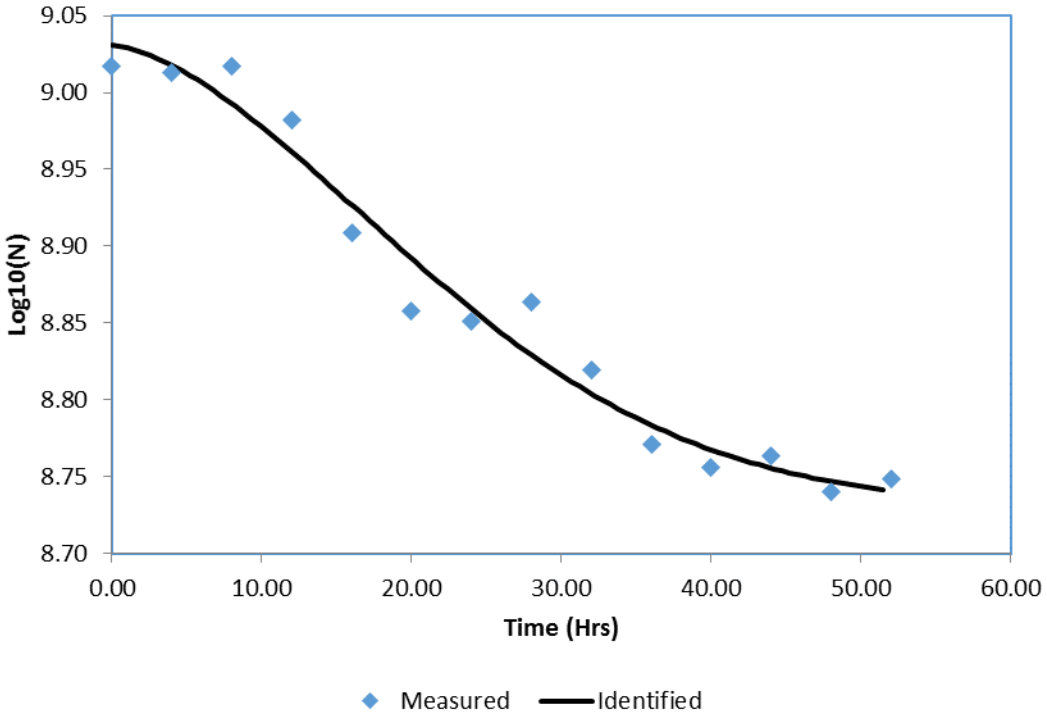


Figure 4.10. Two Mixed Weibull Distributions of Temperature-dependent inactivation of *Campylobacter jejuni* at -4^{0}C.

Table 4.3: Calculated parameters of the Two Mixed Weibull Distributions of Temperature-dependent inactivation of *Campylobacter jejuni* at -4^{0}C.

	Parameter	
Parameters	**values**	**Standard Error**
Alpha	0.00	0.76
delta1	38.98	31.43
P	1.60	0.70
LOG10(N0)	9.03	0.02
Delta 2	26414558.24	6915250879890100.00

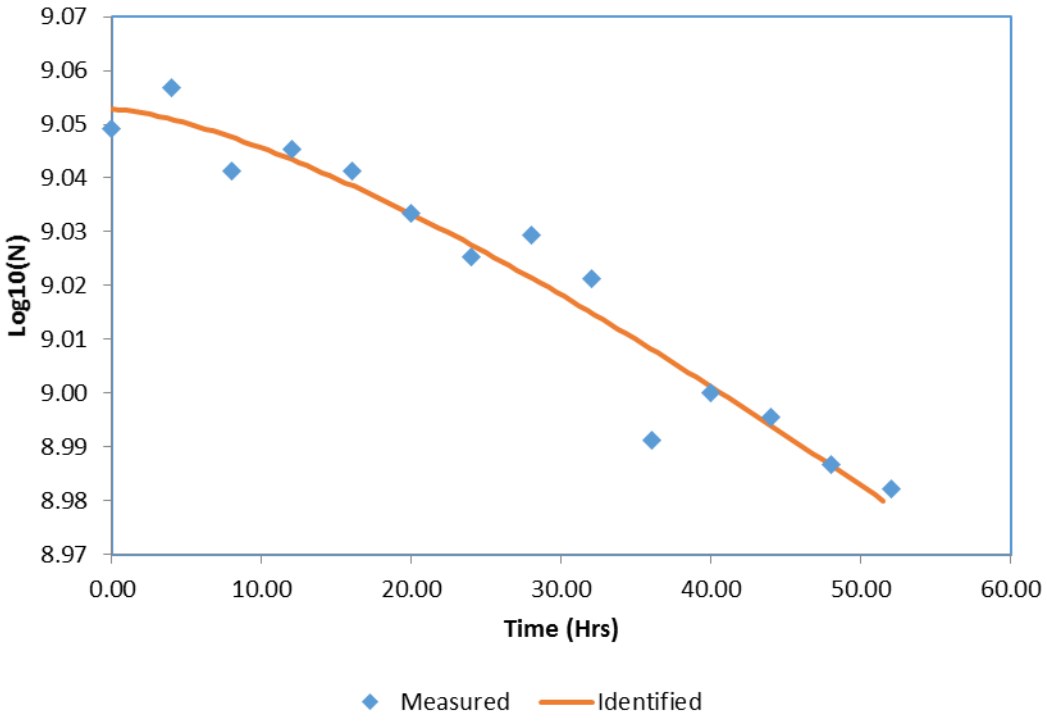


Figure 4.11. Two Mixed Weibull Distributions of Temperature-dependent inactivation of *Campylobacter jejuni* at 4^0C.

Table 4.4: Calculated parameters of the Two Mixed Weibull Distributions of Temperature-dependent inactivation of *Campylobacter jejuni* at -4^{0}C.

Parameters	**Parameter values**	**Standard Error**
alpha	0.00	285.44
delta1	192.46	23415.03
p	1.45	2.19
LOG10(N0)	9.05	0.01
Delta 2	1322.47	2064303.63

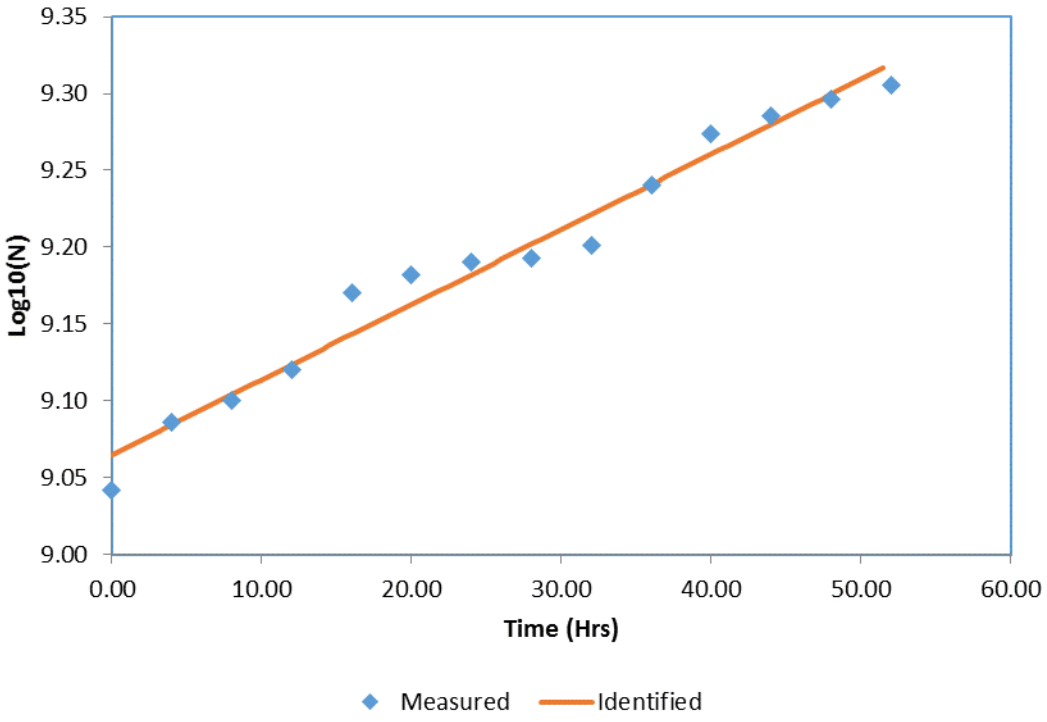


Figure 4.12. Bigelow and Esty's model fitting of growth of *Campylobacter jejuni* at 25^0C.

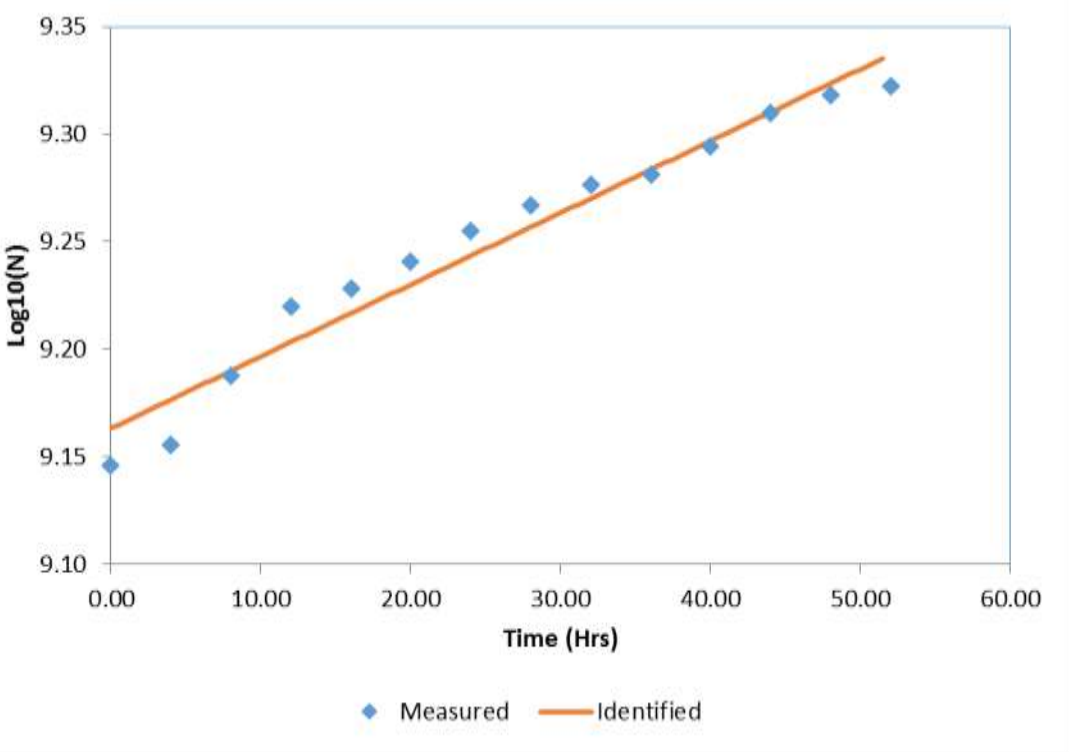


Figure 4.13. Bigelow and Esty's model fitting of growth of *Campylobacter jejuni* at 35^0C.

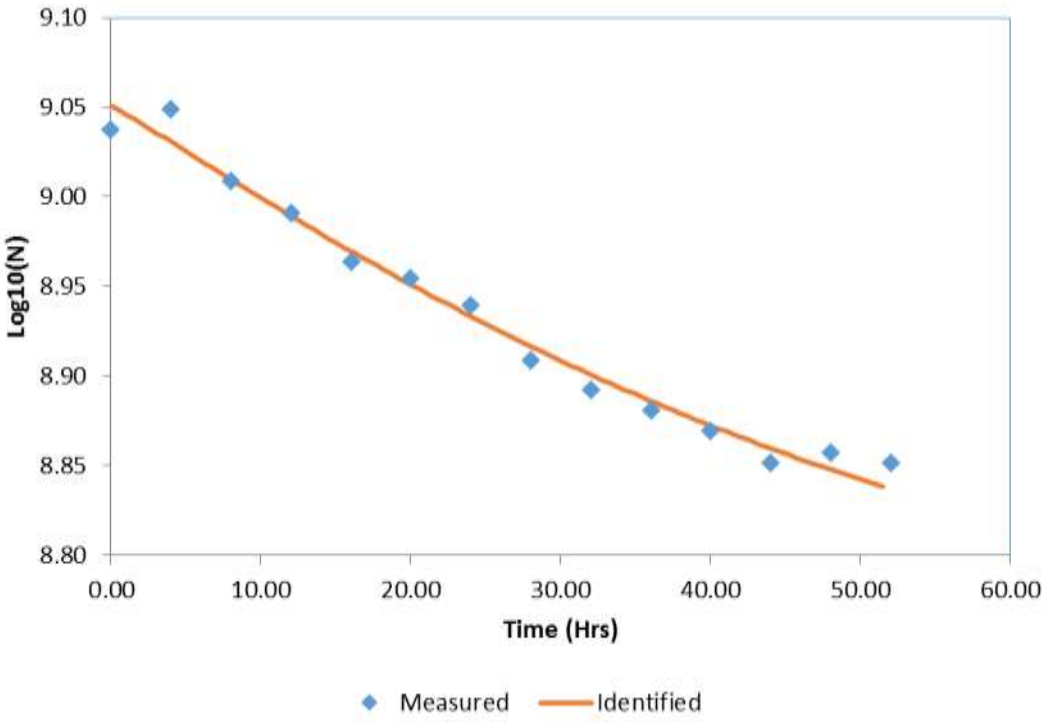


Figure 4.14. Two Mixed Weibull Distributions of Temperature-dependent inactivation of *Campylobacter jejuni* at 45^0C.

Table 4.5: Calculated parameters of the Two Mixed Weibull Distributions of Temperature-dependent inactivation of *Campylobacter jejuni* at 45^0C.

Parameters	**Parameter values**	**Standard Error**
alpha	0.00	5.76
delta1	82.16	440.07
P	1.06	0.59
LOG10(N0)	9.05	0.01
Delta 2	4529.87	661879.20

4.7. Modeling temperature dependent inactivation of *Escherichia coli*

At -4^{0}C the data obtained from the inactivation of *Escherichia coli* also fitted into the Two Mixed Weibull Distributions with an R^2 value of 0.9889. The fitted model is described in figure 4.15. The model identified conform to Weibull Model described in equation 1:

$$N = \frac{N_0}{(1+10^{\alpha})} \times \left[10^{-\left(\left(\frac{t^{-1}}{\partial_1}\right)^{p}+\alpha\right)} + 10^{\left(-\left(\frac{t}{\partial_2}\right)^{p}\right)}\right] \ldots\ldots\ldots\ldots \quad \text{Equation 1}$$

Where the values of each parameter were calculated and outlined in Table 4.6 with values of alpha, delta1, p, and Log_{10} (N_0) as 00.71, 36.38, 2.59 and 9.39 respectively.

It is important to note that Values of alpha, delta and ρ are only derivable for inactivation Models with a negative slope. Where positive slopes exist, the Bigelow and Esty's Model will be adopted and values of K_{max} and Log_{10} (N_0) will be obtained.

At 4^{0}C, 25^{0}C, 35^{0}C, and 45^{0}C the data fitted into the thermal death Model with an R^2 Value of 0.9422, 0.9342, 0.9520 and 0.9419 as shown in Figure 4.16, 4.17, 4.18 and 4.19 respectively. The Model also conformed to:

$$N = N_0 \times e^{-K_{max} \times t}$$

Which when the Logarithmic values of both sides was taken, it transformed to:

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{\ln 10}$$

Which can be further simplified as:

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{2.303} \ldots\ldots\ldots\ldots \quad \text{Equation 2.}$$

This model signifies a positive slopped model with k_{max} and LOG_{10} (N_0) values of 0.00 and 9.40; -0.02 and 9.38; -0.01 and 9.39; and -0.01 and 9.40 for 4^0C, 25^0C, 35^0C, and 45^0C models respectively. This signifies that the inactivation of the isolate is not possible these temperatures using the design adopted and indicates the risk associated with samples displayed openly at market temperatures and at poor processing temperatures.

4.8. Summary of model parameters

Table 4.7 shows the model parameters for microbial inactivation at a different temperature. Data having K_{max} values signifies a positive slope which indicates that the organism was growing under experimental condition. However, at -4 and 4 degrees, the isolates were inactivated. This implies that *Campylobacter jejuni* tested grew at 25 and 35 degrees, while *Escherichia coli* grew at 25, 35 and 45 degrees.

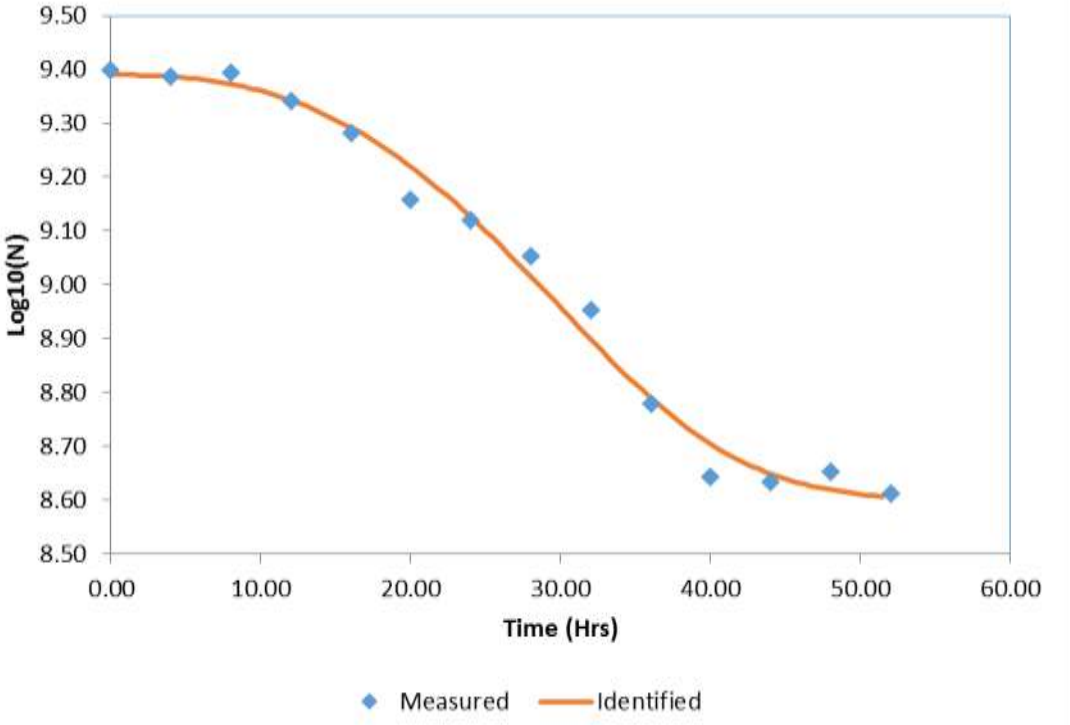


Figure 4.15. Two Mixed Weibull Distributions of Temperature-dependent inactivation of *Escherichia coli* at -4^{0}C.

Table 4.6: Calculated parameters of the Two Mixed Weibull Distributions of Temperature-dependent inactivation of *Escherichia coli* at -4^0C.

Parameters	**Parameter values**	**Standard Error**
alpha	0.71	0.37
delta1	36.38	4.35
p	2.59	0.56
LOG10(N0)	9.39	0.02
delta2	271589.11	136936636072347.00

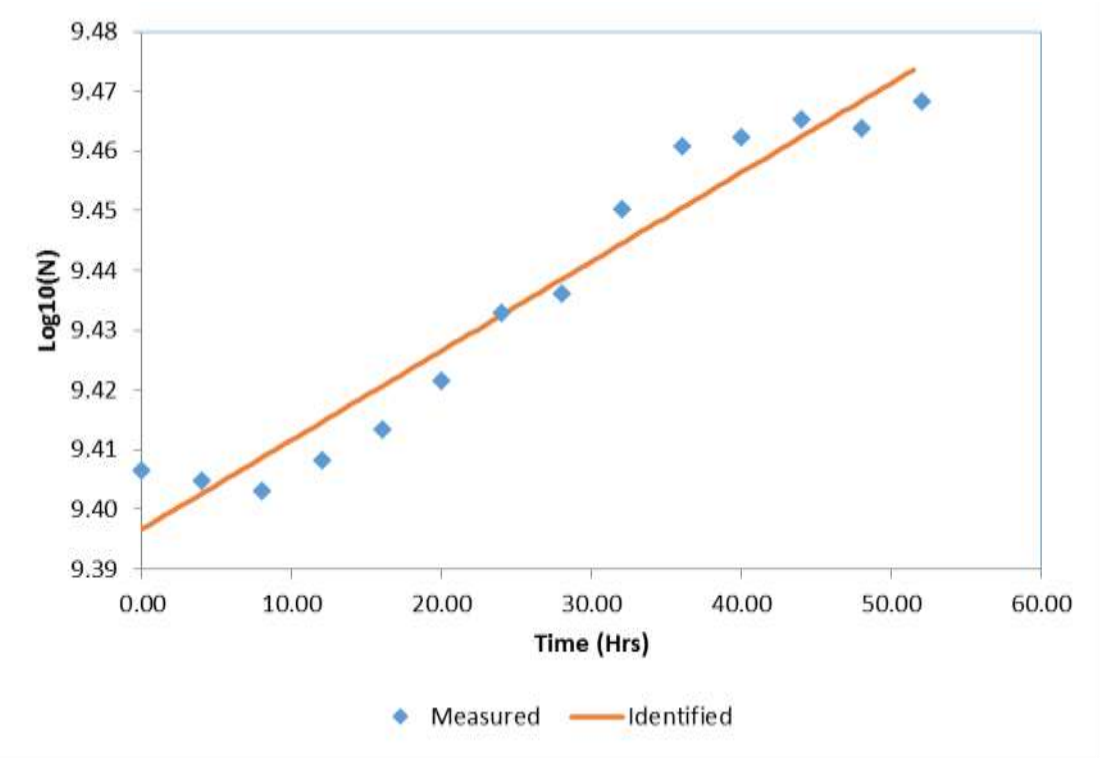


Figure 4.16. Bigelow and Esty's model fitting of growth of *Escherichia coli* at 4^0C.

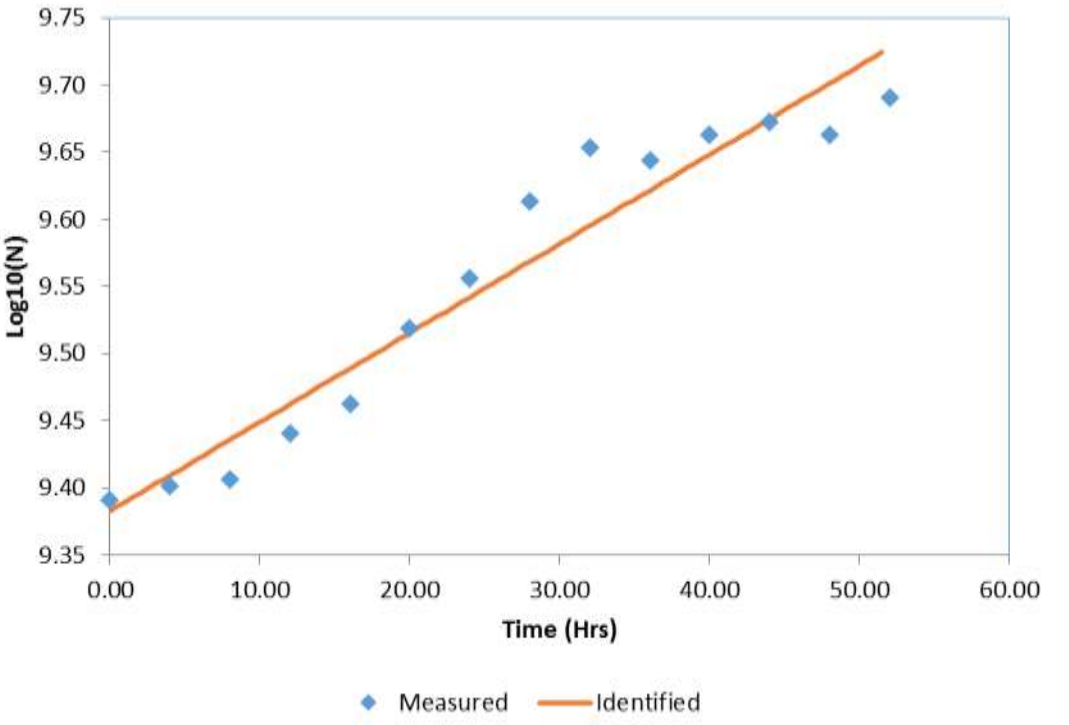


Figure 4.17. Bigelow and Esty's model fitting of growth of *Escherichia coli* at 25^0C.

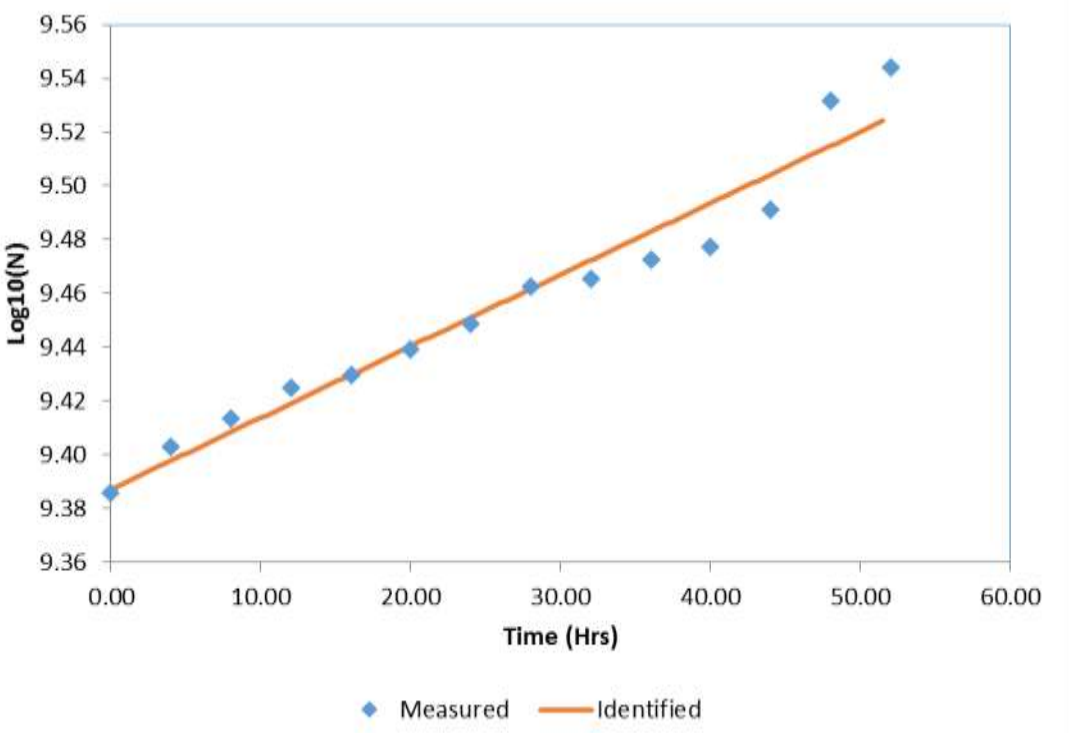


Figure 4.18. Bigelow and Esty's model fitting of growth of *Escherichia coli* at 35^0C.

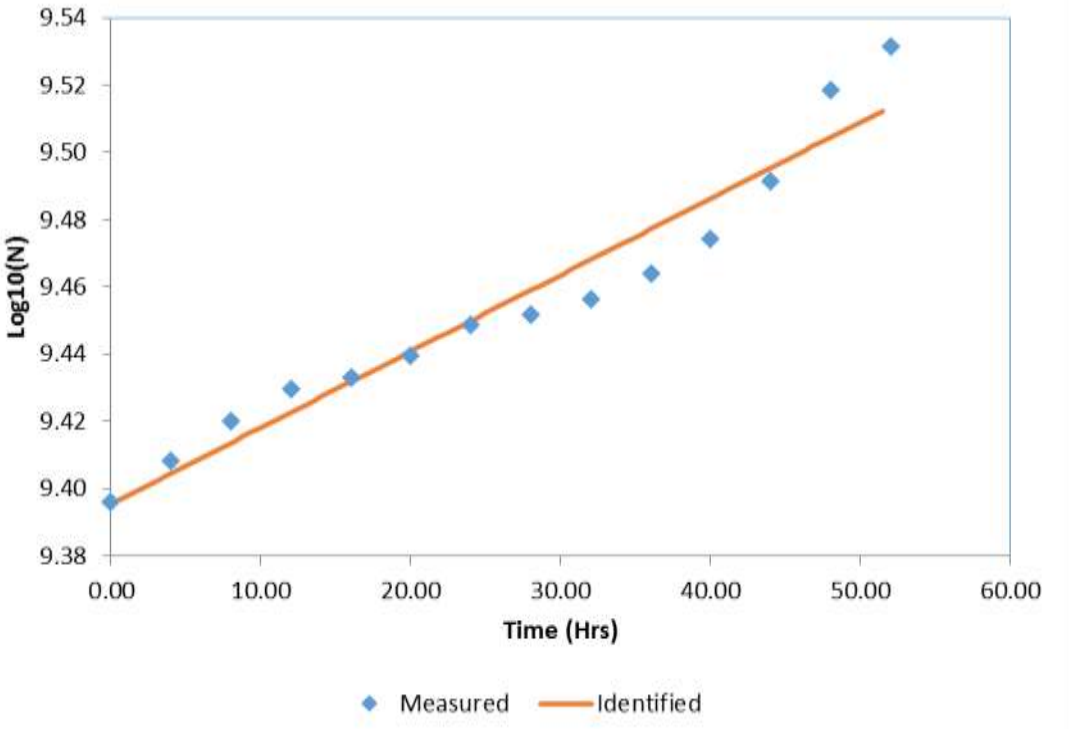


Figure 4.19. Bigelow and Esty's model fitting of growth of *Escherichia coli* at 45^0C.

Table 4.7. Summary of Modelling parameters

	Campylobacter jejuni					*Escherichia coli*				
	Temperature (Degree Celsius)					Temperature (Degree Celsius)				
Parameters	-4	4	25	35	45	-4	4	25	35	45
A	0	0			0	0.71				
δ1	39.98±4.04	192.46±3.66			82.16±2.86	36.38±1.99				
P	1.6±0.01	1.45			1.06	2.59				
Log N	9.03±0.01	9.06±0.01	9.16±0.01	9.16±0.02	9.05±0.01	9.39±0.02	9.4±0.03	9.38±0.03	9.39±0.02	9.419±0.04
K_{max}			-0.01	-0.01			0	-0.02	-0.01	-0.01
R^2	0.968	0.9394	0.9706	0.9573	0.9816	0.9889	0.9422	0.9342	0.952	0.9419
MSE	0.0005	0.0001	0.0002	0.0002	0.0001	0.0014	0	0.0009	0.0001	0.0001

δ_1 =first decimal reduction; N_0 = the initial size of the population; K_{max}=Maximum growth rate; a is defined as the logit of *f*;*p* represents the shape of the curve; MSE=Mean Sum of Squared Error

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Campylobacter jejuni and *Escherichia coli* 0157: H7 are leading causes of gastroenteritis worldwide. It has been reported to be a cause of diarrhea in Nigeria (Adekunle *et al*., 2009). Most infections are sporadic and are associated with eating or handling undercooked poultry or meat; or consumption of unpasteurized milk or contaminated water. More recently, *Campylobacter jejuni* has been identified in meat and meat products sold in Owerri, Imo state Nigeria (Braide *et al*., 2017). *Escherichia coli* 0157: H7 (verotoxin producing *E. coli*) is the most notorious serotype of *Escherichia coli* which is highly infectious and has been a leading cause of mortality and morbidity worldwide (Feng and Monday, 2000; Monday *et al*., 2007).

Methods of isolation and identification of *Campylobacter jejuni* and *Escherichia coli* has been reported by so many researchers (OIE, 2008; Salihu *et al*., 2009a; Olufemi and Oluseye, 2016; Braide *et al*., 2017; Mailafa *et al*., 2017). *Campylobacter* spp has been isolated from several sources locally including Poultry, Beef, goat meat, red meat and meat products (Salihu *et al*., 2009a; Olufemi and Oluseye, 2016; Braide *et al*., 2017). No work so far in Nigeria has been published on the isolation of these isolate from ready to eat vegetables as indicated in this work.

Salihu *et al*. (2009b) and Olufemi and Oluseye (2016) characterized isolate based selected Biochemical tests such as the indoxyl-acetate hydrolysis, hippurate hydrolysis and nitrate reduction and H_2S production. Indeed, Salihu (2009a and b) characterized isolates into biotypes including *Campylobacter jejuni*,

Campylobacter coli, *Campylobacter lari* and *Campylobacter upsaliensis* based on three tests viz., Hippurate, rapid H_2S and DNase test. Braide *et al*. (2017) used the API Campy Kit to characterize the isolates into several subtypes. In developed and some developing countries, methods of characterization of the isolates are usually molecular based (Karshima and Bobbo, 2016; Nwankwo *et al*., 2017; Whiley *et al*., 2017). However, there have been no reports for the combination of the use of ABIS online and the biochemical tests, or ABIS online and the Latex agglutination tests for verification of *Escherichia coli* 0157: H7, following a well-recognized colonial morphology on CT-SMAC.

In this research, a 100% prevalence of *Campylobacter jejuni* was recorded for Poultry meat samples, 66% prevalence of beef and 44.7% prevalence from vegetable samples. Previously, Salihu *et al.* (2009a) reported that *C. jejuni* was isolated in 340 (49.9%) raw poultry samples. In addition, Olufemi and Oluseye (2016) reported a 96.4% prevalence *of Campylobacter jejuni* from poultry samples. The reported prevalence was relatively lower than the prevalence observed in this research. This could be due to the method adopted in this work. However, these reports also agree with previous reports by Olufemi and Oluseye (2016) that *Campylobacter jejuni* is prevalent in selected food samples in Nigeria. The prevalence of this isolate in vegetables can be implicated in horizontal contamination from water sources contaminated with exudates from reservoirs of this isolates such as poultry (Braide *et al*., 2017) and faecal samples (Karshima and Bobbo, 2016; Whiley, 2017). Indeed, water has been reported to be an effective vehicular source of contamination resulting in major outbreaks, and as such, should be regarded as a pathogen of global concern (Marja-Liisa *et al*., 2003; Richardson *et al*., 2007). In addition, the microbial (Bacterial) populations recorded for all samples for *Campylobacter jejuni* and *Escherichia coli* 0157: H7 exceeds the

required limit of 5.0 x 10^2 Cfu/g for meat and ready-to-eat vegetables as specified by USDA (2018).

Stephen and Nduka (2009) reported the prevalence of the pathogenic *Escherichia coli* 0157: H7 in Benin City, Nigeria. In their report, cabbage, lettuce, and tomatoes had 48(66.67%), 68(94.99%) and 64(88.8%) samples with *E. coli* respectively. However, none of the *Escherichia coli* isolated was the enteropathogenic strain. On the other hand, Reuben and Makut (2014) also reported that the occurrence of *E. coli* O157:H7 in vegetables such as cabbage, cucumber, spinach and bitter leaf was 20% (2), 10% (1), 30% (3) and 10% (1) respectively in Lafia, located in Northern Nigeria. Their methods were also based on colonial and biochemical characterization in addition to Latex agglutination tests. This research, however, recorded a prevalence of 12%, 4%, and 16% among samples of vegetables, Poultry meat, and beef respectively. The method of characterization also differed as ABIS online was used in addition to Latex agglutination which could be responsible for the varied prevalence.

Extracts of pepper and ginger have been reported to have antimicrobial activities with a broad spectrum of activity (Neihaya *et al*., 2015; Ruth and Elijah, 2015; Nassan and Mohamed, 2016; Nizar, 2016). However, their combined effect and single effects have been poorly discussed. Kirby Bauer method of assaying for antimicrobial agents have also been identified to have limitations of its sensitivity at low concentrations of the antimicrobial agent (Adeleye *et al*., 2018). Therefore, the factorial design coupled with the inhibition of Dehydrogenase activity as the measure was adopted. The findings from this work demonstrated that a highest inhibitory growth of 88.85% and 98.5% at 12% salt concentration; and a maximum non-inhibitory effect of 12.38% and 108% at 1200 µg/ml ginger, 400 µg/ml pepper and 4% salt for *Campylobacter jejuni* and *Escherichia coli* 0.57:H7 respectively.

Extracts of pepper and ginger have been reported to have antimicrobial activities against *Escherichia coli* but, the paucity of information is available on their activity against *Campylobacter jejuni*.

5.2 Conclusion and recommendation

The inactivation models reported depicts that it is possible to set standardized critical control points in the production line if *Campylobacter jejuni* or *Escherichia coli* is suspected as a contaminant. In addition, this work has demonstrated that the tested spices have single and combined effects against tested isolates. However, the progression for their single effects follows the trend from salt, ginger to pepper. However, there is a need to extract the active ingredients/phytochemicals in pepper and ginger and test them in their purified form. Also, the isolates are supposed to be banked for future studies but due to the absence of a culture bank, subsequent studies cannot be carried out on these tested isolates. This calls for the need to power a culture collection center through collaborative funding among Nigerian Universities for research purposes.

Future work can also test the combination of the spices and temperature treatment in inactivation studies. Also, the gene that is responsible for the resistance to environmental stress can be identified as well as the metabolites produced during stress conditions for the purpose of manipulation and its usefulness respective

5.3 Limitations of Study

The absence of a culture collection center in Nigeria hamper several inactivation research. This is because most isolates that are subjected to inactivation are meant to be typed and banked in culture collection centers. This will enable future validation of experimental results as well as a means of creating a database for other inactivation based research

Also, the absence of the bank limit the progress in the creation of an online database which can be used for subsequent model validation processes. As this work is reported, there is the absence of a local database that can be used to validate models and hence the MSE and R^2 were adopted for model Validation.

Funding also was a major limitation. Access to funding for postgraduate studies in Nigeria is Poor. This is a major challenge to the Academic staff of Nigerian Universities. Research is hampered by poor funding. The recommended prospective research that could emanate from this work could have been completed if funding was available within the space of time of the experiment. This also challenges the staff and postgraduate students to design quality research that could attract foreign Funding since the local funding is poorly accessible.

References

Adekunle, O.C., Coker, A.O. and Kolawole, D.O. (2009). Incidence, isolation, and characterization of *Campylobacter* species in Osogbo. *Biology and Medicine,* **1** (1): 24-27.

Adeleye, S. A., Braide W., Mike-Anosike, E. E., Uzor, B. C. and Korie, M. C. (2018). Inhibition of Dehydrogenase activity of *Escherichia coli* and *Staphylococcus aureus* by Extracts of *Vernonia amygdalina* and *Moringa oleifera*. Research *Journal of Pharmaceutical, Biological, and Chemical Sciences*, **9**(2): 951-956.

Adeleye, S. A., Oguoma O. I., Braide, W. and Egbadon, E. O. (2016) Phytochemistry and Antimicrobial Properties of 2:1 and 1:2 Ethanol-methanol Extracts of *Tetrapleura tetraptera*. *American Journal of Current Microbiology*, **4**(1): 1-9.

Agunos, A, Waddell, L., Léger, D. and Taboada, E. A (2014) systematic review characterizing on-farm sources of *Campylobacter* spp. for broiler chickens. *PLoS ONE* **9**: e104905

Allos, B. (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. *Clinical Infectious Diseases,* **32**: 1201-1206.

Amalaradjou, M.A.R., Narayanan, A., Baskaran, S.A. and Venkitanarayanan, K. (2010). Antibiofilm effect of trans-cinnamaldehyde on uropathogenic *Escherichia coli*. *The Journal of Urology*, **184** (1): 358–363.

Anderson, W. A., McClure, P. J., Baird-Parker, A. C. and Cole, M. B. (1996). The application of a log-logistic model to describe the thermal inactivation of *Clostridium botulinum* 213B at temperatures below 121.1 °C. *Journal of Applied Bacteriology,* **80**: 283-290.

Angesom, H. and Mahendra, P. (2013). Detection of *Campylobacter jejuni* from food and its epidemiology. *Journal of Public Health and Epidemiology*, **5**(9): 357-361.

Armstrong, G.L., Hollingsworth, J. and Morris, J.G. Jr. (1996). Emerging foodborne pathogens: *Escherichia coli* 0157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiologic Reviews,***18**: 29–51.

Arthur, T.M., Barkocy-Gallagher, G.A., Rivera-Betancourt, M., and Koohmaraie, M. (2002). Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Applied and Environmental Microbiology*, **68**: 4847–4852.

Asimi, O.A., Sahu, N.P. and Pal, A.K. (2013) Antioxidant capacity of crude water and ethylacetate extracts of some Indian species and their antimicrobial activity against *Vibrio vulnificus* and *Micrococcus luteus*. *Journal of Medicinal Plants Research,* **7**(26):1907-1915.

Augustin, J. C., Carlier, V. and Rozier, J. (1998). Mathematical modeling of the heat resistance of *Listeria monocytogenes*. *Journal of Applied Microbiology*, **84**: 185-191.

Bahrndorff, S., Rangstrup-Christensen, L., Nordentoft, S. and Hald, B. (2013). Foodborne disease prevention and broiler chickens with reduced *Campylobacter* infection. *Emerging Infectious Diseases,* **19:**425–430.

Baker, D.A. and Genigeorgis, C (1990). Predicting the safe storage of fresh fish under modified atmospheres with respect to *Clostridium botulinum* toxigenesis by modelling length of lag phase of growth. *Journal of Food Protection*, 53: 131–140.

Balchin, M.L., and Deans, S.G. (1997). Bioactivity of selected plant essential oils against *L. monocytogenes*, *Journal of Applied Microbiology*. **82**:759-762.

Bang, D. D., Pedersen, K., and Madsen, M. (2001). Development of a PCR assay suitable for *Campylobacter* species mass screening programs in broiler production. *Journal of Rapid Methods & Automation in Microbiology*, **9**:97–113.

Banji, D., Banji, O.J.F., Pavani, B., Kranthi, K.C. and Annamalai, A.R. (2014) Zingerone regulates intestinal transit, attenuates behavioural and oxidative perturbations in irritable bowel disorder in rats, *Phytomedicine*, **21**(4):423-429.

Baranyi, J. and Pin, C. (2001). A parallel study on bacterial growth and inactivation. *Journal of Theoretical Biology*, **210**: 327-336.

Baranyi, J. and Roberts, T.A. (1995). Mathematics of predictive food microbiology. *International Journal of Food Microbiology*, 26: 199-218.

Baranyi, J., Pin, C. and Ross, T. (1999). Validating and comparing predictive models. *International Journal of Food Microbiology*, 48(3):159-66.

Baranyi, J., Robinson, T.P., Kaloti, A. and Mackey, B.M. (1995). Predicting growth of Brocothrix thermosphacta at changing temperature. *International Journal of Food Microbiology*, 27: 61-75.

Beatriz, O. and Ana, H. (2011). Emerging Thermo Tolerant *Campylobacter* Species in Healthy Ruminants and Swine. Department of Animal Health, NEIKER-Instituto Vasco de Investigacio´n y Desarrollo Agrario, Derio, Bizkaia, Spain. *Foodborne pathogens and Diseases*, **8**:1-8.

Behringer, M., Miller, W.G. and Oyarzabal, O.A. (2011) Typing of *Campylobacter jejuni* and *Campylobacter* coli isolated from live broilers and retail broiler

meat by flaA-RFLP, MLST, PFGE, and REP-PCR. *Journal of Microbiological Methods*, **84**: 194-201.

Bell, C. (2002). Approach to the control of entero-hemorrhagic *Escherichia coli* (EHEC). *International Journal of Food Microbiology,* **78**: 197–216.

Berg, J., McAllister, T., Bach, S., Stilborn, R., Hancock, D., and Le Jeune, J. (2004). *Escherichia coli* O157: H7 excretion by commercial feedlot cattle fed either barley-or corn-based finishing diets. *Journal of Food Protection,* **67**:666–671.

Bettelheim, K.A. (2007). The non-O157 Shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. *Critical Reviews in Microbiology,* **33**: 67–87.

Bhaduri, S. and Cottrell, B. (2004). Survival of cold-stressed *Campylobacter jejuni* on ground chicken and chicken skin during frozen state. *Applied Environmental Microbiology*, **70**:7103-7109.

Bigelow, W.D., and Esty, J.R. (1920). The thermal death point in relation to typical thermophilic organisms. *Journal of Infectious Diseases*, **27**, 602.

Braide, W., Adeleye, S.A ., Ohanusi, I., Ibegbulem, C.R., Mike-anaosike, E.E. and Madu, L.C. (2017). Incidence Of Campylobacter Species In Poultry Products And Beef Sold In Owerri, Imo State, Nigeria. *EC Microbiology*, **10** (4): 147-155

Brandl, M.T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annual Review of Phytopathology,* **44**: 367–392.

Brito-Argáez, L., Moguel-Salazar, F., Zamudio, F., González-Estrada, T. and Islas-Flores, I. (2009) Characterization of a *Capsicum* chinense Seed Peptide

Fraction with Broad Antibacterial Activity. *Asian Journal of Biochemistry* **4**: 77-87.

Buchanan, R.L. (1993). Predictive food microbiology. *Trends Food Science and Technol*ogy, **4**: 6-11.

CDC (2009a). Recommendations for diagnosis of Shiga toxin-producing *Escherichia coli* infections by clinical laboratories. *MMWR Recomm Rep*. **58:**1–14.

CDC (2009b). Multistate outbreak of *E. coli* 0157:H7 infections linked to eating raw refrigerated, prepackaged cookie dough. Update June 30, 2009. http://www.cdc. gov/ecoli/0619.html (accessed Feb 15, 2018).

Centers for Disease Control and Prevention (CDC) (2008). Preliminary Food net data on the incidence of infection with pathogens transmitted commonly through food in 10 states, Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report **57**:366–370.

Chaban, B., Ngeleka, M. and Hill, E. (2010). Detection and quantification of 14 *Campylobacter* species in pet dogs reveals an increase in species richness in faeces of diarrheic animals. *BioMed Central Microbiol*ogy, **10**:73.

Chai, S.J., Cole, D., Nisler, A. and Mahon, B.E. (2017).Poultry: The most common food in outbreaks with known pathogens, United States, 1998–2012. *Epidemiology & Infection*, **145**: 316–325.

Chang, Y.P., Liu, C.H., Wu, C.C., Chian, C.M., Lian, J.L. and Hsieh, S.L(2012). Dietary administration of zingerone to enhance growth, non-specific immune response, and resistance to *Vibrio alginolyticus* in Pacific white shrimp *(Litopenaeus vannamei)* juveniles. *Fish Shellfish Immunology*, **32**(2):284-290.

Chase-Topping, M.E., McKendrick, I.J. and Pearce, M.C. (2007). Risk factors for the presence of high-level shedders of *Escherichia coli* O157 on Scottish farms. *Journal of Clinical Microbiology,* **45**: 1594–603.

Cheesbrough, M. (2009). *District Laboratory Practice in Tropical Countries*, Part 2. *Cambridge University Press, Cambridge*. Pp. 137-150.

Chen, I.N., Chang, C.C., Ng, C.C., Wang, C.Y., Shyu, Y.T. and Chang, T.L. Antioxidant and Antimicrobial Activity of Zingiberaceous Plants in Taiwan. *Plants Foods for Human Nutrition*. **63**(1):15-20.

Chen, J. and Griffiths, M.W. (1996). Luminescent Salmonella strains as real time reporters of growth and recovery from sublethal injury in food. *International Journal of Food Microbiology*, 31: 27-44.

Chick, H. (1908). An investigation of the laws of disinfection. *Journal of Hygiene*, **8**: 92-158.

Chigor, V., Umoh, V. and Smith, S. (2010). Occurrence of *Escherichia coli* O157 in a river used for fresh produce irrigation in Nigeria. *African Journal of Biotechnology,* **9**: 178–182.

Cichewicz, R.H., and Thorpe, P.A. (1996). The antimicrobial properties of chile peppers (*Capsicum* species) and their uses in Mayan medicine. *Journal of Ethnopharmacology,* **52**: 61-70.

Cole, M. B., Davies, K. W., Munro, G., Holyoak, C. D. and Kilsby, D. C. (1993). A vitalistic model to describe the thermal inactivation of *Listeria monocytogenes*. *Journal of Industrial Microbiology*, **12**:232-239.

Coroler, l., Leguerinel, I., Mettler, E., Savy, N. and Mafart, P. (2006). General Model Based on Two Mixed Weibull Distributions of Bacterial Resistance

for Describing Various Shapes of Inactivation Curves. *Applied and Environmental Microbiology*, **72**: 6493-6502.

Corradini, M. G., and Peleg, M. (2003). A model of microbial survival curves in water treated with a volatile disinfectant. *Journal of Applied Microbiology.* **95**:1268-1276.

de Haan, C.P.A., Kivistö, R., Hakkinen, M., Rautelin, H. and Hänninen, M.L. (2010) Decreasing trend of overlapping multilocus sequence types between human and chicken *Campylobacter jejuni* isolates over a decade in Finland. *Applied Environmental Microbiology,***76**:5228–36.

de Perio, M.A., Niemeier, R.T., Levine, S.J., Gruszynski, K. and Gibbins, J.D. (2013). *Campylobacter* infection in poultry-processing workers, Virginia, USA, 2008–2011. *Emerging Infectious Diseases.***19**:286–8.

Dean, S. G., Simpson, E., Noble, R.C., MacPherson, S. and Penzes, L. (1992) Natural antioxidant from *Thymus vulgaris* (thyme) volatile oil. *Acta Horticulturae,* **322**: 171-182.

Dearlove, B.L., Cody, A.J., Pascoe, B., Méric, G., Wilson, D.J. and Sheppard, S.K. (2016) Rapid host switching in generalist *Campylobacter* strains erodes the signal for tracing human infections. *ISME Journal*, **10**(3):721-9.

Deckert, A.E., Taboada, E., Mutschall, S., Poljak, Z., Reid-Smith, R.J., Tamblyn, S., Morrell, L., Seliske, P., Jamieson, F.B., Irwin, R., Dewey, C.E., Boerlin, P. and McEwen, S.A. (2014) Molecular epidemiology of *Campylobacter jejuni* human and chicken isolates from two health units. *Foodborne Pathogens and Disease,* **11**:150–5.

Draper, N.R. (1988). *Response surface designs*. In: Encyclopedia of Statistical Sciences, Wiley, New York. Pp. 107–119.

Dugasani, S., Pichika, M.R., Nadarajah, V.D., Balijepalli, M.K., Tandra, S., and Korlakunta, J.N. (2010) Comparative antioxidant and anti-inflammatory effects of [6]- gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. *Journal of Ethnopharmacology*. **127**(2):515-520.

Duh, Y. H. and Schaffner, D.W. (1993). Modeling the effect of temperature on the growth rate and lag time of *Listeria* innocua and *Listeria monocytogenes*. *Journal of Food Protection*, 56: 205–210.

EFSA Panel on Biological Hazards (BIOHAZ) (2011). Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*,. **9**:2105–246.

EFSA Panel on Biological Hazards (BIOHAZ) (2013). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA Journal*, **13**:51–8.

Eleazu, C.O. and Eleazu, K.C. (2012) Physico-chemical properties and antioxidative potentials of 6 new varieties of ginger (Zingiber officinale). *American Journal of Food Technology*, **7**(4):214-221.

Ellström, P., Hansson, I., Söderström, C., Engvall, E.O. and Rautelin, H. A. (2014). Prospective follow-up study on transmission of *Campylobacter* from poultry to abattoir workers. *Foodborne Pathogens and Disease*, **11**:684–8.

FCJ refugee center Community kitchen (2008). The around the world cookbook. Available at www.fcjrefugeecentre.org/wp-

content/uploads/2013/04/Cook-Book-2008.pdf. Retrieved 6:08pm on March 5, 2018.

Feng, P. and Monday, S.R. (2000). Multiplex PCR for detection of trait and virulence factors in enterohemorrhagic *Escherichia coli* serotypes. *Molecular and Cellular Probes,* **14**: 333–337.

Fisher, I. and Meakins, S. (2006). Surveillance of enteric pathogens in Europe and beyond: Enternet annual report for 2004. http://www.eurosurveillance.org/ViewArticle.aspx? Hussein, .S. and Bollinger, L.M. (2005). Prevalence of Shiga toxin-producing *Escherichia coli* in beef. *Meat Science,* **71**:676–89.

Food and Drug Adminiatration (2013). Foodborne Pathogens Resources for Medical Professionals from Food Safety for Moms to Be. Available at https://www.fda.gov/food/resourcesforyou/healtheducators/ucm091681.htm

Fu, B., P. S, Taoukis, and T. P. Labuza (1991). Predictive microbiology for monitoring spoilage of dairy products with time-temperature integrators. *Journal of Food Sciences,* 56: 1209-1215.

Garcia-Migura, L., Hendriksen, R.S., Fraile, L., and Aarestrup, F.M. (2014) Antimicrobial resistance of zoonotic and commensal bacteria in Europe: the missing link between consumption and resistance in veterinary medicine. *Veterinary Microbiology*, **170**:1–9.

Geeraerd, A.H., Valdramidis, V.P. and Van Impe, J.F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, **102** (1): 95-105.

Gibbs, P.A., Williams, A.P. (1990). In: Using Mathematics for Shelf Life Prediction, *Food Technology*, Int, Europe, Pp. 287–290

Gibson, A. M., Bratchell, N., and Roberts, T.A. (1998). Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride, and storage temperature. . *International Journal of Food Microbiology*, **44**: 49-68.

Gibson, A.M., Bratchell, N. and Roberts, T.A. (1988) Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *International Journal of Food Microbiology*, 6: 155-178.

Giffel, M.C., Zwietering, M.H. (1999) Validation of predictive models describing the growth of Listeria monocytogenes. *International Journal of Food Microbiology*, 46: 135–149.

Gill, C.O. Jones, S.D.M., Tong, A.K.W. (1991b). Application of a temperature function integration technique to assess the hygienic efficiency for spray chilling beef carcasses. *Journal of Food Protection,* 54:731-6.

Gill, C.O., Harrison, J.C.L. Phillips and D.M. (1991a) Use of a temperature function integration technique to assess the hygienic efficiency of a beef carcass cooling process. *Food Microbiology*, 8:83-94.

Grzanna, R., Lindmark, L., and Frondoza, C. Ginger. (2005). A herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food,* **8**(2):125-132.

Guptha, S. and Ravishankar, S. A.(2005). Comparison of the antimicrobial activity of garlic, ginger, carrot, and turmeric pastes against *E. coli* in laboratory buffer and ground beef. *Foodborne Pathogens and Disease*, **2**(4):330-340.

Hadush, A. and Pal, M. (2013). Detection of Campylobacter from foods and its epidemiology. *Journal of Public Health and Epidemiology,* **5**: 357-361.

Hajmeer, M. H., Basheer, I. A. and Najjar, Y. M. (1997). Computational neural networks for Predictive Microbiology: II. Application to microbial growth. *International Journal of Food Microbiology,* **34**: 51-66.

Hajmeer, M., Basheer, I., Hew, C. and Cliver, D. O. (2006). Modeling the survival of *Salmonella* spp. in chorizos. *International Journal of Food Microbiology,* **107**: 59-67.

Harriet, W., Ryan, M. and Kirstin, R. (2017). Detection of *Campylobacter jejuni* in Lizard Faeces from Central Australia Using Quantitative PCR. *Pathogens*, ***6***(1): 1

Hauschild, A.H.W. (1982). Assessment of botulism hazards from cured meat products. *Food Technology*, 36: 95–104.

Hussein, H.S. and Sakuma, T. (2005). Prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *Journal of Dairy Sciences,* **88**:450–65.

Janisiewicz, W., Conway, W., Brown, M., Sapers, G., Fratamico, P., and Buchanan, R. (1999). Fate of *Escherichia coli* O157: H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Applied and Environmental Microbiology*. **65**: 8-11.

Jeffrey, J.S., Hunter, A. and Atwill, E.R. (2000). A field–suitable, semisolid aerobic enrichment medium for isolation of *Campylobacter jejuni* in small numbers. *Journal of Clinical Microbiology,* **38**: 1668-1669.

Jones, F.A.(1996). Herbs – useful plants. Their role in history and today. *European Journal of Gastroenterology and Hepatology*. **8**(12):1227-1231.

Jones, N.L., Shabib, S. and Sherman, P.M. (1997). Capsaicin as an inhibitor of the growth of the gastric pathogen *Helicobacter pylori*. *FEMS Microbiology Letters*, **146**: 223-227.

Jorgensen, F., Ellis-Iversen, J., Rushton, S., Bull, S.A., Harris, S.A., Bryan, S.J., Gonzalez, A. and T. J. Humphrey.(2011). Influence of season and geography on *Campylobacter jejuni* and C. coli subtypes in housed broiler flocks reared in Great Britain. *Applied Environmental Microbiology*, **77**:3741–8.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. and Man, S.M.() Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**: 687–720.

Kaper, J.B. (2005). Pathogenic *Escherichia coli*. *International Journal of Medical Microbiology*, **295**: 355–356.

Karshima, S.N. and Bobbo, A. A (2016). Isolation and PCR Characterisation of Thermophilic *Campylobacter* Species in Dogs Presented to Selected Veterinary Clinics in Jos, Nigeria. *Alexandria Journal for Veterinary Sciences* . **50**(1): 70-77. 8p.

Kawai, T. (1994). Anti-emetic principles of *Magnolia obovata* Bark and *Zingiber officinale* Rhizome, *Planta Medica*, **60**(1):17-20.

Khan, M. and Siddiqui, M. (2007). Antimicrobial activity of Piper fruits. *Natural Product Radiance*, **6**:111-113.

Khani, M., Muhamad, A.R., Rahmani, M. and Rezazadeh, S.H. (2011) .Tropical medicinal plant extracts against rice weevil, *Sitophilus oryzae* L. *Journal of Medicinal Plants Research*, **5**: 259-265.

Kittl, S., Heckel, G., Korczak, B.M. and Kuhnert, P. (2013). Source attribution of human *Campylobacter* isolates by MLST and Fla-typing and association of genotypes with quinolone resistance. *PloS One*. **8**:e81796.

Komanapalli, I. and Lau, B. (1996). Ozone-induced damage of *Escherichia coli* K-12. *Applied Microbiology and Biotechnology*, 46(5-6):610-4.

Kovanen, S.M., Kivistö, R.I., Rossi, M., Schott, T., Kärkkäinen, U.M., Tuuminen, T., Uksilaf, J., Rautelinh, H. and Hänninena, M.(2014). Multilocus sequence typing (MLST) and whole genome MLST of *Campylobacter jejuni* isolates from human infections in three districts during a seasonal peak in Finland. *Journal of Clinical Microbiology,* **52**:4147–54.

Kumar, G., Kathie, L. and Rao, K. V. B. (2011). A review on pharmacological and phytochemical properties of *Zingiber officinale* Roscoe (Zingiberaceae). *Journal of Pharmacy Research*. **4**(9): 2963-2966.

Law, D. (2000). Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli*. *Journal of Applied Microbiology,* **88**: 729–745.

Lawes, J.R., Vidal, A., Clifton-Hadley, F.A., Sayers, R., Rodgers, J., Snow, L., Evans, S.J. and Powell, L.F. (2012). Investigation of prevalence and risk factors for *Campylobacter* in broiler flocks at slaughter: results from a UK survey. *Epidemiology & Infection*, **140**:1725–37.

Leotta, G.A., Milibesky, E.S. and Chinen, I. (2008). Characterisation of Shiga-toxin producing *Escherichia coli* O157 strains isolated from humans in Argentina, Australia, and New Zealand. *BMC Microbiology,* **8**:46.

Lévesque, S., Fournier, E., Carrier, N., Frost, E., Arbeit, R.D. and Michaud, S. (2013) Campylobacteriosis in urban versus rural areas: a case-case study

integrated with molecular typing to validate risk factors and to attribute sources of infection. *PloS One*, **8**:e83731.

Line, J.E., Oakley, B.B. and Stern, N.J. (2013). Comparison of cumulative drip sampling with whole carcass rinses for estimation of *Campylobacter* species and quality indicator organisms associated with processed broiler chickens. *Poultry Science*, **92**, 218–224.

Mackey, B. M. and Kerridge, A. L. (1988). The effect of incubation temperature and inoculum size on growth of salmonellae in minced beef. *International Journal of Food Microbiology*, 6: 57-65.

Mailafia, S., Madubuike, S. A., Raji, M. A., Suleiman, M. M., Olabode, H.O.K., Echioda-Egbole, M. and Okoh, G. P. R. (2017) Phenotypic identification of *Escherichia coli*O157:H7 isolates from cattle at Suleja Abattoir, Nigeria. *African Journal of Microbiology Research*, **11**(21): 845-850, 7

Mandrell, E., Miller, G., Motarjemi, Y. and Adams, C. (2006). *Campylobacter*. In: Emerging foodborne pathogens. *Woodhead Publishing Ltd* pp. 476521.

Manning, S.D., Motiwala, A.S., Springman, A.C. (2008). Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. *PNAS 2008:* **105**:4868–4873.

Marja-Liisa, H., H. Haajanen., T. Pummi., K. Wermundsen., M.L. Katila., H. Sarkkinen., I. Miettinen. and H. Rautelin. (2003). Detection and Typing of *Campylobacter jejuni* and *Campylobacter* coli and Analysis of Indicator Organisms in Three Waterborne Outbreaks in Finland. *Applied and Environmental Microbiology*, **69**(3):1391–1396

Martin, K.W., Mattick, K.L., Harrison, M. and Humphrey, T.J. (2002). Evaluation of selective media for *Campylobacter* isolation when cycloheximide is replaced with amphotericin B. *Letters in Applied Microbiology* **34**:124–129.

McClure, P.J., Baranyi, J., Boogard, E., Kelly, T.M. and Roberts, T.A. (1993). A predictive model for the combined effect of pH, sodium chloride and storage temperature on the growth of *Brochothrix thermosphacta*. *International Journal of Food Microbiology*, 19: 161– 178.

McDonald, K. and Sun, D. W. (1999). Predictive food microbiology for the meat industry: a review. *International Journal of Food Microbiology,* **52**: 1-27.

McMeekin, T. A., Olley, J., Ross, T., and Ratkowsky, D.A. (1993). *Predictive Microbiology: theory and application.* Taunton, UK*: Research Studies Press.* 340 p

McMeekin, T., Brown, J., and Krist, K. (1997). Quantitative microbiology: a basis for food safety. *Emerging infectious diseases*, **3**(4): 541-9.

Mike-Anosike, E.E., Braide, W., Adeleye, S.A., Lugbe, P.B., Akien-Alli, I.J., Uzor, B.C., Nto, C. T. and Korie, M.C. (2018). Inhibition of Dehydrogenase Activity of Selected Clinical Isolates by Extracts of Cymbopogon Citratus (Lemon Grass) and Moringa Oleifera. *International Journal of Research in Pharmacy and Biosciences,* **5**(1): 1-6.

Milnes, A.S., Stewart, I. and Clifton-Hadley, F.A. (2008). Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica* in cattle, sheep, and pigs. *Nature Reviews Microbiology*, **6**:904–912.

Molina-Torres, J., García-Chávez, A. and Ramírez-Chávez, E. (1999). Antimicrobial properties of alkamides present in flavouring plants

traditionally used in Mesoamerica: affinin and capsaicin. *Journal of Ethnopharmacology,* **64**: 241-248.

Monday, S.R., Beisaw, A. and Feng, P.C.H. (2007). Identification of Shiga toxigenic *Escherichia coli* seropathotypes A and B by multiplex PCR. *Molecular and Cellular Probes,* 21: 308–311.

Mortensen, J.M. and Mortensen, J.E. (2009). The Power of Capsaicin. *Journal of Continuing Education*, **11**: 8-13.

Mukherjee, S., Babitzke, P. and Kearns, D.B. (2013). FliW and FliS function independently to control cytoplasmic flagellin levels in *Bacillus subtilis*. *Journal of Bacteriology* **195**: 297-306.

Nassan, M.A., and Mohamed, E.H. (2016). Immunopathological and Antimicrobial Effect of Black Pepper, Ginger and Thyme Extracts on Experimental Model of Acute Hematogenous Pyelonephritis in Albino Rats. *International Journal of Immunopathology and Pharmacology*, **27**(4):531-41.

Neihaya, H. Z., Rasha, M. S. and Tahreer, H. (2015). Antibacterial Effect Of Ginger And Black Pepper Extracts (Alone And In Combination) With Sesame Oil On Some Pathogenic Bacteria. *World Journal of Pharmacy and Pharmaceutical Sciences*, **4** (03): 774-784.

Niemira, B., Sommers, C. and Fan, X. (2002). Suspending lettuce type influences recoverability and radiation sensitivity of *Escherichia coli* O157: H7. *Journal of Food Protection*, **65**: 1388–1393.

Nizar, I. A. (2016). Antimicrobial Effect of Several Oil Extracts on *Escherichia coli*. *American-Eurasian Journal of Agricultural and Environmental Sciences*, **16** (6): 1159-1163.

NSM. (2007). National Standard Methods, QSOP 49. Safe use of plastic bags for incubation of food samples, London. *Health Protection Agency*, pp. **1**-12.

Nwankwo, I.O., Faleke, O.O., Salihu, M.D., Musa, U., Magaji, A.A., Audu, B. and Ngulukun, S. (2017). Prevalence and molecular identification of *Campylobacter* species isolates from poultry and humans in Sokoto, Nigeria. *Sokoto Journal of Veterinary Sciences*. **15**: 1-8

OECD (2015), Meat consumption. Available at http://www.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook_19991142

OIE. (2006). Manual of diagnostic tests and vaccine for terrestrial animals OIE, Paris, France,http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrialanimals/. Accessed May 3rd, 2018.

OIE-Office of International Epizootics. (2008). Vero cytotoxigenic *Escherichia coli*. Terres. Man. Chapter 2.9.11. pp. 1295-1298.

Okorondu S. I., Adeleye, S. A. Okorondu, M. M. O. (2015). Review on medicinal plants. *Nigerian journal of Microbiology*, **29**:3167-3183.

Olufemi, O. and Oluseye, O. (2016). Prevalence and Antibiotics Resistance of *Campylobacter jejuni* in Retail Chickens in Oyo State, Nigeria. *Food Science and Quality Management*. **48**: 7-11.

Olumide, A.O. and Florence A.B. (2016). Harnessing the potentials of Predictive Microbiology in microbial food safety and quality research in Nigeria. *Future Science*, **2**(1), FSO91

Ozbey, G. and Tasdemi, B. (2014). Seasonality and antibiotic resistance of *Campylobacter* in Turkish chicken meat. *Veterinaria Italiana*, **50**:277–83.

Pal, M. (2007). *Zoonoses*. 2nd edition Satyam Publishers, Jaipur, India.Pp 234

Pal, M. (2013). Public health concern due to emerging and re-emerging zoonoses. *International Journal of Livestock Research* **3**: 56-62.

Pal, M. (2014). Impact of emerging bacterial foodborne pathogens on human health. Ph.D. Lecture Notes. Addis Ababa University, College of Veterinary Medicine, Debre Zeit, Ethiopia. pp. 1-15.

Pal, M. and Mahendra, R. (2015). Sanitation in Food Establishments. 1st ed. LAMBERT Academic Publishing, Saarbrucken, Germany. p 246

Paton, J.C., and Paton, A.W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clinical Microbiology Reviews*, **11**:450–479.

Peleg, M. and Cole, M. B. (1998). Reinterpretation of microbial survival curves. *Critical Reviews in Food Science*, **38**: 353-380.

Penas, E., Gomez, R., Frı´as, J. and Vidal-Valverde, C. (2008). Application of high-pressure treatment on alfalfa (Medicago sativa) and mung bean (Vigna radiata) seeds to enhance the microbiological safety of their sprouts. *Food Control*.**19**: 698–705.

Persson, S., Olsen, K.E.P., Ethelberg, S. and Scheutz, F. (2007). Subtyping method for *Escherichia* Shiga Toxin (Verocytotoxin) 2 variants and correlation to clinical manifestations. *Journal of Clinical Microbiology*,**45**: 2020–24.

Pollock, K.G.J., Stewart, A. and Beattie, T.J. (2009). From diarrhoea to haemolytic uraemic syndrome—when to seek advice. *Journal of Medical Microbiology*. **58**:397–98.

Rajan, I., Narayanan, N., Rabindran, R., Jayasree, P.R. and Kumar, P.R.M. (2013). Zingerone protects against stannous chloride- induced and hydrogen peroxide- induced oxidative DNA damage *in vitro*. *Biological Trace element Research*.**155**:455-459.

Rangel, J.M., Sparling, P.H., Crowe, C., Griffin, P.M. and Swerdlow, D.L. (2005). Epidemiology of *Escherichia coli*O157:H7 outbreaks, United States, 1982–2002. *Emerging Infectious Diseases.* **11**: 603–09.

Ratkowsky, D. A., Olley, J., McMeekin, T.A., and Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, **149**: 1-5.

Ratkowsky, D.A., Ross, T., Macario, N., Dommett, T.W. and Kamperman, L. (1996), Choosing probability distributions for modeling generation time variability. *Journal of Applied Bacteriology*, 80:131-7.

Reuben, C.R., and Makut, M.D. (2014). Occurrence of *Escherichia coli* O157:H7 in vegetables grown and sold in Lafia metropolis, Nigeria. *World Journal of Microbiology*. **1**(3):017-021.

Richardson, G., Thomas, D.R., Smith, R.M., Nehaul, L., Ribeiro, C.D., Brown, A.G, Salmon, R.L. (2007). A community outbreak of *Campylobacter jejuni* infection from a chlorinated public water supply. *Epidemiology & Infection* **135:** 1151–1158.

Roberts, T.A. (1989). Combinations of antimicrobials and processing methods. *Food Technology*, 43: 156–163.

Roels, J. A. and Kossen, N. W. F. (1978). On the modeling of microbial metabolism. *Progress in Industrial Microbiology*, 14: 95-203.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Microbiology*. **81**: 501-508.

Rosso, L., Lobry, J. R., Bajard, S., and Flandrois, J. P. (1995). Convenient model to describe the combined effects of temperature and pH on microbial growth. *Applied Environmental Microbiology,* **61**: 610-616.

Ruth, T. S. O., and Elijah, I. O. (2015). Antimicrobial Effect Induced by Fresh Ginger Root Extracts in Broilers. *British Biotechnology Journal*. **9**(1): 1-6,

Sakuma, M., Urashima, M. and Okabe, N. (2006) Verocytotoxin-producing *Escherichia coli,* Japan, 1999-2004. *Emerging Infectious Diseases.***12**:323–25.

Salihu, M. D., Junaidu, A. U., Magaji, A. A., Abubakar, M. B., Adamu, A. Y. and Yakubu, A. S. (2009a). Prevalence of *Campylobacter* in poultry meat in Sokoto, Northwestern Nigeria. *Journal of Public Health and Epidemiology*. **1**(2): 041-045

Salihu, M.D., Junaidu, A.U., Oboegbulem, S.I. and Egwu, G.O (2009b). Prevalence and Biotypes of *Campylobacter* Species Isolated from Sheep in Sokoto State, Nigeria. *International Journal of Animal and Veterinary Advances*. **1**(1): 6-9.

Shane, M. (2000). *Campylobacter* infection of commercial poultry. *Review on Scientific Techniques.* **19**:376–395.

Shukla, Y. and M. Singh. Cancer preventive properties of zinger: *a brief review*, *Food and Chemical Toxicology*. **45**(5), 683–690.

Skinner, G.E., and Larkin, J.W. (1994). Mathematical modeling of microbial growth: a review. *J. Food Safety***. 14**: 175-217.

Smith, D.R., Moxley, R.A., Peterson, R.E., Klopfenstein, T. J., Erickson, G.E. and Clowser, S.L. (2008). A two-dose regimen of a vaccine against *Escherichia*

coli O157: H7 type III secreted proteins reduced environmental transmission of the agent in a large-scale commercial beef feedlot clinical trial. *Foodborne Pathogens and Disease,* **5**: 89–598.

Snedeker, K.G., Shaw, D.J., Locking, M.E. and Prescott, R. (2009). Primary and secondary cases in *Escherichia coli* 0157 outbreaks: a statistical analysis. *BMC Infectious Diseases,* **9**:144.

Spencer, S.E.F., Marshall, J., Pirie, R., Campbell, D., Baker, M.G. and French, N.P. (2012). The spatial and temporal determinants of Campylobacteriosis notifications in New Zealand, 2001–2007. *Epidemiology & Infection,* **140**: 1663–77.

Stahl, A.L., Sartz, L., Nelsson, A., Bekassy, Z.D. and Karpman, D. (2009). Shiga toxin and lipopolysaccharide induce platelet-leukocyte aggregates and tissue factor release, a thrombotic mechanism in hemolytic uremic syndrome. *PLoS One,* **4**: 69-90.

Stephen, A. E., and Nduka, U.(2009). Enterohaemorrhagic *Escherichia coli* 0157:H7 Prevalence in meat and vegetables sold in Benin City, Nigeria. *African Journal of Microbiology Research*, **3**(5): 276-279

Strachan, N.J.C., Rotariu, O., Smith-Palmer, A., Cowden, J., Sheppard, S.K., O'Brien, S.J., Maiden, M. C. J., Macrae, M., Bessell, P. R., Matthews, L., Reid, S. W. J., Innocent, G. T., Ogden, I. D., and Forbes, K. J.(2013). Identifying the seasonal origins of human Campylobacteriosis. *Epidemiology & Infection,* **141**:1267–75.

Stumbo, C.R., Prokit, K.S., Ramarkrishnan, T.V., Evans, D.A. and Franaas, F.J. (1983). CRC handbook of lethality guides for low acid canned foods. 1. Boca Raton (FL). CRC Press.

Subash, K. G., and Anand, S. (2014). Medicinal properties of *Zingiber officinale* Roscoe - A Review. *IOSR Journal of Pharmacy and Biological Sciences*, **9**(5): 124-129

Sunilson, J.A.J., Suraj, R., Rejitha, G., Anandarajagopa, K. (2009). In vitro antibacterial evaluation of *Zingiber officinale*, Curcuma longa and Alpinia galangal extracts as natural foods preservatives, *American Journal of Food Technology*. **4**(5):192-200.

Suzuki, H. and Yamamoto, S. (2009) *Campylobacter* contamination in retail poultry meats and by-products in Japan: A literature Survey of Food Control. *Journal of Food Control,* **20**: 531-537.

Sweetman, S.C. and Martindale, N (2002). *The complete drug Reference*, 33nd ed. Pharmaceutical Press, London Pp. 1115-1127.

Tajkarimi, M.M., Ibrahim, S.A. and Cliver, D.O. (2010) Antimicrobial herb and spice compounds in food. *J Food Control* **21**:1199-1218.

Tarr, P.I., Gordon, C.A. and Chandler, W.L. (2005) Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet.***365**:1073–86.

The King James version Holy Bible containing the old and new testament. (1979). Genesis 1:29, *Authorized King James version with the words of Christ in red*, maps, and selected helps to bible study. Holman Bible Publishers, Nashville, Tennessee.

United States Department of Agriculture (2018) Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Retrieved from http://www.fsai.ie/uploadedFiles/Reg2073_2005(1).pdf

Van Boekel, M. A. (2002). On the use of Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, **74**: 139-159.

Vereecken, K. M., Dens, E. J., and Van Impe, J. F. (2000). Predictive modeling of mixed microbial populations in food products: evaluation of two-species models. *Journal of Theoretical Biology*, **205**: 53-72.

Von Baum, H. and Marre, R. (2005). Antimicrobial resistance of *Escherichia coli* and Therapeutic implications. *International Journal of Medical Microbiology*, **295**: 503-511.

Vugia, D., Cronquist, A., Hadler, J., Tobin-D'Angelo, M., Blythe, D., Smith, K., Thornton, K., Morse, D., Cieslak, P., and Jones, T. (2006). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2005. *Morbidity and Mortality Weekly Report*, **55**: 392–395.

Wagenaar, J.A., French, N.P. and Havelaar, A.H. (2013) Preventing *Campylobacter* at the source: Why is it so difficult? *Clinical Infectious Diseases*, **57**: 1600-1606.

Walker, S.J., Archer, P. and Banks, J.G. (1990). Growth of *Listeria monocytogenes* at refrigeration temperatures. *Journal of Applied Bacteriology*, 68: 157-62.

Weibull, W. (1939). A statistical theory of the strength of materials. *Ingeniörsvetenskapsakademiens handlingar* **151**: 1-45.

Whiley, H., Ryan, M, and Kirstin, R. (2017) Detection of *Campylobacter jejuni* in Lizard Faeces from Central Australia Using Quantitative PCR. *Pathogens*, 6(1):1-6

White, D.G., Zhao, S., Simjee, S., Wagner, D., and McDermott, P.F. (2002) Antimicrobial resistance of foodborne pathogens. *Microbes and infection*. **4**:405-4.

Whiting, R. C., and Buchanan, R. L. (1993). A classification of models in Predictive Microbiology- a reply to K.R. Davey. *Food Microbiology*, **10**: 175-177.

Whiting, R.C. (1995). Microbial modeling in foods. *Critical Reviews in Food Science and Nutrition*, **35**: 467-94.

Whiting, R.C. and Buchanan R.L. (1994). Microbial modeling. *Food Technology* **48**(6):113-119.

WHO (2012). The global view of Campylobacteriosis: report of an expert consultation, Utrecht, Netherlands, 9-11 July 2012.

WHO (2016) *Campylobacter* Fact Sheet. World Health Organization, Geneva, Switzerland.

Wieczorek, K. and Osek, J. (2013). Antimicrobial resistance mechanisms among *Campylobacter*. *BioMed Research International*, **20**:34-42.

Williams, M.S., Golden, N.J., Ebel, E.D, Crarey, E.T. and Tate, H.P (2015). Temporal patterns of *Campylobacter* contamination on chicken and their relationship to Campylobacteriosis cases in the United States. *International Journal of Food Microbiology*, **208**:114–121.

Woldemariam, T., Asrat, D, and Zewde, G (2009). Prevalence of Thermophilic *Campylobacter* species in carcasses from sheep and goats in an abattoir in Debre Zeit area, *Ethiopia*. *Ethiopian Journal of Health Development*. **23**:229-233.

Xiong, R., Xie, G., Edmondson, A.E. and Sheard, M.A. (1999). A mathematical model for bacterial inactivation. *International Journal of Food Microbiology*, 46: 45-55.

APPENDIX 1:

META DATA FOR MICROBIAL INACTIVATION MODELS FOR *ESCHERICHIA COLI* AND *CAMPYLOBACTER JEJUNI*

INACTIVATION MODELS FOR *ESCHERICHIA COLI*

-4 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.40	9.39	0.00
4.00	9.39	9.39	0.00
8.00	9.39	9.37	0.00
12.00	9.34	9.34	0.00
16.00	9.28	9.29	0.00
20.00	9.16	9.22	0.00
24.00	9.12	9.13	0.00
28.00	9.05	9.02	0.00
32.00	8.95	8.90	0.00
36.00	8.78	8.79	0.00
40.00	8.64	8.70	0.00
44.00	8.63	8.65	0.00
48.00	8.65	8.62	0.00
52.00	8.61	8.61	0.00
Least Sum of Squared Error			0.01

Parameters	Parameter values	Standard Error
Alpha	0.71	0.37
delta1	36.38	4.35
P	2.59	0.56
LOG10(N0)	9.39	0.02
delta2	271589.11	136936636072347.00

Mean Sum of Squared Error	0.0014
Root Mean Sum of Squared Error	0.0376
R-Square	0.9889
R-Square adjusted	0.9856

$$N = \frac{N_0}{(1 + 10^{\alpha})} \times \left[10^{-\left(\left(\frac{t-1}{\partial_1}\right)^p + \alpha\right)} + 10^{\left(-\left(\frac{t}{\partial_2}\right)^p\right)} \right]$$

Inactivation model identified

N=N0/(1+10^alfa)*(10^(-((t-1)/delta1)^p+alfa)+10^(-((-1)/delta2)**p))

For identification purposes reformulated as

LOG10(N)=log10(10**N0/(1+10**alfa)*(10**(-(t/delta1)**p+alfa)+10**(-(t/delta2)**p)))

as can be derived from

Coroler *et al*. (2006). General Model Based on Two Mixed Weibull Distributions of Bacterial Resistance for Describing Various Shapes of Inactivation Curves. *Applied and Environmental Microbiology*, **72**: 6493-6502

4 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.41	9.40	0.00
4.00	9.40	9.40	0.00
8.00	9.40	9.41	0.00
12.00	9.41	9.41	0.00
16.00	9.41	9.42	0.00
20.00	9.42	9.43	0.00
24.00	9.43	9.43	0.00
28.00	9.44	9.44	0.00
32.00	9.45	9.44	0.00
36.00	9.46	9.45	0.00
40.00	9.46	9.46	0.00
44.00	9.47	9.46	0.00
48.00	9.46	9.47	0.00
52.00	9.47	9.47	0.00
Least Sum of Squared Error			0.00

Parameters	Parameter values	Standard Error
Kmax	0.00	0.00
LOG10(N0)	9.40	0.00
Mean Sum of Squared Error		0.0000
Root Mean Sum of Squared Error		0.0064
	R-Square	0.9422
	R-Square adjusted	0.9373

$$N = N_0 \times e^{-K_{max} \times t}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{\ln 10}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{2.303}$$

Inactivation model identified

N= N0 * exp(-kmax * t)

For identification purposes reformulated as

LOG10(N)=LOG10(N0)-kmax*t/LN(10)

as can be derived from

W.D. Bigelow and J.R. Esty 1920. The thermal death point in relation to typical thermophylic organisms. Journal of Infectious Diseases, 27, 602

25 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.39	9.38	0.00
4.00	9.40	9.41	0.00
8.00	9.41	9.44	0.00
12.00	9.44	9.46	0.00
16.00	9.46	9.49	0.00
20.00	9.52	9.52	0.00
24.00	9.56	9.54	0.00
28.00	9.61	9.57	0.00
32.00	9.65	9.60	0.00
36.00	9.64	9.62	0.00
40.00	9.66	9.65	0.00
44.00	9.67	9.67	0.00
48.00	9.66	9.70	0.00
52.00	9.69	9.73	0.00
Least Sum of Squared Error			0.01

Parameters	Parameter values	Standard Error
Kmax	-0.02	0.00
LOG10(N0)	9.38	0.02

Mean Sum of Squared Error	0.0009
Root Mean Sum of Squared Error	0.0307
R-Square	0.9342
R-Square adjusted	0.9287

Inactivation model identified

N= N0 * exp(-kmax * t)

For identification purposes reformulated as

LOG10(N)=LOG10(N0)-kmax*t/LN(10)

as can be derived from

W.D. Bigelow and J.R. Esty 1920. The thermal death point in relation to typical thermophylic organisms. Journal of Infectious Diseases, 27, 602

35 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.39	9.39	0.00
4.00	9.40	9.40	0.00
8.00	9.41	9.41	0.00
12.00	9.42	9.42	0.00
16.00	9.43	9.43	0.00
20.00	9.44	9.44	0.00
24.00	9.45	9.45	0.00
28.00	9.46	9.46	0.00
32.00	9.47	9.47	0.00
36.00	9.47	9.48	0.00
40.00	9.48	9.49	0.00
44.00	9.49	9.50	0.00
48.00	9.53	9.51	0.00
52.00	9.54	9.53	0.00
Least Sum of Squared Error			0.00

Parameters	Parameter values	Standard Error
Kmax	-0.01	0.00
LOG10(N0)	9.39	0.01
Mean Sum of Squared Error		0.0001
Root Mean Sum of Squared Error		0.0104
	R-Square	0.9520
	R-Square adjusted	0.9479

$$N = N_0 \times e^{-K_{max} \times t}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{\ln 10}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{2.303}$$

Inactivation model identified

N= N0 * exp(-kmax * t)

For identification purposes reformulated as

LOG10(N)=LOG10(N0)-kmax*t/LN(10)

as can be derived from

W.D. Bigelow and J.R. Esty 1920. The thermal death point in relation to typical thermophylic organisms. Journal of Infectious Diseases, 27, 602

45 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.40	9.40	0.00
4.00	9.41	9.40	0.00
8.00	9.42	9.41	0.00
12.00	9.43	9.42	0.00
16.00	9.43	9.43	0.00
20.00	9.44	9.44	0.00
24.00	9.45	9.45	0.00
28.00	9.45	9.46	0.00
32.00	9.46	9.47	0.00
36.00	9.46	9.48	0.00
40.00	9.47	9.49	0.00
44.00	9.49	9.50	0.00
48.00	9.52	9.50	0.00
52.00	9.53	9.51	0.00
Least Sum of Squared Error			0.00

Parameters	Parameter values	Standard Error
kmax	-0.01	0.00
LOG10(N0)	9.40	0.00

Mean Sum of Squared Error	0.0001
Root Mean Sum of Squared Error	0.0098
R-Square	0.9419
R-Square adjusted	0.9371

$$N = N_0 \times e^{-K_{max} \times t}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{2.303}$$

Inactivation model identified

N= N0 * exp(-kmax * t)

For identification purposes reformulated as

LOG10(N)=LOG10(N0)-kmax*t/LN(10)

as can be derived from

W.D. Bigelow and J.R. Esty 1920. The thermal death point in relation to typical thermophylic organisms. Journal of Infectious Diseases, 27, 602

INACTIVATION MODELS FOR *CAMPYLOBACTER JEJUNI*

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.02	9.03	0.00
4.00	9.01	9.02	0.00
8.00	9.02	8.99	0.00
12.00	8.98	8.96	0.00
16.00	8.91	8.93	0.00
20.00	8.86	8.89	0.00
24.00	8.85	8.86	0.00
28.00	8.86	8.83	0.00
32.00	8.82	8.80	0.00
36.00	8.77	8.78	0.00
40.00	8.76	8.77	0.00
44.00	8.76	8.76	0.00
48.00	8.74	8.75	0.00
52.00	8.75	8.74	0.00
Least Sum of Squared Error			0.00

Parameters	Parameter values	Standard Error
Alpha	0.00	0.76
delta1	38.98	31.43
P	1.60	0.70
LOG10(N0)	9.03	0.02
delta2	26414558.24	6915250879890100.00

Mean Sum of Squared Error	0.0005
Root Mean Sum of Squared Error	0.0216
R-Square	0.9680
R-Square adjusted	0.9584

$$N = \frac{N_0}{(1 + 10^{\alpha})} \times \left[10^{-\left(\left(\frac{t^{-1}}{\partial_1}\right)^p + \alpha\right)} + 10^{\left(-\left(\frac{t}{\partial_2}\right)^p\right)} \right]$$

Inactivation model identified

N=N0/(1+10^alfa)*(10^(-((t-1)/delta1)^p+alfa)+10^(-((-1)/delta2)**p))

For identification purposes reformulated as

LOG10(N)=log10(10**N0/(1+10**alfa)*(10**(-(t/delta1)**p+alfa)+10**(-(t/delta2)**p)))

as can be derived from

Coroler *et al*. 2006. General Model Based on Two Mixed Weibull Distributions of Bacterial Resistance for Describing Various Shapes of Inactivation Curves. Applied and Environmental Microbilogy, 72, 6493-6502

4 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.05	9.05	0.00
4.00	9.06	9.05	0.00
8.00	9.04	9.05	0.00
12.00	9.05	9.04	0.00
16.00	9.04	9.04	0.00
20.00	9.03	9.03	0.00
24.00	9.03	9.03	0.00
28.00	9.03	9.02	0.00
32.00	9.02	9.01	0.00
36.00	8.99	9.01	0.00
40.00	9.00	9.00	0.00
44.00	9.00	8.99	0.00
48.00	8.99	8.99	0.00
52.00	8.98	8.98	0.00
Least Sum of Squared Error			0.00

Parameters	Parameter values	Standard Error
alpha	0.00	285.44
delta1	192.46	23415.03
p	1.45	2.19
LOG10(N0)	9.05	0.01
delta2	1322.47	2064303.63

Mean Sum of Squared Error	0.0001
Root Mean Sum of Squared Error	0.0071
R-Square	0.9394
R-Square adjusted	0.9212

$$N = \frac{N_0}{(1+10^{\alpha})} \times \left[10^{-\left(\left(\frac{t^{-1}}{\partial_1}\right)^p+\alpha\right)} + 10^{\left(-\left(\frac{t}{\partial_2}\right)^p\right)}\right]$$

Inactivation model identified

N=N0/(1+10^alfa)*(10^(-((t-1)/delta1)^p+alfa)+10^(-((-1)/delta2)**p))

For identification purposes reformulated as

LOG10(N)=log10(10**N0/(1+10**alfa)*(10**(-(t/delta1)**p+alfa)+10**(-(t/delta2)**p)))

as can be derived from

Coroler *et al*. 2006. General Model Based on Two Mixed Weibull Distributions of Bacterial Resistance for Describing Various Shapes of Inactivation Curves. Applied and Environmental Microbilogy, 72, 6493-6502

25 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.04	9.06	0.00
4.00	9.09	9.08	0.00
8.00	9.10	9.10	0.00
12.00	9.12	9.12	0.00
16.00	9.17	9.14	0.00
20.00	9.18	9.16	0.00
24.00	9.19	9.18	0.00
28.00	9.19	9.20	0.00
32.00	9.20	9.22	0.00
36.00	9.24	9.24	0.00
40.00	9.27	9.26	0.00
44.00	9.29	9.28	0.00
48.00	9.30	9.30	0.00
52.00	9.31	9.32	0.00
Least Sum of Squared Error			0.00

Parameters	Parameter values	Standard Error
kmax	-0.01	0.00
LOG10(N0)	9.06	0.01

Mean Sum of Squared Error	0.0002
Root Mean Sum of Squared Error	0.0148
R-Square	0.9706
R-Square adjusted	0.9681

$$N = N_0 \times e^{-K_{max} \times t}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{\ln 10}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{2.303}$$

Inactivation model identified

N= N0 * exp(-kmax * t)

For identification purposes reformulated as

LOG10(N)=LOG10(N0)-kmax*t/LN(10)

as can be derived from

W.D. Bigelow and J.R. Esty 1920. The thermal death point in relation to typical thermophylic organisms. Journal of Infectious Diseases, 27, 602

35 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.15	9.16	0.00
4.00	9.16	9.18	0.00
8.00	9.19	9.19	0.00
12.00	9.22	9.20	0.00
16.00	9.23	9.22	0.00
20.00	9.24	9.23	0.00
24.00	9.26	9.24	0.00
28.00	9.27	9.26	0.00
32.00	9.28	9.27	0.00
36.00	9.28	9.28	0.00
40.00	9.29	9.30	0.00
44.00	9.31	9.31	0.00
48.00	9.32	9.32	0.00
52.00	9.32	9.34	0.00
Least Sum of Squared Error			0.00

Parameters	Parameter values	Standard Error
kmax	-0.01	0.00
LOG10(N0)	9.16	0.01

Mean Sum of Squared Error	0.0002
Root Mean Sum of Squared Error	0.0123
R-Square	0.9573
R-Square adjusted	0.9537

$$N = N_0 \times e^{-K_{max} \times t}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{\ln 10}$$

$$\log N = \log N_0 - \frac{e^{-K_{max} \times t}}{2.303}$$

Inactivation model identified

N= N0 * exp(-kmax * t)

For identification purposes reformulated as

LOG10(N)=LOG10(N0)-kmax*t/LN(10)

as can be derived from

W.D. Bigelow and J.R. Esty 1920. The thermal death point in relation to typical thermophylic organisms. Journal of Infectious Diseases, 27, 602

45 DEGREES

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	9.04	9.05	0.00
4.00	9.05	9.03	0.00
8.00	9.01	9.01	0.00
12.00	8.99	8.99	0.00
16.00	8.96	8.97	0.00
20.00	8.95	8.95	0.00
24.00	8.94	8.93	0.00
28.00	8.91	8.92	0.00
32.00	8.89	8.90	0.00
36.00	8.88	8.89	0.00
40.00	8.87	8.87	0.00
44.00	8.85	8.86	0.00
48.00	8.86	8.85	0.00
52.00	8.85	8.84	0.00
Least Sum of Squared Error			0.00

Parameters	Parameter values	Standard Error
Alpha	0.00	5.76
delta1	82.16	440.07
P	1.06	0.59
LOG10(N0)	9.05	0.01
delta2	4529.87	661879.20

Mean Sum of Squared Error	0.0001
Root Mean Sum of Squared Error	0.0108
R-Square	0.9816
R-Square adjusted	0.9760

Inactivation model identified

N=N0/(1+10^alfa)*(10^(-((t-1)/delta1)^p+alfa)+10^(-((-1)/delta2)**p))

For identification purposes reformulated as

LOG10(N)=log10(10**N0/(1+10**alfa)*(10**(-(t/delta1)**p+alfa)+10**(-(t/delta2)**p)))

as can be derived from

Coroler *et al*. (2006). General Model Based on Two Mixed Weibull Distributions of Bacterial Resistance for Describing Various Shapes of Inactivation Curves. *Applied and Environmental Microbiology*, **72** 6493-6502