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PREDICATION OF SHELF STABILITY OF BEEF MUSCLES SLAUGHTERD IN ADDIS ABEBA SLAUGHTER HOUSE

By

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**A Thesis Submitted to Addis Ababa University College of Natural Science in Partial
Fulfillment of the Requirements for the Degree of Master of Science in Food Science
and Nutrition.**

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Approved by Examining Board:

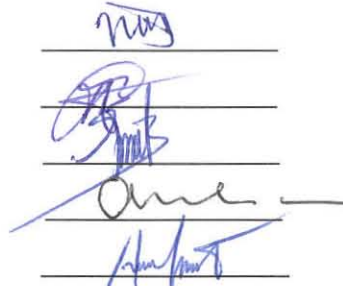
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Declaration

This thesis is my original work and has not been presented for a degree in any other university, and all sources of the material used for the thesis have been appropriately acknowledged.

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List of Abbreviations and acronyms

ANOVA	Analysis of variance
a_w	Water activity
CFU	colony forming units
Cm^2	Centimeter square
CO_2	Carbon dioxide
$\text{C}_4\text{H}_6\text{O}_2$	Diacetyl
$\text{C}_2\text{H}_6\text{S}$	Dimethylsulphide
CSI	Chemical spoilage index
COP	cut- off point
DFD	Dark Firm and Dry
DMRT	Duncan multiple range test
E.coli	<i>Esteritia coli</i>
E_h	Oxidation-reduction potential
EHNRI	Ethiopian health and nutrition research institution
HACCP	Hazard Analysis Critical Control Point
H_2O_2	Hydrogen peroxide
KOH	Potassium hydro oxide
LAB	Lactic acid bacteria
LPD	Lag phase duration
ML	Milliliter
metMb	Metmyoglobin

MASL	Meters above sea level
MAP	Modified atmosphere packing
MSE	Mean Square error
NH ₃	Ammonia
NaCl	Sodium chloride
PCA	Plate count agar
PFM	predictive food microbiology
pH	Potential of hydrogen
PSU	Pseudomonas
PSE	Pale, Soft and Exudative meat.
RH	Relative humidity
R ²	regression coefficient
SSO	specific spoilage organism
SE	standard error
SI	Sensory index
TVBN	Total volatile basic nitrogen

Abstract

The objective of the present study was the development of predictive shelf life model for beef muscle based on the growth of specific spoilage organism (SSO) as affected by temperature and pH.

Beef muscle was stored at four isothermal temperatures (2, 5, 8 and 11 °C) and the samples were investigated within two days interval for total of fourteen days to quantify the influence of temperature and pH on shelf life. The Microbial analysis made for the spoilage microorganisms (total viable count, E.coli and Pseudomonas) which are dominantly found on muscle meat parallel to sensory evaluation and pH determination. The collected growth data were converted into log base and have been used as a basis for the development of shelf life model.

The result of the present study showed that the growth of the spoilage bacteria were increased with time and the most rapidly growing bacteria was found to be Pseudomonas with the highest growth rate (0.01823 to 0.1833 log(CFU/ml) h⁻¹) followed by total viable count (0.0097 to 0.179 log(CFU/ml) h⁻¹) while E.coli (0.0093 to 0.169 log(CFU/ml) h⁻¹) grow slowly. Pseudomonas bacteria also significantly correlated with sensory attributes (p<0.05) indicated that it was the specific spoilage organism (SSO) of beef muscle during aerobic storage. Predictive shelf life model was established based on its growth. The shelf life was estimated at the time at which the sensory score reached the value of 3 point which is considered to be the rejection point. The number of bacterial count at the end of shelf life was estimated using the primary growth model and setting time equal to shelf life. With this method the samples stored at 2, 5 and 8 °C were found to retain their sensory qualities until the experimental time (192, 165.5, 144 hours respectively) while the sample stored at 11°C retained these qualities for only 77.75hours). The Gompertz equation predict the growth of Pseudomonas bacteria and the R² value was greater than 0.95 and MSE was between (0.026-0.23).

Key words Shelf life, predictive model Beef muscle, spoilage bacteria, Sensory evaluation

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1 Introduction

Shelf-life is defined as the period of time between packaging of a product and its end use when product properties remain acceptable to the product end user. Shelf-life properties may include appearance, texture, flavor, color and nutritive value. Simply shelf-life is the amount of time that passes before food becomes unpalatable or unfit for human consumption because of inappropriate storage conditions and growth of spoilage organisms (Bruckner, Raab et al. 2009).

The glycogen content of animal muscles is reduced when the animal is exposed to pre-slaughter stress which changes the pH of the meat to higher or lower levels depending on the production level of lactic acid it produced due to the breakdown of glycogen content of animal muscles via anaerobic glycolytic pathway. Higher levels of pH (6.4-6.8) result in Dark Firm and Dry (DFD) meat (Barbut, Sosnicki et al. 2008). Long term stress causes DFD meat which has a shorter shelf life. Severe short term stress results in a Pale, Soft and Exudative (PSE) meat. PSE meat has a pH lower than normal ultimate value of 6.2 which is responsible for the breakdown of proteins providing a favorable medium for the growth of bacteria (Foote, Horst et al. 2004).

Generally, there are three main mechanisms for meat and meat products spoilage after slaughtering and during storage: microbial spoilage, lipid oxidation and autolytic enzymatic spoilage (Ghaly, Dave et al. 2010). During slaughtering and processing the meat surface is contaminated with a variety of microorganisms (Haushaltwissenschaften and Bruckner 2010).

Meat is a highly perishable food product unless properly stored, processed, packaged and timely distributed for consumption. Meat spoils quickly and becomes hazardous particularly due to microbial growth. Uncooked meat can have some level of microbial contamination present and cannot be expected to be safe unless further processed. The Potential load of the microbial contamination is influenced by the condition of Pre-slaughter handling of livestock abattoir practices, extent of hygienic handling and subsequent storage conditions, and post slaughter handling of meat. This play an important part in deterioration of meat quality (McDonald and Sun 1999).

The main bacteria implicated in the spoilage of refrigerated beef include *Pseudomonas spp.*, *Brochothrix*, *Lactobacilli spp*, *Leuconostoc spp.*, *Carnobacterium spp.*, and *E.coli*. Only one of these microorganisms is responsible for the spoilage of fresh meat during chill storage. This organism is called specific spoilage organism (SSO). The growth of the SSO is influenced by several factors which are broadly categorized into intrinsic (properties of the food), extrinsic (storage environment), processing (treatments during processing) and implicit factors (microbial interactions) (Haushaltwissenschaften and Bruckner 2010).

The combination and interaction of the intrinsic and extrinsic factors determines the microbiology of meat. Among these some factors are especially influential to microbial growth in meats. The intrinsic nature of uncooked meat is characterized with high water activities (0.98), moderate pH (5.5-6.5) and readily available carbon and other nutrients sources of energy makes meat ideal for microbial growth. The most important factor influencing microbial growth is temperature and it is the primary extrinsic controlling factor.

This is evident in most Hazard Analysis Critical Control Point (HACCP) systems for meat products where temperature is a Critical Control Point (McDonald and Sun 1999). In order to prevent food spoilage very important and valid method need to be developed based on the microbial growth and the properties of specific food. However, there is no valid method published information on meat produced in Addis Ababa slaughter house.

Traditionally the shelf life of a product has been mainly determined via challenge tests. In these tests the effects of specific conditions on the growth and proliferation of the SSO were tested. To estimate the shelf life of meat, challenge tests are mainly too, expensive labor intensive, time consuming and only valid for the product and conditions tested. But data generations with challenge tests are the basis for models which can predict the growth or decline of microorganisms. Generally Predictive microbiology is used to describe microbial behavior in order to prevent food spoilage as well as food-borne illnesses (Caleb, Mahajan et al. 2013). The behavior of microbial populations in foods (growth, survival or death) is determined by the properties of food and the storage conditions. The effect of these properties predicted by models derived from quantitative studies on microbial populations (Rahman and Noor 2013).

Predictive microbiology models have immediate practical applications to improve microbial food safety, quality and the development of a quantitative understanding of the microbial ecology of foods. While models are very useful decision-support tools and models are best only if they are simplified representation of reality. (Morshed, Uddin et al. 2011)

For the successful development of a predictive shelf life model to be applicable for fresh meat several points have to be considered.

At first a detailed knowledge of the spoilage process is required as it relates to various influencing factors. These includes the knowledge of specific spoilage organism of the product as well as the population level of the SSO at which spoilage occurs (minimal spoilage level) and the range of environmental conditions over which a particular SSO is responsible for spoilage (Fakruddin, Mazumdar et al. 2011).

1.1 Statement of the Problem

Consumption of meat is continuously increasing worldwide. Especially fresh beef is considered high-energy type of food to be the food of choice due its high nutritional value including vitamins and minerals and provides an important source of high quality protein (Rahman and Noor 2013). However, it has a short shelf life of one day or less at ambient temperature (15-30°C) and a few days at refrigerated temperature (0-10°C) due to microbial spoilage of both pathogenic and non-pathogenic and lipid oxidation. The maximum shelf life of fresh beef depends on several factors such as pH, water activity, microbial growth and temperature (Abbas, Mohamed et al. 2008). Since fresh beef has unique biological and chemical properties and its nutrients composition represents an optimum medium for microbial growth It undergoes deterioration progressively from slaughter until consumption (Mohamed, Jamilah et al. 2008).

Different types of spoilage and pathogenic microorganisms may be introduced into and on the surface of fresh beef during slaughtering and processing which causes rapid spoilage, great loss of valuable protein and also affect human health (Jamilah, Abbas et al. 2008) .

Therefore to extend the shelf life of fresh beef effective shelf life extender need to develop.

But for model development storage tests should be conducted at specified environmental conditions. These storage tests are often carried out in laboratory media like nutrient broth. The problem is models based on microbiological growth data generated in broth often under- or overestimate microbial growth in real food.

For example (Baranyi, Pin et al. 1999) developed a microbial growth model based on growth data of a mixed microbial population in broth. In their study they showed that the overall error of this model was 53.5 % when comparing the predictions of the model with the observations in naturally spoiled food.

For the development of model the microbial data used for validation However, only a few models have been published which were on the one hand developed in fresh meat instead of laboratory media and on the other hand validated. Accordingly the model of (Koutsoumanis and Nychas 2000) for the growth of *Pseudomonas* sp. in ground meat, the model of (Margeirsson, Gospavic et al. 2011) for *Pseudomonas* sp. in poultry and the models of (Mataragas, Skandamis et al. 2008) as well as (Zhang, Li et al. 2011) for lactic acid bacteria in modified atmosphere-packed (MAP) cooked sliced ham. Hence, in the present study predictive model was developed and validated based on the growth of the dominant spoilage bacteria found in the specimens of this investigation.

1.2 Significance of the study

As mentioned before Fresh meat with high moisture content, a moderate pH and readily available sources of energy, carbon and other nutrients provides an ideal matrix for microbiological growth. Its shelf life is mainly limited by the growth of specific spoilage organism (SSO). Thus estimation of the growth of spoilage organism and thereby shelf life study is highly relevant in meat as it allows optimizing the storage management and reducing economic losses.

The use of mathematical models can help to reduce the need for storage trials, challenge tests, product reformulations and process modifications, which are labor intensive, time consuming and expensive. Developed model have also great value to food industry to satisfy expectations of different stakeholders and demonstrate science-based assurance of beef safety while maintaining the original sensorial characteristics.

While traditional microbiological methods are only relevant to the particular conditions under which they were tested, the predictive food microbiology (PFM) using mathematical models could overcome these limitations and become a very effective part of meat production and prevention of food. Unnecessary spoilage of product could be controlled and reduced with its application(McDonald and Sun 1999).

1.3 Objectives

This research was planned and executed with general objective of developing predictive shelf life model for beef muscle based on the growth of specific spoilage organism.

The Specific objectives are:

1. To develop predictive shelf life model for beef muscle based on the growth of specific spoilage organism.
2. To study the effect of pH and temperature on the growth of specific spoilage organism during storage.
3. To estimate the limit of shelf life under different temperature condition based on the sensory acceptability test parallel to microbial count.

2 Literature review

2.1 Composition of meat

Meat described as the most perishable food and its moist nutritious surface is conducive to the growth of a wide range of spoilage bacteria. (Giannuzzi, Pinotti et al. 1998). From the legislative perspective meat is defined as parts of warm-blooded animal in fresh or processed form which are suitable for human consumption. Based on the color meat can be categorized as red meat (e.g. pork, beef, lamb) and white meat (poultry). The difference in color is caused by a different content of myoglobin in the muscle. Red muscles have myoglobin rich fibers whereas white muscle has myoglobin poor fibers

(Haushaltwissenschaften and Bruckner 2010). However, the basic composition of both meat types is comparable. As shown in table 1.1, the main component is water (> 70 %), followed by protein (around 20 %), lipids (< 10 %) and ash (around 1 %). Carbohydrates are only present in very low concentrations of 0.05 – 2 % (Karpas, Tilman et al. 2002).

Table 1.1 Average compositions of cuts of beef, pork and chicken

Meat	Cut	Moisture	Protein	Fat	Ash
Pork	Boston butt	74.9	19.5	4.7	1.1
	Lion (M.psoas major)	75.3	21.1	2.4	1.2
	Cutlet chops	54.5	15.2	29.4	0.8
	Ham	75.0	20.2	3.6	1.1
	Side cuts	60.3	17.8	21.1	0.85
Beef	Shank	76.4	21.8	0.7	1.2
	Sirloin steak	74.6	22.0	2.2	1.2
Chicken	Hindleg (thigh+drum stick	73.3	20.0	5.5	1.2
	Breast	74.4	23.3	1.2	1.1

Source: (Haushaltwissenschaften and Bruckner 2010)

In contrast to fruits and vegetables meats are composed mainly of protein and fats rather than carbohydrates. Water content is 71–76% (Haushaltwissenschaften and Bruckner 2010).

2.2 Meat spoilage

Highly perishable foods like meat provide excellent conditions for the growth of hazardous microorganisms. With the harsh environment and the absence of refrigeration the shelf life of such meat is expected to be very short. Microbial contamination can lower the quality of fresh meat and shorten its shelf life results in economic loss and probably health hazards(Nychas, Skandamis et al. 2008). Low temperature storage is one of the primary preservation methods to maintain meat freshness because the rates of microbiological chemical and biochemical changes are reduced at decreased temperatures(Gill, Greer et al. 1997). During slaughtering the oxygen supply of the muscles breaks down as a result of the death of animal. The degradation of glycogen changed from the aerobic pathway to anaerobic glycogen is catabolised via pyruvate to lactic acid.

The accumulation of lactic acid leads to a decrease of the pH of the muscle in meat 24 hours after slaughtering. (Vaikousi, Biliaderis et al. 2009) When meat is considered as spoiled it is no longer acceptable for human consumption this is mainly attributed to sensory changes in color, odor, aroma and texture. These changes during storage are mainly caused by microbiological growth as the characteristics of fresh meat is (high moisture content, moderate pH and readily available sources of energy, carbon and other nutrients) makes it ideal for microbiological growth (Limbo, Torri et al. 2010).

2.3 Shelf life of meat product

Food is naturally perishable depending on its physical and chemical properties and the storage conditions. There is a point in which the quality of a given product becomes unacceptable or harmful to the consumer. At this point it has reached the end of its shelf-life and the ability to predict the end of shelf life has great importance to the food industries and handlers (Ross, Dalgaard et al. 2000).

Muscle foods are described as spoiled if organoleptic changes make unacceptable to the consumer. These organoleptic characteristics include changes in appearance and discoloration, the development of off-odors, slime formation or any other characteristic which makes the food undesirable for human consumption. It is known that endogenous enzymatic activity within muscle tissue and post-mortem activity can contribute to changes during storage. However, it is generally accepted that detectable organoleptic spoilage is a result of decomposition and the formation of metabolites caused by the growth of microorganisms. The changes which take place also vary according to the species of microflora present, the characteristics of the meat, processing methods, product composition and the environment in which the food is stored (Ammor, Argyri et al. 2009).

2.4 Factors affecting meat spoilage

It is very important to identify factors which determine the shelf-life of the product. These may be microbiological, chemical or physical depending on the product type, the process and the storage and packaging conditions in which the product passes from the production till it reaches to the end user (Argyri, Panagou et al. 2010). Physical changes can be caused by the mishandling during harvesting, processing and distribution.

Chemical changes as a result of enzymatic action, oxidative reactions and non-enzymic browning can all lead to spoilage of a product. The use of chemical kinetics and the study of the rates of mechanisms in which one chemical species converts to another and the Arrhenius relationship that describes the influence of temperature on the reaction rate constants have been used to model changes in food quality (Subramaniam 2000).

2.4.1 Microbial effect of spoilage

Muscles of healthy animals do not contain any bacteria but as soon as animals are slaughtered meat is exposed to contaminants and good sanitation practices are essential to produce high quality meats. The number of spoilage organisms on meat just after slaughter is a critical factor in determining shelf life (Haushaltwissenschaften and Bruckner 2010).

The surface of beef carcasses may contain from log 1 to log 7 CFU/ml bacteria most of which are psychotropic bacteria. Chopping and grinding of meats can increase the microbial load as more surface area is exposed and more water and nutrients become available. A large variety of microbes are commonly found on fresh meat however, different microbes become dominant during spoilage depending on pH, composition, texture the storage temperature and packaging atmosphere (McMeekin and Ross 2002).

Most foods serve as good growth medium for many different microorganisms. The microorganisms will cause changes in appearance, flavor, odor and other qualities of foods.

The degradation processes includes

Putrefaction: Protein + proteolytic microorganism \longrightarrow amino acids + amines + ammonia + H_2S .

Fermentation: Carbohydrate + saccharolytic microorganism \longrightarrow organic acids + alcohol + gases.

Rancidity: Fatty foods + lipolytic microorganisms' \longrightarrow fatty acids + glycerol.

Microorganisms cause spoilage not only by degradation of foods but also by synthesis of various products like pigments and polysaccharides leading to discolorations and formation of slimes (Clerk 2013). Meat is the most perishable foods, because of abundance of nutrients and moisture content. Fresh meats contain a large group of potential spoilage bacteria. The extent of spoilage of meat depends upon the availability of nutrients, presence of oxygen, storage temperature, pH, storage time and the generation time of the spoilage microorganisms under a given environment.

Psychotropic bacteria are the most predominant types in uncooked meat at low temperature. To reduce spoilage of fresh meats, storage at low temperatures modified atmosphere packaging and vacuum packaging are extensively used. Several other methods to reduce initial microbial load and slow down growth of gram-negative bacteria have been used which include the addition of small amounts of organic acids to lower the pH of meat, drying of meat surfaces (to reduce a_w) and a combination of the above factors including lower storage temperature (McMeekin and Ross 2002). However the effect of microorganisms can be positive or negative. Positive microbial effects include cheese, yoghurt, and wine, which can be seen as increasing the nutritional value.

Negative aspects of microbial growth include food deterioration and spoilage by decaying and food poisoning, thus releasing enzymes into the liquid surrounding and absorb the products of external digestion(ElMasry, Sun et al. 2013).

During unfavorable condition several species of bacteria can produce resistant cells called endospore which is highly resistant to heat, chemicals, desiccation (drying out) and ultraviolet light. The endospore may remain dormant for long periods of time. When conditions become favorable for growth (thawing of meats) the endospore germinate and produce viable cells that can begin exponential growth(Ammor, Argyri et al. 2009).

2.4.1.1 Microbial spoilage Processes

The colonization and growth of microorganisms on meat surfaces occurs in stages the first of which involves the attachment of bacterial cells. This process described as a loose and reversible sorption, the second and irreversible stage of attachment involves the production of a glycocalyx by the bacterium that consists of an adhesive extracellular polysaccharide layer. Other factors may also influence the attachment of bacteria to meat surfaces and these include surface morphology, temperature, growth phase, motility and other bacteria previously present (Valero, Carrasco et al. 2012). (Cardelli and Labuza 2001) reported that the spoilage organisms primarily belong to the *genus Pseudomonas* (most commonly found species are *Pseudomonas. fragi*, *Pseudomonas Fluorescens* and *Pseudomonas putrefaciens* as compared with several other spoilage bacteria that have been observed to attach more rapidly to meat surfaces. The dominant spoilage micro florae are generally Gram-negative bacteria and non-motile aerobic rods (Huff-Lonergan and Lonergan 2005).

2.4.2 Chemical changes causing Food Spoilage

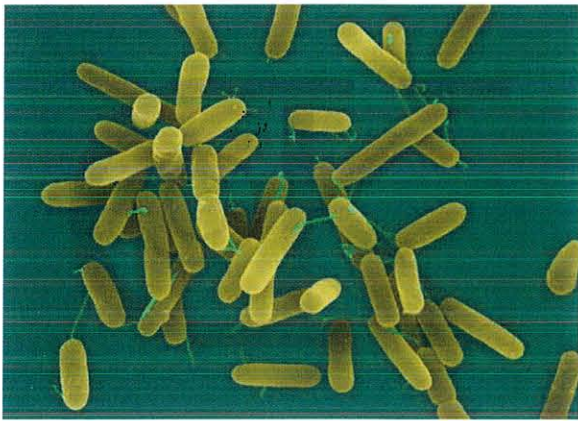
Food spoilage occurs due to reaction or breakdown of the chemical components of the food, including its proteins, lipids, and carbohydrates. The rate at which the chemical reaction takes place depends on many factors which are water activity, temperature, pH, exposure to light or oxygen.

Protein degradation can involve reactions with protein and other ingredients brought about by enzymatic activity. Protein hydrolysis is achieved by enzymes collectively called proteases. Protease enzyme in a meat product causes the meats to become mushy. Degradation of meat protein is also by the oxidation of proteins overexposure to oxygen can cause myoglobin and oxymyoglobin to oxidize into meat myoglobin causing the change in meat color from bright red to brown which renders the meat not appealing to the consumer (Rodríguez-Caturla, Valero et al. 2012).

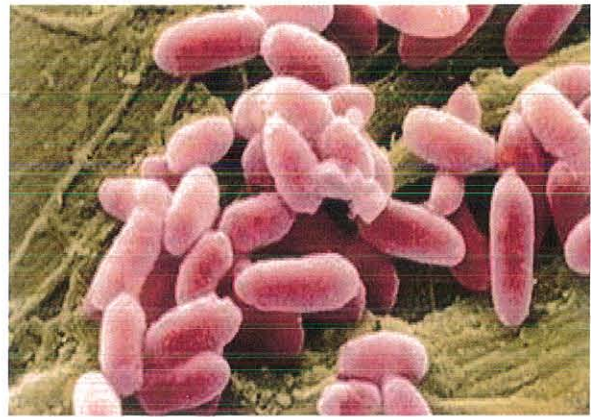
2.5 Specific spoilage organisms

The muscles of healthy animals are sterile at the point of slaughter. However, during slaughtering and processing the meat surface is contaminated with a variety of microorganisms. Only one of these microorganisms is responsible for the spoilage of fresh meat during chill storage. This organism is called specific spoilage organism (SSO)(Raab, Bruckner et al. 2008). *Pseudomonas* is the fastest growing bacteria which can utilize glucose at refrigeration temperatures (Bruckner, Albrecht et al. 2013). At levels of $\log 10^7$ CFU starts producing off-odors and once the surface population of bacteria reached 10^8 CFU/ml and recognizable off-odors develop leading to sensory spoilage.

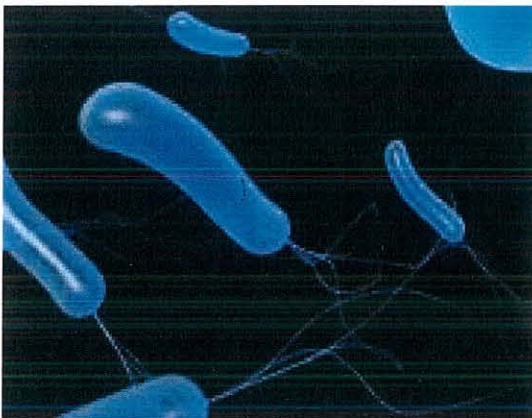
The development of off-odors is dependent upon the extent to which free amino acid utilization has occurred and the odors have been described as 'dairy type aroma' at 10^7 cfu/ml and sickly sweet/fruity aroma at 10^8 cfu finally putrid odour at 10^9 cfu /ml.(Bruckner, Albrecht et al. 2012).When the number of bacteria approaches to (10^8 cfu cm^2) glucose has been utilized and other substrates are used sequentially until nitrogenous compounds lead to the formation of fragrant substances such as ammonia (NH_3), dimethylsulphide (C_2H_6S) and diacetyl ($C_4H_6O_2$).



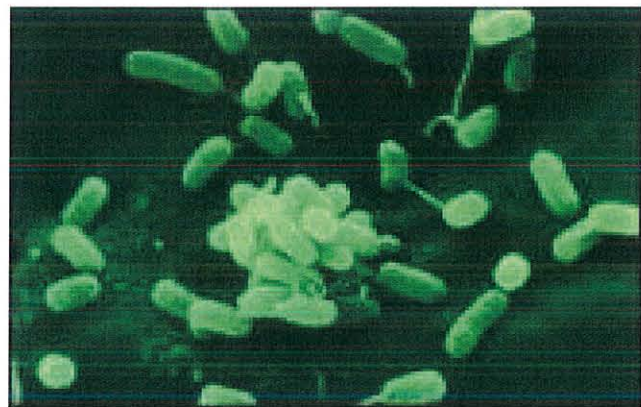
Pseudomonas aeruginosa



Pseudomonas lundensis



Pseudomonas fluorescens



Pseudomonas fragi

Figure 2.1 *Pseudomonas* species commonly present on meat product (Wikipedia)

For fresh aerobically stored beef, pork, poultry *Pseudomonas* sp. has been identified as SSO *Pseudomonas fragi*, *Pseudomonas fluorescens* *Pseudomonas lundensis* and *Pseudomonas aeruginosa* are the main species which are detected during the aerobic spoilage of meat. To determine the SSO for a product two requirements must be met; first it must be the dominant organism over a prolonged period and it must be highly correlated with spoilage of the product (Dilbaghi, Reader et al. 2007).

From the initial micro flora of fresh meat less than 10 % is capable of growing at refrigeration temperature while the proportion of the SSO is even lower (Khajeh and Ghanbari 2011). However, during storage the SSO grows faster than the rest of the micro flora producing metabolites responsible for off-odors or slime which leads to the sensory rejection of the meat. At the point of spoilage which is the point of sensory rejection the cell concentration is termed minimal spoilage level. Shelf life is defined as the time from beginning of storage until the SSO reaches the minimal spoilage level. (Lebert, Baucour et al. 2005) Besides the determination of the minimal spoilage level the concentration of the metabolites produced by the SSO can also be used for the estimation of shelf life. The metabolite which corresponds to spoilage caused by the growth of the SSO can be regarded as being a chemical spoilage index (CSI) (Lebert, Baucour et al. 2005)

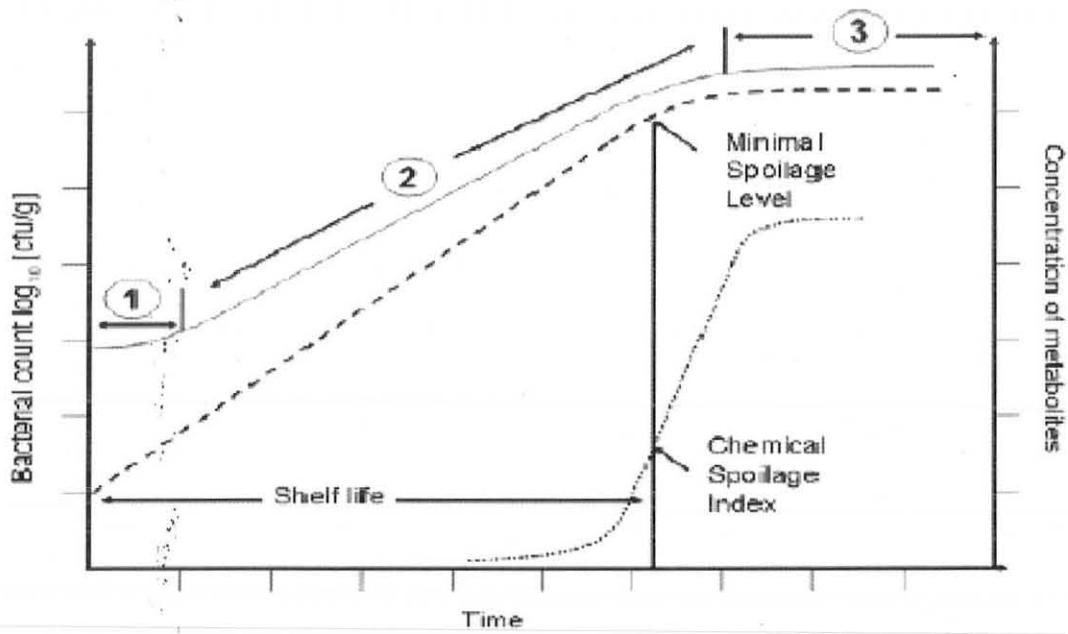


Figure 2.2 General pattern of microbial spoilage with different growth phases as well as the relations between changes in the total micro flora, the SSO and the metabolites. (—) total micro flora, (- - -) SSO, (.....) metabolites. (Haushaltswissenschaften and Bruckner 2010)

Glucose is the first substrate utilized by *Pseudomonas* sp. during the spoilage of fresh meat. The dominance of these bacteria partly explained by its metabolism of glucose via the Entner-Doudoroff metabolic pathway (an alternative to glycolysis). In this pathway glucose is converted to the less commonly used 2-ketogluconate or gluconate which provides an extracellular energy source for *Pseudomonas* sp. and cannot be metabolised by other bacteria. After the depletion of glucose the *pseudomonas* sequentially catabolise lactate, pyruvate, gluconate and at the end amino acid.

The metabolism of nitrogenous compounds such as amino acids finally leads to the sensory changes which occur at the point of spoilage (Foods 2010).

2.6 Factors affecting the growth of spoilage bacteria

During storage the growth of the spoilage bacteria is affected by several factors which are divided into four groups according to (Bruckner, Albrecht et al. 2012). Intrinsic factors which are characteristics of the food product itself (physical and chemical properties) pH, moisture content and nutrients are some of the examples of intrinsic factors. Extrinsic factors are those properties of the environment that exist outside of the food product which affect both the food and their microorganisms (E.g. storage temperature, gas atmosphere). The third one is processing factors which are the physical or chemical treatments during processing of the food (e.g. heat treatment) and the fourth is implicit factors which describe synergistic or antagonistic influences among the organisms. Factors which are considered as being relevant for fresh meat are the intrinsic factors such as water activity (aw), inherent pH of the meat surface and nutritional content as well as the extrinsic factors like storage temperature and oxygen availability (Barbin, ElMasry et al. 2013). Changes of these parameters influencing microbiological growth during storage have been investigated by several authors. (Barbin, ElMasry et al. 2013) analyzed the effect of pH-value, glycolytic potential and their mutual correlations in fresh pork but without considering their relationship to shelf life. (Bee, Anderson et al. 2007) Compared different parameters in fresh pork and beef during storage and found high correlations between D-glucose as well as L-lactate and bacterial counts (total plate count and psychotropic bacterial count) in fresh pork. The Correlations between pH and bacterial counts found to be medium magnitude and the SSO were not investigated in their study (Huff-Lonergan, Baas et al. 2002).

2.6.1 Effect of temperature

One of the best examples of extrinsic factor is Storage temperature. It is the most important environmental factor of microbial growth and shelf life of fresh meat (Ellouze and Augustin 2010). The higher the temperature during transportation and storage the faster the rate of microbial growth. Microorganisms individually and as a group grows over a wide range of temperatures. It is important to know the temperature growth ranges for organisms of importance in foods in order to select the proper temperature for a product storage (Margeirsson, Lauzon et al. 2012).

The cold-loving organisms are psychotropic with the ability to grow at lower temperature. They are the scourge of food stored in refrigerators. In food microbiology mesophilic and psychotropic organisms are of greatest importance (Jouki and Khazaei 2011).

2.6.2 Effect of pH

Most microorganisms grow best at pH values around 7.0 (6.6–7.5) whereas few grow below a pH of 4.0. Bacteria tend to be more fastidious (complex nutritional or cultural requirements for growth) in their relationships to pH than molds and yeasts with the pathogenic bacteria are the most fastidious one. (Lonergan, Stalder et al. 2007). The pH of living tissue is about 7.0 but after rigor the pH of meat is about 5.5 to 5.7, increased acidity of post-mortem muscle results from the accumulation of lactic acid. Lactic acid is formed as glycogen is degraded (anaerobic glycolysis) to produce ATP. This change in pH during the conversion of muscle to meat is the most important event because it affects so many chemical, Physical and a sensory trait of meat products (Barbut, Sosnicki et al. 2008).

If the pH of the meat decline rapidly and reaches 5.5 to 5.8 while the muscle temperature is still high (more than 36 °C) the meat become PSE (Pale, Soft and Exudative). This condition is compounded by slow chilling. Conversely, if the animal's energy supply (glycogen) is exhausted before slaughter the muscle pH will be abnormally high (6.0 to 7.0) making the meat appear DFD (Dark Firm and Dry).The pH fails to decline since there is no enough glycogen present in the muscle at death to form normal levels of lactic acid in the meat. This muscle goes into rigor rapidly and more susceptible to bacterial spoilage than normal or PSE meat(Zhang, Lonergan et al. 2006).

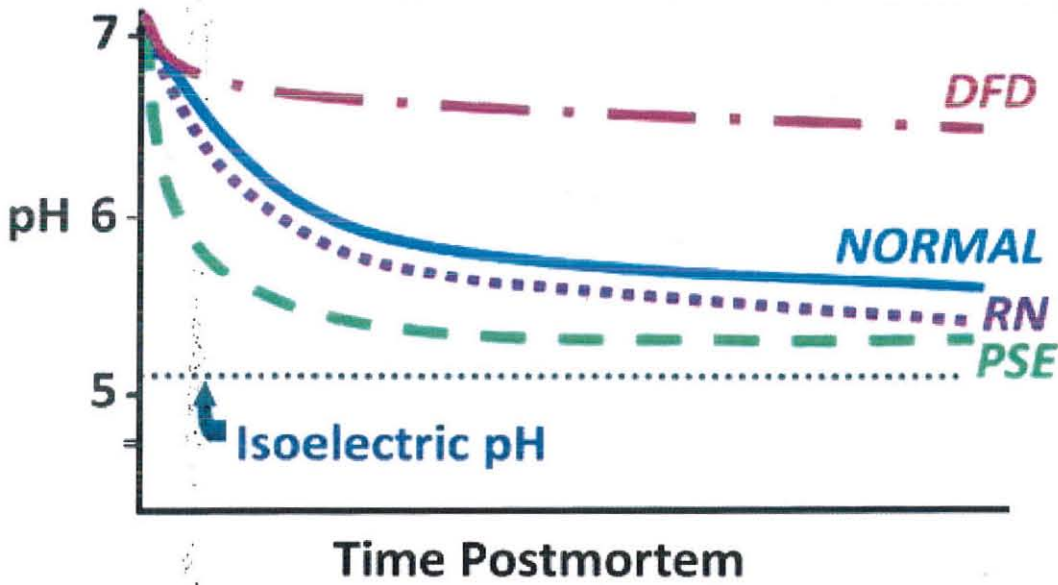


Figure 2.3 Change in pH of post mortem muscle. DFD (dark firm and dry meat), PSE (Pale, Soft and Exudative). Source: (Newton and Gill 1981).

The extent of the pH decline varies slightly between muscles. Muscle with more red fibers (darker red color, smaller, slower contracting, more aerobic cells) will have a slightly higher pH than muscles that are more "white" (lighter color, larger, faster contracting, more anaerobic cells) will be slightly lower (Huff-Lonergan and Lonergan 2005).

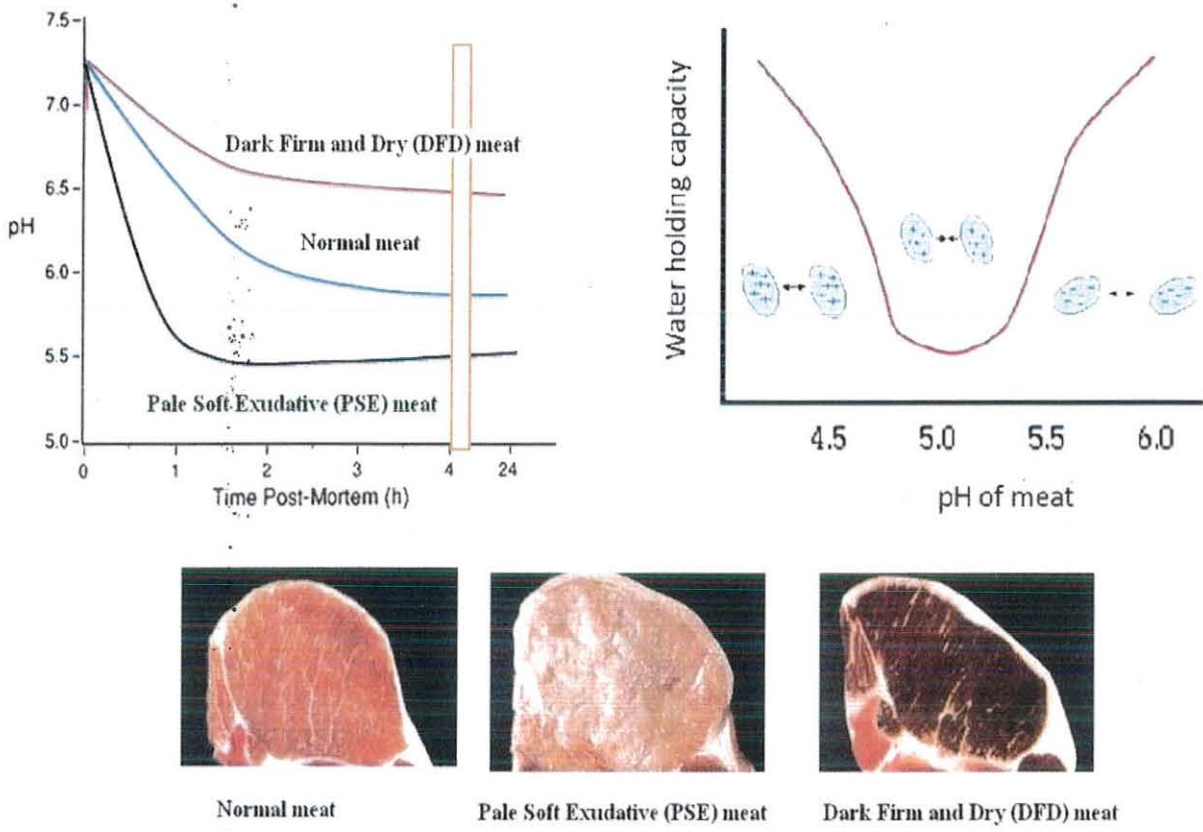


Figure 2.4 color of normal, PSE and DFD meat. Source: (Wikipedia the free encyclopedia)

More over Pre-slaughter stress leads to increased excretion of faeces, which will increase the risk of carcass contamination during slaughter, including contamination with food-borne pathogens. The increased excretion of faecal material contributes to high microbial loads on bedding, floors and walls, which can in turn contaminate the skin of other animals.

2.6.3 Moisture content (water activity [aw])

Water activity is an important property that can be used to predict food safety, stability and quality. Water activity scale extends from 0 to 1.00 (pure water). Most foods have a water activity in the range of 0.2 for very dry foods to 0.99 for moist fresh foods (Slade, Levine et al. 1991).

Most molds and yeasts can grow at a minimum water activity value of 0.80. Thus a dry food like bread is generally spoiled by molds and not bacteria. In general, the water activity requirement of microorganisms decreases in the following order Bacteria > Yeast > Mold. Below 0.60, no microbiological growth is possible. Thus the dried foods like milk powder cookies, biscuits are more shelf stable (Slade, Levine et al. 1991).

Factors that affect water activity requirements of microorganisms are nutritive value of culture medium, temperature, oxygen supply, pH, and inhibitors. Water activity of the foods can be reduced by several methods such as by the addition of solutes or hydrophilic colloids, drying and dehydration or by concentration which restrict microbial growth to make the food microbiologically stable and safe. The Water activity of a food is described by the ratio between the vapor pressure of the food with the surrounding air and the vapor pressure of pure water under identical conditions. Water activity in practice is measured as Equilibrium Relative Humidity (ERH) and is described by the formula (Slade, Levine et al. 1991).

$$\text{Water Activity } (a_w) = \text{ERH} / 100 \text{ (Slade, Levine et al. 1991)}$$

One of the oldest methods of preserving food is drying or desiccation. The preservation of food by drying is a direct removal or binding of moisture content from a food product.

The various applications of water activity includes maintaining the chemical stability of foods, minimizing non enzymatic browning reactions and spontaneous autocatalytic lipid oxidation, prolonging the desired activity of enzymes and optimizing the physical properties of foods such as texture(Dilbaghi, Reader et al. 2007).

2.6.4 Nutrient content

Nutrients in food determine the type of organism that will grow (Talens, Mora et al. 2013).

The presence of easily utilizable nutrients will encourage faster growth. For example, a food with easily utilizable sugars will allow better growth than one which contains polysaccharides. Most foods contain enough peptides and amino acids that they can meet the nitrogen requirement of most organisms. Some organisms are also proteolytic and can grow on proteins containing food. (Kamruzzaman, ElMasry et al. 2012).

The ability to utilize a major component of the food material limit their growth and it is as a competitive advantage compared to those that cannot(Huff-Lonergan and Lonergan 2005) .

In general molds have the lowest requirement followed by yeasts gram-negative bacteria and gram-positive bacteria. Many food microorganisms have the ability to utilize sugars, alcohols and amino acids as sources of energy. Few others are able to utilize complex carbohydrates such as starches and cellulose as sources of energy. Some microorganisms can also use fats as the source of energy but their number is quite less(Zhang, Xiao et al. 2010).The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids.

Also, other nitrogenous compounds which can serve this function are proteins, peptides and nucleotides (Huff Lonergan, Zhang et al. 2010) . In general simple compounds are utilized first by a majority of microorganism such as amino acids. The same is true for fats and polysaccharides. In general gram negative bacteria and molds are able to synthesize most or all of their requirements. The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances that have antimicrobial activity. Nisin and other bacteriocins are good examples (Kim, Huff-Lonergan et al. 2010)

2.6.5 Oxidation-reduction potential (E_h)

The oxygen tension or partial pressure of oxygen and the reducing and oxidizing power of food (E_h) influences the growth of organisms (Leistner 2000). In relation to oxygen, bacteria can be aerobic, anaerobic or facultative. A high (E_h) potential favors the growth of aerobic and facultative organisms. Most fresh foods have a low (E_h) potential in their interior but have a higher (E_h) outside. Fresh meat could support the growth of aerobic organisms in the exterior and the growth of anaerobic organisms inside.

Microorganism has different degrees of sensitivity to the oxidation-reduction potential (E_h) of their growth medium (Jay 1995). The (E_h) value at which microorganisms will grow determines whether they require oxygen for growth or not. According to their E_h value microorganisms can be classified into the three groups Aerobes (+500 to +300 mV), Anaerobes (+100 to -250 mV) and Facultative anaerobes (+300 to -100 mV). E_h as inhibitory factor is especially important in meat product (Leistner 2000). The E_h values of a product are highly variable depending on the pH of the food, extent of microbial growth,

packaging conditions, oxygen partial pressure in the storage environment and ingredients. (Lund, Baird-Parker et al. 2000).

2.6.6 Other microorganisms Activities

The inhibitory effect of some members of the food microorganisms on others microbes is entrenched. Some food borne organisms produce substances that are either inhibitory or lethal to others. These include antibiotics, bacteriocins, hydrogen peroxide, and organic acids (such as lactic acid). Generally microbial interference is a phenomenon that refers to nonspecific inhibition or destruction of one microorganism by other members of the same habitat or environment. The mechanism for this interference is competition for nutrients, competition for attachment/adhesion sites unfavorable alteration of the environment and combinations of these are some of the intervention among the microbes of the same habitat (Jahan 2008).

2.7 Microbial modeling

Depending on the product's process and storage conditions the microbiological shelf-life determined by either the growth of spoilage or pathogenic microorganisms. Traditional methods for the determination of shelf life include storage of the product at different temperatures and determining spoilage by sensory evaluation or microbial count (Kreyenschmidt, Hübner et al. 2010).

Previously the shelf life of a product has been determined via challenge tests it include the study of the effects of specific conditions on the growth and proliferation of the specific spoilage organism (SSO) was tested. To estimate the shelf life by challenge tests is too expensive, labor intensive, time consuming and only valid for the product and conditions

tested (Walker, 1994; Roberts, 1995) however; data generation with challenge tests is the basis for mathematical models which can predict the growth or decline of microorganisms. This field of research is called predictive microbiology or predictive food microbiology. Although the term predictive microbiology is relatively new, the concept of mathematically describing microbial responses to the environment is not. For more than 75 years the safe production of canned food has been determined based on thermal destruction models for *Cl. botulinum* (Korkeala, Alanko et al. 1989). With the increasing capabilities and widespread availability of personal computers predictive microbiology has become an abundant area for research and software development and application. Multifunctional models which enable the quantification of the interactions between two or more factors and allow the interpolation of combinations of factors which were not clearly tested formerly can be used easily by food microbiologists now a days (Le Marc, Pin et al. 2005)

2.7.1. Significance of predictive models

To control food spoilage it is necessary to understand the growth and reproduction patterns of microorganism that causes spoilage and predicate their growth at different temperatures. Microbiological predictions can do this quickly and effectively (Miller et al., 1998). Determining the product's specific spoilage organism (SSO) by using appropriate media for microbial growth and selecting effective mathematical equations are the foundation for a good microbiological prediction model. Mathematical models can also help to reduce the need for storage trials, challenge tests, product reformulations and process modifications which are labor intensive, time consuming and expensive (Nychas, 2008)

2.7.2 Classification of predictive models

There are two categories of predictive models. Mechanistic and Empirical model. Mechanistic or deterministic models are built up from theoretical bases and allow interpretation of the microbial response in terms of known phenomena and processes and it consider the complexity of microbial physiology.

The current level of understanding these types of models are rare. In contrast there is excess of empirical models to describe mathematically the data. Empirical models describing the data in convenient mathematical relationship or curve fitting method.

Empirical models can be further subdivided into probabilistic and kinetic models.

Probabilistic model used for predicting the probability that organisms grow and produce toxin within a given period of time. Probability models indicate only the probability of growth or toxin production does not indicate the speed at which it occurs. Probabilistic models are most relevant for determining whether certain microorganisms will grow or not when they are close to their growth boundaries. This type of model is commonly used to predict the growth or toxin formation by *Cl. botulinum* (Korkeala and Bjorkroth 1997)

Kinetic models are considered the rates of response of growth or death. Examples include the Gompertz and square root models which describe the rates of response of lag time, specific growth rate and maximum population density or inactivation survival models that describe destruction or survival over time. Another generally accepted classification of predictive food models is the classification in primary, secondary and tertiary models proposed by (Buchanan 1993).

2.7.2.1 Primary modeling

Primary models describe the change of microbial numbers with time. The response can be measured directly by the microbial count (colony forming units (CFU)) per ml, toxin formation, substrate levels or metabolic products and indirectly by absorbance, optical density or impedance (Le Marc, Pin et al. 2005). The change of microbial count, especially the count of the SSO can be described by plotting the data with a primary model and sigmoidal functions are used variously. The mathematical equation describes the change in Response over time. The most widely used Primary models are the Logistic Model and the Gompert model which are Comparable in applicability and accuracy (Zwietering, Jongenburger et al. 1990).

(Baranyi and Roberts 1994) reparameterized the equations of Logistic and the Gompertz function to include biologically relevant parameters lag time and specific growth rate. The modified Gompertz function was found to be statistically adequate to describe the microbiological growth and easy to use. (Buchanan 1993) also stated that the Gompertz function is easy to use with good curve-fitting software. Some of the primary models are growth models, the growth decline model D-values or thermal inactivation or survival models, the exponential growth rate and first-order thermal inactivation (model. developmen.aspx.)

2.7.2.2. Secondary Model

Secondary models are used to describe the response of one or more parameters of the primary model with respect to environmental or cultural factors such as (temperature, pH, aw) (Buchanan and Phillips 1990).

As temperature is considered as the most important influence factor secondary models mainly describe the temperature dependency of model parameters. Well-known secondary models are the Arrhenius model and the square root model as well as their modified forms. The simple Arrhenius equation is used in predictive microbiology but it is only accurate over a limited temperature range for microbial growth therefore modified versions have been developed to achieve better fits with extreme temperature (Kreyenschmidt and Ibaldo 2012). However, these modified forms have been reported as being complex and not widely used and successful applications of the simple Arrhenius model are available for many different meat types and meat products (Kreyenschmidt, Hübner et al. 2010). Another frequently used secondary model is the Square root model which was first successfully applied by (Li, Xie et al. 2008) to describe statistically the relationship between microbial growth rate and temperature

2.7.2.3 Tertiary models

The incorporation of primary and secondary models in user-friendly computer software to provide a complete prediction tool to model users (non modelers) in the forms of applications software and expert systems is called tertiary model. These programs may include algorithms to calculate microbial responses to changing conditions on the growth and survival of microorganisms and compare microbial behavior under different conditions and graph the growth of several microorganisms simultaneously. Tertiary models end-user can enter a set of product characteristics and receive a prediction of growth parameters (Le Marc, Pin et al. 2005).

However, only a few predictive tertiary models are available for use in industry. General predictive microbiology software with database are for example ComBase (<http://www.combase.cc>) and the Seafood Spoilage and Safety Predictor (SSSP) (<http://sssp.dtuaqua.dk/>) (Baranyi and Tamplin 2004) which can be freely accessed worldwide via the internet. The use of tertiary models for the prediction of shelf life and remaining shelf life in the industry facilitates used to make better informed decisions regarding further storage and the distribution of the product.

Table 2.1 Primary, secondary and tertiary models used for describing the growth microorganism

Classification	Model	Source
Primary model	Gompertz function	Gibson et al. (1987)
	Modified Gompertz	Zwietering et al. (1990)
	Logistic model	Jason (1983)
	Baranyi model	Baranyi et al. (1993)
Secondary models	Belehradek model(square-root model)	Belehradek(1930)
	Ratkowsky model(square-root model)	Ratkowsky et al.(1982)
	Arrhenius model	Arrhenius(1889)
	Probability models	Hauschild(1982)
Tertiary models	Food Spoilage Predictor (Pseudomonas Predictor)	Neumeyer et al.(1997) Blacburn(2000)
	Seafood Spoilage and Safety Predictor(SSSP)	Dalgaard et al .(2008) http://sssp.dtuaqua.dk/
	ComBase	Baranyi & tamplin (2004)
	Temperature History Evaluation for Raw Meats(THERM)	Ingham et al.(2007,2009) http://WWW.meathaccp.wise.edu/them/
	Growth predicator	http://WWW.ifr.ac.uk/Safety/GrowthPredictor

2.7.2.4 Validation of models

The microbial growth data used for the development of the model as well to validation of the model. However, only a few models have been published which were developed In fresh meat instead of laboratory media and validated. These are e.g. The model of (Koutsoumanis, Stamatiou et al. 2008) for the growth of *Pseudomonas* sp. in ground meat, the model of for *Pseudomonas* in poultry and the models of (Mataragas, Skandamis et al. 2007)

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	Growth predictor	http://WWW.ifr.ac.uk/Safety/GrowthPredictor

Source: (Haushaltwissenschaften and Bruckner 2010)

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Skandamis et al. 2007) as well as (Kreyenschmidt, Hübner et al. 2010) for lactic acid bacteria in modified atmosphere-packed (MAP) cooked sliced ham. Models cannot be used with confidence until validation is done. Statistical measures like Root Mean-square Error (RMSE) and coefficient of determination (r^2) values were used to assess the reliability of predictive equations developed. This term has been used to mathematically compare observed and predicted values and smaller value indicates a model better predicts the observed response (Le Marc, Pin et al. 2005). Two important factors for validation of predictive models are accuracy factor and bias factor introduced by (Baranyi, Pin et al. 1999). Accuracy and bias factors are the mean square differences between predictions and observations (Pin, Sutherland et al. 1999).

Development of predictive microbial models

Data generation



Growth curves are generated in model systems for combination
Of environmental factors (temp., pH; NaCl, etc)

Primary modeling



Growth curves are fitted by sigmoidal growth models

Secondary modeling



The effect of controlling factor(s) on kinetic parameters
(e.g. the lag phase and the growth) is modeled

Productive validation



Predicted values of kinetic parameters are compared to
Values obtained in products and challenge tests

Tertiary modeling

Validated models are included in application software

Source:(Fakruddin, Mazumdar et al. 2011)

3. Materials and Methods

3.1. Study Area

The research was conducted in Addis Ababa the capital city of Ethiopia. It is located on elevation of 2000 to 3000 m.a.s.l. The mean annual rainfall is 1800 with a bimodal pattern and the days mean annual minimum is 14 °C and maximum is 21°C temperature(Avery 2004). In Addis Ababa city there are 1369 butcher's shops and 120 supermarkets that are retailing beef and beef by-products from slaughter houses. Most of the butcheries sold only beef. Only 5.8 percent sold sheep meat and 2.9 percent sold poultry, in addition to beef. No butcheries reportedly sold goat meat. Butcher's shops sell on a retail basis but also serve meat on their premises as raw meat (kurt) as well as roasted products.

3.2 Sampling and sample preparation

The beef animals were slaughtered in Addis Ababa slaughter house and their carcasses were aseptically distributed to butcheries. At the butcheries, the carcasses were randomly selected and appropriate samples randomly collected. Samples were aseptically brought to the laboratory under chilled and controlled environment for analysis. Chilled environment was achieved by keeping the samples in an ice box containing ice bags. The samples were transferred to temperature regulated refrigerator and kept there until start of analysis. The laboratory analysis was conducted in Ethiopian health and research institution.

In the laboratory every muscle were divided into 100g slices under aseptic condition. Each beef muscle slice were placed on an individual tray and over wrapped with falcon

polyethylene film packaging .Time between slaughtering and the first investigation were less than 15 hours

3.3 Experimental design

Four Muscles with initial pH 5.61, 5.52, 5.62 and 5.36 were tested. One kg from each four carcass taken from hind quarter part the Amharic version is *nekel*; local people use this part to prepare kitfo. Each sample 100g and over-wrapped. Samples were stored at four different temperature set up (2, 5 8 &11 ° c).Initial analysis for microbial count and pH measurement made at time zero and then samples were tested every 48 hours(2 days) intervals for about 14 days. Triplicate packages from each storage temperature were taken at appropriate time intervals (0, 48, 96,144, 192, and 240, 286 and 336 hours) and the samples were analyzed for microbial counts of spoilage bacteria (total plat count, *Pseudomonas* sp. and E coli) pH and sensory evaluation for color, aroma, texture and overall acceptability.

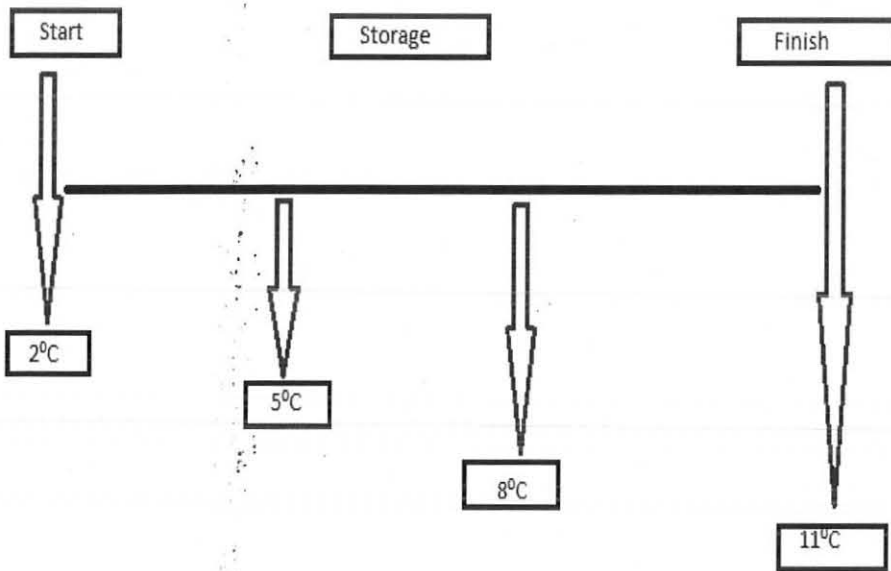


Figure 3.1 Experimental procedure

3.4 Analytical method

3.4.1 Determination of bacterial count

For microbial analysis a representative sample of 25 g was sliced using sterile surgical blend. The sample digested in side of the stomacher-bag which was filled with 225 ml saline peptone diluents (0.85% NaCl with 0.1% saline peptone) homogenized for 60 sec and 1 ml of homogenized solution transferred to 10-fold serial dilution and 0.2 ml of dilution spread on the surface of the media for enumeration of *pseudomonades bacteria*. For total aerobic viable count and *E colia* 0.5ml of the sample poured in to agar media .(i) total aerobic viable count on plate count agar (Merck 1.05463) incubated at 35°C for 48 hours for *E coli* count. (Violat Red Bile Agar) incubated at 35°C for 48 hours and for *pseudomonas* on pseudomonas agar incubated at 30°C for 48h during the storage period microbial count was carried out periodically.

3.4.1.1 Pseudomonas count

Pseudomonas bacteria are Gram negative aerobic bacteria belonging to the family *Pseudomonadaceae* containing 191 described species. As a result of their metabolic diversity ability to grow at low temperatures and ubiquitous nature many *Pseudomonas* sp. can cause food spoilage, off flavors and physical defects in foods .Their growth rates are highly temperature dependent their presence indicates a high potential for spoilage during extended storage. In the laboratory analysis *Pseudomonas* detection was carried out using spread plate techniques. It is one of the most common methods for enumerating aerobic microorganisms.

According to the procedure described on the agar container media was prepared in the proportion of (47g of agar per 1000 ml of distilled water) pseudomonas agar powder mixed with distilled water as per needed for a single day analysis and sterilized in autoclave (Astel pioneer sterilizer) at 121°C for 30 minutes. 20 ml of pseudomonas agar poured to each Petri dish and allowed to solidify then 0.5-mL of sample spread on the surface of the agar plates using a glass spreader which is sterilized by alcohol and flame before each spreading. The drop of sample taken from three dilution tests for initial analysis was from 10^2 , 10^3 and 10^4 dilution factors (the dilution factors are different for all analysis days because when the number of bacteria increases the dilution factor should also increase unless the number of colonies can't be counted clearly) started with the highest dilution and proceeds to the lowest a separate spreader for each plate was used and allowed the surfaces of the plates to dry for 15 minutes. Plates were incubated for two days at 30°C in an inverted position.

3.4.1.2 Total viable count and *E. coli* count

Pour plate technique commonly used for total viable count and *E. coli* count. It is an alternative method for using agar plates to obtain isolated colonies. Pour plate used when it is necessary to know the number of organisms present per unit volume of specimen sample. When a specific portion of the sample is placed in the Petri dish a count of the colonies that grow after incubation reveals their concentration in the original sample. The sample were diluted in 10 fold ratio. The sample were poured in 15-20 mL of melted agar which has been cooled to 50°C first then mixed well with the sample and let to solidify on a flat table top. The agar inverted and incubated to develop colonies.

3.4. 2 Identification and characterization

All plate were examined visually for typical colony types and morphology characteristics associated with each growth medium and the selectivity of each medium was checked routinely by Gram staining and microscopic examination of smears from randomly selected colonies of each media. From plate count agar a single colony were picked and smeared on clean microscopic slide and covered with cover slip. The preparation was observed under light microscope using oil immersion objective. The observed cell shapes and cell arrangements were recorded

3.4.2.1 Characterization

Catalase test

A drop of 3% solution of hydrogen peroxide (H_2O_2) placed on a clean microscope slide and pure colony on plate count agar were transferred and mixed using sterile wire loop. formation bubble were considered as a positive test (catelase positive) (Medlyn, Dreyer et al. 2002)

Gram reaction (KOH test)

The KOH(potassium hydro oxide) test was undertaken according to(Mayer and Hillebrandt 1997).Twenty-four hours old pure Culture colony picked from agar plate and put on clean slide and stirred with two drop of 3% KOH for about 2 minutes. The Gram-negative mass allowed rise with inoculating needle followed the loop to raise 0.5 to 2cm or more whereas the Gram positives did not show slime.

3.5 Instrumental analysis

3.5.1 Determination of pH

For accurate pH readings the pH-meter were calibrated before use and adjusted to the room temperature. The pH-value measured by a portable pH meter the instruments has battery drive and glass electrodes it used for also direct measurement by inserting glass electrode in meat tissue. The operation requires some fluidity in the sample and the glass electrode should be thoroughly wet. The amount of water necessarily will not alter the pH. 50 g of raw meat homogenized in distill water before each measurement and the electrode rinsed with distilled water. The pH-value recorded and measurements were made in triplicate and average pH value calculated for each sample.

3.6 Sensory Analysis and Shelf-life Estimation

In order to determine the shelf-life of the product various sensory estimation method were used by many authors. According to (rodríguez-pérez, zurera-cosano et al. 2003) some of the methods are evaluation of each sensory attributes independently. The other one is overall sensory quality estimation in order to estimate overall sensory quality (Q) the mean of each attribute (p_i) were multiplied by a factor (f_i) based on the degree of importance attributed by the panelists using the formula $Q = \frac{\sum (p_i * f_i)}{\sum f_i}$. Attributes jointly is also one of the methods that the mean of the values of all the attributes together versus time. General acceptability is the study of the general acceptability of samples over time. Weibull method(Weibull 1951) is a statistical design for evaluating shelf-life based on the assumption that at short shelf-life periods there is a very low probability that the product will be rejected by the panelist.

This probability remains low until nearer the end of the product's shelf-life. This may be applied to both hedonic scales and acceptance/ rejection tests. In this case by employing a scale between 1 and 9 rejection corresponded to scores below 5 this valuation was interpreted in terms of acceptable (+) or reject able (-). Data were subsequently fitted to a polynomial regression in order to estimate shelf-life. The most commonly used method is Smell and tastes it is study of the development of smell and taste as the most influential attributes in the shelf-life period(Labuza and Schmidl 1988).

3.6.1 Sensory evaluation

In the present study the meat samples stored in isothermal temperatures evaluated for acceptability by panelists. 100 g of a sample individually was wrapped with falcon polyethylene plastic and stored. First samples evaluated freshly and every two days for 14 days. Fifteen panelists were participated from the staff member of EHNRI institution based on their availability, interest and their physical conditions during the sensory evaluation period. Orientation was given for the panelists about the parameters to be tested appearance (color), aroma, texture and overall acceptability. The samples were provided to panelists on dishes coded with 3-digit random numbers. Acceptability of uncooked meat was evaluated using a 5-point hedonic scale, where 5 = like extremely, 4 = like moderately, 3 = neither like nor dislike, 2 = dislike moderately and 1 = dislike extremely. 3 were considered as the limit of acceptability. The sensory evaluation was conducted at room-temperature in the sensory laboratory. The same meat samples were evaluated over storage times. The order of presentation of the products to each panel was different in a random sequence then the panelists asked to state their judgment of the products' acceptability on the given attributes score sheet.

The shelf life limit was defined as the point when the average of the score was less than 3 and 5 was considered the highest quality score. Therefore the score of 3 was the limit of acceptance.

3.7 Statistical design and data analysis

Regression equation was used to study cause and effect of the independent variable on dependent variable while correlation analysis was used to examine the relationship between sensory index, pH, temperature, *Pseudomonas* count, Total viable and *E.coli* cont. The data were analyzed using SPSS (v. 19). Fitness of Gompertz models were compared by the mean Square error (MSE) and coefficient of regression (r^2).

3.8 Modeling of microbial growth

The growth data of the all the tree spoilage bacteria of beef muscle were changed into log base (\log_{10} CFU /ml) and modeled as a function of time using the Gompertz model to describe the growth of microorganisms with time and the kinetic parameters (μ_{\max} and λ) were estimated. An Arrhenius secondary model was used to model the effect of growth parameters.

3.8.1 Primary modeling

In order to evaluate the microbial growth Kinetic, models are used. It takes parameters which describe how fast a microorganism grows such as duration of lag phase and growth rate. The predictions are used as a primary growth model to describe the microbial growth curve and to predict the change in microbial number over time. In building experimental

values, lag phase and growth rate are derived by fitting microbial count data to a mathematical function.

The model may quantify colony forming units per ml which are direct measures of the response the most common Gompertz equation used whose expression is

$$N(t) = A + C \cdot e^{-e^{-B(t-M)}}$$

with $N(t)$: microbial count [\log_{10} cfu/g] at time t , A : lower asymptotic line of the growth curve (initial bacterial count), C : difference between upper asymptotic line of the growth curve (N_{\max} = maximum population level) and the lower asymptotic line; B : relative growth rate at tie M [$1/h$], M : time at which maximum growth rate is obtained (reversal point), t : time [h].

3.8.2 Secondary modeling

The Arrhenius secondary model used to study the growth parameter as affected by the storage temperature and pH of the sample.

$$\ln B = \ln F - \left(\frac{E_a}{T \cdot R} \right)$$

B relative growth rate at time M [$1/h$], F pre-exponential factor [$1/h$], E_a : activation energy for bacterial growth [kJ/mol], R : gas constant [$8.314 J/mol \cdot K$], T : absolute temperature [K]

The parameter maximum growth rate and lag phase duration estimated using the formulas $\mu = B \cdot C / e$ ($\log(CFU/ML) \text{ day}^{-1}$) with ($e = 2.7182$) and lag phase duration ($LPD = M - (1/B)$) (days) respectively.

3.9 Validation of the growth model and reliability assessment:

Bias factor (B_f) and accuracy factor (A_f) were determined according to (Ross 1996) to evaluate the precision of the model by comparing predicted and actual microbial counts.

$$B_f = \frac{\sum(N_{predicted} - N_{actual})}{n}$$

$$A_f = \frac{\sum|N_{predicted} - N_{actual}|}{n}$$

Bias and accuracy factor for the model were calculated with the predicted and observed (actual) values. If the bias factor is 1.00 indicate an exact agreement between predicted and actual microbiological count. As for the bias factor an accuracy factor of 1.00 shows a perfect agreement between observed and predicted values. The larger the accuracy factor the less accurate the mean values which are estimated (Ross, 1996).

4. Result and Discussion

In the present study simple model was developed to predict beef muscle shelf-life when stored under aerobic conditions over variety of temperatures. Gompertz model was used primary model to describe the growth of microorganisms with time. The lag phase was modeled based upon the assumption of its significant contribution on the overall shelf-life of the beef muscle. The growth of *Pseudomonas* bacteria was fitted using nonlinear regression by the statistical software Origin 6.0G (OriginLab Corporation, Northampton, USA).

This model was developed after observations of different spoilage microorganisms of beef muscle (Total Viable Count, *E.coli* count and *Pseudomonas* count) growing aerobically at four temperatures (2, 5, 8 and 11°C). A primary model of microbial growth is just a mathematical description of microbial concentrations over some period of time.

4.1 Change in microbial number with time

The changes in microbial count of beef muscle with initial pH ranging from 5.36 to 5.6 were monitored at different isothermal storage temperatures (0 to 11 °C).

Table 4.1 Change in Total viable count with time (CFU/ml)

Time (h)	2±°c	5±°c	8±°c	11±°c
0	4.521 ^a ±0.05	5.983 ^b ±0.04	6.505 ^c ±0.02	7.001 ^d ±0.005
48	5.482 ^a ±0.01	6.298 ^b ±0.05	6.888 ^c ±0.005	8.597 ^d ±0.05
96	5.711 ^a ±0.06	6.575 ^b ±0.02	7.702 ^c ±0.06	9.762 ^d ±0.005
144	5.909 ^a ±0.04	6.592 ^b ±0.05	9.2999 ^c ±0.03	10.869 ^d ±0.06
192	6.254 ^a ±0.02	9.584 ^b ±0.04	9.814 ^c ±0.03	11.39 ^d ±0.021
240	6.936 ^a ±0.03	10.482 ^b ±0.03	10.915 ^c ±0.07	19.99 ^d ±0.065
286	8.591 ^a ±0.05	17.673 ^b ±0.02	23.958 ^c ±0.04	24.58 ^d ±0.005
336	10.12 ^a ±0.01	25.58 ^b ±0.003	29.125 ^c ±0.01	33.998 ^d ±0.01

Values in the same column followed by different superscripts are significantly different (p < 0.05). Each value is a mean of three replicates ± SD

The mean log values of TVC from samples of fresh meat and the change of microbial number during 14 days of storage at four isothermal temperatures were shown in Table 4.1. During storage the number of TVC significantly increased due to temperature variation at each sampling hour. The initial count of all the sample stored at different temperature were significantly different due to handling processes starting from slaughtering house until delivered to the butchers it was (4.5-7.0) log cfu/ml also the source of variation was the difference of the buttheries and there handling method.

For each condition tested the number of total viable count increased as the storage time increased. The increment was based on the difference in the initial count and storage temperature variation. Sample stored at 2°C increases through the storage hours from 4.5 log CFU/ml to 10.1 log CFU/ml and sample stored at 5°C was increased slightly for the first 6 days and the rate of growth is drastically changed. Similarly, for samples stored at 8 and 11°C change of bacterial count is very significant at the end of the storage period.

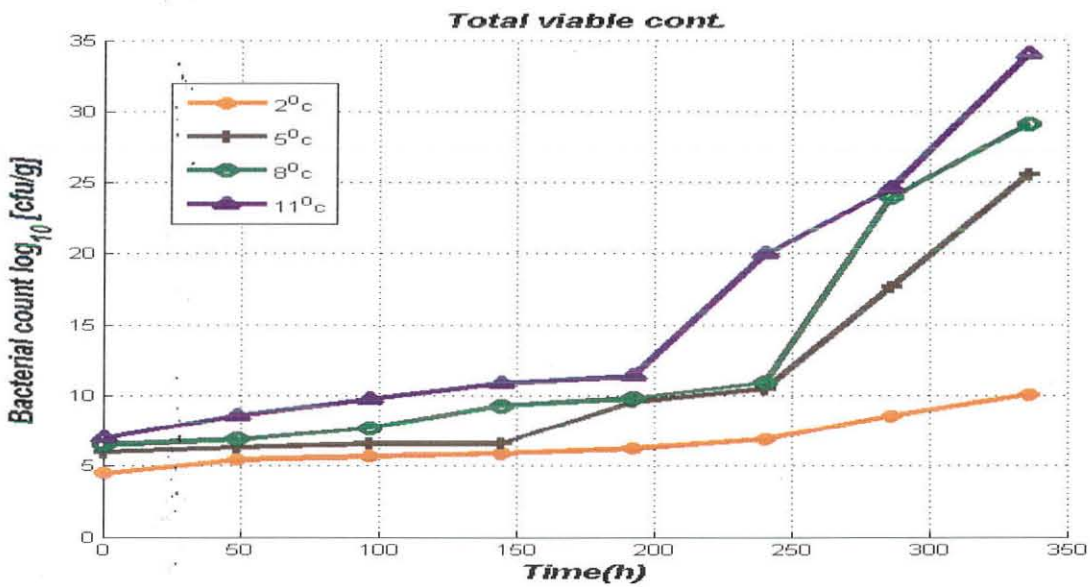


Figure 4.1 Total viable count fitted with the Gompertz model

Table 4.2 Change in number of *E.coli* with time log (CFU/ml)

	2±°c	5±°c	8±°c	11±°c
0	4.195 ^a ±0.03	4.296 ^b ±0.07	5.778 ^c ±0.03	7.176 ^d ±0.05
48	4.231 ^a ±0.04	4.832 ^b ±0.09	6.579 ^b ±0.06	8.255 ^d ±0.02
96	4.291 ^a ±0.06	4.871 ^b ±0.07	7.342 ^c ±0.04	9.380 ^d ±0.01
144	4.834 ^a ±0.07	5.765 ^b ±0.04	8.176 ^c ±0.05	10.217 ^d ±0.05
192	4.983 ^a ±0.02	7.283 ^b ±0.02	8.318 ^c ±0.01	10.311 ^d ±0.04
240	5.629 ^a ±0.01	11.732 ^b ±0.005	13.697 ^c ±0.02	16.843 ^d ±0.03
286	7.856 ^a ±0.01	16.301 ^b ±0.02	20.903 ^c ±0.05	21.631 ^d ±0.02
336	9 ^a ±0.02	23.041 ^b ±0.05	28.322 ^c ±0.04	32.623 ^d ±0.01

Values in the same column with different superscript are significantly different ($p < 0.05$)

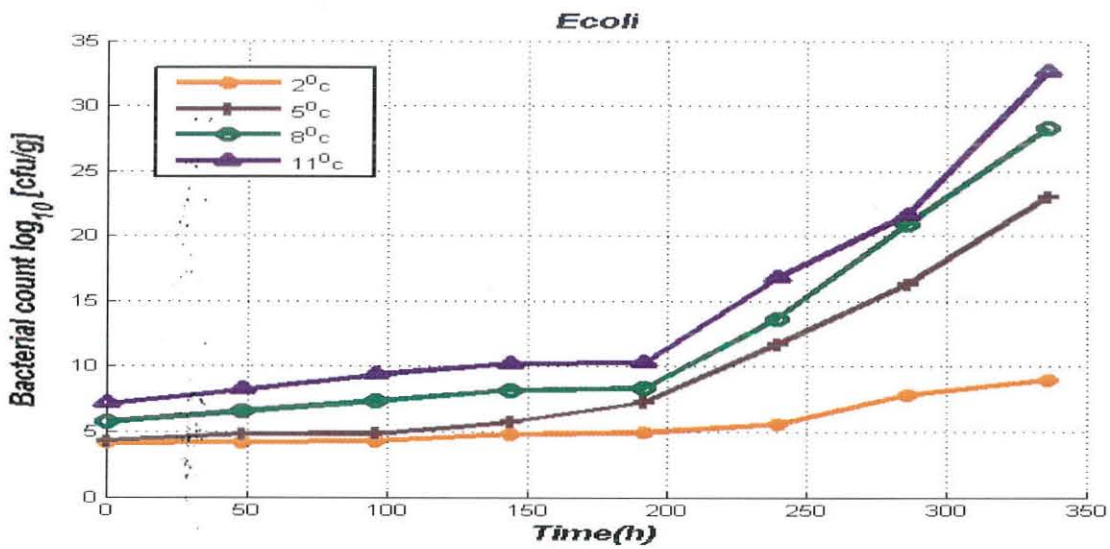


Figure 4.2 *E.coli* fitted with the Gompertz model

Table 4.3: Change in number of *Pseudomonas sp.* with time log (CFU/ml)

Time(h)	2±1°C	5±1°C	8±1°C	11±1°C
0	3.7324 ^a ±0.105	5.7521 ^b ±0.0005	6.08278 ^c ±0.001	7.0170 ^d ±0.001
48	4.8692 ^a ±0.01	6.235528 ^b ±0.14	7.36921 ^c ±0.003	7.9138 ^d ±0.04
96	5.4313 ^a ±0.005	7.167317 ^b ±0.03	9.6989 ^c ±0.210	8.2154 ^d ±0.02
144	6.0253 ^a ±0.001	7.361728 ^b ±0.001	10.0792 ^c ±0.001	11.5575 ^d ±0.12
192	6.2455 ^a ±0.03	11.14613 ^b ±0.004	11.5211 ^c ±0.004	13.4653 ^d ±0.202
240	6.9334 ^a ±0.04	11.5092 ^b ±0.005	13.1673 ^c ±0.005	17.2922 ^d ±0.003
286	8.9159 ^a ±0.001	22.41497 ^b ±0.006	24.7784 ^c ±0.005	26.1818 ^d ±0.004
336	9.3010 ^a ±0.002	23.93651 ^b ±0.005	29.8669 ^c ±0.001	33.7161 ^d ±0.05

Values in the same column with different superscript are significantly different ($p < 0.05$)

For beef muscle Competition occurs among members of an anaerobic gram-negative flora (mainly *pseudomonas sp.* and *shewanella putrefaciens*). Of the psychrotrophic bacteria *pseudomonas spp.* are gram negative bacteria dominate at refrigeration temperatures and considered as one of the main spoilage microorganisms in meat and poultry (Jay, 2000).

In beef muscle *Pseudomonas* are numerous than the other microorganisms in the microbial flora because they grow faster and have greater affinity for oxygen than the others (Jay, 2000).

The growth of *pseudomonas* bacteria as affected by temperature difference and duration of storage time is shown in (Table 4.3). At each temperature the growth of *pseudomonas* bacteria increasing constantly. The temperature difference on the *pseudomonas* growth had a significant effect ($p < 0.05$). The microbial profile described above has also been

reported in other studies on aerobically stored meat the results of the study showed that the development of microbial association during storage was increased with time with *Pseudomonas* dominating the microbial populations. According to (Koutsoumanis, Stamatiou et al. 2006) the increment pseudomonas was relatively more during the first eight days corresponding to the lag and exponential growth phases. After eight days the bacterial growth rate was fairly steady. The number of *Pseudomonas* organisms and total bacteria at day 8 was significantly different from those at day 10.

However, in the present study the microbial growth profile shows the reverse. As shown in the table 4.1, 4.2 and 4.3 the growth of bacterial count continually increases until the final storage time. About the last storage hour the bacterial count increases ultimately. The mean log value of a graphical comparison of the model developed in this study and in the previously published *Pseudomonas* spp. models of (Neumeyer, Ross et al. 1997) and (Willocox, Mercier et al. 1993) shows that the same general trend and the spread in predicted growth rates expands with increasing temperatures. The predictions of the present model developed using experimental data.

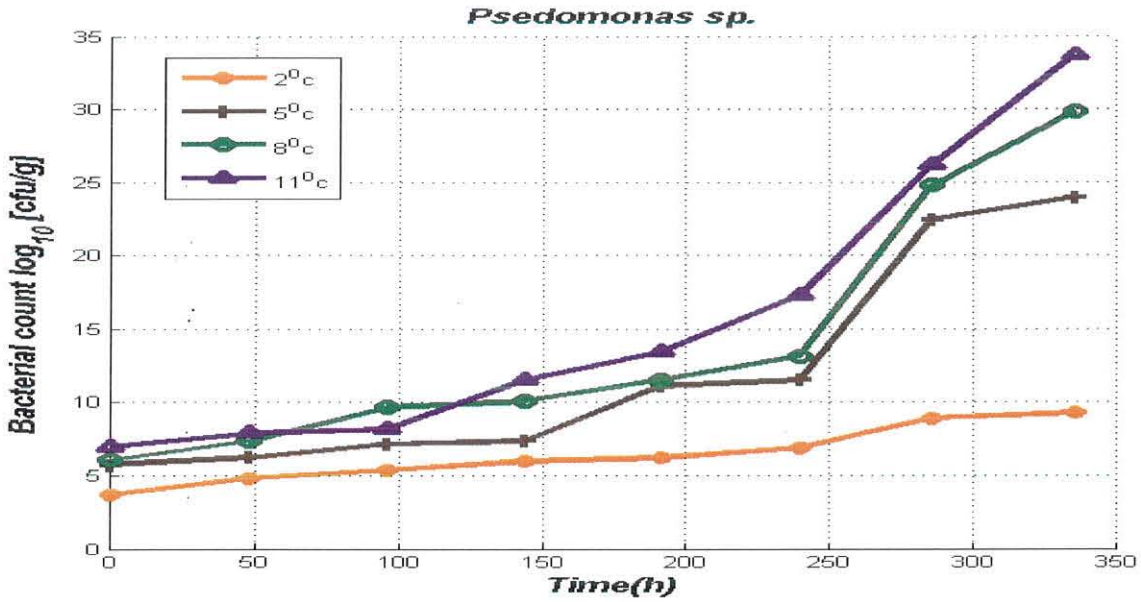


Figure 4.3 The growth of *Pseudomonas* count at constant storage temperatures from 2 – 11°C fitted with the Gompertz model.

As shown in Figure 4.3 the model fits with the growth of *Pseudomonas*. The growth curve is slightly similar with the typical S-type microbial growth. The growth was significantly affected by temperature ($p < 0.005$). As the temperature increased the amount of time needed to reach the spoilage limit decreased approximately, 192 hours required at 2°C but only 77.7 hours necessary at 11°C and the Initial log number of *E. coli* and TVC count were slightly higher than *Pseudomonas* Counts it was 4.1 log cfu/ml-7.0 log cfu, 4.5 log cfu/ml-7.0 log cfu and 3.7 log cfu/ml-7.0 log respectively. As the storage hour increases the relative growth rate was higher for *Pseudomonas* however at the end of storage the maximum growth among the three bacteria did not show relevant differences independent of temperature and initial bacterial count. (Giannuzzi, Pinotti et al. 1998), reported that increasing temperature led to a faster growth with *Pseudomonas* bacteria.

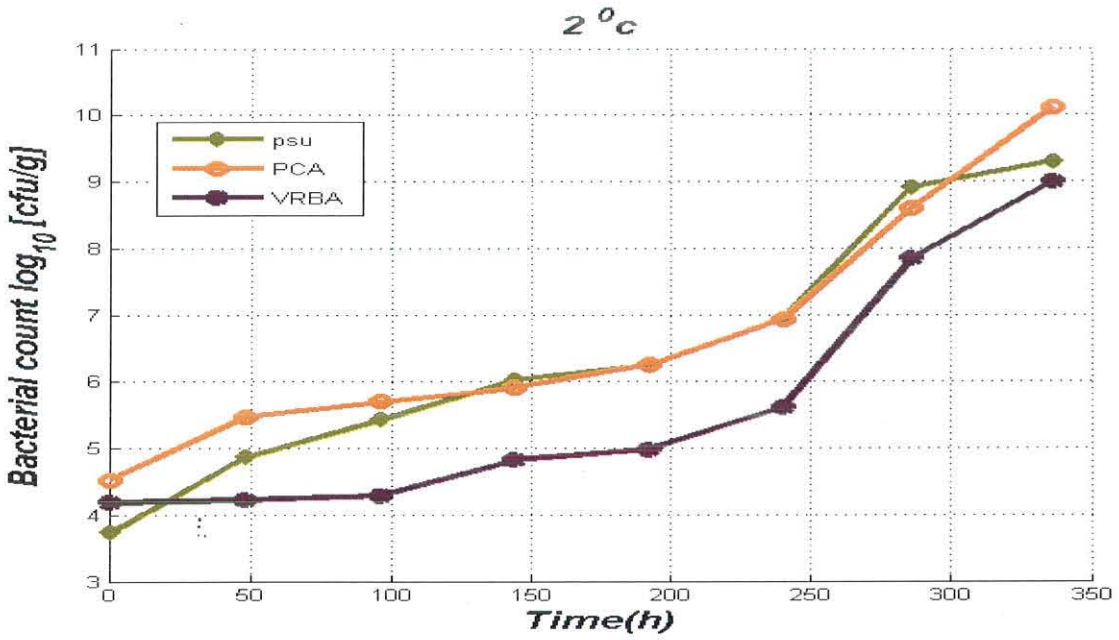


Figure 4.4 The growth of PSU (pseudomonas) , PCA(plate count agar) and VRBA:voilate red bile agar for (E.coli) bacteria at temperature 2°C

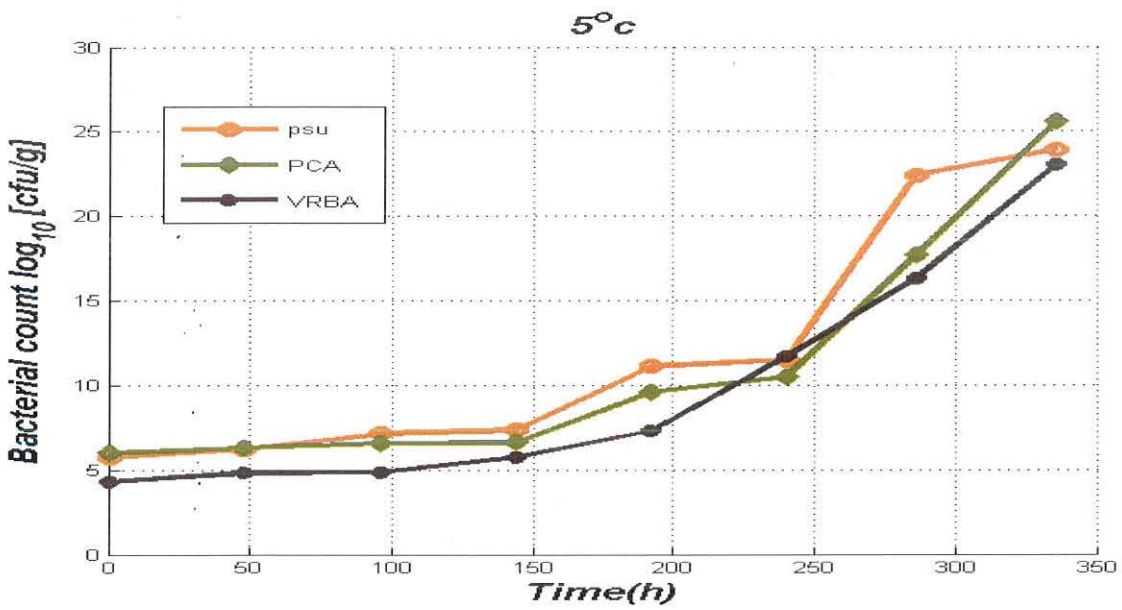


Figure 4.5 the growth of The growth of PSU (pseudomonas) , PCA(plate count agar) and VRBA:voilate red bile agar for (E.coli) bacteria at temperature at 5°C

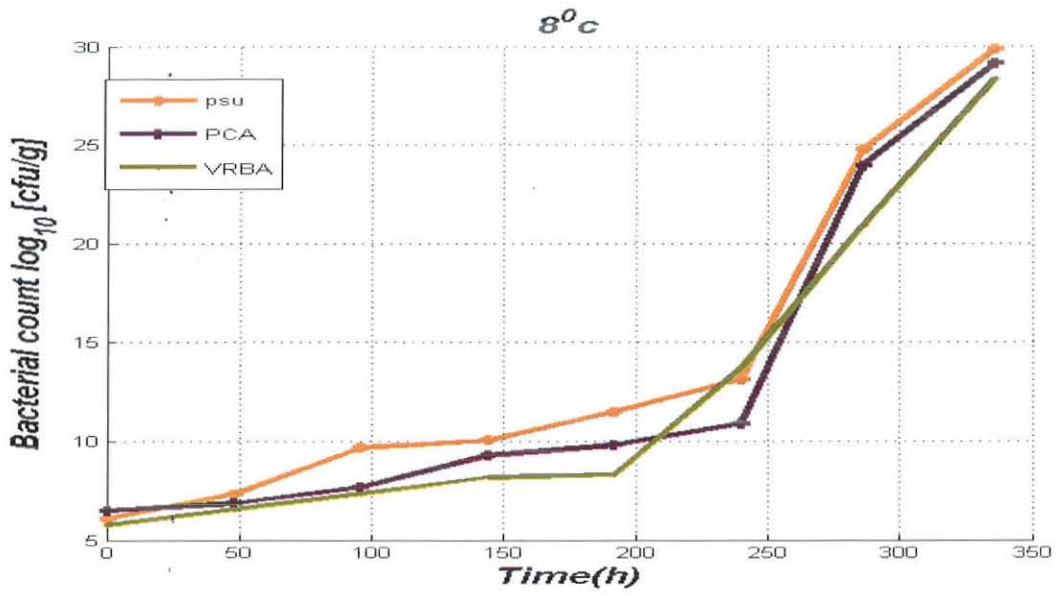


Figure 4.6 the growth of The growth of PSU(pseudomonas) , PCA(plate count agar) and VRBA:voilate red bile agar for (E.coli) bacteria at temperature at 8°C

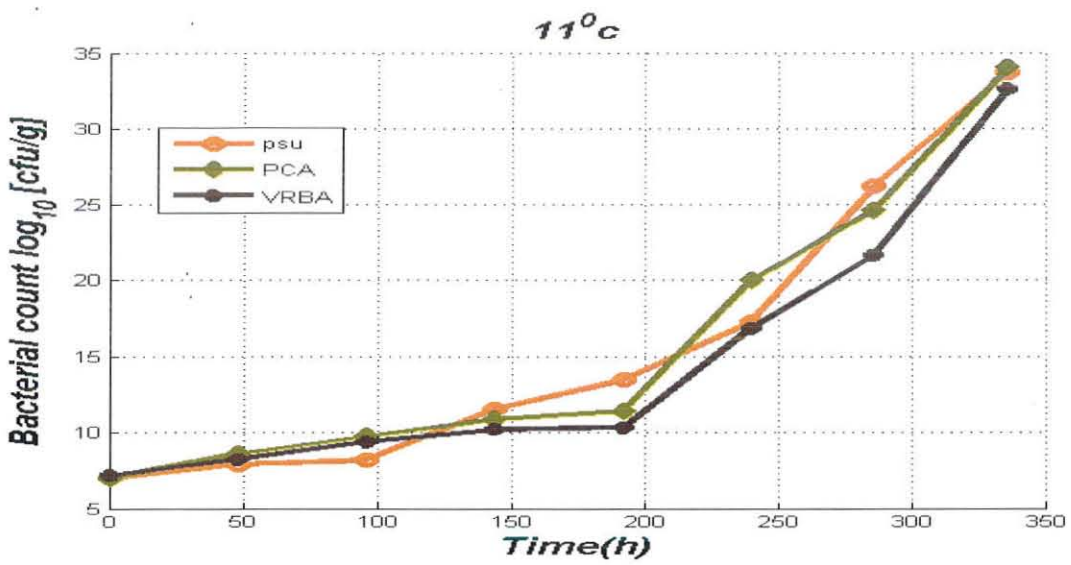


Figure 4. 7 The growth of PSU(pseudomonas) , PCA(plate count agar) and VRBA:voilate red bile agar for (E.coli) bacteria at temperature at 11°C

The results obtained are summarized in figure 4.4 4.5, 4.6 and 4.7 which present comparison of all the tree spoilage bacteria in different temperature. During the first storage temperature the graph shows relatively minimum growth. It is observed that pseudomonas and plat count were nearly overlapped each other however, pseudomonas slightly higher at the beginning and at the end but the graph shows minimum growth for VRBA. Table 4.7 shows the rapid growth of all bacteria's at 11°C due the higher storage temperature and the maximum number of initial bacterial amount. At all conditions tested pseudomonas was the dominant bacteria followed by Total plate count and E.coli (VRBA) remained at third levels.

The results of the study showed that the development of microbial association during storage was different for all pH and temperature tested with pseudomonades dominating the microbial populations. With respect to the environmental parameters to include in the model that significantly influence the growth rate have been considered. However, prior research has concluded that within the pH range of normal poultry meat or muscle Pseudomonas spp. was predominate as the spoilage microorganism without affecting its growth rate (McMeekin and Ross 1996). This clearly represents an optimum environment for Pseudomonas growth and as expected Pseudomonas spp. was found to be the predominant spoilage microorganism (Pooni and Mead 1984). The mean log values of TVC, pseudomonas and E.coli from samples of beef muscle during storage at $5 \pm 1^\circ\text{C}$ (in which the spoilage processes start) are shown above. During storage these microorganisms significantly increased. TVC of beef muscle was about 5.983 log cfu/ml initially and the number of count increased as the storage time increased after 6 days total viable counts exceed the limit of shelf life.

Pseudomonas reached 7.635 log cfu/ml at day 6 these organisms grow faster and have greater affinity for oxygen than the others (Jay,2000).The number of E.coli counts increased 5.765log cfu /ml on day 6 and to 7.383 log cfu/g on day 7 respectively. In this study the number of E. coli and TVC found to lower in log count than pseudomonas in all samples throughout the storage period. For sample stored at 2°C the increment was slightly as compared to the rest of the temperature storage due to the initial count is lower

4.2 Effect of pH on bacterial growth

Table 4.4 Determination of pH of the sample

Time(h)	2±1°C	5±1°C	8±1°C	11±1°C
0	5.61 ^c ±0.05	5.52 ^b ±0.04	5.62 ^c ±0.34	5.36 ^a ±0.31
48	5.51 ^a ±0.06	5.62 ^c ±0.01	5.58 ^b ±0.01	5.68 ^d ±0.02
96	5.51 ^b ±0.01	5.41 ^a ±0.03	5.65 ^c ±0.03	6.07 ^d ±0.07
144	5.54 ^b ±0.03	5.43 ^a ±0.03	5.70 ^c ±0.04	6.58 ^d ±0.03
192	5.53 ^a ±0.09	5.55 ^a ±0.01	5.84 ^b ±0.02	5.96 ^c ±0.04
240	5.54 ^a ±0.005	5.76 ^b ±0.01	6.89 ^c ±0.01	7.25 ^d ±0.005
286	5.61 ^a ±0.04	7.34 ^c ±0.01	6.90 ^b ±0.01	6.88 ^d ±0.005
336	5.54 ^a ±0.001	6.05 ^b ±0.04	7.56 ^c ±0.005	7.02 ^d ±0.003

The pH of the meat samples in combination with temperature conditions during meat storage presented in Table 4.4 Comparisons showed significant differences (p < 0.05).

The observed value of the Initial PH of beef sample were consistent with generally observed pH values of fresh beef in the literature (5.30– 5.70) (Koutsoumanis, Stamatiou et al. 2006). The pH-value were increasing slightly even if some degree of fluctuation occurs.

The difference between initial and end value was significant ($p < 0.05$) for all sample type. The results of (Ross, Ratkowsky et al. 2003) also indicated an increasing pH during storage time. The pH of the first sample stored at 2°C decreased first and increased again at the end of the storage time. In general initial pH of meat can vary significantly depending on animal feeding and handling or other factors affecting rigor mortis (Koutsoumanis, Stamatiou et al. 2006).

The higher pH-values have been associated with a faster microbial spoilage of meat (Borch, Kant-Muermans et al. 1996). However in contrast to this, (Gill and Newton 1982) have demonstrated that the growth rate of *Pseudomonas* sp. on fresh meat was the same at a pH of 5.5 as well as 6.4 which is consistent with the findings of who also observed no effect of the pH on the growth rates of *Pseudomonas* sp. in the pH range of 5.3 – 7.8

Table 4.5 Correlations of pH with microbial count

Temp(°c)	Pseudomonas	TVC	E. coli
2	0.468	0.284	0.481
5	0.619	0.396	0.417
8	0.629	0.331	0.647
11	0.575	0.417	0.567

In the present study the results were confirmed by the correlations between pH-value and bacterial cont. The Correlations was significant ($p < 0.05$) for all storage temperature and spoilage bacteria however their magnitudes were low. (Allen et al. 1997) also reported significant but low correlations between pH and bacterial counts .Therefore, pH as an intrinsic factor can be disregarded concerning its influence on *Pseudomonas* bacteria and not included in the modeling session.

4.3 sensory evaluations

The shelf-life of a food must be determined by means of both sensory and microbiological analysis. (rodríguez - pérez, zurera - cosano et al. 2003) With regard to microbiological analysis a number of authors have proposed different criteria for determining shelf life and expiry date

(Notermans, Wijtzes et al. 1993) reported a good correlation between shelf-life and the time taken for the mesophilic microorganism count to reach 10^6 cfu/mL. Other authors report a better correlation between organoleptic changes and the time taken for lactic acid bacteria counts to reach between 10^6 - 10^8 cfu/mL (Zurera-Cosano, Rincon-Leon et al. 1988).. Sensory analysis was performed at the same time as microbiological analysis). (rodríguez - pérez, zurera - cosano et al. 2003).Five-point hedonic scale was used to comprehensively evaluate the shelf life of beef muscle .Fifteen Panelists were participated from the staff member of EHNRI institution each panelist was provided with a work sheet listing the main quality characteristics of the product. Aroma, Color Texture and overall acceptability and a scale from maximum 5 to minimum 1in the present study Aroma is considered as the most important of all sensory evaluation parameter to determine the shelf life of given product Meat color is also one of the criteria consumer considers when choosing a product (Seyfert, Mancini et al. 2007). It is an important indicator of meat quality, hygiene and freshness. The color depends not only on the meat's myoglobin and hemoglobin content but also on the chemical states of both myoglobin and hemoglobin (Mancini, Hunt et al. 2005).

The growth of *Pseudomonas* constantly reduces the partial pressure of oxygen and gradually promotes the formation of metmyoglobin (metMb), resulting in a gradual change in meat color. In general, it is important to evaluate specific quality characteristics and their corresponding critical thresholds to determine the shelf life of a product (Brooks et al., 2008). A product will not be of acceptable quality if a specific characteristic surpasses its critical threshold. In addition, the number and types of microorganisms must be considered when determining the shelf life of a product (Brooks et al., 2008).

Table 4.6 Average value and standard deviation of sensorial analysis of muscle beef at (2°C)

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Color	4.3±0.48 ^a	4.06±1.1 ^c	4.12±0.6 ^d	3.57±0.78 ^{bc}	4.0±0.0 ^d	3.22±0.83 ^b	3.0±0.0 ^a	1.8±1.1
Marbling	4.1±0.69 ^a	3.82±1.46 ^c	3.69±0.8 ^c	3.5±1.77 ^c	2.77±0.71 ^c	2.57±0.83 ^{cb}	2.4±0.54 ^c	2.0±0 ^c
Texture	4.43±0.53 ^a	4.12±1.2 ^{ab}	3.94±1.1 ^{ac}	3.71±0.98 ^{bd}	3.0±1.41 ^{ab}	3.55±0.72 ^a	2.6±1.14 ^c	3.0±0 ^{ab}
Overall acceptability	4.43±0.53 ^c	4.25±0.7 ^{dc}	4.0±0.9 ^{bd}	3.42±0.78 ^{ad}	3.0±0.00 ^d	2.66±0.86 ^a	2.2±1.09 ^{dc}	2.0±0 ^d

Table 4.7 Average value and standard deviation of sensorial analysis of muscle beef at (5°C)

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Color	4.3±0.48 ^a	3.12±1.15 ^{ab}	4.00±0.5 ^{ac}	2.50±0.5 ^b	3.0±0.97 ^d	3.00±0.70 ^a	3.0±0.0 ^a	2.2±0.83 ^b
Marbling	4.1±0.37 ^a	4.02±0.93 ^d	3.88±0.83 ^d	3.5±0.71 ^{cd}	3.0±0.0 ^c	2.57±1.32 ^d	2.5±1.1 ^{cd}	2.4±0.71 ^d
Texture	4.42±0.53 ^a	3.62±1.25 ^b	4.0±0.70 ^d	3.14±0.8 ^b	3.5±0.71 ^b	3.44±1.01 ^a	1.8±1.09 ^a	3.5±0.71 ^d
Overall acceptability	4.14±0.37 ^a	3.50±0.97 ^{ab}	4.0±0.50 ^{ac}	2.42±0.9 ^a	4.0±0.00 ^b	2.88±0.78 ^b	1.6±0.5 ^{ab}	2.0±0.0 ^{ac}

Table 4.8 Average value and standard deviation of sensorial analysis of muscle beef at (8°C)

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Color	4.6±0.53 ^b	3.68±1.0 ^{bc}	3.47±1.0 ^a	2.40±0.84 ^a	2.0±0.0 ^b	3.7±0.83 ^{db}	3.0±0.0 ^a	3.4±0.9 ^{dc}
Flavor	4.1±0.69 ^a	3.76±1.1 ^{ab}	3.37±0.8 ^b	3.00±1.57 ^b	2.44±0.0 ^b	2.00±0.72 ^b	2.0±0.89 ^b	1.5±0.71 ^b
Texture	4.85±0.4 ^c	3.87±0.9 ^{ab}	3.58±0.71 ^a	3.14±1.2 ^{bc}	2.5±2.12 ^a	3.44±1.01 ^a	2.4±0.6 ^{ab}	3.0±0.0 ^c
Overall acceptability	4.43±0.6 ^c	3.68±0.9 ^{cb}	3.58±0.79 ^a	3.14±1.1 ^{ab}	2.0±0.00 ^c	3.33±1.1 ^{ad}	2.6±1.34 ^c	1.0±0.71 ^b

Table 4.9 Average value and standard deviations of sensorial analysis of muscle beef at (11°C)

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Color	4.7±0.48 ^c	2.50±1.36 ^a	3.70±1.1 ^b	4.0±0.57 ^d	3.55±1.1 ^{ca}	3.0±0.0 ^a	2.4±0.89 ^{ca}	1.5±0.71 ^a
Flavor	4.3±0.48 ^b	3.13±1.4 ^a	2.63±1.37 ^a	2.14±1.06 ^a	2.11±0.0 ^a	1.2±1.08 ^a	1.00±0.4 ^a	1.0±0.0 ^a
Texture	4.71±0.5 ^b	3.58±1.5 ^a	3.88±0.93 ^b	3.00±1.0 ^a	1.5±0.71 ^a	3.44±1.01 ^a	3.0±0.70 ^d	2.5±0.71 ^a
Overall acceptability	4.28±0.5 ^b	2.87±1.36 ^a	3.68±1.05 ^b	3.42±1.13 ^c	1.5±0.71 ^a	2.88±1.3 ^{ac}	1.8±0.9 ^{ad}	1.0±0.0 ^a

Values in the same row with different superscript are significantly different ($p < 0.05$)

The shelf life was determined for the sample stored at four temperatures based on the score provided the samples evaluated accordingly (5 = like extremely, 4 = like moderately, 3 = neither like nor dislike, 2 = dislike moderately and 1 = dislike extremely). In the present study the cut-off point (COP) determined when the sensory score reach ≤ 3 for shelf life limit the score below 3 point was considered rejection based on consumers acceptability. According to (Hough, Langohr et al. 2003) one of the first authors to propose the COP methodology was (Gacula 1975) the shelf life limit estimation was based on regression of

the panel scores versus storage time using an arbitrary 2.5 cut-off point on 1 (none) to 7 (very strong) off flavor scale. Also (rustom, lópez - leiva et al. 1996) used 1 (dislike extremely) up to 7 (like extremely) for acceptability test to determine shelf life limit of ultra-high temperature-sterilized peanut beverages defining 4 as the acceptability limit.

To study the combined effects of modified atmosphere packaging and antioxidants on the storage stability of pork sausages (Martínez, Cilla et al. 2006) used a 5-point scale to measure off-odor the scale was from 1 = none to 5 = extreme with 3 = being considered as a limit of acceptability. The sensory attributes are shown on the above tables the result shows that all the samples were scored above 4 initially. The samples stored at 2 °c relatively has longer shelf life Lasting for up to day 8 the score was coincide with the shelf life limit 3.00 point .The Sample stored at 5 °c remain acceptable up to the first 6 days the score was 3.5 at day 6 the samples stored at 8 °c also remain acceptable up to day six but the score was exactly coincide with the cut-off point. However the sample stored at 11°c was with very short shelf life it was remain acceptable only for the first two days also other attributes were analyzed the score shows variation based on the panelist's choice. The score for color and overall acceptability were decreased parallel to aroma however texture shows slight fluctuation it was continuously decreases for the first 8 days then increased again for sample stored at 5 and 8 °c. But it was only decreases for the first two days then increases and decreases again for the sample 2 and 11 °c. considering sensory evaluation scores the products are acceptable for consumption for the first eight days of storage for stored at temperature 2 and 5 °c, and 6 and 2 days for sample stored at 8 and 11 °c respectively.

Table 4.10 The correlation between the sensory attributes with sampling days

Temperature °C	Aroma	Color	Texture	Overall acceptability
2	0.950	0.55	0.413	0.602
5	0.972	0.605	0.623	0.987
8	0.984	0.171	0.44	0.847
11	0.923	0.227	0.55	0.831

There was significant Correlations ($p < 0.05$) for all attributes however, the magnitude were relatively lower for texture and color of the samples it was ($r^2=0.41-0.55$ and $0.55-0.22$) respectively. Although correlation was relatively higher for Overall acceptability ($r^2=0.602-0.831$) the most significant correlation was found due to change in flavor ($r^2=0.950-0.923$) it is assumed as the most influential attributes in the shelf-life period. In view of these results it is concluded that flavor was found to be the most important factors in determining shelf-life it is a practical and simple method and the panelists also familiar with the organoleptic property of the product

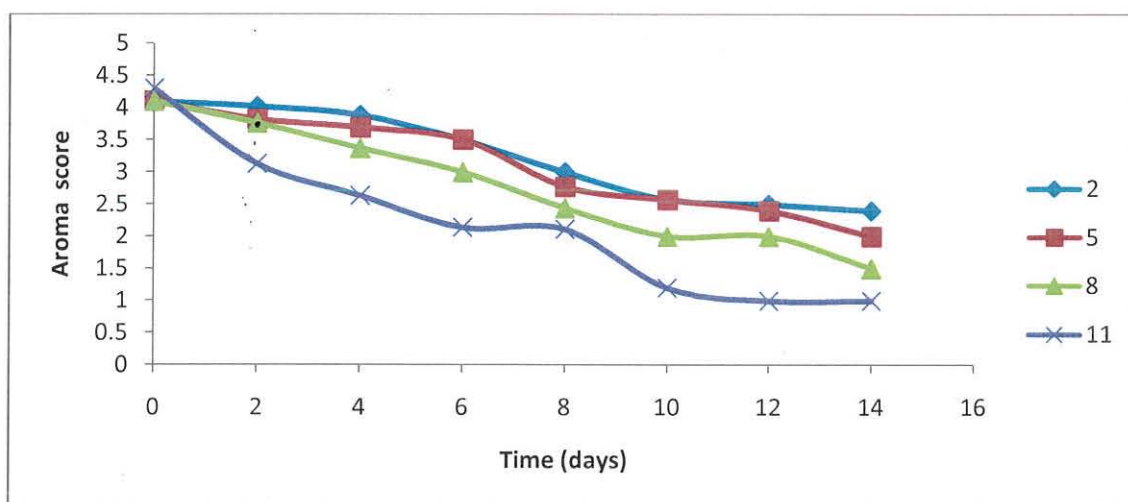


Figure 4.8 The correlation of Aroma score verses time

(rodríguez - pérez, zurera - cosano et al. 2003) Reported that flavor as the most important sensory defects for cooked meat products. (Dalgaard and Koutsoumanis 2001) also used of all the methods listed in the literature review part, method based on mean of smell and taste was selected to estimate the shelf-life of cooked chicken breast since these parameters had a greater impact on the shelf-life of the product.

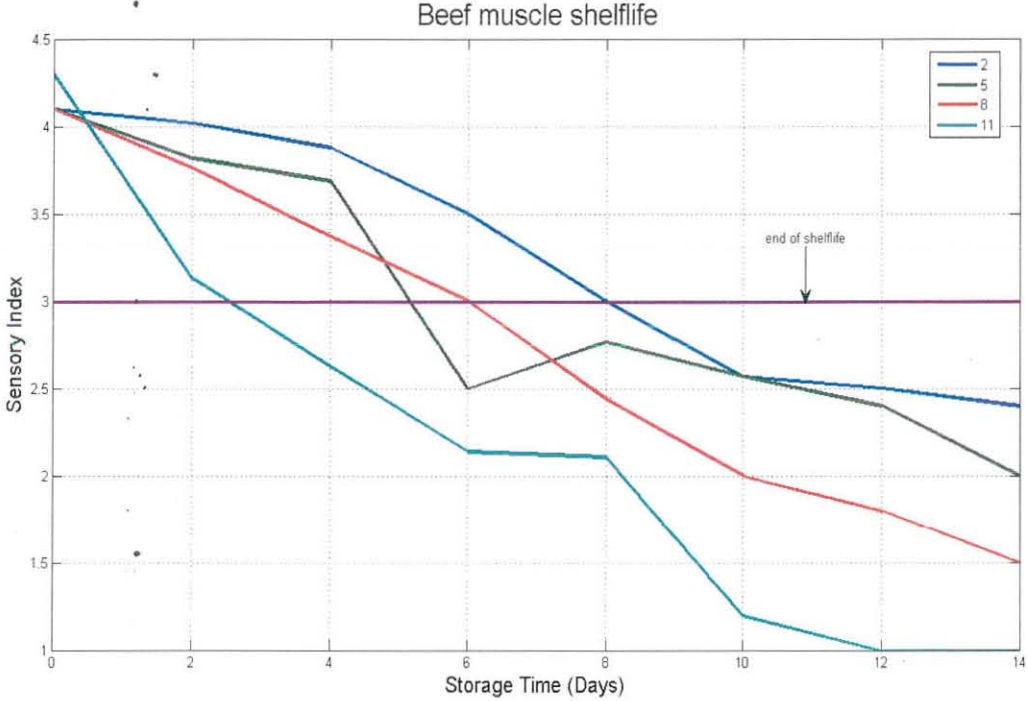


Figure 4.9 The sensory score for beef muscle at different constant temperature (End of shelf life ≤ 3)

The sensory index decreasing linearly with increasing temperature as similar with the find of (Al-Sheddy, Al-Dagal et al. 1999). At all investigated constant storage temperature very high significant correlation ($p < 0.05$) were obtained between the count of Pseudomonas and the SI (sensory index) ($r^2 = 0.970$ to 0.997) for TVC the correlation was ($r^2 = 0.902$ to -0.889) as well as for E.coli count ($r^2 0.850-0.874$). Correlation between Pseudomonas and

spoilage index were greater than those between the total viable count and E.colia count

Thus, it is concl

4.4 Modeling of microbial growth

4.4.1 Primary model

Primary model describe changes in microbial numbers with time. The growth data from the enumeration of different microbial count changed into log base and was modeled as a function of time to calculate the specific growth rate and the Lag phase duration.

Gompertze model was used as a primary model. The expression is

$$\log N = A + C \cdot e^{-e^{-B(t-M)}}$$

where log N is the logarithm of microbial counts [log (CFU/ ml)] at time t, A taken as the initial microbial count, C is difference between upper asymptotic line of the growth curve (N_{\max} =maximum population level) and the lower asymptotic line, B is the relative growth rate], M is the time required to reach the maximum growth rate and e is the base of Napierian logarithm (2.718281).

To calculate the log N the parameters C, B and M were predicated using Origin 6 software. The graphs were drawn by MATLAB (version7.0). To calculate the growth parameters for each temperature, the predicted data obtained were fitted to the Gompertz function at time t the modified Gompertz model is expressed by the given equation. Using the above equation it is possible to predict the bacterial log count and the probability for how long the beef muscle will stay without spoilage at a given temperature for any time needed to calculate

$$N(t) = A + C.e^{-e^{-B(t-M)}}$$

$$2^{\circ}\text{C} \quad N(t) = 3.7324 + 9.3482e^{-e^{-0.0053(t-218.2638)}}$$

$$5^{\circ}\text{C} \quad N(t) = 5.7521 + 55.4775e^{-e^{-0.0060(t-344.5530)}}$$

$$8^{\circ}\text{C} \quad N(t) = 6.0828 + 117.5054e^{-e^{-0.0044(t-441.5239)}}$$

$$11^{\circ}\text{C} \quad N(t) = 7.0170 + 101.6844e^{-e^{-0.0049(t-394.7537)}}$$

Using this parameters the specific growth rate $\mu = B.C/e(\log(\text{CFU/ML})\text{day}^{-1})$ with $e=2.7182$ and lag phase duration (LPD= $M-(1/B)$ (day s)) (Giannuzzi, Pinotti et al. 1998) were derived. These models numerically fitted to experimental data of pseudomonas bacteria collected at various temperatures

4.4.1.1 Application of primary model for prediction of microbial count

Table 4.11 Experimental and predicated value for pseudomonas count at 2°C

2°C					
Sampling hrs	Experimental value	Predicted value	Residuals	MSE	R ²
0	3.7324	4.1249	0.3925		
48	4.8692	4.5312	-0.338		
96	5.4314	5.1187	-0.3127		
144	6.0253	5.8586	-0.1667	0.026	0.98
192	6.2455	6.6955	0.45		
240	6.9335	7.5657	0.6322		
288	8.9159	8.3792	-0.5367		
336	9.301	9.1984	-0.1026		
			0.018		

Table 4.12 Experimental and predicted value at 5°C

5°C					
Sampling hrs	Experimental value	Predicted value	Residuals	MSE	R ²
0	5.7521	5.7751	0.023		
48b	6.2355	5.9117	-0.3238		
96	7.1673	6.4360	-0.7313		
144	7.3617	7.7929	0.4312	0.103	0.854
192	11.1461	10.3920	-0.7541		
240	11.5092	12.3521	0.8429		
288	21.1970	22.4149	1.2179		
336	23.9365	23.1217	-0.8148		
				0.109	

Table 4.13 Experimental and predicted value at 8°C

8°C					
Sampling hrs	Experimental value	Predicted value	Residuals	MSE	R ²
0	6.0828	6.2008	0.118		
48	7.3692	6.5192	-0.85		
96	9.6989	8.3426	-1.3563		
144	10.0792	9.0576	-1.0216	0.523	0.890
192	11.5211	12.0522	0.5311		
240	13.1673	13.5807	0.4134		
288	23.3889	24.7781	1.3892		
336	29.8669	30.1208	0.2539		
				0.5223	

Table 4.14 Experimental and predicted value at 11°C

11°C					
Sampling hrs	Experimental value	Predicted value	Residuals	MSE	R ²
0	7.0170	7.1047	0.0877		
48	7.9138	7.4067	-0.5071		
96	8.2154	8.2810	0.0656		
144	11.5575	10.2138	-1.34337	0.161	0.954
192	13.4653	13.6615	0.1962		
240	17.2922	18.8479	1.5557		
288	26.1818	25.3510	-0.8308		
336	33.716	33.7086	-0.0074		
			0.283		

Table 4.11, 4.12, 4.13 and 4.14 shows the experimental and predicted values obtained using Gompertz function and their residuals. The sum of the residuals for all temperature (2, 5, 8 and 11°C) found to be 0.018, 0.109, 0.5223 and 0.283 respectively which is close to zero as a result it is considered that good agreement between experimental data obtained from practical analysis and predicted value by the Gompertz function.

4.4.1.2 Statistical evaluation of the model

To evaluate the model comparisons of experimental and predicted value were made using Mean square error (MSE) and regression coefficient (R²). The lower MSE defines the better adequacy of the model to describe the data (Sutherland, Bayliss et al. 1995). The experimental data showed better fit for temperature 2 and 11°C with MSE 0.026 and 0.161 respectively. However, the MSE was higher for temperature 5 and 8°C it was 0.103 and 0.523 respectively.

The best fitting model will have the smallest MSE and maximum R² values. Because the R² values were greater than 0.95 and the MSE were relatively small the model has been determined as well predicated

4.4.1.3 Estimation shelf life under different temperature conditions

Table 4.15 Levels of the spoilage bacteria at the time of rejection Estimated by primary model

Temperature (°C)	Shelf life (h)	PSU log ₁₀ cfu/ml	TVC log ₁₀ cfu/ml	E.coli log ₁₀ cfu/ml
2	192	6.713	6.13	4.88
5	165.65	8.73	7.77	6.01
8	144	8.982	7.101	5.23
11	77.75	8.917	7.912	7.01

The shelf life was estimated at the time at which the score reached the value of 3 which was the rejection point. The number of spoilage bacterial count at the end of shelf life was estimated using the primary growth model and setting time equal to shelf life. With this method the samples stored at 2, 5 and 8 were found to retain their sensory qualities until the experiment time (192, 165.5, 144 hours respectively) whereas at 11°C they retained these qualities for only 77.75 hours. Predicted shelf life value was estimated at the time when the spoilage microorganisms multiply from the initial to the spoilage level. The bacterial levels at the end of shelf life are shown in (table 4.15) in most cases the level of pseudomonades at the end of shelf life was constantly close to log₈ CFU/ml. The level of TVC was also relatively constant but always at least 1 log CFU/ml lower than pseudomonades. The rest spoilage bacteria (E. coli) at the end of shelf life ranged from log₄ to log₇ CFU/ml depending on the storage temperature.

The observation that pseudomonades were the dominant organisms at the end of shelf life with a constant population level can lead to their characterization as a good spoilage index for aerobically stored beef muscle.

According to (Borch, Kant-Muermans et al. 1996) spoilage of aerobically stored meat cuts occurs when pseudomonas reach 10^7 to 10^8 CFU per ml. (Gram and Huss 1996) also reported that spoilage based on the growth of Pseudomonas leads to off-odours and slime-production when the bacterial numbers reach 7 – 8 log₁₀ cfu/ml. In the present study the spoilage limit of the pseudomonas bacteria found to be closer to log 8 CFU/ml corresponding to the time in which the panelists reject the sample that is 3 point. According to the quality policy and standards of a meat industry (Baranyi, Robinson et al. 1995) any value above the limit considered as a spoilage

4.4.2 Secondary modeling

Secondary models describe the responses by the parameters of the primary models to changes in environmental conditions such as temperature, pH and water activity. Since these three factors are the major factors affect microbial growth it is very important to study their effect however, the water activity parameter can't be included in these experiment because of the instrument which measure the a_w of meat product did not measure properly the digital screen shows the measurement result above 1.00 it is invalid. Only the a_w of pure water is 1.00. In the present study Arrhenius model is used as a secondary modeling function. The parameters maximum growth rate (μ) and the lag phase duration (LPD) estimated from the primary model expressed as a function of temperature to study the effect of temperature on these parameter.

4.4.2.1 Effect of temperature on the growth on specific growth rate

The effect of incubation temperature on the growth parameter of spoilage bacteria is studied using modified Arrhenius equation to describe the temperature relationships. The primary growth model used to estimate the major parameters of bacterial growth the maximum growth rate (μ) and the lag phase duration (LPD) .Temperature dependence of growth parameters was modeled using the Arrhenius equation.

$$\ln B = \ln F - \left(\frac{E_a}{T.R} \right)$$

B relative growth rate at time M [1/h], F pre-exponential factor [1/h], E_a : activation energy for bacterial growth [kJ/mol], R: gas constant [8.314 J/mol K], T: absolute temperature [K]

The Arrhenius equations described the experimental data when logarithms of maximum growth rate plotted with temperature linear dependence was found

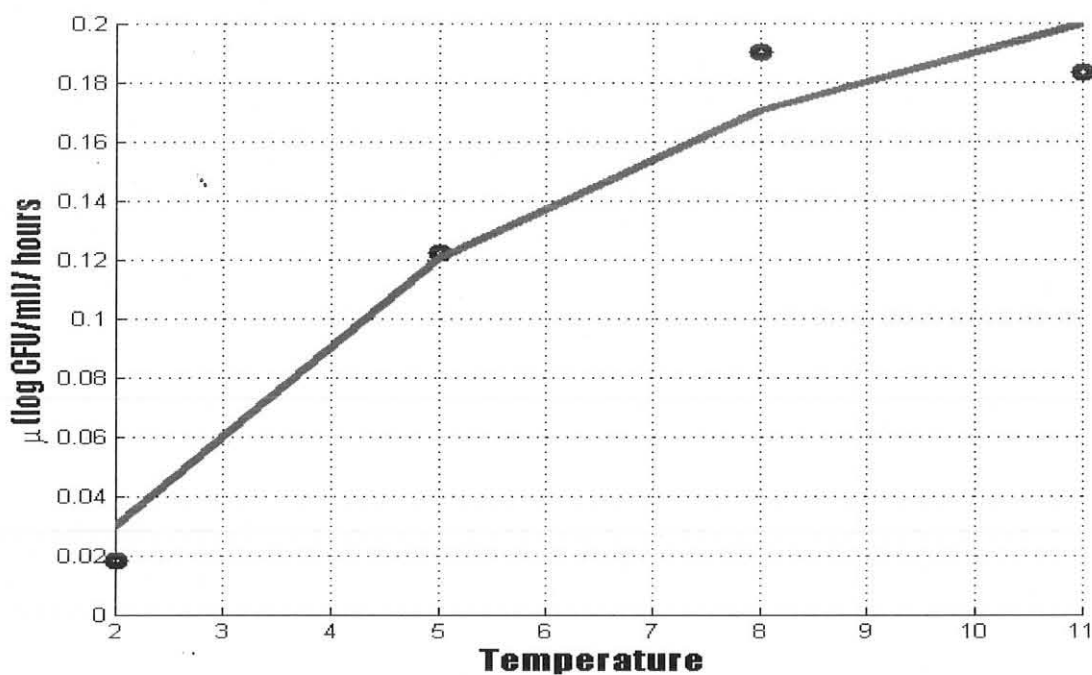


Figure 4.10 Temperature versus specific growth rate

4.4.2.2 Effect of temperature on the lag phase duration

Temperature variation influenced lag phase duration. It is apparent that as incubation temperature decreased lag time increased. The lag phase duration is affected by many variables and bacterial responses to changes in the environment are complex and difficult to characterize. The majority of mathematical models for spoilage microorganisms have been focused on the effect of the environmental factors on maximum specific growth rate without taking into account the lag phase. However, the lag-phase duration of the SSO can be a significant part of the total shelf life of foods thus ignoring lag phase may lead to underestimated shelf life predictions with significant economic losses for the food industry(Liu, Wang et al. 2012).The lag phase determined as the ratio between the amount of work that a cell has to perform in order to adapt to its new environment and the rate at which it is able to perform that work. The temperature had a significant effect on pseudomonas bacteria lag time.

Table 4.16 Specific growth rate (μ) and lag phase duration (LPD)

Temperature (°C)	$\mu = B.C/e$ (log(CFU/ml) h ⁻¹)			LPD =M-(1/B)(h)		
	Pseudomonas	Total viable count	E.coli	Pseudomonas	Total viable count	E.colia
2	0.01823	0.0097	0.0093	214.250	217.66	220.74
5	0.1225	0.0195	0.182	190.672	190.565	195.984
8	0.1902	0.1550	0.161	177.886	185.901	182.98
11	0.1833	0.179	0.169	29.584	32.989	35.963

The impact of temperature on the growth parameter of spoilage organisms are described in the above Table. *Pseudomonas* sp. grow at the highest rates with The specific growth rate (μ) increased with rising temperatures it was ranging from 0.01823 to 0.1833 (h^{-1}), LPD diminished from 214.250 to 29.584 h for 2 and 11 $^{\circ}\text{C}$ respectively. In the case of Total viable count μ values changed from 0.0097 to 0.179 h^{-1} and LPD diminished from 217.66 to 32.989 days respectively when temperature increased from 2 to 11 $^{\circ}\text{C}$. While *E. coli* bacteria grow relatively at lowest rate with μ values from 0.0093 to 0.169 h^{-1} and LPD values between 220.74 to 32.989 h at 2 and 11 $^{\circ}\text{C}$ respectively .which is different from the observation of (Coll Cárdenas, Giannuzzi et al. 2008).where they found that the specific growth rate was not significantly different at 4 and 10 $^{\circ}\text{C}$. However, the present results were similar to those obtained in a study by (Pal, Labuza et al. 2008) ,which showed that temperature is an important factor in microbial growth. If the temperature is too low the plasma membrane freeze and unable to transport nutrients form a proton gradient then growth is not possible. When the temperature increases to the organisms' growth temperature range (0 to 25 $^{\circ}\text{C}$) the intracellular enzymatic reactions can be performed the metabolic rate increases and the growth rate is accelerated (Ellouze and Augustin 2010). Therefore, as the temperature increases the specific growth rate of spoilage bacteria between 2 to 11 $^{\circ}\text{C}$ increases and the lag phase duration decreases.

4.5 Model Verification

To evaluate the performance of the model the bias and the accuracy factor calculated according to (Baranyi, Pin et al. 1999). For microbial counts of each constant temperature. Bias factors provide as a measurement index for the average variation between the predicted and observed values it indicate the systematic over or under prediction (Huss 2007). The accuracy factor is a measurement to estimate the accuracy of an established model. In this study a large accuracy factor indicates inaccurate results and a value of closer to 1 accurate.

$$B_f = 10^{(\sum(N_{predicted} - N_{actual}))/n}$$

$$A_f = (\sum|N_{predicted} - N_{actual}|)/n$$

N_{actual} is the number of microorganisms in the experiment, $N_{predicted}$ is the number of microorganisms predicted by the microbial growth model and n is the Number of experiment.

Table 4.17 presents the bias factors (B_f) and accuracy factors (A_f).

Temperature (°c)	Bias factor	Accuracy factor
2	1.0315	1.085
5	0.9410	1.661
8	0.7183	1.137
11	0.8984	1.7521

Accuracy and bias factors provided an indication of the average deviation between the model predictions and observed results and the closeness to a value of 1 was an effective and practical measure of predictive model validity.

5 Conclusions and Recommendation

5.1 Conclusions

In the present study the growth of the spoilage bacteria of beef muscle (TVC, *Pseudomonas* and *E.coli*) were examined at the storage temperature (2, 5, 8 and 11°C).

The sensory analysis was carried out parallel with microbial count and the spoilage limit determined for each storage temperature. Correlation between the growths of the spoilage bacteria and sensory quality was evaluated. However, the growth of *Pseudomonas* and sensory evaluation was significantly correlated ($p < 0.05$) and the highest growth rate is observed for *Pseudomonas* (0.01823 -0.1902). Therefore, *Pseudomonas* is considered to be the specific spoilage organisms for beef muscle and the results fitted with the primary Gompertz equation and established a model based on its growth. Thus it provides an equation to predict the shelf life of beef muscle that the initial number of *Pseudomonas* organisms (N_0) and the actual temperature are known. The developed model used to determine the effect of both initial *Pseudomonas* spp. concentration and storage temperature on shelf-life meat under aerobic storage conditions. The Arrhines secondary model also used to consider the effect of temperature and pH on the growth parameter of the spoilage bacteria and both factors are significantly correlated ($p < 0.005$) with spoilage bacterial growth, however, the correlation level for pH was found to be lower in magnitude.

5.2 Recommendations

- ✚ In the butcheries' the meat was packaged in newspaper pieces that are stored on the floor. And the butcheries are also rarely equipped with refrigeration. Most of the butcheries stored their meat in a room temperature, open-air environment. However the raw meat should be stored at lower temperature (between one and four degrees Celsius) in order to slow the growth of spoilage microorganisms
- ✚ Further research is needed in order to evaluate the effect of other factors such as meat composition and the combined effect the most important growth factors (PH ,aw and temperature)
- ✚ Further work must be carried out on the development of user-friendly software which can make the application of the model easier for end user without detailed mathematical knowledge.

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Sensory Acceptability Test

Evaluate the samples according to the numbers indicated and give value from 1-5 for each attribute based on the key given. If you have any question please ask the server.

Keys

5 = Like extremely

4 =Like moderately

3= neither like nor dislike

2 =Dislike moderately

1 =Dislike extremely

Attributes	501	602	703	804
Color				
Aroma				
Texture				
Over all acceptability				

Preference ranking test

In front of you are seven coded samples. Taste each sample

Please indicate you are preference by placing

501----- 602----- 703----- 804-----

1st choice beside the sample that you prefer most

2nd choice beside the sample that you prefer second

3rd choice beside the sample that you prefer third

4th choice beside the sample that you least prefer

General Comments: -----
