PREPARATION OF A SANDWICH

WHICH HAS A SHELF LIFE OF TWO WEEKS

PREPARATION OF A SANDWICH WHICH HAS A SHELF LIFE OF TWO WEEKS

By

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DECLARATION

The work described in this thesis was carried out by me at the Keels Food Products Ltd and Faculty of Applied Sciences under the supervision of Mrs K M Somawathie and Ms Swarnamala Perera A report on this has not been submitted to any other university for another degree

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DEDICATED TO

MY LOVING PARENTS

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ABSTRACT

The objective of the research is to prepare a nutritionally rich sandwich, which has a shelf life of two weeks

The sandwich consists of two bread slices with a butter slice and a specially prepared meat slice in between them Two meat loaves (one is of low moisture content than the other) were prepared to take meat slices Two kinds of sandwich samples were prepared with these two kinds of meat loaves and packaging with N_2 gas flush was done Few samples of prepared sandwiches were kept at ambient temperature and visually inspected and others were stored in a chill room Prepared samples were microbiologically tested after four seven and eleven days respectively

A fungal growth could be visually inspected in both samples kept at ambient temperature after four days and increased with the time Butter started to melt after one day For the samples stored in the chill room these changes could not be inspected

Up to four days both samples made with high and low moisture content meat were microbiologically acceptable When it comes to the 7^{th} day only the sample which has the meat slice of low moisture content was microbiologically acceptable

When it comes to the 11^{th} day, both kinds of samples were microbiologically unacceptable

Since by the 7^{th} day the sandwich with the meat slice of low moisture content was microbiologically acceptable that meat loaf was selected for the preparation of another batch

So the sandwich which is prepared from bread butter and meat is nutritionally rich and has a shelf life of eleven days

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INTRODUCTION

Today the society is more complex and complicated. All in the society are busy. In most families both parents do jobs to double their income level. Hence they have limited time to spend at home. They do not have enough time to prepare their meals. So they seek for something convenient. i.e. easy and speedy ways of preparing their meals. Thus consumers of today want convenience in preparation and serving of food. . The market changes in order to suit the consumers' preferences. Recent marketing trends also show increased sales of convenient foods. The large market penetration of microwave oven, which is a quick way of food preparation, is one such change.

But today most of the people are having meals, which are nutritionally disastrous. When both convenience and nutritional value are considered, sandwiches can be taken as one of the best solutions. Sandwiches are an excellent source of nutrition because they may contain variety of fillings and represent good value for money.

Sandwiches are not cooked at the point of eating of course, and for this reason they are a 'high-risk' food and must be stored, displayed and sold in clean, safe surroundings. 'High –risk' foods are those that are more likely to contain higher levels of bacteria and are not necessarily cooked before being eaten.

Many sandwiches are made for packed lunches at school or work. In areas of large towns or cities of developed countries where there are a huge number of offices, shops or factories, and there are often several sandwich shops and many are thriving businesses.

The popularity of sandwiches within the take-away food sector has increased and sandwiches have become an important part of production for today's Sri Lankan baker

Preparation of a sandwich as a solution for above requirements was done in this research

Preparation of a ready-to-eat sandwich with a considerable shelf life increases the interest of producers while a nutritious sandwich increases the interest of consumers The present study was carried out at the Keels Food Products Ltd to fulfil this need Objectives of the study were to

1. Prepare a sandwich, which has a shelf life of two weeks
2. Make the sandwich nutritionally rich

CHAPTER 2

2.0 LITERATURE SERVEY

2.1 Sandwich; a ready-to –eat food

The sandwich has been produced by the fourth Earl of sandwich in the eighteenth century. The sandwich has become increasingly popular since that time, and has been developed to suit changing life styles and eating habits. Many sandwiches are quite substantial, and are sufficiently nutritious to provide a small, transportable meal. We can now buy sandwiches ready-made in plastic packages, many with exotic fillings on whole meal and specialty breads.

Sandwiches are not cooked at the point of eating. For this reason they are 'High Risk' foods and must be stored, displayed and sold in clean, safe surroundings. High-risk foods are those that are more likely to contain higher levels of bacteria and are not necessarily cooked before being eaten. Langers (refrigerated display cabinets) are used to store high-risk products at a temperature of below 5^0c (Fuller, 1994).

2.2 Sandwich bread

A standard loaf of sandwich bread may contain, in addition to the four essential ingredients some fat, an emulsifier at about 1% and a proprietary improver. There are wide variations of this bread for sandwiches. The flour can be white or whole wheat. It can have added soft wheat grains, malted wheat; Kibbled, rolled part-cooked or milled rye; oats, barley, maize or other cereal or any combination of these. And it can have nuts and seeds or a whole range of other flavour and texture ingredients that are suspended by the gluten in the wheat flour in order to keep the bread light, soft and palatable (Anonymous, 1999 Sep)

2.2 .1 Extension of shelf life

Mor-Life ™ functions as a dough conditioner. It is an enzyme-based product that gives 7 to 10 day extension of shelf life with freshness after baking. It improves the volume and appearance of the bread too. By using this dough conditioner we can save on dough costs by reducing the amounts of emulsifiers and dough conditioners used (Anonymous, 1999 Aug).

The freshness and quality of meat foods combined with the use of natural preservation methods and minimal processing are highly valued and demanded by the meat consumers around the globe. To meet these demands, new packaging techniques such as modified atmosphere packaging have been developed.

Freezing is the only known way of keeping most kinds of sandwiches fresh. One of the chief reasons for the remarkable retention of freshness is the fact that freezing retards staling of bread better than any other method of preservation. On the other hand, when exposed to a dry atmosphere, slices of many kinds of bread become dry and unpalatable in a very short time. This indicates the great importance of rapid preparation and the sealing of the sandwiches in moisture-vapor-proof sheeting or packages. From the above it might be assumed that no problems are encountered in freezing sandwiches. However the fillings of a number of kinds of sandwiches are undesirably changed by freezing and subsequent storage.

Sandwiches made from day-old bread had greater acceptability and were more uniformly moist and fresh appearing than those made from fresh bread.

2.3 Modified Atmospheric Packaging

Modified-Atmosphere Packaging (MAP) is the enclosure of food in a package, inside which the atmosphere is modified with respect to CO_2, O_2, N_2, water vapour and trace gases. This modification is generally achieved using one of two processes i.e. gas flush packaging or vacuum packaging.

MAP can significantly increase the shelf life without losing the fresh quality of muscle foods. MAP is a process by which the shelf life of a fresh product is increased significantly by enclosing it in an atmosphere which slows down the degradative processes particularly growth of microorganisms.

Vacuum packaging is a form of MAP but disadvantageous because of deformation of cuts by film tightening. However use of appropriate gas mixtures for MAP offers an alternative to vacuum packaging.

Packaging material plays a crucial role for the success of MAP technology. The correct atmosphere induced initially cannot maintain long, if the gases pass through the barrier material. The requirements of machine for packaging are governed by packaging methods. Thermoformed rigid or semi rigid base material is required for thermoformed packaging. The basic material is thermoformed into a tray after loading of the product, the tray passes into a chamber in which the air is evacuated and the desired gas(es) introduced into it. Similarly a top lidding material is drawn over the tray and sealed to lip edge.

This system has the advantage of being able to evacuate air from the package before flushing with gas and a low residual oxygen level in the package can be achieved.

The pillow wrap or horizontal form-fill-seal machine employs a single reel of flexible packaging material which is formed into a tube and the two edges are heat sealed. The product is passed into this tube which is vented of air with a flushing gas prior to sealing (Bharti, Sahoo, 1999).

2.4 N_2 in the gas packaging

As an inert gas, N_2 is used for gas packaging. Nitrogen gas is generally considered as a neutral filler gas as it influences neither the colour of the meat nor its microbiological quality.

To guard against pack collapse, on initial or progressing dissolution of CO_2, it is common practice to include some N_2, which is physiologically inert, as ballast in the pack atmosphere. Even when the pack is properly constructed and filled to an appropriate gas volume to meat weight ratio, changes in the atmosphere composition are inevitable during storage.

The effect of temperature on off odour production was significant in all atmospheres. The spoilage pattern of pathogenic bacteria at 5^0c was attributed to high growth of *Bacillus thermosphacta*, *Pseudomonas*, *Enterobecteriaceae*, although it has been shown that members of lactic acid bacteria (LAB) can also produce off odours.

The effect of N_2 packaging on cooked, cured meat loaves and found remarkably improved appearance by retarding the greenish discoloration.

N_2 has two roles in gas packaging: first, it displaces O_2. With regard to moist foods, displacement of O_2 by N_2 retards the growth of aerobic bacteria as Pseudomonas and Yeasts and moulds, the oxidation of fats and colors and the development of off-flavours. Nitrogen it self is and does not interact directly with microorganisms.

Nitrogen's second role in gas packaging is to act as an inert filler, which keeps flexible packages from developing a vacuum This is a problem particularly associate with moist food products packed in CO_2 (Sahoo and Anjaneyulu, 1995)

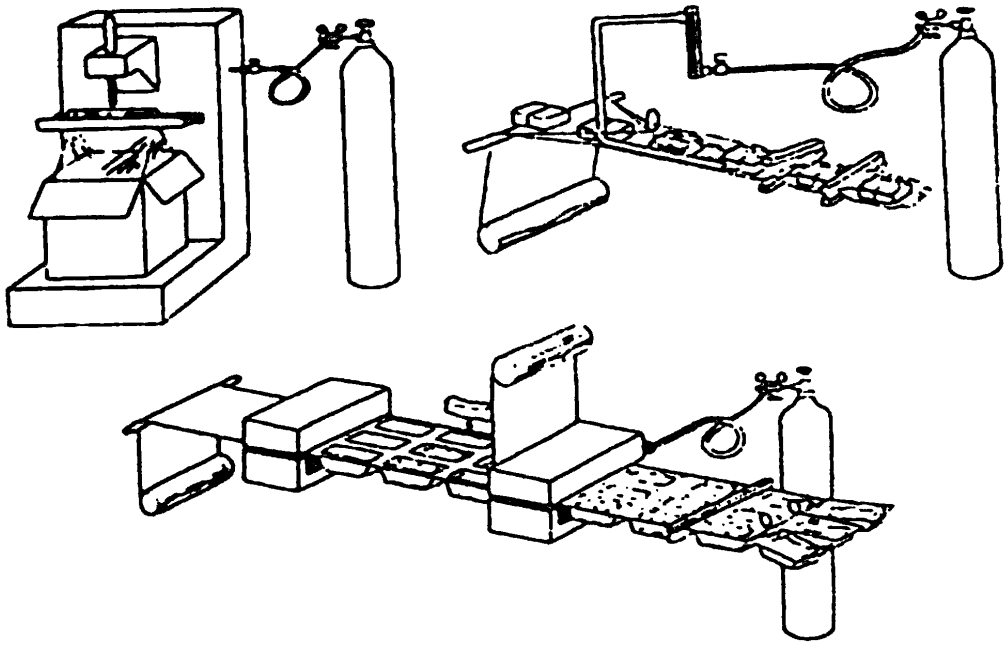


Figure 1 Systems for Modified Atmospheric Gas Packaging

2.5 Packaging Materials

The choice of packaging material is an important factor in any MAP operation. A low water vapour transmission rate, together with a high gas barrier must generally be achieved. Generally, all MAP packages are based on thermoplastic polymers. All packages made from such plastic materials allow some gas transmission, even at chill temperature The packaging material need to have mechanical strength to withstand machine handling and subsequent storage, distribution and retailing. Materials in use are laminations or co extrusions of polyethylene with polyester or nylon with or without the addition of a high barrier layer of vinylidene chloride-vinylchloride co-polymer or ethylene-vinyl alcohol co-polymer, depending on the barrier required

With regard to the microbiological and sensory shelf life and quality of gas-packed foodstuffs, appropriate packaging materials are more important than the type of the packaging machine, although the packaging technique also affects the appearance of a product e.g. a vacuum before gas flushing cannot be used for some products due to crushing and compression which has already been mentioned Several factors must be taken into account in determining the combination of packaging material properties required

for each specific product and market. Naturally the basic requirements are the same as generally set for food packaging materials, e.g. the material must be suitable for use in contact with foodstuffs from the health point of view. Particularly with regard to gas packaging, the important factors are the following:

The gas barrier properties; in most gas packaging applications, excluding vegetables and fruits, it is desirable to maintain the atmosphere initially injected into the package for as long a period as possible. The correct atmosphere at the start will not serve for long if the packaging material allows it to change too rapidly. Some of the polymers currently used include PE (polyethylene), PETP, metalized PETP, PP (poly propylene), PS, PVC (poly vinil chloride), PVdC (poly vinilidene chloride), PA, EVA and EVOH (ethylene vinil alkohol). Their oxygen and water vapour permeability are given below; (Ooraikul, Stiles, 1991).

Table 2.1. Barrier properties of some selected polymers. The film thickness is 25 micro meters.

POLYMER	OXYGEN PERMIABILITY (cm^3/m^2 24 h 101.3 kPa) 23°c, 75% RH	WATER VAPOR PERMIABILITY (g/m^2 24 h) 38°c, 90% RH
EVAL	0.16-1.4 (0% RH) 10.1-17.8 (100% RH)	21.7-58.9 (40°C)
PA 66	31	93-155 (40°C)
OPA 6	18 6	155
PA 6	40.3	341
PP	2325	3.9-10.9
PET	74.4-139.5	27.9-46.5
PVC (ngid)	77.5-310	14 0-79 1
HDPE	2325	4 7-6 2
LDPE	6510	15 5-23 3
PS	5425	108 5-170 5
PVDC	0 31	0 31

Source: Modified Atmospheric Packaging, 1991

These polymers are normally used as laminated or co-extruded multiplayer materials in order to have the barrier properties required. The inner layer is usually polyethylene or its co-polymer, which forms the food contact and heat seal medium. Polyethylene or ethylene vinyl acetate alone is not suitable for gas packaging because of their high gas permeability.

As a rule packaging materials with oxygen permeability lower than $100cm^3/m^2$ 24h 101.3 kPa are suitable for the quality of gas packed products.

In choosing packaging materials for gas packaging one has also to pay attention to how resistant to mechanical stresses (e.g. puncture), humidity and temperature (frozen or chilled) the material needs to be. If a material is of poor mechanical strength, the mechanical stresses, humidity and low temperature during storage, transport and handling can damage the package and cause leakage.

The adequate integrity of the seal is important in order to maintain the correct atmosphere in the package. The seal however must not be to tight; the right balance between tightness and security of the closure and ability to peel back a lidding material must be determined.

The type of package to be used; rigid or semi rigid, lidding tray or flexible film pouch, has to be taken into consideration when choosing packaging materials.

In order to improve the appearance of the packages in retail outlets the polyethylene in the packaging laminates can be specially treated to prevent condensation of water which fogs the package and prevents the consumer examining the product.

The microwave ability of packaging materials will be emphasized more and more in the future, and this is a factor that should also be considered in gas packaging, particularly in the case of the ready-to-eat food products. For instance, the low melting point of PVC makes the PVC-LDPE-lamination or co extrusion film much used as a base web material in deep-draw machines unsuitable for microwave oven heating. This means that base material in deep drawing should be changed to another material if deep-draw packaging is to be used for gas-packed food products intended to be heated in a microwave oven in their package

The only additional expenses are the gas mixture itself and its related equipments. The packaging speed may also slow down to some extent.

In most MAP applications, it is desirable to maintain the atmosphere initially introduced into the package for as a long period as possible and to keep the gas ratio unchanged. The better the seal, the more difficulty there will be in opening the pack. The right balance between tightness and security of the closure and the ability to peel back a lidding material must be determined. The heat sealing is considered best for MA packs.

Packages can be designed to reduce the total exposed area by adding labels, printing on the film, and increasing film thickness. However all these techniques decrease the ability of customers to view the product inside the package (Ooraikul and Stiles, 1991).

If bread is packed in polythene without modifying the atmosphere, the bread is spoilt by microbial activity after 3 days.

For MAP, a laminated packaging material with high gas barrier polymer such as ethylene vinyl alcohol or polyvinilydene chloride should be used and when the bread is packed with the inert gas N_2, the shelf life can be extended to 5 days.

Both 99.5% CO_2 and 73% CO_2/ 27% N_2 combination extend shelf life to 14 days.

MAP offers increased shelf life due to significant reductions of spoilage organisms, the cost of this type of packaging makes it prohibitive in most commercial circumstances. The cost of the high barrier laminates makes them more suited to higher value products.

2.6 Na/K lactates

Sodium and potassium lactates are preservatives, which can be used in meat, poultry and seafood products for extending shelf life, enhancing flavors and controlling pathogens.

Na/K lactate is the neutral Na/K salt of natural L (+) lactic acid, which is a natural component of muscle tissues. It has a mild saline taste. Due to the mild saline taste, usage of 2% level (in the final product) hardly affects the saltiness of meat products. It does not affect the color of the meat. It is pH neutral (6.5-8.5).

Shelf life of meat products is improved not only from anti microbial activity by lowering water activity, but also by the specific bactericidal effect of the lactate ion. Na-lactate is widely applied in processed meat products to extend the shelf life. The extent of shelf life extension has been as much as 2-4 days in ground poultry products. In cooked comminuted products shelf life can be extended with 30-100% depending on product formulation and usage level.

It improves the yield by improving water-binding capacity and due to that the product becomes firm in texture.

Recently it was discovered that this ingredient inhibits pathogenic microorganisms like *Staphilococcus aureus*, *Salmonella*, *Listeria* and particularly Clostredium botulinum. Studies have shown that Na-lactate possesses strong anti botulinal functionality thereby reducing the risk of growth of Cl.botulinal spores in uncured vacuum-packed poultry products.

For comminuted products, during mixing/chopping, Na-lactate (PURASAL^{R}) can be added Generally 1.5-3.5% (1-2% dry solids) of Na-lactate is recommended for addition to meat and poultry products based on final weight of the product Processors may consider the reduction of the normal NaCl content by 0.1-02% when using the Na salt of lactic acid. There is a possibility of lowering the NaCl content, which allows a better taste without the risk of a decreased shelf life It does not contribute to any significant effect on the pH also (Manual of Purasal ^{TM}).

2.7 Microbiological Status

The number, type and growth of microorganisms are dependent upon presence or absence of oxygen Restricting oxygen will generally retard the growth of *Pseudomonas* while enhancing the growth of *Lactobacilli* or *Brochothrix*. Lowering oxygen level in MA environment helps in extending the shelf life but the outgrowth of anaerobic pathogens may be stimulated. With a high partial pressure, O_2 free CO_2 extends the lag phase, the generation time and slow the growth rate of all spoilage organisms.

Many theories regarding the mechanism by which CO_2 acts on bacterial cells, include alteration of intracellular pH, disruption of the internal enzymatic equilibria, entry of mass action equilibria for enzymatic decarboxylation, changes in cell membrane functions and the physico-chemical properties of protein and toxicity of carbonic acid in its un dissociated form. CO_2 is also inhibitory to various bacterial enzymatic and biochemical pathways.

The temperature not only determines whether microbial numbers increase or decrease but also influence the nature of the flora which becomes dominant. In spite of all good packaging conditions, the long life of meat cannot be achieved unless special emphasis is provided to temperature during storage, distribution and retailing. At chill temperatures, the storage life is determined mainly by concentration of oxygen.

The increased solubility of CO_2 at lower temperature is undoubtedly a major factor. However, if bacteria have commenced their growth phase, CO_2 has almost no inhibitory effect at all (Man, 1994)

2.8 Shelf life

The shelf life is the time it takes for the product to become unacceptable to the consumer. Analysis shows how the sensory qualities change with time and how long it takes for these changes to make the product unacceptable The results determine shelf life Food

must remain safe to eat and be in the best possible condition, up to and a little beyond the 'Best Before' date.

Quality of food can be evaluated both with human senses and with instruments. Human senses are used to perceive the sensory properties of foods and instruments are used to quantify physical properties contributing to the sensory and non-sensory characteristics of food quality (Larmend, 1997)

2.8.1 Investigational testing; Scope of the microbiological tests

To determine if a microbiological hazard is present or indicated.
To establish microbiological trends.
To conduct challenge testing to predict safety in use or predicted abuse situations.
To conduct challenge testing to predict shelf life.

2.8.2 Sampling for microbiological tests

To be meaningful, the information given by microbiological examination must be fair and accurate assessment of the microbiological status of the material being examined. There are two main aspects to achieving this aim:

1. The sample must be as representative as possible of the environment or of the product
2. The sample must be taken aseptically, that is without any contamination occurring during sampling, sample handling between the time that it is taken and the time that it is examined should prevent significant microbiological multiplication or death, as any change of numbers will give an incorrect result

The changes of microbiological populations in environments or products are complex. Growth and death can occur rapidly and at different rates with different kinds of organisms leading to major changes in the make-up of population. This means there is no opportunity to resample at leisure; so it is important to 'get it right first time'

As well as taking representative samples of product it is also prudent to sample ingredients according to a plan which will enable the probability of accepting or rejecting an

unsatisfactory lot to be quantified. When examine a food for microorganisms, homogenous distribution of microbes is seldom found, even in liquids.

As contamination may introduce serious errors, which could lead to the wrong action being taken, aseptic handling must be applied from the time that the sample is taken until the microbiological examination is complete. All the apparatus used for sampling and laboratory examination must be sterile.

Once the samples are taken they must be sent to the laboratory immediately, or stored away from the production area. To minimize microbial population changes, dry samples may be held at ambient temperature provided this is less than 20^0C.

All samples should be examined as soon as possible after receipt. Examination of samples usually means preparing serial dilutions, so that accurate counts of microorganisms may be made. For each dilution used in making the dilution series a separate sterile pipette is used.

2.8.3 Dilution of samples

In the preparation of microbial examination all solid and most liquid food or ingredient samples will be diluted in a liquid. The choice of diluent is important and depends on the particular type of examination and on the composition of the food. The method of mixing sample and diluent is also important. Mixing must be efficient, but it may also cause clumps of bacteria to come apart and mold hyphae to break. The count will therefore be affected. A standard mixing procedure must be adopted so that result may be comparable both between different workers and between different batches of the same product.

Enrichment is a technique, which is used when low numbers of a specific group of microorganisms are being looked for, and generally these microorganisms are pathogens. A known quantity of food or liquid is added to a given volume of broth (usually the ratio is 1:10 sample to broth), the inoculated broth is incubated, and then examined for the pathogen under test. This makes the added food material a significant part of the culture medium, and may explain why some protocols or formulations are more successful than others for a particular application.

The colonial appearance is important for identification of a type or kind of microorganism. The final growth and the appearance of the organism are the interaction of culture media and environmental factors.

This method is used when it is anticipated that the food or liquid contains microorganisms at levels in excess of 10 per g or ml. The food or liquid is diluted, a 1ml

sample of each dilution pipetted on to a plate, an agar growth medium added at 42°C to 45°C and the colonies (which develop after incubation from each microorganism or clump of microorganisms) are counted. The count of microbes in the food or liquid can be calculated as follows:

Colony count per plate * the dilution factor plated = the count per g or ml

1. It is easy to make a mistake in the dilution factor especially when an 'unusual' or 'unfamiliar' one is used.
2. In counting where the food itself, e.g. some spices is inhibitory, the organisms may only be able to grow at high dilutions, e.g. 10^{-3} or 10^{-4} Where an unfamiliar or inhibitory material is under examination it is therefore prudent to take the dilution series further than would be necessary for routine counting.

2.8.3.1 Choice of diluent

Dilutions of the samples are made through a series of tubes or containers. Usually the dilutions are decimal dilutions, and may be made as 1 in 10 dilutions or as 1 in 100 dilutions at each stage Whatever the volume used for each dilution step, the choice of diluent is important and so is the total time taken to complete the procedure. During dilution there should be no death of microorganisms, nor should multiplication occur. This means that the diluent should contain salt, and possibly peptone, at a level, which prevents any osmotic shock damaging the microbes and possibly killing them It also means that the time between making the original dilution of the sample, and adding the growth medium to the plate should be not more than 30 minutes

If diluted samples are held longer than this, then multiplication could occur in the dilution tubes in which there is more food present (1 in 10 or 1 in 100 dilution tubes), whereas in the dilution tubes of 1 1000 or greater, even though the diluent is designed to prevent osmotic shock, some microorganisms may die Others such as *Pseudomonas* species may in fact be able to multiply even in diluent To overcome these problems therefore the dilution series procedure should be completed as soon as possible

However for most applications a simple peptone/saline diluent is used for a wide variety of foods and liquids, which may not be the best possible diluent for ever food stuffs, but which when used correctly, gives a satisfactory rate of microbes from food samples Always check to see that peptone diluent is non-turbid before use Turbidity indicates either

gross multiplication of microorganisms or poor preparation of diluent. Sterility of diluent batch should always be checked.

2.8.4 Colony count

The most usual method used to estimate the count of microorganisms in foods or liquids is the colony count, which is both simple and versatile. This has the advantage that by using a series of dilutions it is possible by a single examination to obtain a result even though the anticipated microbiological status of the sample is not known. It is also possible to obtain counts for specific microorganisms. A single dilution series may be plated on to several selective agars, for example, Violet Red Bile Agar (VRBA) for coliforms; Baird-parker Agar for *Staph. aureus*; and Acidified Malt Extract Agar or Chloramphenicol Rose Bengal Agar for yeasts and/or molds. By carrying out this procedure, often accompanied by plating on a non-selective agar and incubating aerobically and/or anaerobically at different temperatures to obtain the 'total' bacterial count, much information is available. This enables a judgment to be made of the microbiological status of the food indicating both the efficiency of processing and the hygiene of food handling process.

The colonies, which arise from microbial growth, must be carefully counted and the correct dilution factor applied so that no wrong conclusions are made regarding the microbial status of the foodstuff under test. Also the media and apparatus used during preparation of the sample must be sterile and the technique used must be completely aseptic, otherwise colonies will develop from contaminating organisms, not just from those present in the foodstuff. To confirm sterility and asepsis, control plates should be incubated, that is plates containing the agar only, or agar with diluent.

It is difficult to count a plate when there are more than 250-300 bacterial colonies present, or where there are more than 150-200 yeast colonies. Although it is possible to count colonies when there are more present, the colony size is smaller because of the overcrowding, and colonies over-lie on another so that undercounting can result in as much as tenfold reduction of the correct count.

Bacterial spoilage of different products is largely influenced by the nature of meat together with environmental factors and the degree and composition of initial micro flora

Some of the microorganisms may be present in low numbers and in several cases the numbers sought are below the sensitivity of the direct plate method. For example, *Salmonella*-absent in 25g samples *E.coli* absent in 10g samples (Man, 1994).

Table 2.2. Microbiological standards for unprocessed/semi processed foods

TEST	MAXIMUM COUNT (cfu/g)
Staphilococcus aureus count	$< 10^4$
Total Coliform Count	$< 10^4$
Yeast and Mould count	$< 10^4$
Total Plate Count	$< 10^7$
E.coli	Absent in 10g of the sample
Salmonella	Absent in 25g of the sample

Source: Working Instructions, Keels Food Products Ltd.

Sensory qualities also deteriorate with the time. After determining the acceptable time period for the sandwich by doing microbiological tests, the acceptability of sensory qualities should also be checked by performing a sensory analysis. For that a simple paired comparison test should be done

A pair of coded samples is presented for comparison on the basis of some specified characteristic such as sweetness. Fewer samples are required and there is less tasting, but the statistical efficiency is not as great. The probability of a panelist selecting a sample by chance is 50%. Roessler *et al* published tables for the rapid analysis of the results of paired comparison tests. Two tables for paired comparisons data are presented. One table is used for directional difference tests or one-tailed test, when only one answer is correct. The other table is used in preference tests or two-tailed tests, when either response can be correct.

Paired comparison tests give no indication of the size of the difference between the two samples but determine whether there is a detectable difference or not (Larmond, 1977).

2.9 Staphylococcus aureus

Many foods provide a good medium for the growth of Staphylococci. Examples include milk and cream, custard and cream-filled baked goods, meat and potato salads and some cooked meats such as baked ham.

Staphylococci are also often present on raw poultry including chicken. Not all of the Staphylococci isolated from raw meat are S. aureus but many are and a number of them are enterotoxigenic.

Foods such as meat or milk that are naturally contaminated are seldom involved in food poisoning, because these foods are contaminated with other competitive microorganisms

Butter is an unlikely food to be involved in food poisoning, because of the small amount eaten at one time.

Staphylococci are not good competitors unless they out number the other organisms present. Usually meats are cooked or baked at temperatures that kill any Staphylococci associate with them (Michael, 1989).

2.9.1 Source of contamination

Contamination may be occurred from a cough or sneeze by a food handler who happens to carry an enterotoxigenic Staphylococci in the throat or nose, contamination by a food handler with some type of Staphylococci infection, such as an infected cut on the hands

Staphylococci are ubiquitous, although their major reservoirs are humans and animals. Food poisoning outbreaks do occur from foods that have been contaminated by healthy Staphylococcal carriers. Most food poisoning outbreaks result from contamination by food handlers. This is because most outbreaks result from Staphylococci growing in foods after the food has been heated. Heat treatment generally will destroy any Staphylococci present before there is adequate growth for enterotoxin production. Enterotoxin is produced in the food by Staphylococci introduced on to the food after heating (Michael, 1989)

2.9.2 Prevention and control

Staphylococci are ubiquitous organisms that are impossible to eliminate from the environment. At least 50% of individuals carry these organisms from time to time in their nasal passages, throat or on their hands. Any time a food is exposed to human handling, there is a good possibility to contaminate with Staphylococci. Not all strains are enterotoxigenic but a large percentage may be. Heating food after handling normally would ensure against food poisoning unless the food is held unrefrigerated for several hours before heating. Heating meat until the internal temperature reaches 73.9-76.7 ^{0}C is sufficient to inactivate any Staphylococci present. Heating the meat at 51.1 ^{0}C for 12 h completely inactivate any Staphylococci present. Heating is the most effective method for inactivating Staphylococci in food. Unfortunately enterotoxin is not inactivated by pasteurization. No *S. aureus* survive at microwave heating. Heating the foods to an internal temperature of 74-77 ^{0}C does not consistently eliminate viable *S. aureus*. A mean internal temperature of nearly 100 ^{0}C is needed to ensure that all portions will reach a minimum of 74-77 ^{0}C. If food poisoning does occur, it is usually due to contamination occurring after cooking (Michaell, 1989).

If enterotoxin is produced in food, heating might not be sufficient to destroy the toxin. In many instances, foods are not processed further after handling, and unless proper care is taken, the organisms may grow and produce enterotoxin. To prevent Staphylococcal food poisoning, it is important to keep susceptible foods refrigerated at all times except when being prepared and while being served. Foods should be refrigerated in a manner to facilitate quick cooling of the entire food mass. Most food poisoning out breaks could be prevented if this simple precaution were taken (Michael, 1989)

2.10 Escherichia coli

It is presumed that humans are the major reservoir of *E coli*. *E coli* is often present in feces of asymptomatic human carriers

E coli infection is principally acquired by ingestion of contaminated food or water. *E coli* is not readily transmitted to healthy individuals by direct person to person contact. Chickens may also be a reservoir of the organism (Michael, 1989)

2.10.1 Control and prevention

Symptomatic and asymptomatic human carriers are believed to be a principle reservoir and source of *E.coli* strains involved in human illness. These organisms are present in the intestinal tract of carriers and hence are excreted in the feces. Food may be contaminated by infected food handlers who practice poor personal hygiene or by contact with water contaminated by human sewage. Important measures to prevent food poisoning include educating food workers in safe food handling techniques and proper personal hygiene (such as washing hands after defecation), properly heating foods to kill pathogens and holding foods under appropriate conditions to avoid bacterial multiplication (e.g. chill leftover foods rapidly). Additionally unchlorinated water should be used for cleaning food processing equipment and food contact surfaces (Michael, 1989).

In contrast, the intestinal tract of cattle and likely other animals (such as chickens) used in the production of food, is an important reservoir of *E.coli*. Hence raw foods of animals origin may be contaminated by the organism through fecal contact during slaughter or milking procedures Prevention of fecal contamination during recovery and processing of foods derived from animals is paramount to control food born infections of *E.coli*. Additionally, foods of animal origin should be heated sufficiently to kill the organism before consumption Although the infectious dose of *E.coli* is not known, measures should be taken to control its growth on meats by rapid cooling to less than 7^{0}C after slaughtering and processing (Michael ,1989).

2.11 Yeasts and moulds

The medium recommended for the detection and enumeration of yeasts and moulds is Potato Dextrose Agar Minerals present in agar could influence the pigment formation of certain fungi where pigment production is a critical part of the identification of the fungus It is clearly important to stabilize this characteristic The agar used in Potato Dextrose Agar is carefully screened to ensure correct pigment production by fungus (Alphaprint, Alton, Hants,1990)

2.12 Coliforms

With *Coliforms* bile salts are commonly incorporated in the culture media as the selective agent. Examples of selective media used for this purpose are MacConkey agar and Violet Red Bile Agar. The former additionally contains a nutrient source (peptone), lactose, which is dissimilated by *Coliforms* with the production of acid (and gas) and a pH indicator (neutral red); the latter medium contains crystal violet as an additional selective agent but is otherwise based on similar principles (Michael,1989).

Like most nonpathogenic gram negative bacteria, the *Coliforms* grow well on a large number of media and in many foods. These organisms have been reported to grow as low as $-2\ ^{0}C$ and as high as about $50\ ^{0}C$ (Michael ,1989).

They are capable of growing in the presence of bile salts, which inhibit the growth of gram positive bacteria. Advantage is taken of the latter fact in their isolation from various sources Unlike most bacteria they have the capacity to ferment lactose with the production of gas and this characteristic alone is sufficient to make presumptive determinations of *Coliforms*. By incorporating lactose and bile salts into culture media (such as MacConkey agar), it becomes possible to differentiate between these organisms and most others that may be present in foods as well as water (Michael ,1989).

It is not difficult, however to some times demonstrate the presence of these organisms in air, dust, on the hands and in and on many foods The problem then is not simply the presence of *Coliforms* but their relative numbers. While the presence of large numbers of *Coliforms* in foods is highly undesirable, it would be virtually impractical to eliminate all of these organisms from fresh and frozen foods (James,1978)

2.13 Total Viable Count

One of the most common microbiological tests carried out on foods is the total viable count, which is also known as the standard plate count or aerobic plate count In this, suitable dilutions of the food sample are plated on or in a agar based media containing complex nutrients which support the growth of as wide a range of microorganisms as possible Nutrients included in, for example, nutrient agar (Oxoid) are beef extract, yeast extract and peptone (a proteolytic enzyme digest of fresh meat containing a variety of

inorganic salts, growth factors and peptides) The pH of the medium is usually adjusted to 7-7.4 so that bacteria rather than yeasts or moulds are recovered (James,1978).

The individual bacterial cells transferred to the plate in the diluent divide in the normal way during incubation Thus an estimate of the total number of viable cells (i e cells capable of growth in the recovery medium) in the dilution plated out, can be calculated by counting the total number of bacterial colonies which develop following incubation Under normal aerobic conditions obligate aerobes and facultative anaerobes will grow (James,1978).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Materials for the preparation of meat loaf

3.1.1.1 Equipments

Bowl chopper
Mincer
Balances, which are capable of weighing ingredients in kilos and grams
Casings (brown colored; 90mm in diameter/50 cm in length)
Filler
Stands to hang loaves
Cooking chamber
Chill room

3.1.1.2 Ingredients

Chicken meat
Cereal binders
Soya protein isolates
Sodium lactate
Ice/water
Nitrite salt
Phosphates
Ascorbic acid
Sodium Ascobate
Mono Sodium Glutamate

Mace
Coarse pepper
Chili powder
Nutmeg
Mustard

3.1.2 preparation of sandwich

3.1.2.1 Equipments

Slicer

Sharp knife, Plastic chopping board

Packaging materials

N_2 gas cylinder with a connected tube

Sealer

3.1.2.2 Ingredients

Bread, Butter, Cooked meat

3 1 3 Microbiological tests

3.1.3.1 Equipments and glassware

Dry sterilization	hot air oven
Wet sterilization	automatic and portable autoclaves
Electronic balance	0 01g sensitivity
Water bath	44 +/- 0 5 °c
Distilled water apparatus	
Incubator	35 +/- 0.5°c
Laboratory consumables (knife/pair of scissors, forks etc)	
Pipettes	(1ml/10ml)
Medical flat bottles	(300ml)
Test tubes	(16mm diameter)
Petri dishes	
Stomacher bags	(sterilized)
Stain less steel racks	(to hold stomacher bags at sample introduction)
Cotton wool	
Sterilization box/Al foil paper	
Beaker	(1L)
Mc Cartney bottles	(28 ml)
Durham tubes	(4mm diameter)
Glass spreaders	(sterilized)

3.1.3.2. Diluent, Reagent, Media

Media	Violet Red Bile Agar, Potato Dextrose Agar, Mac conkey Broth Purple, Tryptone water, Standard Plate Count Agar (APHA), Baird Parker
Diluent	Buffered peptone water
Reagent	Indole

3.2 Methods

3.2.1 Preparation of the meat loaf

Using the Mincer, chicken meat was minced and using the bowl chopper the meat mixture was prepared.

chicken meat A — 5 rounds — Fast chop, Fast bowl

↓

addition of salt, spices, Na-lactate — 15 rounds — Fast chop, Fast bowl

↓

addition of soya protein isolates, 1/3 ice/water — 20 rounds — Fast chop, Fast bowl

↓

addition of chicken meat B fat emulsion, balanced ice/water — 30 rounds — Fast chop, Fast bowl

↓

addition of cereal binders — 10 rounds — Fast chop, Fast bowl

↓

addition of chicken meat C — 15 rounds — back chop

↓ — 2-3 rounds — slow chop, slow bowl

meat mixture

Figure 2 Flow chart of preparing meat mixture

Prepared meat mixture was filled into casings to prepare meat loaves. They were cooked in the cooking chamber by using steam for 2 ½ hours until core temperature comes up to 74^{0}C) After that the cooked loaves were cooled by showering treated water for nearly 15 minutes and were stored in a chill room

Following above procedure, another meat loaf, which is 2% low in moisture content, was prepared.

For the preparation of sandwich, slicing of the meat loaf and butter were done by using the Slicer.

3.2.2 Preparation of the sandwich

A cooked meat slice and a butter slice were kept in between two bread slices. The margins of the sandwich were removed by cutting them with a knife.

Sandwiches were packed in poly ethylene teraphthalate (PET) packages and N_2 gas was flushed into the package and at the same time heat sealing was done at equal points Two samples from prepared sandwiches were kept at ambient temperature and others were stored in a chill room at about 5^{0}C.

3.2.3 Microbiological Examinations

A sample was selected randomly from the batch and following microbiological tests were done in every four days and results were tabulated.

Another batch of sandwiches was prepared and for that one also microbiological tests were done

Buffered peptone water was the diluent used for all the microbiological tests It was prepared as follows

Buffered peptone water	20 00g
Distilled water	1000 00ml

This mixture was heated until the solids are dissolved and autoclaved at 121^{0}C for 20 minutes. (Before sterilization the diluent was poured into bottles and tubes and the mouths were closed by inserting an Al screw cap and cotton wool plugs)

3.2.3.1 DETERMINATION OF TOTAL PLATE COUNT (TPC)

3.2.3.1.1 Standard Plate Count Agar (APHA) medium preparation

Standard Plate Count Agar	23.5g
Distilled water	1000.00ml

Mixture was heated until the solids are dissolved and autoclaved at 121^{0}C for 20 min. (before sterilization the media was poured into bottles and the mouth was closed by inserting an Al screw cap)

3.2.3.1.2 Sample innoculation and preparation of dilution

- A sample of 90ml of pre sterilized buffered peptone water was taken into a stomacher bag (sterilized) and 10g of test sample was introduced aseptically
- The sample was blended.
- A sample of 1ml of the (1/10 dilution) was aseptically introduced to a test tube containing 9ml of buffered peptone water to prepare dilutions

3.2.3.1.3 Preparation of the TPC Agar plates and innoculation

A sample of 1 ml of any pre determined dilution was aseptically introduced into the sterilized plate and approximately 15-20ml of TPC Agar was poured Lid was closed and was shaken gently for even distribution and kept for 30 min at room temperature for solidifying

3.2.3.1.4 Incubation and counting colonies

The solidified plates were placed upside down in the incubator and temperature was set at 33-35 +/- 0 5^{0}C. They were incubated for 48 hours.

Colonies appearing in the media were counted by using the colony counter and the results were recorded

3.2.3.2 DETERMINATION OF TOTAL COLIFORM COUNT

3.2.3.2.1 Violet Red Bile Agar medium preparation

Pre prepared powder (OXOID)	51.5g
Distilled water	1000.00ml

Mixture was heated until the solids are dissolved and autoclaved at 121^{0}C for 20 min (before sterilization the medium is poured into bottles and the mouth was closed by inserting an Al screw cap).

3.2.3.2.2 Sample innoculation and preparation of dilutions

- A sample of 90ml of pre sterilized buffered peptone water was taken into a stomacher bag (sterilized) and 10g of the test sample was introduced aseptically
- The sample was blended
- A sample of 1ml was aseptically introduced (1/10 dilution) to a test tube containing 9ml of buffered peptone water to prepare dilutions

3.2.3.2.3 Preparation of the Violet Red Bile Agar plates and inoculation

A sample of 1ml of any pre determined dilution was aseptically introduced into the sterilized plate and approximately 15-20ml Violet Red Bile Agar was poured The lid was closed and it was shaken gently for well distribution and kept for 30 min at room temperature for solidifying

3.2.3.2.4 Incubation and counting colonies

The solidified plates were placed upside down in the incubator and the temperature was set at 35 +/- 0.5^{0}C and incubated for 24 hours.

Colonies appearing in the media were counted by using the colony counter and the results were recorded.

3.2.3.3 DETERMINATION OF POTATO DEXTROSE AGAR COUNT (YEASTS AND MOULDS)

3.2.3.3.1 Potato Dextrose Agar medium preparation

PDA	30.00g
Distilled water	1000.00ml

Mixture was heated until the solids are dissolved and autoclaved at 121^{0}C for 20 min (before sterilization the medium was poured into bottles and the mouth was closed by inserting an Al screw cap).

3.2.3.3.2 Sample inoculation and preparation of dilution

- A sample of 90ml of pre-sterilized buffered peptone water was taken into a stomacher bag (sterilized) and 10g of the test sample were introduced aseptically
- The sample was blended.
- A sample of 1ml of the dilution (1/10 dilution) was aseptically introduced to a test tube containing 9ml of buffered peptone water to prepare dilutions

3.2.3.3.3 Preparation of the PDA plates

Aseptically introduce 1 ml of any pre determined dilution into the sterilized plate and pour approximately 15-20 ml of Potato Dextrose Agar and close the lid and gently shake for well distribution and keep for 30 min at room temperature for solidifying

3.2.3.3.4 Incubation and counting colonies

The solidified plates were placed upside down in normal room temperature at 25 +/- 5°C and incubated for 48 hours

The colonies which appeared in the media were counted by using the colony counter and results were recorded

3.2.3.4 DETERMINATION OF E.COLI

3.2.3.4.1 Media preparation

3.2.3.4.1.1. Mac Conkey broth purple medium preparation

Prepared powder (OXOID)	40.00g
Distilled water	1000.00ml

Mixture was heated until the solids are dissolved. The medium was poured into Mc Cartney bottles and Durham tubes were placed in them an inverted direction. They were autoclaved at 121°C for 20 min. (note - the screw cap was not tightened till sterilization was complete)

3.2.3.4.1.2 Tryptone water

Pre prepared powder (OXOID)	15 00g
Distilled water	1000 00ml

Mixture was heated until the solids are dissolved and autoclaved at 121°C for 20 min (before sterilization the medium was poured into bottles and the mouth was closed by inserting an Al screw cap)

3.2.3.4.1.3. Indole reagent (KOVACS)

P-diethyl amino benzaldehyde	05 00g
N- Amyl Alcohol	75 00ml
Conc Hydrochloric acid	25 00ml

P-diethyl amino benzaldehyde was dissolved in alcohol and the acid was added slowly.

3.2.3.4.2 Sample inoculation and preparation of dilutions

- A sample of 90ml of pre-sterilized buffered peptone water was taken into a stomacher bag (sterilized) and 10g of the test sample were introduced aseptically.
- The sample was blended.

3.2.3.4.3 Introduction of the samples to Mac conkey broth and incubation

1. A sample of 1ml of the dilution (1/10 dilution) was aseptically introduced to the Mac Cartney bottle.
2. The bottles were incubated at 35^{0}C for 24 hours The color change of the media and gas formation were checked.
3. If colour was changed from purple to yellow and gas formation was observed, the sample was considered positive for presumptive *E.coli*.
4. If colour was changed from purple to yellow and no gas formation was observed, the sample was considered positive for presumptive *E coli*
5. If no colour change was observed, the sample was considered as *E coli* negative

3.2.3.4.4 Introducing the presumptive *E.coli* positive sample to tryptone water

1. An inoculum of 1ml from presumptive positive samples was aseptically introduced to 9ml of sterile tryptone water
2. The test tubes were incubated at 44 +/- 0 5 ^{0}C for 24 hrs
3. Three drops of indole reagent (KOVACS) was introduced and was shaken gently A violet color ring formation was observed on the top of the tube

3.2.3.4.5 Recording results

1. If above violet ring occurred, the sample was considered as *E coli* present If color changed from purple to yellow the sample was considered positive for presumptive *E coli*
2. If there was no color change in the Mac Conky broth bottles the sample was considered as negative for *E coli*

3.2.3.5 DETERMINATION OF STAPHILOCOCCUS AUREUS

3.2.3.5.1 Baird Parker medium preparation

Pre prepared powder (OXOID)	6.30 g
Distilled water	100 ml

Mixture was heated until the solids are dissolved and autoclaved at 121^{0}C for 20 min (before sterilization the medium was poured into tubes and bottles and the mouth was closed by inserting an Al screw cap). It was cooled to 50^{0}C and 50ml of Egg-York Tellurite Emulsion SR 54 was added aseptically and mixed well before pouring onto sterilized plates.

3.2.3.5.2 Sample inoculation and preparation of dilutions

1 A sample of 100ml of sterilized buffered peptone water was taken in to a stomacher bag (sterilized) and 10.0g of the test sample were introduced aseptically.
2 The sample was blended
3 Serial dilutions were prepared when necessary

3.2.3.5.3 Introducing the samples to Baird-Parker plates and incubation

1. A sample of 0.1 ml of the dilution was aseptically introduced onto two Baird-Parker plates. This quantity was carefully spread as quickly as possible over the surface of the Baird parker plates
2. The plates were incubated at 35+/- 0.5^{0}C for 24-48 hours

3.2.3.5.4 Counting colonies and recording results

Colonies which appeared in the media were counted and results were recorded (*S aureus* colonies are gray black in color. They are shiny convexes and surrounded by white entire margin)

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Results

Table 4.1. Microbiological Results of ingredients (First Batch)

INGREDIENT	TCC	STAPH	TPC	PDA	E.COLI
BREAD	< 10	$< 10^2$	$9.0 * 10^3$	$8.0 * 10^3$	ABS
BUTTER	$< 10^2$	$<10^2$	$2.2 * 10^4$	$1.2 * 10^3$	PE+
MEAT 1 (HIGH MOISTURE CONTENT)	$< 10^2$	$< 10^2$	$5.3 * 10^4$	$1.75 * 10^2$	PE+
MEAT 2 (LOW MOISTURE)	$< 10^2$	$<10^2$	$3.0 * 10^4$	$1.1 * 10^2$	ABS
	$<10^4$	$<10^4$	$<10^7$	$<10^4$	- satisfied

Table 4.2 Microbiological Results of the samples After 4 days

COMBINATION	TCC	STAPH	TPC	PDA	E.COLI
Sample (meat 1)			$3.715 * 10^5$	$1.455 * 10^3$	PE+
Sample (meat 2)			$3.275 * 10^5$	$1.05 * 10^3$	PE+
			$<10^7$	$<10^4$	- satisfied

Table 4.3 Microbiological Results of samples After 7 days

COMBINATION	TCC	STAPH	TPC	PDA	E.COLI
Sample (meat 1)	$4.95 * 10^4$	$2.0 * 10^2$	$1.288 * 10^7$	$5.4 * 10^5$	PE+
Sample (meat 2)	$1.235 * 10^3$	$< 10^2$	$9.24 * 10^6$	$4.4 * 10^3$	PE+

meat 1	$>10^4$	$<10^4$	$>10^7$	$>10^4$ - unsatisfied
meat 2	$<10^4$	$<10^4$	$<10^7$	$<10^4$ - satisfied

Table 4.4 Microbiological Results of the samples After 11 days

COMBINATION	TCC	STAPH	TPC	PDA	E.COLI
Sample (meat 1)	$<10^2$	$<10^2$	$9.6 * 10^7$	$5.9 * 10^6$	PE+
Sample (meat 2)	$<10^2$	$<10^2$	$3.27 * 10^7$	$5.6 * 10^6$	PE+

meat1	$<10^4$	$<10^4$	$>10^7$	$>10^4$ - unsatisfied
meat 2	$<10^4$	$<10^4$	$>10^7$	$>10^4$ - unsatisfied

Table 4.5 Microbiological Results of the samples (second batch) After 11 days

TCC	STAPH	TPC	PDA	E.COLI
1 0 * 102	< 102	5.6 * 104	1 21 * 103	PE+
$<10^4$	$<10^4$	$<10^7$	$<10^4$	- satisfied

Table 4.6 Temperature Variations in the chill room (First batch)

DATE	TEMPARATURE (^{0}C)
24/8/00	5.2
25	4 5
26	5 1
27	5.5
28	5 5
29	5 25
30	5 2
31	5 0
1/9/00	5 5
2	4 9
3	5 7
4	5 2

Average temperature = 5 2 ^{3}c

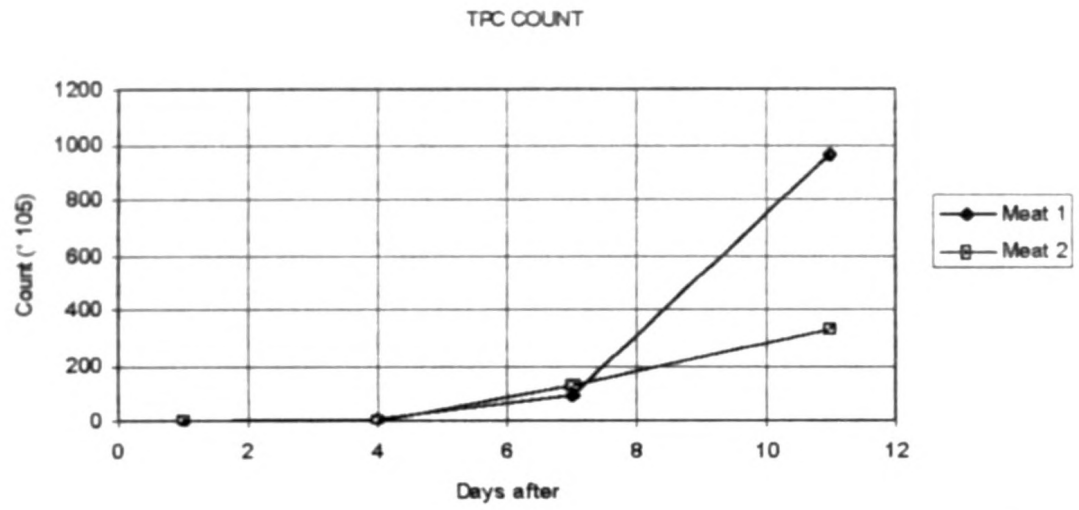
Table 4.7 Temperature variations in the chill room

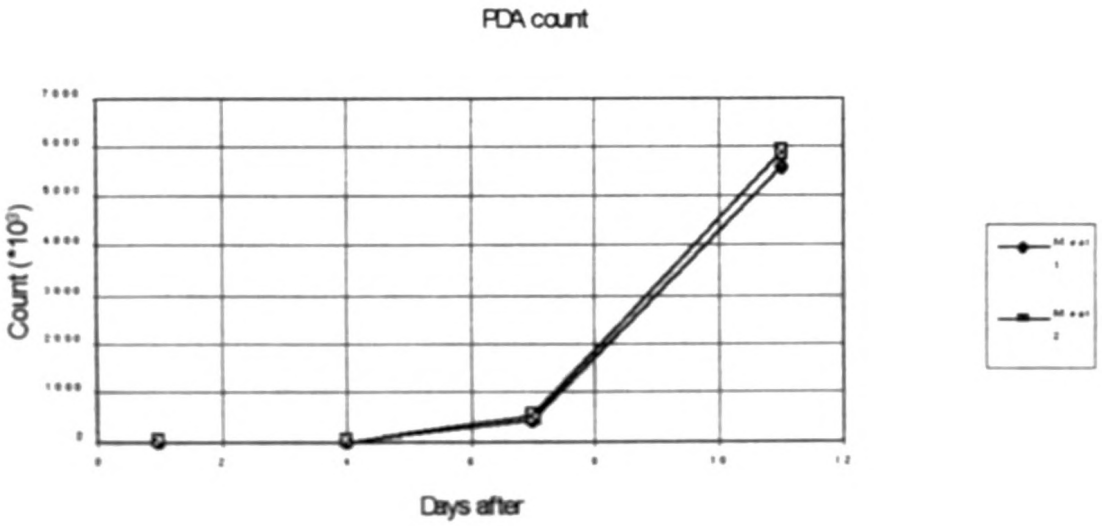
DATE	TEMPARATURE (^{0}C)
6/10/00	5.2
7	4.9
8	4.7
9	5.0
10	5.3
11	5.0
12	4.5
13	5.5
14	5.1
15	4.7
16	4.8
17	5.6

Average temperature = 5 0 ^{0}c

Table 4.8 Observations of the samples kept at ambient temperature

DAY	OBSERVATION
1	No change
2	Butter started to melt
3	Slightly melted butter
4	Slightly melted butter
5	White fungus on bread, melted butter
6	Increased fungal growth, melted butter
7	Further increase of fungal growth
8	Growth of yellow black fungus also Spread to melted butter also





The sandwich consists of bread, butter and chicken meat. These ingredients have following nutritional composition.

Bread	Carbohydrates
	Protein
	Vit. Thiamin, riboflavin, Niacin
	Ca, Fe
Butter	Fat 80%
	Carbohydrates, Vit. A, D
Cooked chicken meat	Protein
	Fat
	Fe, Ca, B1, B2, Niacin
	Added Vit. C

So the sandwich is nutritionally rich.

4.2 DISCUSSION

On selective agars, which often contain diagnostic agents in their formulation, too many colonies results in loss of the correct diagnostic change. A good example of this is VRBA with *coliforms*. When the number of colonies is less than 100 per plate, the *coliform* colonies are purple red in color and surrounded by a zone of precipitated bile acids. When the colonies are overcrowded, no purple-red color develops in the colonies, neither is there any precipitation, and so the sample would be recorded as *coliform* free, when in fact it had an unacceptably high count.

The background of food material on the plate is not possible to achieve when low dilutions of food sample are plated, and when the particles are small. Fine particles resemble colonies and these particles may be counted as colonies. One recommendation to overcome this problem is to incorporate 2,3,5 triphenyltetrazolium chloride (TTC) into the agar medium immediately before the plates are poured. Most bacterial colonies are colored red and so can be easily counted However TTC can be inhibitory to colony development of certain Gram-positive bacteria and not all the colonies, which develop, give a red color, so would be missed rather than counted. This lack of red color may also occur when the TTC is added to the plates following incubation.

Another method is, to pour an extra plate and to refrigerate this plate during the time, which the other plates are incubating. When the plates are counted, a careful comparison of the incubated plates with the refrigerated plate for each sample should enable a colony count to be made However it must be recognized that counting of these foods which have small particles is not easy and it would be wise for more than one experienced technician to check the counts where these problems occur. Some microorganisms never form large colonies and the use of magnification is important on plates to enable a count to be made The plate must be closely examined as a superficial examination makes it likely that the colonies will not be seen and so not counted

When 'spreading' (spread of colonies across the agar surface) occurs, colonies of other microorganisms may be prevented from developing or they may not be seen This happens occasionally in the thermophilic spore count when spreaders producing ammonia give the plate a clear appearance In either case undercounting of the sample results Although there are agar formulations which inhibit the formation of spreaders, they can also have the effect of reducing the colony count of the sample so should not be adopted without

investigation on any food stuff which is causing problems. Providing the spreader does not cover more than half of the area of the plate equal to half the area of the plate and count obtained as , Colony count * 2 * dilution factor = count per ml or g

Carrying of inhibitors from the food. Spices are examples of such food-mainly cinnamon, nutmeg, ginger and cloves. Inhibition may be overcome by using higher dilutions A neutralizing agent can be added to the agar but checks must be made to show that the neutralizing agent itself is not inhibitory.

Media may be insufficiently tempered i.e. poured too hot (above 45°C) thus pasteurizing the organisms on the plate.

Spread plates may be over-dried. This results in poor, slow or no growth.

The expected growth pattern may be altered if diluent or medium is used directly from refrigerated storage because of shortage of time.

Any medium has a shelf life. Over-aged medium, whether dehydrated or made up, may not have its designed properties.

Selective media by their nature, work by discouraging (inhibiting) growth of some organisms and allowing the wanted one(s) to grow on. The more highly selective the medium, the more this is so and for some strains of a wanted organism it may be over-selective and they may be suppressed

Incubation temperature is a compromise between the ideal and the available This may affect growth. Incubation time is usually the minimum time needs to get results. It may be too short for some strains and/or when cells are stressed.

Leaving media tempering in a water bath (usually about 45°C to 50°C) for some hours or overnight is likely to cause hydrolysis and therefore poor setting of acid agars and unknown changes in other media Good practice sets a maximum holding limit of 4 hours

Microorganisms other than *Staph aureus* can grow on Baird-Parker agar and since all *S aureus* strains do not give the typical classical reaction, microscopic examination of colonies should be carried out Some *Bacillus* species give the typical black, shiny colonial appearance on Baird-Parker agar and in this case a microscopic examination prevents a wrong decision being made regarding the status of the sample with reference to the *S aureus* count

A personal bias, which is responsible for different counts on the same plate by different persons There is a tendency for individuals to be consistently high, medium or low

counters A person variability shown when the same plate is counted again by the same person

N. gas used in gas flushing was not in food grade Therefore sensory evaluation was not done

A good packaging material for MAP should have a low water vapour transmission rate, together with a high gas barrier property

The most suitable material for MAP packaging is laminations or co-extrusions of polythene/nylon/EVOH or PVdC

If this packaging material was used for the sandwich packaging we can have a satisfied length of shelf life

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUTIONS

- The sandwich prepared with cooked chicken meat of low moisture content has a shelf life of 11 days
- The sandwiches prepared from both low moisture content and high moisture content chicken meats can be kept for 4 days
- The ingredients used in preparation of sandwich i e bread, butter and chicken meat make it nutritionally rich

5.2 RECOMMENDATIONS

- Shelf life was not checked after 11 days due to the time limitations. Hence further studies are needed to determine the shelf life
- Food grade N_2 gas should be used in gas flushing and sensory evaluation should be carried out to check the consumer acceptability for the product
- If the processing system is automated, the product can be prepared more hygienically and this may result a longer shelf life
- For MAP, a packaging material laminated with high gas barrier polymer such as ethylene vinyl alcohol should be used

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