

**EFFECT OF ROOM TEMPERATURE CURING
ON MICROBIAL POPULATION OF
CURED PORK PRODUCTS**

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RODERICK KWABENA DADDEY-ADJEI

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FACULTY OF AGRICULTURE
LEGON**

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THESES ROOM

ABSTRACT

A total of 64 fresh bacons of average weight 1.3kg and 64 fresh hams of average weight 4.6kg were used in the project. The bacons were randomly divided into 2 groups of 32. Sixteen 32 bacons were divided equally and dry cured under room temperature (DCRT) for 2,4,5 and 6 days respectively. There were 4 replicates in each treatment. The rest of the 16 bacons which were also dry cured for 2,4,5 and 6 days but under cold temperature (DCCT), served as control. The second group of 32 bacons were similarly divided into 2 groups and pickle cured at room temperature (PCRT) and cold temperatures (PCCT) respectively for 2, 4, 5 and 6 days. The mean room temperature and cold-room temperature for curing were 28°C and 0°C respectively. All the fresh hams were treated in a similar manner. There were four replicates in each treatment.

All products were smoked with hard wood shavings for ten hours at an average temperature of 59°C and left intact in the smokehouse for storage under average ambient temperature of 31°C and relative humidity of 68°C and relative humidity of 68%.

Random sampling of deep muscle tissues of fresh hams and bacons for microbiological analysis was done before curing. Sampling for microbiological analysis was carried out after 1, 4 and 8 days of storage. Standard bacteriological techniques were used to enumerate and identify microorganisms.

Staphylococcus sp., *Streptococcus sp* and *Proteus sp* were isolated from the dry and pickle cure before their use on the fresh bacons and hams. *Proteus sp* and *Staphylococcus sp.* were isolated from all cured hams and bacons. *Streptococcus sp.* were however isolated from only two DCCT 14 bacons, two DCCT and one PCRT hams under ambient storage conditions. With the exception of bacons dry cured for two days, all room temperature and cold-room temperature cured bacons and hams carried *Bacillus sp.*, *Escherichia coli.*, *Serratia sp.*, *Citrobacter sp.* and *Pseudomonas sp.* *Enterococcus sp.* was isolated from only bacons dry cured at cold-room temperature for 5 and 6 days and hams dry cured at cold room temperature for 4 and 5 days and bacon pickle cured under cold room temperature for 2 days. Apart from *Monilia* species which was isolated from dry cured room temperature bacons after 2 days of curing, no other fungus was observed growing on all the products during the curing. Fungi however grew on all cured products except the 2-day dry cured bacons under room and coldroom temperature conditions.

As the days of curing of dry bacons and hams at room temperature increased, there was a corresponding increase in total viable counts (TCVs) of bacteria. The odour of the dry cured products also deteriorated leading to the termination of curing on the 6th day. Similarly, the TVCs obtained for bacons and hams pickle cured under room temperature increased with days of curing leading to worsening of the off-odour. This led to termination of the curing on the 5th days.

On the contrary, increasing the days for dry curing or pickle curing under cold-room temperatures resulted in decreased TVCs when analysed after smoking and storage for one day under ambient conditions.

Comparison of the TVCs of room temperature hams and bacons to their respective controls did not usually show very large differences in magnitude during storage. Hams that were dry cured under room temperature or cold-room temperature respectively had higher TVCs than bacons cured under similar conditions.

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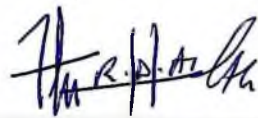
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DEDICATION

This work is dedicated to my parent Mr. E.F.K. Adjei, Miss F.A. Ofosu, Mrs M.Y. Adjei, my brothers, especially Samuel T. Adjei and Literary Club of the University Christian Fellowship, in appreciation of their help, guidance and deep concern for my welfare and education.

DECLARATION

I do hereby declare, that except for other people's work, which have been quoted and acknowledged, this work is my original research, and this thesis has not been presented for another degree elsewhere, either in part, or as a whole



Roderick Kwabena Daddey-Adjei

SUPERVISORS



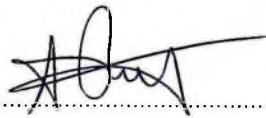
Dr. B.D. Akanmori

Noguchi Memorial Institute for

Medical Research

Head of Immunology

Legon.



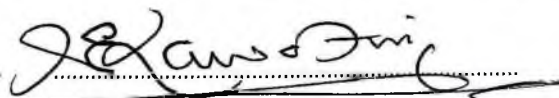
Mrs. A.R. Barnes

Department of Animal Science

and Head of Meat Science Unit

University of Ghana

Legon.



Dr. E. K. Awotwi

Head of Department

of Animal Science ,Legon

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CHAPTER 1

INTRODUCTION

The pig has presently become the central attraction in the country's bid for quick provision of animal protein. This is because it possesses merits such as high prolificacy, high dressing out percentage (70% average) and short generation interval. Pork is also versatile compared to other meats. Cured and smoked bellies (bacon) and hams (thighs) are varieties of processed pork. In Ghana, smoked pork, locally called "Domedo" is common and popular.

It was reported as early as in 1956 by Baker that bacon and ham are excellent sources of animal protein, B – vitamins and inorganic elements. In comparison with other meats, pork is also recognised as a major source of thiamine. The thiamine content is approximately 1.18mg/100g of pork compared to 0.13mg/100g of beef and 0.21mg/100g of mutton (Kayang, 1987). Pork also contains appreciable amounts of riboflavin and niacin, (Ronald and Ronald, 1991). Although pork products could easily be the solution to Ghana's animal protein requirement, the problem of their low consumption exists. This among other factors, is due to ineffective marketing of pork, (Obimpeh, 1986; Andah, 1990; and Barnes, 1990). Fresh pork, is not relished as much as processed pork which consumers find tastier. Both religious and non-religious beliefs militate against the consumption of pork for a considerable number of people in Ghana. However it is of particular interest that such claims and arguments for abstinence, are not strictly adhered to by their proponents, more so when they try the products for themselves. Considering this and the numerous advantages of pork, it can be concluded

that with effective marketing and proper preservative methods, bacon and ham may be widely accepted by the Ghanaian populace.

The flat profile, high fat content and thin thickness are notable attributes that greatly contribute to the better preservation of bacon. In general like all meats pork spoils rapidly at ambient temperatures with putrid odour, (Wood, Evans and Razvi, 1972). However bacon spoils less rapidly (1-2 weeks) at ambient temperatures of 20°C, (Spencer, 1969). The advantage of using bacon as a quick and short term solution to supply the average Ghanaian with animal protein cannot therefore be reaped if the meat product has a short shelflife under ambient conditions as exists in Ghana. Cold storage of meat products in the rural setting, where protein deficiency problems are most prevalent, is largely hindered by the fact that electric power is not readily available due to the high capital investment required in electrification. There is therefore the need to evolve a simple but effective processing method for meat which could be adopted anywhere in the country. According to Mann, (1963), four general processing methods widely used in meat preservation are sun drying, smoking, curing with common salt (sodium chloride) and spices, refrigeration and heat sterilization with respect to canning. The process used in meat preservation are principally concerned with inhibiting microbial spoilage, although modes of preservation are sought which minimize concomitant depreciation of the quality of the commodity, (Lawrie, 1985).

Of the four general processes of food preservation methods, the combination of cure application (sodium chloride and nitrites) and drying by smoking are easy to apply and can give a desirable cumulative effect. Bratzler *et al*, (1969), reported that wood smoke exerted a drying effect as well as a bactericidal and anti-oxidant effect to increase the stability of the processed meat. Most important is the ability of smoke to delay oxidative rancidity which would otherwise occur as a result of brining and partial drying treatments, (Foster and Simpson, 1961; Price and Schweigert, 1971 and Lawrie, 1985). Smoke also effects the organoleptic characteristics of meat products by imparting characteristic smell (odour/flavours) and desirable colour to meat. The art of meat preservation by curing is addition of sodium chloride and potassium or sodium nitrate and/or nitrite to meat, (Patton, 1971). He stated further that these curing salts act in dual capacity as preservatives and tenderisers to improve the palatability and acceptance of meat. The combination of smoke and cure would therefore have effect on the shelflife of pork products. The shelf life would be indicated by how rapidly the organoleptic characteristics, change with time during storage. The degree to which these attributes are maintained will be determined by how effective the applied cure can combat the activities and effects of microorganisms under ambient conditions.

The objectives of this work are;

1. to determine the maximum number of curing days possible for bacon and ham dry cured or pickle cured under room temperature.

2. determine the microbial load of the bacons and hams cured under ambient and coldroom temperatures.
3. identify microorganisms associated with bacons and hams.

CHAPTER 2

LITERATURE REVIEW

2.1 TYPES OF MEAT

The definition for meat varies and tends to include and exclude certain animal species. FAO, (1992) defined meat as the flesh and organs of animals and fowls. Potter and Hotchkiss, (1995) expanded this definition to embrace fish. Earlier, Forrest *et al*, (1975) defined meat to be animal tissue, which are suitable for use as food. This was modified as the flesh of animals used for food, (Lawrie, 1985). Often it is widened to include, as well as the musculature, organs such as liver and kidney, brains and other edible tissue. Lawrie, (1985) indicated that, the bulk of meat consumed in the world include that from sheep, goats, cattle, pigs and poultry. Ziegler (1966), Price and Schweigert, (1971), Lawrie (1974) and Gracey, (1981) have made mention of a wide host of animals used as meat.

Meat as an entity can be subdivided into four general categories or types, (Forrest *et al*, 1975). These are "red meat", poultry meat, sea foods and game meat. "Red" meat, in terms of consumption, is the largest type and includes beef, pork, mutton and veal. However, horse, goat, eland, llama, camel, water buffalo and rabbit meats are commonly used. Poultry meat, the flesh of domestic birds, includes that of chickens, turkeys, ducks, geese and guinea fowl. Sea foods include the flesh of aquatic animals, of which the bulk are fish with the flesh of clams, lobsters, oysters, crabs, and many other species. Game meat is the fourth type and according to Krostitz, (1996), it

has been one of man's main sources of food in prehistoric times and now plays a relatively modest role in total food and meat consumption worldwide. Kordylas, (1990) however included game and wildlife along with crustaceans, and molluscs or shellfish in this fourth category.

In Ghana both reared and wild animals are considered sources of meat. This includes antelopes, wild rodents (rats), rabbits, snakes and birds of various species.

2.2 **IMPORTANT MICROFLORA OF FRESH AND CURED PORK**

The bacteriology of fresh pork has been investigated by several workers, (Ayres, 1960; Gardner and Carson, 1967; Gardner *et al*, 1967). The flora can be divided into five groups. These are the species of the *Pseudomonas – Achromobacter* group, *Enterobacter- Hafnia* group, *Kurthia* group, *Lactobacillus* group and *Microbacterium thermosphactum*. The first group is numerically the most important and at a storage temperature of 2°C, can represent over 95% of the flora. After storage of pork at higher temperatures above 15°C, the *Pseudomonas – Achromobacter* group account for perhaps less than 20% of the flora, the remaining organisms being predominantly species of *Kurthia* and *Enterobacter – Hafnia* groups. A similar pattern has been observed on pork stored in the presence of carbon dioxide, except that under these conditions the incidence of species of *Enterobacter – Hafnia* group increased and that of members of the *Pseudomonas – Achromobacter* group decreased. Hechelmann and Kasprowiak, (1992) found that in the presence of air, *Pseudomonadecae* are predominant in refrigerated raw meat. Earlier, Gill and Newton, (1978); indicated that, strains of *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Bronchothrix thermosphacta* were most common on fresh meat with other psychotrophic flora.

The microflora of bacon is also well documented, (Hansen, 1960, Ingram, 1960; Cavett, 1962; Tonge, Baird-Parker and Cavett, 1964; Spencer, 1967). Both fresh and vacuum-packed bacon carry a microflora comprised mainly of micrococci and lactobacilli. Under aerobic conditions the micrococci are dominant and putrefactive spoilage results, whilst in vacuum packs, lactobacilli are dominant and souring spoilage occurs.

Microflora of fresh pork include the *Enterobacteriaceae* to which pathogenic organisms such as salmonellae, *Arizona*, and *Escherichia coli* belong. The *Bacillaceae* which include the genera *Bacillus* and *Clostridium* are also associated with meat. These are spore formers and can survive the heat treatment of meat products. (Hechelmann and Kasproviak, 1992). Schuppel, Salchert and Schippel, (1996), indicated that, cases of mastitis can lead to endogenous contamination of meat with *Streptococci sp.*; (*Streptococcus dysgalactiae*; *Streptococcus uberis*, *Streptococcus agalactiae*. Non differentiated streptococci), *Escherichia coli*, coliform bacteria, staphylococci (coagulase – negative), *Clostridium perfringens* and *Actinomyces pyogenes*. Shuppel, Salchert and Schippel, (1996) in an experiment stressed that, of the *E.coli* isolated from meat samples, nine strains proved to be virulent after allocation to various serotypes. They possessed heat-stable enterotoxin II and P-fimbriae.

Gracey, (1981) published a more exhaustive list of microflora of fresh carcasses. This included major bacteria or carcass contaminants such as *Salmonella sp.*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium botulinum*, *Proteus sp.*, *Pseudomonas sp.*, *Providencia sp.*, *Citrobacter sp.*, *Aeromonas hydrophila*, *Yersina enterocolitica*, *Campylobacter sp.*, and *Shigella sp.*

Important microflora of cured pork are usually similar to those that contaminate fresh pork through improper handling and processing of pork carcasses. These species also have the additional ability of surviving the altered conditions in the cured meat. Any investigation of this type would be complicated by variations in the properties of the meat and of its initial flora and curing procedure, (Riddle, Hibbert and Spencer, 1969). Jay, (1978) earlier, made an extensive list of organisms associated with cured and uncured meats. He stated that, the flora of fresh and cured meats, including fresh poultry and fresh seafood's, included the following genera of bacteria: *Alcaligenes*, *Bacterium*, *Bacteriodes*, *Brevibacterium*, *Clostridium*, *Corynebacterium*, *Escherichia*, *Flavobacterium*, *Halobacterium*, *Lactobacillus*, *Leuconostoc*, *Micrococcus*, *Spirillum*, *Photobacterium* and *Vibrio*. Jay, (1978) and Ingram, (1960) reported that, bacteria of the genera, *Streptococcus*, *Lactobacillus* and *Micrococcus* are capable of growing well on certain types of bacon such as Wiltshire bacon while *Streptococcus faecalis* is very dominant in several other types of bacon and cured ham.

Vacuum-packed bacon tends to undergo souring due primarily to micrococci and lactobacilli. Vacuum-packed, low-salt bacon stored above 20°C may be spoiled by *Staphylococcus sp.*, (Tonge, Baird-Parker and Cavett, 1964). Bacteria such as *Achromobacter*, *Bacillus-Pseudomonas*, *Proteus* and *Micrococcus*, have been strongly associated with the souring of cured hams, (Jay, 1978). In their study of vacuum-packed sliced bacon, Cavett (1962) and Tonge, Baird-Parker and Cavett, (1964) found out that, when higher-salt bacon is held at 20°C for 22 days, the catalase positive cocci dominated the flora while at 30°C the coagulase negative staphylococci became

dominant. In low salt bacon held at 20°C, the micrococci as well as *S. faecalis* were dominant.

Mould and yeasts are also associated with fresh and cured pork. The mould genera of *Thamnidium*, *Penicillium*, *Sporotrichum*, *Cladosporium*, *Mucor*, *Aspergillus*, *Atternaria*, *Oidium*, *Fusarium*, *Botrytis* and *Rhizopus* were identified while yeasts genera such as; *Candida*, *Debaromyces*, *Saccharomyces*, *Torulopsis*, *Rhodotorula*, and *Torula* have also been reported, (Jay, 1978). He also indicated that, when spoiled meat products are examined, only a few of these yeast and mould are found, and in almost all cases, one or more genus is found to be characteristic of the spoilage of a given type of meat product. The presence of the more varied flora on non-spoiled meats, then, may be taken to represent the organisms that existed in the original environment of the products in question or contaminants picked up during processing, handling, packaging and storage, (Jay 1978).

2.2.1 **SOURCES OF MICROORGANISMS OF MEAT AND MEAT PRODUCTS**

Meat is a good medium for microbial growth and susceptible to invasion by them, (Cudjoe, 1986). In the healthy and physiologically normal animal, those organs which have no direct contact with the exterior may introduce bacteria to the blood, tissues and organs, (Gracey, 1981). Effective control of microorganisms by the animal's defence mechanisms starts to fail immediately after slaughter or post-mortem. Microorganisms gaining access to the meat post-mortem are therefore able to perform their physiological functions unhindered. The method of access to the meat is varied.

Lawrie, (1985) indicated that, the organisms which spoil meat, gain access through infection of the living animal (endogenous disease) or by contamination of the meat post-mortem (exogenous disease).

2.2.1.1 **Exogenous Contamination of Meat and Meat Products**

Majority of microbes associated with meat carcasses are derived from the environment, (Cudjoe, 1986). The microbial load of meat is an important factor in determining the shelflife and acceptability of all meat products. Initial microbial contamination of meat may result from the introduction of microorganisms into the vascular system when unsterile knives are used for exsanguination, (Forrest *et al*, 1975). Since blood continues to circulate for a short period of time following sticking, microorganisms introduced through sticking with an unsterile knife, may be disseminated throughout much of the animals' body. During slaughtering, hoisting, flaying, cutting, processing, storage and distribution of meat, subsequent contamination occurs. Contamination of the carcass occurs with contact to hides, feet (trotters), manure, dirt and punctured viscera, equipment used for every operation performed, the clothing and hands of personnel, washing and scalding water for dehairing, brine used in curing, airborne microorganisms in the chilling room, storage and aging coolers, in the processing and packaging rooms; (Forrest *et al*, 1975; Jay, 1978; Gracey, 1981; Lawrie, 1985, Hechelmann and Kasproviak, 1992. Woltersdorf and Mintzloff; 1996). Irrespective of the standard of hygiene some surface contamination of carcasses are almost impossible to avoid, (Cudjoe, 1986). Non-specific primary contamination does not usually contribute to the ultimate microbial deterioration, (Mossel, Dijkann and Snijder, 1975). They stated further that, selection occurs, and this leads to the proliferation of viable organisms which eventually endanger the health of

the consumer or lead to deterioration phenomenon with the upsurge of food poisoning by bacteria or fungi, (Lawrie, 1985).

Some bacteria involved in exogenous contamination include, *Salmonella sp.*, *Arizona sp.*, *Eschericia coli*; *Bacillus sp.*, *Clostridium sp.*, *Streptococci sp.*, *Actinomyces sp.*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Staphylococci sp.*, *Proteus sp.*, *Pseudomonas sp.*, *Providencia sp.*, *Citrobacter sp.*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, and *Shigella sp.*, (Gracey, 1981; Lawrie, 1985; Hechelmann and Kasprowiak, 1992; and Schuppel, Salchert and Schippel, 1996).

2.2.1.2 **Endogenous Contamination of Meat and Meat Products**

Contamination from infections arise from established disease in the live animal and may involved both parasitic worms and bacteria, (Dolman, 1957; Lawrie, 1985) and some may be diseases and infections which are naturally transmitted between vertebrate animals and man (zoonotic), (Gracey, 1981). These include anthrax, brucellosis, contagious pustular dermatitis, erysipelas, leptospirosis, listeriosis, louping-ill, ornothosis, psittacosis, Q-fever, ringworm, streptococcal meningitis and tularaemia. Most infections arise mainly through contact whiles others are airborne. Some bacteria involved in endogenous contamination include, anaerobes like *Clostridium sporogenes* and other *Clostridium sp.*, *Bacillus sp.*, *Pseudomonas sp.*, and coliforms, (Haines, 1941; Callow arid Ingram, 1955 and Cosnett *et al*, 1956).

2.3 **SPOILAGE MANIFESTATION OF CURED MEAT PRODUCTS**

What one individual describes as spoiled might well be considered edible by another, (Forrest *et al*, 1975). Undesirable changes in flavour, odour and colour may also result in stored meat, (Kayang, 1987), and ultimately affect the palatability in a negative way. Meat spoilage does not necessarily imply decomposition or putrefaction, though they may be strongly linked. It is not due to solely microbial action but also to such factors as insects, intrinsic enzymatic and oxidative reactions as well, (Forrest *et al*, 1975), or involve proteolytic changes, discolorations and slime production, (Ulrich and Halvorson, 1951).

Surface slime is a sign of spoilage and it is the superficially observable effect of the coalescence of a sufficiently large number of individual colonies of microorganisms like *Proteus sp* and some cocci. The further apart these colonies are apart, the longer the time it will take slime to form, (Haines, 1937). Slime growth also results in a greyish appearance of the surface, (Kayang, 1987). This adversely affects the aesthetic value of the product.

Chemical changes involves the degradation of proteins, lipid, carbohydrates and other complex molecules into simpler ones and is accomplished by the action of endogenous hydrolytic enzymes that are present in the meat as well as enzymes of microbes, (Forrest *et al*, 1975). Undesirable odours and taste are also evidence of spoilage. This is due to gases produced during proteolytic changes by microorganisms,

(Price and Schweigert, 1971; Forrest *et al*, 1975; Lawrie, 1985; Kayang, 1987, Flores and Bermell, 1996). Lawrie, (1985), stated further that, free amino acids present are attacked by deaminases with the production of hydrogen sulphide, carbon dioxide and ammonia. Flores and Bermell, (1996) reported that, acidification also leads to loss of typical sensory characteristics because of souring and indicated that, meat proteins lose their solubility during acidification and develop a gel texture which affects meat consistency. Putrid odours produced during spoilage involve gases such indole, methylamine, skatole and hydrogen sulphide. These foul-smelling substances tend to be liberated particularly under reducing conditions. For example, *Pseudomonas fragi* if present during the storage of pork at 2 – 10°C, cause proteolysis of myofibrillar proteins and this will raise the emulsifying capacity of the meat, (Lawrie, 1985).

Spoilage in meat, may be manifested by colour change. Such change mostly lead to a lowering of the aesthetic acceptance of the product. Discoloration may be due to the alteration or destruction of meat pigments, (Lawrie, 1985). It was further reported that, myoglobin may be oxidized to brown metmyoglobin, and may combine with hydrogen sulphide produced by bacteria, to form sulphmyoglobin or be broken down to form yellow or green pigments by microbially produced hydrogen peroxide. Whiteley and D'Souza, (1989) reported that *Streptococcus faecium subsp. casseliflavus* caused a yellow discoloration (carotenoid in nature), both under aerobic and anaerobic conditions. Similarly, hydrogen peroxide – producing lactic acid bacteria is known to cause green discoloration of cooked cured meat products, (Stekelenburg, Zomer and Moulder, 1990). Discoloration may also be due to the elaboration of foreign pigments by the microorganisms themselves, (Lawrie, 1985). For example, *Pseudomonas sp.*, produce blue-green pigments whiles micrococci, sarcinae, and yeast produce pink pigments. B.

prodigiosus produce red pigments. Pigments produced by *Cladosporium sp.*, *Sporotrichum sp.* and *Penicillium sp.* are black, white and blue-green respectively. Halophilic pseudomonads produce black or red discolouration in salted meat and meat products. *Listeria viridescens* produce green cores in sausages.

Jensen, (1949) reported that many types of micro-organisms cause spoilage by producing free fatty acids and yellow or green pigments, from the superficial fat in meat, and indeed, such changes are frequently the limiting factors in storage.

The sources of bacteria causing spoilage in cured meat products are evidently similar to those implicated in the exogenous and endogenous contamination of meat. For example, the spores of aerobic saprophytic *Bacillus sp.* may easily be carried from the soil by wind and settle on uncovered meat product and thrive.

2.4 **CURING**

Curing has an age-old history, (Mottram and Rhodes, 1973). Curing serves to reduce the growth of microbes, enhances colour and enriches flavour of meat products, (Callow, 1956). Forrest *et al.*, (1975) reported that meat curing involves the application of salt, colour fixing ingredients and seasonings to meat in order to impart distinct properties to the product. Similarly, Mottram and Rhodes, (1973) reported that, the distinctive flavour of cured meat may be due to the salt, sugar, nitrite or smoke or both, applied during the curing process. The curing salts act in a dual capacity as preservatives and tenderizers, to improve the palatability and acceptance of meat, (Patton, 1971). Curing also lowers the moisture content of meat and meat products.

The low moisture contents thus becomes a limiting factor for microbial growth and hence retards meat spoilage. Nitrite is a bacteriostatic agent for the control of *Clostridium botulinum*, (Greenberg, 1972).

2.4.1 TYPES OF CURE

Application of cure is achieved either through the rubbing of the curing mixture in dry form over the surface of the meat or through the application of a solution of the ingredients of the cure. The development of curing technology in the last century has concentrated, albeit fortuitously, on two distinct but interrelated objectives; the reduction in the curing or maturation period and the increased yield of product from the raw material, (Patton, 1971). For any type of curing, the primary function of the maturation period is to permit sufficient time for the curing salts to diffuse throughout the meat. It follows that the rate of curing is directly related to the method of adding the curing salts. The ageing period also allows the developments of characteristic flavour, (Patton, 1971).

2.4.1.1 DRY CURE

According to Kitchel I and Ingram, (1965) pork was originally "dry cured". This was a slow and laborious process. Sodium nitrate was rubbed by hand into the surface of pork legs and boned out "spencers". These cuts were then stacked up and covered with dry sodium chloride and left to mature for several months. They were then washed in warm water to remove excess salt and dried at a temperature of 80^o – 90^oF. (22 – 27.5^oC) for 1 – 2 days, incurring a weight loss of 7 – 10%. Curing by this method

involved diffusion of curing salts from the surface to the centre of a cut which necessitated a long maturation period. Only well rested pigs, produced consistently good results, i.e. absence of bone taint. Dry-curing and aging were formerly done using ambient temperatures, (Kemp *et al*, 1974). However, most commercial dry-cured hams had been cured and aged under conditions of controlled temperature and relative humidity and with a decreased aging time, (Cecil and Woodroof, 1954: Christian, 1960, Skillely, Kemp and Varney 1964 and Varney, 1967).

2.4.1.2. **PICKLE CURE**

A modified dry cure still practiced involves the maturation process being shortened by injecting pickle into the carcass, particularly the shoulder and leg (the thickest cut) before salting, and this reduces the curing time to three weeks. Tank curing or the English Version Wiltshire style cure is the most popular of pickle curing and yield can be increased by 10%, (Patton, 1971). The butchered whole side is injected with freshly prepared pickle in a strictly controlled system (usually 25 stiches) and immersed in a tank of pickle of similar composition. Originally this was for a period of five days and it was followed by a period of 2 – 5 weeks maturation. This made a total of one month approximately for curing.

2.4.2. **SODIUM CHLORIDE**

Salt is probably the most widely used of food preservatives, (Ingram and Kitchell 1967). It dates from antiquity and indeed the use of the salt as a preservative has been recorded as early as 300BC., (Patton, 1971). Fundamentally, its success depends on the

fact that, it imparts desirable taste in meat and also it inhibits objectionable putrefaction and dangerous microorganisms.

2.4.2.1. **Diffusion of sodium chloride into meat**

The preservation of meat by salt curing depends on salt reaching all parts of the meat, including the fatty tissues and the bones, (Wood, 1966). He further indicated that, the rate-determining step in salt curing is the diffusion of the salt through the tissue. For a given tissue, the rate of diffusion is largely governed by the concentration gradient and the temperature. Diffusion of salt in the tissue causes liquid transport due to osmosis and changes in the protein structure. Similar changes also occur in the drying of fish muscles, (Jason, 1958 and 1965). Jason, (1965) stated that, the effect of temperature was complex and over the range of temperature (5 – 30°C), usually involved in the curing processes, considerable changes are likely to occur in the physical properties of the tissue. Diffusion may vary in different directions relative to the muscle fibres. (Wood, 1966). Kormendy and Gartner, (1958) Wistreich, Moore and Kenyan (1960), worked with leg muscles which were cylindrically cut and found out that the salt uptake per unit area of exposed surface correlated with time of immersion by an empirical log-log plot of uptake against time. Wood, (1966) worked with tissues which were isotropic to salt uptake and recorded that the salt uptake was linear with the square root of the time and was also proportional to the concentration. The diffusion co-efficient was independent to the brine concentration.

2.4.2.2 **Role of sodium chloride on microbial species and population**

Sodium chloride has many effects on the microbial content of meat, (Yu, Siaw and Idris 1982). Solomon, Ekanem and Okubanjo, (19994) noted that, increasing the level of sodium chloride in a pork belly product, (unam inung,) decreased the level of moisture in the cured meat. The effect of salt is often similar to that of drying, (Wood, 1966; Ingram and Kitchell, 1967). This is explained by the fact that salt in food imparts an osmotic withdrawal of water, which in turn affects the water activity in the food. Hence many microbes cannot obtain sufficient water to meet their physiological functions. In a salted food, where salt is the main solute, the water activity is often expressed as the so-called "brine-concentration", (Hankins *et al*, 1950). This is only an approximation, since there is an additional contribution by soluble constituents of the food itself, (Scott, 1957). There are however, occasions when certain organisms tolerate or require high concentrations of sodium chloride which cannot be substituted by other salts.

The reason for the specific requirements for sodium chloride is partly accounted for by the salt requirements of enzymes extracted from the cells, (Ingram, 1957). For example, some forms of cocci are very salt-tolerant and thus thrive in high salt concentration. Mohr and Larsen, (1963) also reported that some microbes require a lot of sodium chloride to maintain the stability of their cell wall.

The action of salt on micro-organisms is varied. Flores and Bermell, (1996) reported that, when salt is used in concentrations of between 2.5 and 3.0% in the

manufacture of cured sausages, it inhibits the growth of spoilage organisms but does not affect the growth of acidifying organisms unless its concentration rises above 3%. Not only does it have an effect upon the ability of growth of microbes, but upon other properties of microbes. For example, high concentration of salt depresses certain properties of microbes like retention of viability, (Fabian and Winslow, 1929), respiration, (Ingram, 1940), fermentation and motility for sporogenesis, (Fabian and Bryan, 1933). There are many important differences in detail with respect to the action of salt on microbes. For example, by increasing salt concentration, motility may be stopped before growth, so that on lightly salted meat, the bacteria are still able to grow but these colonies remain isolated instead of spreading to form a continuous slime. This is because salting diminishes the amount of free liquid at the meat surface, (Ingram and Kitchell, 1967). Salt has more influence on the proteolytic activities of bacteria than on their growth, (Rockwell and Ebertz, 1924). They continued that, low concentration of salt diminish the production of putrid odours and flavours from meat more than it seeks to be explained by the change in the bacteria flora.

2.4.2.3 **Role of Sodium Chloride and Temperature on Microbial species and population**

Temperature plays a critical role in microbial growth. It also has a great influence on the action of salt, (Ingram and Kitchell, 1967) and hence microbes. The lethal action of salt, like that of other disinfectants is less at low temperatures. Shipp, (1958) and Buttiaux and Moriamex, (1958) reported that, *Salmonella sp.* for example, are rapidly destroyed in curing brines at room temperatures but survive for weeks at low temperatures. Ingram, (1958) also reported that *E. coli* behave similarly. Bardley and Taylor, (1960) and Blanche Koelensmid and Van Rhee, (1964), also demonstrated the

same effect of temperature on the action of salt on the survival of salmonellae on culture media and vacuum packed bacon respectively. The difference of the influence of temperature and salt concentration combination as inhibitory to microbial growth is not clear, (Ingram and Kitchel, 1967). Dumes, (1935) reported the growth of *E. coli* and typhoid bacteria at 25°C with concentration of salt at 5 – 8% while the works of Patton, (1971) proved the contrary that low temperatures lessened the lethal action of sodium chloride. Labrie and Gibbons, (1973) reported that the preservative action of salt on fish increased with reduction of temperature. With moulds however, several authors agree that the greatest tolerance exists at the optimum temperature for growth, (Tomkins, 1929; Heintzeler, 1939) with a much lower tolerance near the temperature limits, (Stille, 1948). The maximum salt concentration permitting growth of *Cl. botulinum type E* is 5.8% at 30°C and 25°C, 5.1% at 20°C, and 4.3% at 15°C, (Ohye, Christian and Scott, 1967). Salt concentrations ineffective at high temperatures may become inhibitory or at least, result in markedly delay outgrowth as the temperature falls to 10°C or below, (Segner, Schmidt and Boltz, 1966). The optimum and maximum temperature for growth are raised when an organism grows in the presence of salt, and the rise is large when the organism tolerates high concentration, (Ingram, 1957). It is not clear whether the same applies to the minimum growth temperature and more research would be needed to indicate this, (Ingram and Kitchell, 1967).

2.4.2.4 Role of Sodium Chloride and pH on microbial species and population

The influence of pH is supplementary to that of salt, (Ingram and Kitchell, 1967). They stated that, it is clearly a general rule that, as the acidity rises, less salt is needed to prevent growth of individual bacteria and yeasts. Sherman and Holm, (1922) reported that with a pure culture of *E. coli*, small concentration of salt were needed to

inhibit cell multiplication at pH values remote from the optimum, and the same is true with *Cl. botulinum*, (Ohye, Christian and Scott, 1967), and salmonellae, (Blanche Koelensmid and van Rhee, 1964).

Salt and pH may act together in another way. It has been noted that among the bacteria from the meats, those which resist salt tend to be unusually sensitive to acidity, and vice versa, (Ingram, 1958). A combination of acidity and salt, are very generally, inhibitory. Unfortunately, exploitation of the principle of more salt application because of higher pH of meat as a means of preservation of meat, is hindered by the fact that acidity, in meat appears to accentuate the flavour of the salt in it, (Ingram, 1949). The action of sodium chloride is synergistic with various inhibitory agents. Benzoic acid, a preservative, when used in lower concentration with salt has a synergistic effect, on microorganisms, (Von Schelhorn, 1951). The addition of salt, even if it is not itself acid in reaction, by raising the ionic strength in solution frequently has the effect of reducing the pH, which later is known to increase the effectiveness of weakly acidic preservatives, like benzoic acid, (Ingram, Ottaway and Coppock, 1956). The concentrations of salt needed to change the pH appreciably are however, comparatively large, and some additional explanation may be necessary. Reports that salt "sensitizes" bacteria to carbon dioxide, (Rockwell and Ebertz, 1924) may arise from similar causes.

2.4.3. EFFECT OF SODIUM CHLORIDE ON ORGANOLEPTIC CHARACTERISTICS OF MEAT AND MEAT PRODUCTS

Sodium chloride has many effects on sensory properties of meat. According to (FAO, 1985), it is the main flavouring agent in the manufacture of sausages, bacon and ham and also increases the water-holding capacity of the product by aiding swelling of

the myofibrillar proteins. Incorporation of salt thus enhances protein extraction and therefore the texture of reformed products. Saltiness plays a role in the acceptance or rejection of meat products. This implies that it plays more part in the overall acceptability of meat and its products, (Mottram and Rhodes. 1973). They reported that salt is a major contributor to bacon flavour. The sensory appreciation of the saltiness of bacon is much less intense than that of aqueous solution of equal strength, an effect due, probably, to fluid binding by the protein or to its slow release during mastication, (Ingram, 1949). However, the Canadian National research (1938-39), showed that the high salt content (7.5%) of Canadian bacon compared with Danish bacon (6.2%) was the major source of dissatisfaction, (Winkler and Cook, 1941). At low concentrations, salt helps to improve the flavour and colour of meat, Daun, (1975) and Meyer, (1978), but at higher concentrations, especially when used alone, salt gives a dry harsh, dark coloured and unattractive product, (Kramlich , Pearson and Tauber, 1973). According to Ellis *et al*, (1968) and Pearson *et al*, (1977), high concentration of salt in meat also accelerate oxidative rancidity and hereby affecting flavour development. This is because salt promotes the activity of lipoxidase in meat. It was further indicated that the concentration of sodium chloride alone necessary to confer stability on bacon might be 4 – 5g/100g water, (Wood, Evans and Razvi, 1972). Bacon is already known to exhibit a masking effect on saltiness, (Ingram, 1949) and suggests that salt is bound into the cured meat structure more firmly than in untreated pork meat, though whether by a chemical or physical mechanisms, is not very clear.

2.4.4 **ROLE OF NITRITE AND NITRATE IN MEAT CURING**

The art of preserving meat by curing in common salt, with or without smoking

has been practiced from remote antiquity , (Kerr *et. al*, 1926). The use of nitrates also dates from ancient times, and has been practiced so long, that its origin is unknown, (Hehner, 1910). The curing of meat and comminuted meat products with salt and an alkali nitrate salt is an ancient process originally intended as a method of preservation, (Wassermann and Talley, 1972). Potassium nitrate has long been used to protect meat from spoilage and colouration, (Hoagland, 1908). Hoagland, (1908) stated further that the original preserving function of meat curing has been changed to one of flavour and colour development to satisfy consumer tastes. Since 1929, the inclusion of higher sodium or potassium nitrite at the rate of 200 ppm has been approved by the U.S. Meat Inspection Division. Changes in the colour and appearance of the product occur in the process. It was many years before the mechanism of the curing process was understood and in fact, many of the intermediate stages had still to be elucidated, (Patton, 1971).

Earlier in 1891, Pollenski demonstrated that the nitrate in the cure was reduced as a result of bacterial action. The red colour of the product, which was considered a characteristic of cured meat, was found by Kisskalt, (1899) to be formed in the presence of nitrite. The use of nitrate or nitrite as a curing agent was reported by Haldane, (1901). Kerr *et. al*, (1926) established limits of sodium nitrite concentration in the cure that would yield a satisfactory product, and these limits are part of the legal requirements for cured meats in use today. The work of Kerr *et al*, (1926) however, was primarily directed towards the development of the cured colour in the product. It was noted that the flavour and quality of the products were equivalent to those prepared in the customary fashion, i.e with nitrate only. The relationship of nitrite to flavour was first described by Brooks *et. al*, (1940) in a study of use of nitrite in the cure

of bacon and ham. Although they presented no taste panel data, the authors stated that the panel showed a preference for meat cured with nitrite. At the same time parallel work of the Research Association of the British Food Manufacturers, (Mascara, 1939) reached similar conclusions.

Barnett *et al*, (1935) reported on an extensive study of the factors affecting cured ham flavour which established the relationship between the amount of nitrite in the cure and amount of cured flavour. In the study on the concentration of nitrite in the pumping pickle, they found that the panel had an equal preference for hams pumped with pickle containing nitrite concentration of 1.5g sodium nitrite/ 1 litre of brine and those in which pickle with 0.1g sodium nitrite/ 1 litre of brine has been used. Cho and Bratzler, (1970) studied the effect of nitrite and smoke on the flavour of cured pork roasts, reaching the conclusion that more cured flavour was present in the roasts cured with nitrite. Nitrite-containing and nitrite-free samples could also be distinguished when the samples were smoked and when sodium chloride was omitted from the cure. However, the result also revealed that on 116 occasions involving 288 individual tasting, no differentiation was made between salted pork and cured meat. A similar conclusion regarding the role of nitrite in flavour formation in frankfurters was obtained by Wassermann and Talley, (1972). Sausages prepared with and without nitrite were examined by Skjelkvale, Valland and Russwurm, (1973). They found out that, the quality of 4500mg/kg, of nitrite as used by Barnett *et al*, (1965) caused a bitter flavour with decreased acceptability but no significant differences were observed between frankfurters cured with 150 or 75mg nitrite/kg, Wassermann and Talley, (1972) or sausages with 80 or 40mg nitrite/kg meat Skjelkvale, Valland and Russwurm, (1973). Saltiness of cure plays some part in the overall acceptability of meat products, (Mottram

and Rhodes, 1973). A comprehensive study of the acceptability of Canadian and Danish bacon was carried out by the Canadian National Research Council in 1938 – 1939. The work revealed that the high salt of Canadian bacon (7.5%) compared with Danish bacon (6.2%) was the major source of dissatisfaction, (Winkler and Cook, 1941). These observations were followed by a laboratory taste panel study in which bacon containing, salt in the range 4.5 – 9.0% was evaluated, (Hopkins, 1947). A preference for bacon with 4.7% salt was observed as best with reference to nitrite free bacon while bacon containing 0.25% nitrate and 4% salt level was preferred in the nitrate-salt bacon group. Nitrate at levels of 50mg/kg did not apparently contribute to saltiness. Mottram and Rhodes, (1973) investigated the effect of varying the concentration of sodium nitrite used in curing pork upon the flavour of bacon. The taste panel used identified the various flavour characteristics and examined products cured under different conditions. As the nitrite concentration was increased from zero to 1000mg/kg, an almost linear increase in the intensity of bacon flavour was found but above 1500mg/kg, further increase in flavour was small. Salt was shown to make a major contribution to bacon flavour but sodium nitrite; had no detectable taste at concentrations similar to those found in bacon. The differentiation between salted pork and bacon in blind comparisons by flavour or odour was remarkably uncertain. Wolf and Wassermann, (1972) suggested a re-appraisal of the curing process because the nitrite may under some conditions react with amino compounds present to give small concentrations nitrosamines. They concluded that, without the use of nitrite, the characteristic cured flavour is not developed in meat products. Simon *et al* (1972) reported meat frankfurters made with beef and pork, scored low in a hedonic taste panel evaluation when they contained no nitrite. Wassermann and Talley, (1972) found the role of nitrite in frankfurter flavour to be complex and depended on the type of evaluation panel used.

They reported that, both smoked and unsmoked frankfurters, showed significant differences between products prepared with or without 156 mg nitrite/kg. However, in tests in which "Frankfurter" flavour was scored, the smoked, non-nitrite-treated sample was rated as highly as the cured sample. Analysis of variance showed an interaction between smoke and nitrite. Komarik, (1951) stated that, the processing and curing of unchilled pork, was reported in the U.S.A. It was claimed that this process called the "Thermocure", could produce satisfactory cured bellies in ten hours from slaughter. Patton, (1971) noted that, the main advantages of this cured unchilled carcasses were that, the hot processing allowed for more rapid production and turnover and lower weight losses. Some researchers obtained increased yields, lower cooking and drip losses, improved colour and tenderness and most reported favourably. However the findings were somehow equivocal and it may be said that few detrimental characteristics were found with hot processed cured meats, and the main advantage must be the saving in time and space in production. Smith, Messier and Tittiger, (1989) reported on the effect "dry cured" products, prosciutto, prosciuttini and Genoasalami had on *Trichinella spiralis*. They reported that, curing of the various productions was shown to destroy the *Trichinella* larvae. Pepsin digestion revealed that larvae progressively became loosely coiled, uncoiled and subject to digestion during the curing process.

2.5 **PRESERVATIVE ROLE OF SMOKE ON MEAT**

Smoking is often combined with curing., (Adjekum, 1997). Smoke is generally produced by the slow combustion of saw-dust derived from hard woods, which averagely consists of about 40 – 60% cellulose, 20 – 30% hemicellulose, and 10 – 30% lignin; and it inhibits microbial growth, retards fat oxidation and imparts flavour to cured

meat, (Lawrie, 1985). The saw-dust or hard wood shavings may include oak and mahogany, (Kayang, 1987). The preservative action of wood smoke on meat has been attributed to some of the numerous complex chemical compounds in the smoke, (Lawrie, 1985). According to Foster and Simpson, (1961) wood smoke consists of two phases which are:- a disperse, liquid phase containing smoke particles and a dispersing gas phase. There are over 200 compounds present in wood smoke, (Wilson, 1963). It is believed that formaldehyde accounts for most of the preservative action of wood smoke, although most of the compound include phenols, organic acids, alcohols, carbonyl compounds and polycyclic hydrocarbons, aldehydes, ketones and cresols, (Forrest *et al*, 1975); formic, acetic, butyric, caprylic, vanillic and syringic acids, dimethoxyphenol, methylglyoxal, furfural, methanol, ethanol, acetaldehyde, diacetyl, acetone and 3, 4 – benzopyrene, (Lawrie, 1985). Some of these compounds exhibit either bacteriostatic or bactericidal properties. (Forrest *et al*, 1975). In addition, phenols also have an antioxidant activity that retards the onset of oxidative rancidity, (Forrest *et al* 1975). They also indicated that the compounds listed above probably contribute to the characteristic flavour of smoked meat. The idea of the preservative role of smoke is also towards the lowering of water activity of the product. The dehydration effects of the smoke on the meat together with its antioxidant and anti-bacterial properties all contribute to the preservative action of the smoke and this is crucial in augmenting the keeping quality of the product, (Bratzler *et al*, 1969).

During smoking, smoke components are absorbed by surface and interstitial water in the product, (Forrest *et al*, 1975). They reported further that in products that have their surface remaining intact, a preservative effect will persist and the reverse is true with respect to a loss in bacteriostatic effect. In most present day processed

meats, smoking contributes little if any preservative action; since the smoke components in no case, penetrate more than a few millimeters. The rate and amount of smoke deposition are affected by factors such as temperature, relative humidity, duration of smoking, smoke concentration, smoke composition, method of smoke production, product composition and air velocity in the smoke house, (Foster and Simpson, 1961 and Ziegler, 1966). Ziegler, (1966) reported further that, the change in colour of the outside surface of smoked meat is hastened by high temperatures and that meat, which have been subjected to four to six days of smoke or until they become brown in colour, have some added keeping qualities. The outside surface of such smoked meat and meat products must be trimmed off before cooking or the meat is likely to cause digestive disorders. This is due to the poisonous effect of too large a quantity of the pyroligneous acid. During high temperatures in the smokehouse, deposited phenols on the meat may volatilize; thus causing them to be reversely absorbed into the gaseous phase, (Forster and Simpson, 1961).

Electrostatic smoking processes have been developed in an attempt to speed smoke deposition, but these processes have not achieved widespread commercial application, (Forrest *et al*, 1975). A second type of smoking involves liquid smoke preparations that have been developed as an attempt to eliminate the smoking process. Liquid smoke preparations are free of carcinogenic compounds, such as 3, 4 – benzopyrene, that have been discovered in low levels in natural wood smoke.

2.5.1 **EFFECT OF SMOKING ON MICROORGANISM POPULATION OF MEAT**

Moisture content of meat is reduced by smoke components. The bacteriostatic and

bactericidal effects of smoke components serve to decrease microbial numbers especially on the surface of meat/products. Smoking to an internal temperature of 58°C is capable of destroying all surviving *staphylococci* on meat. Smoking is often combined with curing and this increases the storage life of products, (Adjekum, 1997). Apparently, this combined effect decreases microbial load especially on the meat surface. The combined effect of time and temperature of smoking on microbes of sausages (frankfurters) showed an inverse relationship between time and temperature of smoking with bacterial counts from the product immediately after smoking and storage, (Heiszler *et al*, 1972). The combination of heat and smoke is usually effective in reducing significantly the surface bacterial population of the product, (Price and Schweigert, 1971). They stated further that, in addition, surface dehydration, protein coagulation, and the deposition of a resinous material resulting from the condensation and formaldehyde and phenol, produce a reasonably effective chemical and physical barrier against microbial growth and penetration of the finished product. Today, few meat foods are however produced, in urban settlements in which smoke constituents play an important role in preserving the product against microbial spoilage. This is because, cold storage (refrigeration) is very effective in minimizing microbial growth and thus spoilage.

CHAPTER 3

MATERIALS AND METHODS

3.1 SITE AND SAMPLE ALLOCATION

The experiment was conducted at the Animal Science Department of the University of Ghana, Legon.

A total of 64 fresh bacons and 64 hams of average weight 1.3kg and 4.6kg respectively were used in the study. These were from Large Whites, which had been slaughtered on a clean slaughter slab after resting them. The bacons were randomly divided into two groups of 32 each. Each group was further divided into 2 equal groups of 16 bacons each. The 16 bacons was further sub-divided into 4 groups of 4 bacons each and dry cured under coldroom temperature (DCCT) and room temperature (DCRT) for 2,4,5 and 6 days. The coldroom temperature cured products served as control. The second group of 32 bacons were similarly divided and pickle cured under room temperature (PCRT) and coldroom temperature (PCCT) for 2, 4, 5 and 6 days respectively. The hams were similarly divided and subjected to the same treatments. The mean room temperature and cold room temperature for curing were 28.0°C and 0°C respectively.

3.2 CURING PROCESS

The curing mixture was according to Ziegler's 8-3-¼ mixture of 8 pounds

(3.6kg) sodium chloride, 3 pounds (1.4kg) sugar and ¼ ounces (7g) sodium nitrite for 45kg. meat.

3.2.1 **DRY CURE (DC)**

The 8-3-¼. dry cure mixture was made up of 70.8% finely ground sodium chloride,, 27.5% sugar and 1.7% sodium nitrite and was used at a ratio of 60g of the 8-3-¼ cure mixture per kilogram of meat. The cure mixture was rubbed onto the meat surface. Four replicates of each treated meat type were prepared and laid in a plastic container and covered with a plastic sheet and left at average room temperature (28°C) or average coldroom temperature of 0°C for 2, 4, 5 or 6 days of curing. The hams and bacons being cured under room temperature were covered with a mesh to prevent flies settling on the meat. The relative humidity and corresponding temperature during curing were recorded every three hours.

3.2.2 **PICKLE CURE (PC)**

The pickle cure was made up of 66.13% water, 24% sodium chloride, 9.3% sugar and 0.57% sodium nitrite. Pickle equivalent to 10% of green weight of ham or bacon was introduced into them through stitch pumping. The rest of the solution was used as a cover pickle for the hams and bacons. Four replicates each of ham and bacon were prepared and left to cure for 2,4,5, and 6 days under average room temperature. (28°C) and 0°C coldroom temperature. The hams and bacons being cured under room

temperature were covered with mesh to prevent flies settling, on the meat. The relative humidity and corresponding temperature during curing were recorded.

3.3 SMOKING PROCESS

The smokehouse, which was built of blocks and concrete was preheated to a temperature of 30°C for thirty minutes. Cured hams and bacons were hung randomly in the smokehouse for an hour for excess liquid on the meat to drip off. Smoking was done at an average temperature of 59°C for ten continuous hours. Smoke was generated by combusting hardwood firewood and shavings. The hardwood included sapele (redwood) and shedua.

At the end of smoking, the meat were left intact in the smokehouse to cool to ambient temperature overnight.

3.4 MICROBIOLOGICAL ANALYSIS

Sampling was aseptically done on the fresh hams and bacons before curing. Samples were also aseptically taken from the interior of each smoked ham and bacon after storage in the smokehouse for 1, 4 and 8 days.

3.4.1. CULTURE MEDIA USED FOR BACTERIOLOGICAL AND MYCOLOGICAL ANALYSIS

The culture media for bacteriological and mycological analysis included Blood



Agar, MacConkey Agar, Nutrient Agar, Selenite Faecal Broth, Sabouraud Dextrose Agar, Plate Count Agar, Oxidative – Fermentative Agar, Triple Sugar Agar, Enterococcus Agar and Petrifilms. The compositions and preparations of these media were as follows:

COMPOSITION OF BLOOD AGAR

1. Blood Agar Bases – Special from Difco Laboratories
Detroit Michigan U.S.A.

Formula in grams per litre

Please arrange in straight line

Beef Heart Infusion 500g

Racto – Tryptose 10g

Sodium Chloride 5g

Selected Peptone mixture 20.0g

Defibrinated Sheep Blood (Sterile) to be added

PREPARATION OF BLOOD AGAR

40gms of Bacto blood agar base (dehydrated) was suspended in 1000ml cold distilled water and heated to boiling to dissolve the medium completely. Solution was sterilized in the autoclave for 15 minutes at 121°C. 5% freshly obtained sheep blood was added to the cooled and sterilized medium at approximately 45°C. Medium was well mixed and dispensed into sterile petri dishes.

COMPOSITION OF MACCONKEY AGAR BASE

2. MacConkey Agar Base – Special from Difco Laboratories
Detroit Michigan U.S.A.

Formula in grams per litre

Bacto – Peptone, Difco	17g
Protease Peptone, Difco	3g
Bacto-Lactose	10g
Bacto-Bile Salts No ₃	1.5g
Sodium Chloride	5g
Bacto-Agar	13.5g
Bacto-Neutral Red	0.03g
Bacto-Crystal Violet	0.001

PREPARATION OF MACCONKEY AGAR

To rehydrate the medium, 50g of the agar was suspended in 1000ml cold distilled water and heated to boiling point to dissolve the medium completely. The medium was sterilized in the autoclave for 15 minutes at 121°C and dispensed into sterile petri – dishes to solidify.

3. COMPOSITION OF NUTRIENT AGAR

Formula and Preparation

Oxide dehydrated medium formula (CM₃)

Lab-Lemco powder	1.0gms/Lt.
Yeast extract	2.0 "

Peptone	5.0 "
Sodium Chloride	5.0 "
Agar	15.0 "

PREPARATION OF NUTRIENT AGAR

To rehydrate the medium, 28gms of the dehydrated nutrient agar base was suspended in 1000ml cold freshly distilled water and heated to dissolve the medium completely. The medium was sterilized in an autoclave for 15 minutes at 121⁰C and 15 pounds pressure. It was dispensed aseptically into sterile petri dishes and test tubes.

4. **COMPOSITION OF SELENITE FAECAL BROTH**

Formula and Preparation	Special from Difco Laboratories
Sodium hydrogen selenite	8g
Peptone	1.0g
Mannitol	0.8g
Di-Sodium hydrogen phosphate anhydrous (Na ₂ HPO ₄)	2.0g

PREPARATION OF SELENITE FAECAL BROTH

With care, the medium was mixed with water and heated to 80⁰C to dissolve. The medium was dispensed in 5ml amounts into test tubes with caps. With caps of test tubes loosened the dispensed medium was sterilised for 20 minutes. Test tubes were tightened after sterilization to cool.

5. **COMPOSITION OF SABOURAUD DEXTROSE AGAR (LAB.M. SABOURAUD DEXTROSE AGAR)**

(A selective medium for the isolation of yeasts and fungi).

<u>Formulation</u>	<i>g/litre</i>
Balanced Peptone NO.1	10.0
Dextrose	40.0
Agar	12.0

PREPARATION OF SABOURAUD DEXTROSE AGAR

62g of the medium was dispersed in 1000mls of deionised water and soaked for 10 minutes. It was swirled to mix and sterilized at 121°C for 15 minutes. Care was taken not to overheat the preparation. At 30°C, the medium was dispensed in 15 mls portions into sterile petri dishes.

6. **COMPOSITION AND PREPARATION OF PLATE COUNT AGAR**

Fluka Biochemika – Plate Count Agar.

<u>Composition</u>	g/litre
Tryptone	9
Yeast Extract	2.5
Dextrose	1.0
Agar	9.0

17.5g of medium was dissolved in 1000mls of deionised water and sterilized at 121°C for 15 minutes. It was then dispensed (15mls) into sterile petri dishes to cool and solidify for use.

7. COMPOSITION AND PREPARATION OF m ENTEROCOCCUS AGAR

Composition: (Difco Laboratories)

	<u>g/litre</u>
Bacto tryptose	20
Bacto Yeast Extract	5
Bacto Dextrose	2
Dipotassium Phosphate	4
Sodium Axide	0.4
Bacto Agar	10g
2,3,5 – Triphenyl Tetrazolium Chloride -	0.1

42g medium was dissolved in 1000mls of sterile deionised water and boiled in a water bath till boiling was initiated. The dissolved medium was poured (15mls) into sterilized petri dishes and allowed to solidify for use.

8. COMPOSITION AND PREPARATION OF OXIDATIVE- FERMENTATIVE AGAR

(Hugh and Leifson)

	<u>g/litre</u>
Peptone	2.0
Sodium Chloride	5.0
Sodium Phosphate	0.3
Thymol Blue	0.03

9.8g of the dry medium was suspended in 1000mls of purified water and heated with agitation till a solution occurred. The solution was then sterilized at 121°C for 15 minutes.

9. COMPOSITION AND PREPARATION OF TRIPLE SUGAR AGAR

(Difco Laboratories)	g/litre
Bacto – Beef Extract	3
Bacto – Yeast Extract	3
Bacto – Peptone	15
Protease-Peptone, Difco	5
Bacto – Dextrose	1
Bacto – Lactose	10
Saacharose – Difco	10
Ferrous Sulphate	0.2
Sodium Chloride	5
Sodium Thiosulphate	0.3
Bacto Agar	12
Bacto-phenol red	0.024

65g of the medium was suspended in 1000mls of distilled water and boiled to dissolve. The solution was poured (15mls) into test tubes with caps and sterilized at 121°C for 15 minutes and slanted.

10 PETRIFILMS – These are already prepared and sterilized medium for identification of *E. coli* and other coliforms.

3.4.2 REAGENTS USED FOR BIOCHEMICAL EXAMINATION

Biochemical examination reagents used included Urea Broth, Gelatin, Kovac's reagent, Catalase, Indole, Mannitol, Oxidase and Peptone water (motility determination).

3.4.3 STAINS USED

Stains used included Lactophenol cotton blue, Lugol's iodine, Safranin Crystal violet and Methylene blue.

3.4.4 SAMPLING

A sample of meat (10g) was taken aseptically from muscle tissues 2cm below the surface of the bacon and butt end of ham of every replicate of raw and cured products (to include deep tissue microbial contaminants) and put into 90mls of 0.1% sterile peptone water and homogenized by shaking vigorously at an acute angle, thirty (30) times. This dilution was a 1 in 10.

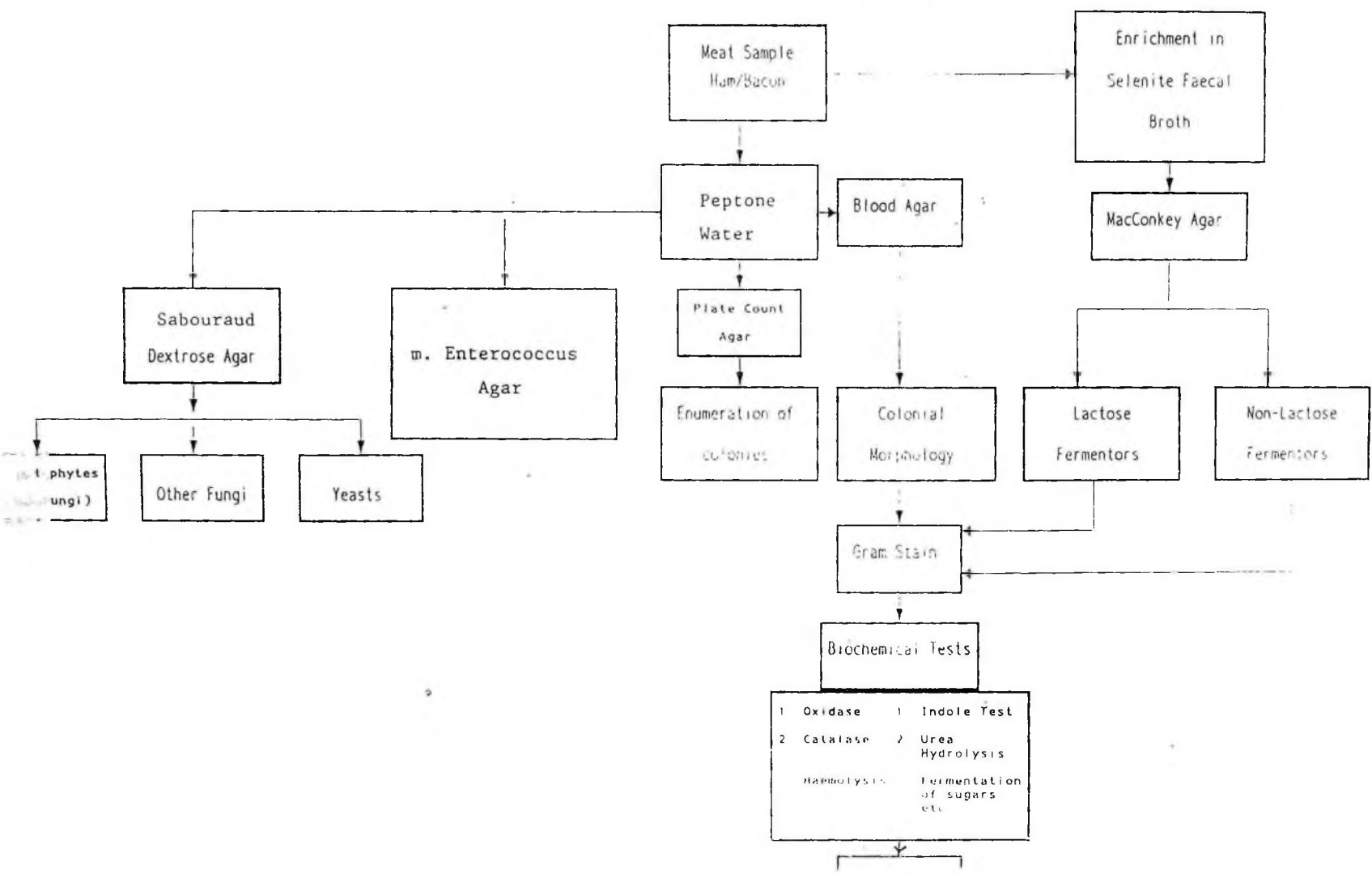
3.4.4.1 SCHEME FOR BACTERIOLOGICAL AND MYCOLOGICAL EXAMINATION OF SAMPLES

Figure 1, illustrates the scheme that was used for bacterial and fungal enumeration, identification and characterization.

FIGURE 1

Scheme for Bacteriological and Fungal Examination

A Yeast and Fungi B Enterococci/Faecal Streptococci C Plate Counts/Total Viable Count D Isolation and Identification



3.4.4.2 TOTAL VIABLE COUNTS (TVC) OR PLATE COUNTS

A volume of 0.1ml of the homogenized solution with 10g of meat and 90mls medium was serially diluted with sterile pipettes before dispensing onto Plate Count Agar. A sterile glass rod bent at its tip at 90° ("hockey stick") was used to spread the diluted inoculum evenly over the Plate Count Agar and incubated at 37°C. Colonies were counted 18 – 24 hours later. Total viable counts (TVCs) of plates that showed counts not exceeding 300 colonies were made using a hand lens and hand tally counter.

3.4.4.3 ENTEROCOCCI/FAECAL STREPTOCOCCO ENUMERATION

Samples were treated as in the case of Plate Count Agar, except it was dispensed onm Enterococcus Agar.

3.4.4.4 FUNGAL AND YEAST IDENTIFICATION

A volume of 0.1ml of the 1 in 10 ratio mixture in section 3.4.4. was dispensed onto Sabouraud Dextrose Agar with a sterile pipette and spread with a sterile "Hockey stick", (glass rod), incubated at 25°C and examined for 24 – 72 hours for fungal growth. Lactophenol cotton blue stain was used to examine yeast, fungal mycelia, hyphae and spores. Fungi and yeast were identified according to Smith, (1969). The staining technique involved the picking of part of a pure colony with a sterile metal pin, teasing the picked colony over a drop of sterile distilled water on a clean glass slide and fixing it over a gentle flame. After drying, a drop of Lactophenol cotton blue stain was poured onto the sample, a minute allowed to elapse and the stain examined under the microscope.

3.4.4.5 Bacteria Identification

10g of meat sample was taken aseptically from deep muscles, 2cm below the surface of the bacon and butt end of ham to include deep tissue microbial contaminants. This was put into 90mls of Selenite Faecal Broth and homogenized and left for 24 hours, after which an inoculum was picked with a sterile inoculation loop and streaked onto MacConkey medium for discrete colonies. MacConkey Agar differentiated non-lactose fermentors (pale colonies) and lactose fermentors (pink colonies).

The mixture from section 3.4.4. was also subcultured on Blood Agar to note the colonial morphology at 37°C in 24 hours.

Colonies from the subcultures of Blood and MacConkey Agars were then Gram stained to note the shape and Gram's reaction. Gram's staining method was as follows:

1. A sterilized metal inoculation loop was used to pick inoculum from a test tube, smear it on a glass slide and air dried.
2. Air dried slides were fixed by slowly passing the slide three times through a spirit flame and allowed to cool.
3. The smear was flooded with crystal violet for three minutes.
4. The smear was then washed with Gram's iodine.
5. The smear was flooded with Gram's iodine for a minute and washed with tap water.
6. The smear was decolourised with methylated spirit till not more crystal violet came out.

7. Tap water was used in washing the smear till the stain was clear.
8. Carbol-fuchsin was washed with tap water, blotted and examined under oil immersion.

Various biochemical tests (section 3:4:2) were employed to ascertain the identity of the bacteria which had been isolated and kept on Nutrient agar.

An already prepared media identification kit (petrifilm) was employed to identify coliforms and other members of the *Enterobacteriaceae*. *Escherichia coli* was identified on the petrifilm medium as blue colonies surrounded by gas bubbles while other coliforms appeared as black colonies with or without gas bubbles. Faecal streptococci were identified with a selective medium called m Enterococcus Agar.

Indole production tests was used to determine the ability of other coliforms apart from *E. coli* to decompose amino acid and tryptophan to indole.

The method employed was as described by Cruickshank, (1970).

Triple Sugar Iron (T.S.I.) Agar was used in the identification of Gram-negative enteric pathogens, particularly members of the *Salmonella – Shigella* group, (Difco Manual, 1965). These microorganisms have the ability of fermenting lactose, saccharose, dextrose with the formation of acid and gas and also produce hydrogen sulphide.

Further reactions with mannitol, urea, gelatin, catalase, Kovac's reagent, oxidase, peptone water (for motility determination) were employed to differentiate closely related bacteria.

Serological tests could not be performed, due to unavailability of reagents.

3.5 STATISTICAL ANALYSIS

Total viable count (TVC) or bacteria counts obtained from the microbial analysis were corrected using the log transformation. This gave standard values for comparisons. Each TVC presented for every cured pork product (C.P.P.) on the different days of storage was the average of the minimum and maximum TVC obtained out of the four replicates from plate counts. The magnitude of the log transformed values (standard values) then showed the rate of bacteria growth with increase in days of storage.

CHAPTER 4

RESULTS

4.1 TEMPERATURE, RELATIVE HUMIDITY RANGES AND SOME MUSCLE PHYSICAL CHANGES DURING CURING AND STORAGE

Temperature and relative humidity ranges during curing under room temperature and coldroom temperature were variable. The maximum and minimum room temperatures during curing were 31.0°C and 26.5°C respectively, while the maximum and minimum relative humidities were 85.0% and 53.0% respectively. The maximum and minimum coldroom temperatures during curing were +4.0°C and -4.0°C respectively, while the maximum and minimum relative humidities were 99.0% and 93.0% respectively. Smoking temperatures ranged between 57 – 62°C.

The maximum and minimum temperatures recorded during storage were +46.0°C and +23.0°C respectively, while the maximum and minimum relative humidities under ambient conditions were 85.0% and 34.4% respectively. Figures a – g of the appendix show the relationship of temperature and relative humidity during storage of the products. The graphs showed in all cases that, as the temperature of curing increased the relative humidity decreased.

Off-odour from all dry and pickle cured hams was observed after two days of curing under room temperature conditions and worsened with increase in days of curing. Curing under room temperature conditions was curtailed after five days for

pickle cured hams and bacons whiles curing was curtailed after six days for dry cured hams and bacons due to intense off-dour of the products at those times.

There was no off-odour detected in all hams and bacons cured under cold room temperature conditions. However the coldroom temperature curing was also ended on the fifth and sixth days for pickle curing and dry curing respectively.

Curing under room temperature conditions, resulted in separation of the muscles of the hams than their controls as the days of curing increased. (figure 2). The increase in separation of the room temperature cured hams was more pronounced over five days than two days. Curing under coldroom temperature resulted in hams and bacons with muscles less separated. (figures 2 and 3). This separation in muscles was more pronounced in hams than bacons.

Observation of 2 day DCRT ham stored over four days showed less turgidity of its muscle when compared to its control. The same was true of 5 day DCRT ham stored over one day against its control.

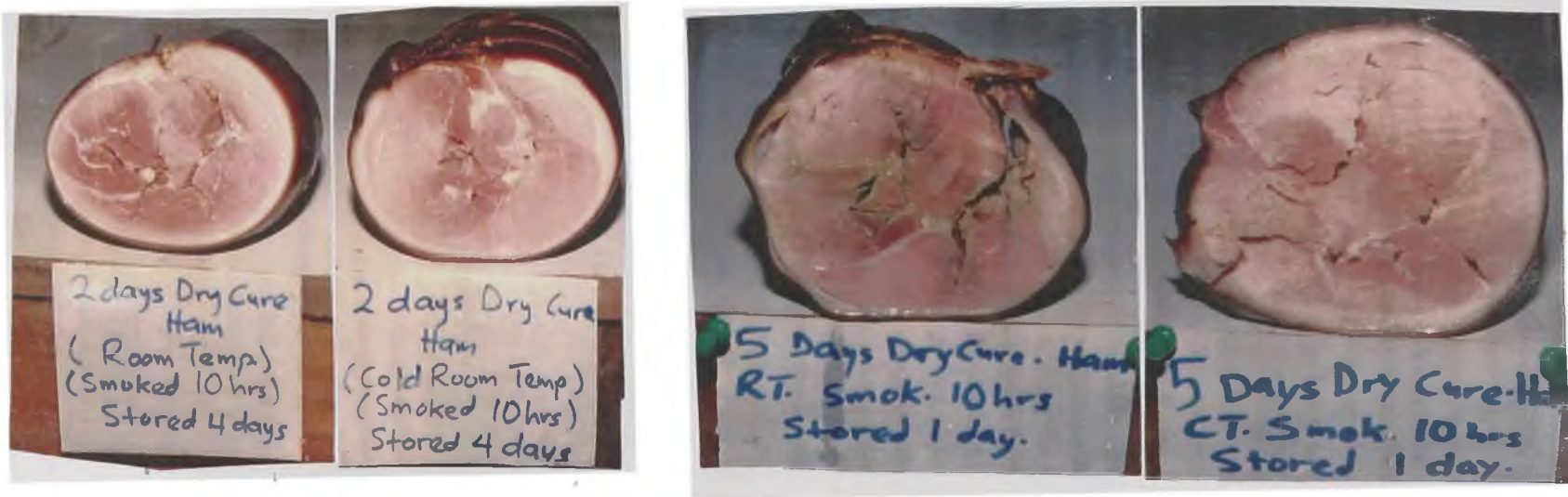


Figure 2: Photographs showing hams dry cured for 2 days and 5 days under room temperature and cold room temperature, smoked at 57°C and stored in the smokehouse. Notice the separation of muscles from the rind (skin) of 5-day dry cure ham under room temperature.



Figure 3: Photographs showing bacons dry cured for 5 days under room temperature and cold room temperature, smoked at 57°C and stored in the smokehouse. No evidence of separation of muscles from the skin in both treatments.

4.2 CHANGES IN MICROORGANISM POPULATION OF HAMS DRY CURED FOR 2,4,5 AND 6 DAYS

Tables 1 –4, present the TVCs for hams dry cured for 2,4,5 and 6 days. In general, increasing days of room temperature curing, showed an increase in TVCs on the first day of storage of the hams in the smokehouse. However, increase in days of curing under coldroom temperature showed a decrease in TVCs on the first day of storage. Comparison of TVCs of hams dry cured under room temperature and hams dry cured under coldroom temperature on the first day of storage, showed that the former products had higher TVCs. For example, table 1 shows TVC for DCRT ham on the first day of storage as 4.403 ad 3.952 for its corresponding DCCT control ham.

The TVCs for both DCRT hams and their corresponding DCCT hams, increased with increase in days of storage under ambient temperatures and relative humidities. Their TVC/day (gradient) were usually positive. The only exception was 5 days DCRT ham (table 3) which decreased in TVC from 5,300 (first day of storage) to 3,190 (fourth day).

Table 1 **CHANGES IN MICROORGANISM POPULATION FOR 2-DAY DRY CURED HAMS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	DCRT		DCCT	
			TVC	TVC/Day	TVC	TVC/Day
27.9	58.6	1	4.403		3.952	
				+0.516		+0.802
28.9	60.0	4	5.950		6.358	
				+0.511		+0.413
29.0	61.4	8	7.994		6.008	

Table 2 **CHANGES IN MICROORGANISM POPULATION FOR 4-DAY DRY CURED HAMS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	DCRT		DCCT	
			TVC	TVC/Day	TVC	TVC/Day
34.3	46.6	1	4.809		2.960	
				+0.266		+0.012
31.0	60.3	4	5.608		5.966	
				+0.322		+0.061
30.1	62.7	8	6.896		6.239	

Table 3 **CHANGES IN MICROORGANISM POPULATION FOR 5-DAY DRY CURED HAMS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	DCRT		DCCT	
			TVC	TVC/Day	TVC	TVC/Day
31.4	64.6	1	5.330		2.452	
				-0.713		+0.590
31.3	59.3	4	3.160		4.223	
				+0.725		+0.325
29.4	73.9	8	6.088		5.521	

Table 4 **CHANGES IN MICROORGANISM POPULATION FOR 6-DAY DRY CURED HAMS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	DCRT		DCCT	
			TVC	TVC/Day	TVC	TVC/Day
25.7	73.3	1	5.609		2.236	

Increase in TVCs of DCCT hams on the fourth day of storage was much higher than the TVCs of DCRT hams, all of which were stored under ambient temperature conditions in the smokehouse.

4.2.1 CHANGES IN MICROORGANISM POPULATION OF BACONS DRY CURED FOR 2,4,5 AND 6 DAYS

Tables 5,6,7 and 8 generally showed that, increasing days of room temperature curing, showed an increase on TVCs on the first day of storage of the bacons in the smokehouse. Increase in days of coldroom temperature curing, however, resulted in a decrease of TVC on the first day of storage of the bacons. The only exception was the relatively high TVC of bacons cured under coldroom temperature for six days (tables 7 and 8). The TVC (2.099) for 6 days DCCT bacon was expected to have been lower than TVC (2.069) for 5 days DCCT bacon by the first day of storage.

Tables 5,6,7 and 8 also showed further that, the TVCs for dry cured room temperature bacons and their controls, increased with increase in days of storage. Their associated gradients (TVCs/day) were positive. The only exceptions were TVCs for 5 days DCRT bacons and 5 day DCCT bacons (table 7), which decreased from 3.224 on the fourth day of the storage to 3.001 on the eighth day of storage; and 3,389 on the fourth day of storage to 2.901 by the eighth day of storage respectively. Their gradients reflected negatively as -0.056 and -0.122 respectively.

Table 5 **CHANGES IN MICROORGANISM POPULATION FOR 2-DAY DRY CURED BACONS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	TVC	DCRT		DCCT	
				TVC/Day	TVC	TVC/Day	TVC/Day
27.9	58.6	1	2.801		2.798		
				+0.399			+0.408
28.9	60.0	4	3.997		4.023		
				+0.499			+0.411
29.0	61.4	8	5.994		5.667		

Table 6 **CHANGES IN MICROORGANISM POPULATION FOR 4-DAY DRY CURED BACONS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	TVC	DCRT		DCCT	
				TVC/Day	TVC	TVC/Day	TVC/Day
34.3	46.6	1	3.160		2.628		
				+0.256			+0.428
31.0	60.3	4	3.224		3.911		
				+0.069			+0.020
30.1	62.7	8	3.001		3.989		

Table 7 **CHANGES IN MICROORGANISM POPULATION FOR 5-DAYS DRY CURED BACONS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	TVC	DCRT		DCCT	
				TVC/Day	TVC	TVC/Day	TVC/Day
31.4	61.6	1	3.160		2.069		
				+0.358			+0.440
31.3	59.3	4	3.224		3.389		
				-0.056			-0.122
29.4	73.9	8	3.001		2.901		

Table 8 **CHANGES IN MICROORGANISM POPULATION FOR 6-DAYS DRY CURED BACONS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	TVC	DCRT		DCCT	
				TVC/Day	TVC	TVC/Day	TVC/Day
25.7	73.3	1	4.626		2.099		

Comparison of TVCs of DCRT bacons and DCCT bacons on the first day of storage, showed that DCRT bacons (table 5) had higher TVC (2.802) than the TVC for DCCT bacon (2.798).

In general however, the TVCs of DCCT bacons increased higher from the first day of storage to the fourth day of storage than their corresponding DCRT bacons in all the respective days of curing (tables 6,7 and 8).

Hams that were dry cured under room temperature or coldroom temperature had higher TVCs than the bacons cured under similar conditions.

4.2.2 CHANGES IN MICROORGANISM POPULATION OF HAMS PICKLE CURED FOR 2.4 AND 5 DAYS

Tables 9, 10 and 11 present the TVCs of pickle cured room temperature hams and their corresponding controls. Similar changes as observed in dry cured hams and bacons were evident. The TVC for both room temperature and coldroom temperature cured hams increased with increase in days of storage. Their associated TVCs/day (gradient) were positive. Some of products were extensively spoiled and gave very offensive odour. These were therefore discarded before the eighth day of storage as indicated in tables, 9, 10 and 11.

Generally, increasing days of room temperature curing, showed an increase in TVCs on first day of storage for PCRT hams while increase in days of curing, decreased TVCs of PCCT products on the first day of storage.

Comparison of TVCs of PCRT ham to PCCT ham on the first day of storage, showed that PCRT hams, usually had higher TVCs than their corresponding PCCT ham. The only exception was 4 days PCRT ham (table 10) which had a lower TVC (2.700) on the first day of storage as against 4 days PCCT ham, which had a higher TVC (2.835) on the same day of storage.

The TVCs of PCCT hams also increased from the first day of storage to higher counts by the fourth day of storage than their corresponding PCRT hams, (tables 9, 10 and 11).

Table 9 **CHANGES IN MICROORGANISM POPULATION FOR 2-DAY PICKLE CURED HAMS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	TVC	DCRT		DCCT	
				TVC/Day		TVC	TVC/Day
31.6	66.5	1	2.801			2.272	
				+0.495			+0.701
27.2	81.7	4	4.287			4.376	
28.9	77.1	8	-			-	

Table 10 **CHANGES IN MICROORGANISM POPULATION FOR 4-DAY PICKLE CURED HAMS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	TVC	DCRT		DCCT	
				TVC/Day		TVC	TVC/Day
30.6	67.0	1	2.700			2.835	
				+0.484			+1.931
29.1	75.0	4	4.151			4.766	
				+0.528			
26.7	80.0	8	6.263			-	

Table 11 **CHANGES IN MICROORGANISM POPULATION FOR 5-DAY PICKLE CURED HAMS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	TVC	DCRT		DCCT	
				TVC/Day		TVC	TVC/Day
35.1	61.8	1	4.053			2.869	
				+0.599			+1.105
29.4	74.4	4	5.849			6.185	
				+0.038			
25.8	79.9	8	6.001			-	

4.2.3 CHANGES IN MICROORGANISM POPULATION OF BACONS PICKLE CURED AFTER 2, 4, AND 5 DAYS

Tables 12, 13 and 14 present changes in TVCs for PCRT bacons and their corresponding PCCT bacons.

The tables also showed similar trends as already seen with earlier treatments. The TVCs for PCRT bacon and the corresponding PCCT bacon, increased with increase in days of storage. Their associated gradients were positive. The only exception was 4 days PCRT bacon, (table 13), where the gradient was negative. (-0.072).

Increasing days at room temperature curing increased the TVCs on the first day of storage for PCRT bacons. Increase in days for coldroom temperature curing, decreased TVCs on the first day of storage for PCCT bacons.

Comparison of TVCs of PCRT bacons to their corresponding PCCT bacons, showed higher TVCs for PCRT bacons when observed on the first day of storage.

The TVCs of PCCT bacon increased in every comparison from the first day of storage of higher counts by the fourth day of storage than their corresponding PCRTbacon. The only exception was 5 days PCCT bacon which had no viable count compared with TVC of 3.469 for 5 days PCRT bacon on the fourth day of storage (Table 14).

Mostly, comparison of PCRT ham to the same-number-of-days-cured PCRT bacon and comparison of PCRT ham to the same-number-of-days-cured PCCTbacon showed the bacons with lower TVCs than hams.

Table 12 **CHANGES IN MICROORGANISM POPULATION FOR 2-DAY PICKLE CURED BACONS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	DCRT		DCCT	
			TVC	TVC/Day	TVC	TVC/Day
31.6	66.5	1	2.040		2.290	
				+0.450		+0.387
27.2	81.7	4	3.389		3.452	
				+0.603		+0.416
28.9	77.1	8	5.801		5.117	

Table 13 **CHANGES IN MICROORGANISM POPULATION FOR 4-DAYS PICKLE CURED BACONS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	DCRT		DCCT	
			TVC	TVC/Day	TVC	TVC/Day
30.6	67.0	1	2.602		1.370	
				-0.072		+1.157
26.1	75.0	4	2.386		4.842	
				+0.691		+0.980
26.7	80.8	8	5.151		5.233	

Table 14 **CHANGES IN MICROORGANISM POPULATION FOR 5-DAY PICKLE CURED BACONS**

Mean Temp. °C	Mean Rel Humidity/%	Days of Storage	DCRT		DCCT	
			TVC	TVC/Day	TVC	TVC/Day
35.1	61.8	1	3.009		0.000	
				+0.153		
29.4	74.4	4	3.469		0.000	
				+0.071		
25.8	79.9	8	3.753	-	-	

4.2.4 ENTEROCOCCI SP. ENUMERATION

Five different products were found to be contaminated after curing, smoking and storage in the smokehouse with *Enterococci sp. (Faecal Streptococci)*. Their log transformed numbers in the different products are as shown in table 15. The highest population (2.813) and lowest population (1.063) of *Enterococci sp.* were found in 5 days DCCT ham and 4 days DCCT ham respectively. From the results, it appears that low temperature (coldroom temperature) selected for *Enterococci sp.* growth. Other products had no *enterococci*.

Table 15: ENTEROCOCCI SP. ENUMERATION

PRODUCT	LOG TRANSFORMED MEAN COUNTS
5 days DCCT Ham	2.813
5 days DCCT Bacon	1.903
2 days PCCT Ham	1.699
6 days DCCT Bacon	1.297
4 days DCCT Ham	1.063

4.3 **BACTERIA ISOLATED IN CURE AND CURED HAMS AND BACONS**

Isolates identified in both dry and pickle cures included *Staphylococcus sp.*, *Streptococcus sp.* and *Proteus sp.*

Isolates from the cured hams and bacons during storage under ambient conditions in the smokehouse are as shown in table 16 in descending order of frequency of occurrence on the 28 cured hams and bacons in the experiment. *Staphylococcus sp.* and *Proteus sp.* were found on all 28 differently cured hams and bacons. *Bacillus sp.* were found on 27 cured products, *Escherichia coli* (7), *Streptococcus sp.* (5), *Enterococcus sp.* (5), *Serratia sp.*, (4), *Citrobacter sp.* (3), *Klebsiella sp.* (2), *Pseudomonas sp.* (2) and *Arizona sp.* (1).

Bacteria isolates found in both dry and pickle cured hams and bacons were similar. These are *Staphylococcus sp.*, *Streptococcus sp.* and *Proteus sp.*

TABLE 16:

**BACTERIA ISOLATES FROM HAMS AND BACONS
STORED UNDER AMBIENT CONDITIONS IN SMOKEHOUSE**

ISOLATE*	CURED HAMS AND BACONS	FREQUENCY
1. <i>Staphylococcus sp.</i>	All samples	28
2. <i>Proteus sp.</i>	All Samples	28
3. <i>Bacillus sp.</i>	All Samples except 2 days DCCT Bacon	27
4. <i>Eschericia coli</i>	4 days DCCT Ham 4 days DCCT Bacon 4 days DCRT Ham 4days DCRT Bacon 2 days PCCT Bacon 4 days PCRT Bacon 4 days PCCT Bacon	7
5. <i>Streptococcus sp.</i>	4 days DCCT Ham 6 days DCCT Ham 6 days DCCT Bacon 6 days DCRT Ham 6 days PCRT Bacon	5
6. <i>Enterococcus sp.</i>	4 days DCCT Ham 5 days DCCT Ham 5 days DCCT Bacon 6 days DCCT Bacon 2 days PCRT Ham	5
7. <i>Serratia sp.</i>	2 days PCCT Ham 2 days PCCT Bacon 2 days PCRT Ham 2 days PCRT Bacon	4
8. <i>Citrobacter sp.</i>	4 days DCCT Ham 4 days DCCT Bacon 4 days DCRT Ham	3
9. <i>Klebsiella sp.</i>	2 days DCCT Ham 2 days DCRT Ham	2
10. <i>Pseudomonas sp.</i>	4 days PCRT Bacon 5 days PCCT Ham	2
11. <i>Arizona sp.</i>	4 days PCCT Ham	1

*Many bacteria were not speciated due to the lack of biochemical reagents

4.3.1 MOULD ISOLATED FROM HAMS AND BACONS DURING CURING

Monilia sp were identified growing on all room temperature cured hams and bacons after the second day of curing. None was observed on any of the hams and bacons being cured under coldroom temperature.

4.3.2 MOULD ISOLATED FROM STORED HAMS AND BACONS

Table 17 presents mould isolated from cured hams and bacons during storage under ambient conditions in the smokehouse.



Mould identified and their frequency of occurrence on the cured hams and bacons were *Monilia sp.* (26), *Rhizopus sp.*, (24), *Penicillium sp.* (3), *Cladosporium sp.* (2) and *Aspergillus sp.* (2). For example, figures 6 and 7 show fungal growth on 2-day pickle cured room temperature (F) and 2 day – pickle cured coldroom temperature (E) hams stored for four days. The fungal growth was more profuse on the room temperature ham than the coldroom temperature ham, stored over a similar period.

4.3.3 YEAST ISOLATED FROM STORED HAMS AND BACONS

Yeast isolated originated from the family *Saccharomycetaceae*. They were isolated on five cured pork products. (Table 18).

**TABLE 17: MOULD ISOLATES FROM HAMS AND BACONS STORED
AMBIENT CONDITIONS IN SMOKEHOUSE**

ISOLATES	CURED HAMS AND BACON	FREQUENCY
1. Monilia sp.	All products except 2 days DCRT and DCCT Bacon	26
2. Rhizopus sp.	All products except 2 days DCRT and DCCT Bacon, 2 Days PCRT and PCCT Bacon	24
3. Penicillium sp.	2 days PCCT Bacon, 4 days PCCT Ham 4 days PCRT Bacon	3
4. Cladosporium sp.	4 days PCCT Bacon 4 days PCCT Ham	2
5. Aspergillus sp..	6 days DCCT Bacon 6 days PDCRT Bacon	2

**TABLE 18: YEAST ISOLATE FROM HAMS AND BACONS STORED
UNDER AMBIENT CONDITIONS IN SMOKEHOUSE**

ISOLATE*	CURED HAMS AND BACON	FREQUENCY
1. Saccharomycetaceae	6 days DCCT Ham 2 days PCCT Ham 2 days PCCT Bacon 4 days PCCT Bacon 4 days PCRT Ham	5

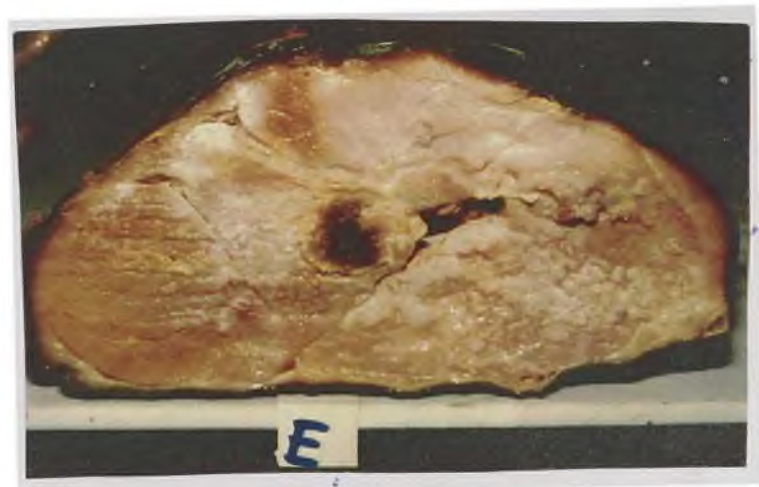
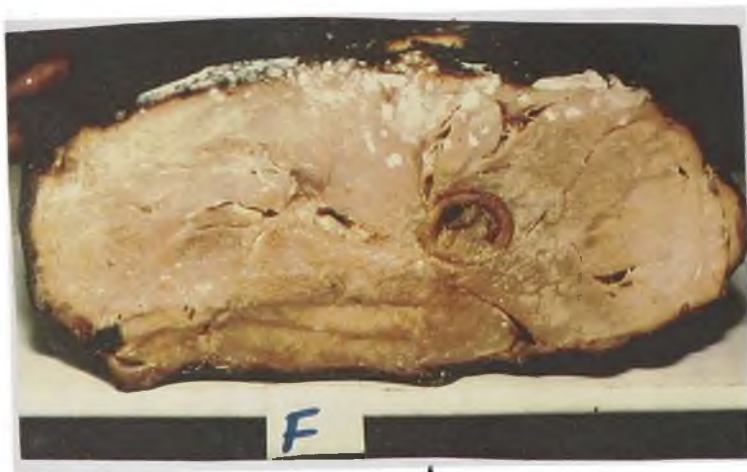


Figure 4: Photographs showing surface of 2 day.pickle cured ham under room temperature (F) and coldroom temperature (E),smoked for 10 continuous hours and stored in smokehouse for four days. There is profuse growth of fungus on (F)but less on (E)

CHAPTER 5

DISCUSSION

5.1 CHANGES IN MICROORGANISM POPULATION (TVC) OF STORED CURED HAMS AND BACONS UNDER AMBIENT CONDITIONS

The increase in TVCs during storage under ambient conditions conforms to the work of Kemp *et al*, (1974), Kayang, (1987) and Anang, (1995). In their work, Kemp *et al*, (1974) reported that, microbial counts increased as storage temperature and time increased. Likewise Kayang, (1987) reported that, increasing the length of storage outside the refrigerator under ambient temperature condition, permitted the proliferation of microorganisms. Most of the TVC/day or gradient of bacteria growth were positive and represent part or parts of the logarithmic phase of the growth curve of bacteria. The positive gradient indicates where bacteria cells had adequate nutrients to meet their physiological needs. However, the results obtained on a few occasions showed negative gradients. This represented the death phase of bacteria growth, where the accumulation of toxic materials from the bacteria pushed survival conditions to a minimum or there could have been incubation of some invading organisms. Increase in bacteria population during curing and even more so with increasing days of curing under room temperature conditions, resulted in subsequent increase in toxic material build up. It could also be due to the depletion of nutrients with increase in days of storage. Low levels of necessary nutrients essential for fastidious and exacting bacteria imply the dying off of those microbes, (Hechelmann and Kasprowiak, 1992). These reasons could possibly have led to the observed negative TVC/day.

The usually higher TVC seen in the coldroom temperature products over their corresponding room temperature products after 4 days of storage, was probably due to the earlier depletion of nutrients in room temperature products during curing. This is explained more by the fact that microbial spoilage was initiated in room temperature products during room temperature curing while their corresponding coldroom temperature products were intact and protected to a large extent against microbial attack by the low temperature environment except for psychrophiles. Hence, during storage under ambient conditions in the smokehouse, microorganisms in cold room temperature cured hams and bacons had the opportunity of proliferating in the hams and bacons during storage under ambient conditions.

Unhindered microbial build up under room temperature curing (where growth conditions were optimal) as compared to a hindered and limited microbial growth under coldroom temperature curing (where low temperature was a limiting factor) may have been the main reason why TVCs of room temperature products were higher by day 1 of storage than their corresponding coldroom temperature products. Although smoking reduces microbial count, (Ulrich and Halvorson, 1951), its effect is known to be more on the surface of the meat product. The deeper tissue of hams especially might have needed more than 10 hours of smoking at 59°C for the heat to be transferred into deep muscles and temperature raised to a over 50°C to reduce the internal microorganism population effectively. This probably was not attained in the experiment. Hence the higher TVCs observed in room temperature cured products over their corresponding coldroom temperature cured products by day 1 of storage. Occasionally coldroom temperature cured products had TVCs that were higher than their corresponding room temperature TVCs. This may be due to contamination of the coldroom temperature

products or carcasses or colonisation of the products by cold storage flora. This includes *Pseudomonas sp.* and *Achromobacter guttatus*, (Price and Schweigert, 1971). The growth of putrefactive spoilage microorganisms, and most enzyme activities are greatly reduced at low temperatures, (Forrest *et al*, 1975). This also explains why TVCs of coldroom products were relatively lower than their corresponding room temperature products. The initial microbial load has a profound effect upon the spoilage life of fresh and processed meat products. However the minimization of further contamination during all subsequent handling, processing, packaging and storage is essential in order to maintain optimum qualitative properties and also prolong shelf-life, (Forrest *et al*, 1975).

Comparing any bacon to its similarly treated ham, showed lower counts for the bacon than the ham. This observation conforms to that of Kayang, (1987). He attributed it to the thinness of the bacon which permits a more effective penetration of smoke and heat, which controlled the growth of microorganisms. An additional reason suggested by Frazier, (1958) is that, the higher fat content of bacon than ham protects it from microbial degradation. This is because fatty acids are antinutritional factors for many microbes.

5.1.1 CHANGES IN MICROBIAL POPULATION AS AN INDICATOR OF SPOILAGE

The definition of spoiled meat is variable among different societies. What one individual describes as spoiled might be considered edible by another, (Forrest *et al*, 1975). They commented further that, spoilage in its usual connotation, is frequently

adequate with the decomposition and putrefaction that results from microorganism activity. Some authors like Jay, (1978) believe that, the build up of microbial population causing the spoilage in the meat products may go along with visible spoilage signs like colour change, softening of meat texture, putrefaction, slime formation and decomposition. For this experiment, very acute off-odour of the cured and stored hams and bacons was the first criteria used to determine the shelflife. With experience, this method is one of the best, (Forrest *et al*, 1975). However it is subjective and there is still a lack of scientific consensus regarding standardization of measurements methods for odour and the method is cumbersome, laborious and costly, (Baker, 1998).

Undesirable changes in colour of the cured hams and bacons as was observed in present experiment with increase in days of storage may have been oxidation of myoglobin to metmyoglobin, Lawrie (1985) or pigments produced by microbes like streptococci, (Whitely and D'souza, 1989)

Other authors have suggested the use of critical TVCs above which meat products may not be consumed. Bacteria counts equal to and above 10^7 represent a food product of terrible and unacceptable microbial count with very low nutrient content and necessary to cause illness, (Smith, 1969). In fact, Anang, (1995) supported this assertion that bacteria counts of the magnitude 10^7 C.F.U./cm² is unacceptable. However, Hechelmann and Kasprowiak, (1992), reported that, TVCs are guide values and recommendations and are not legally binding. They continued that, these values may be useful indicators of the history on the storage of the meat and possible health implications when consumed. These values vary from country to country. For example the recommended maximum level of TVC for heat --processed and smoked meats in

Oregon, U.S.A. is 10^6 C.F.U./1gm of meat, Powers, (1976); while Jay, (1978) proposed limits of a low maximum 10^6 and high maximum of 10^7 C.F.U. for a gramme of fresh and frozen fish, frozen raw and comminuted meat, and non-frozen ground beef. The maximum TVC values in the present experiment were reasonably within the ranges stated by the various authors above. (Tables 1 – 14) These limits could thus have also being the reference values. Presently in Ghana, no such limits of recommended or proposed microbiological limits have been set. It is however anticipated that, the Food and Drugs Board (Ghana), Standards Board (Ghana), the Universities and Research Stations in Ghana will collaborate and come out with some form of recommendations.

5.1.2 EFFECT OF DRY CURE AND PICKLE CURE ON MICROBIAL GROWTH AT ROOM TEMPERATURE

In the majority of the products cured over different lengths of days, the higher TVCs of room temperature products over the cold room temperature products, indicates how less effective room temperature curing was in keeping down bacterial population. Frazier, (1958), Forrest et al, (1975), Jay, (1978) and Anang (1995) reported that, no type of cure of any strength is capable of resisting total microbial growth and spoilage for a long time when extrinsic factors like storage temperature, moisture level (relative humidity) and oxygen levels are optimum. Temperature, especially is a critical factor that will determine the stability and shelflife of any cured hams and bacons. It was clearly realised from the experiment that, no cold room temperature cured hams and bacons emitted any off-odour during curing. This was because, low temperatures, are even lethal to many microbes. No matter the level of hygiene maintained, the processing (curing) of any meat cannot be devoid of microorganisms. The sources of bacteria during room temperature curing were numerous. Literature by Frazier, (1958) Forrest *et al*, (1975), Jay; (1978) and Anang (1995), suggests that, room temperature

(28°C) is high enough to permit spoilage of cured hams and bacons. Very pungent off-odour by curing day two was the result of microbial degradation of the meat. Although no plate counts were done during room temperature curing, off-odour production was enough assessment of the increasing microbial population with increase in days of room temperature. Five days was the maximum number of days possible for both ham and bacon during pickle curing at room temperature. The high moisture content of the injected pickle (66.13%) as well as the total immersion in pickle solution, greatly increased the water activity for microbial spoilage. This fact coupled with the relatively high room temperature (26.5 – 28°C) during curing, high internal moisture content of ham and less fat content, rendered ham greatly vulnerable to bacterial attack and spoilage. Therefore microbial counts from hams were usually higher than their corresponding similarly cured bacons group which had higher fat content, lower moisture content and were thin in thickness and very broad in surface area. The width and broadness of bacon, presented a very wide surface area which led to the quick and effective drying up of the surface of the bacon. This in turn probably lowered the water activity and favoured less microbial growth.

Six days was the maximum number of days possible for room temperature curing. The difference of one day between the maximum of six curing days for dry cured room temperature products and the maximum of five curing days of pickle cured room temperature products was probably due to the lower moisture content in dry cure. The 8-3-¼ sugar mixture in the dry cure represented 70.8% sodium chloride, 27.5% sugar and 1.7% sodium nitrite. The only fluid available for microbial usage were the extracellular and cell fluid of the hams and bacons. This fluid would be far lower in the dry cured products due to exosmosis. The higher moisture content (66.13%) in pickle

cured products (especially ham) plus that of the extracellular and cell fluid, enhanced bacteria to cause spoilage quicker than it would be in dry cured products. Another explanation, by Forrestt et al, (1975) suggests that, increasing the salt content of meat lowers the isoelectric point and thus the pH. It seems that, the magnitude of the pH shift depends on the amount of sodium chloride added and leads to water oozing out of the meat products. It is therefore not surprising that, dry cured products with 70.8% sodium chloride appeared drier than pickle cured (24%) products.

On the whole, it may be summarized that any type of room temperature curing has a limited effect in controlling microorganism population. This control is within the first few days of the curing, after which spoilage is complete.

5.2 MICROORGANISMS ISOLATED IN CURE AND CURED HAMS AND BACONS

Many of the bacteria isolated are of great public health importance to man. This is because they cause illness by the ingestion of the bacteria or their toxins which results in food contamination. Many of the microbes cause gastroenteritis. The illness caused render their victims a liability to their dependants. Secondly, the bacterial activities in many of the food cause spoilage amounting to huge financial losses in the food industry. Some can lead to death.

5.2.1 BACTERIA IN CURE

The three microorganisms isolated in the cure were *Staphylococcus sp.*, *Streptococcus sp.* and *Proteus sp.* Their physiological activities initiated off-odour. Random sampling of microbial contaminants in the deep tissues of ham and bacons

mostly revealed no microbes before curing. Identification of microbes in deep muscle tissues with petrifilms on the few occasions bacteria were isolated showed them to belong to the family *Enterobacteriaceae* but excluded *Eschericia coli*. This implies that, the likely source of *Staphylococcus sp.* and *Streptococcus sp.* is the cure.

The isolation of staphylococci in the cured hams and bacons, conforms with the works of Kemp *et al*, (1974); Jay, (1978); Kayang, (1987); and Hechelmann and Kasprowiak, (1992). Earlier, Castellani and Niven, (1954) and Kemp, (1974) had confirmed that many varieties of staphylococci, grow quite readily on cured meats. Some were coagulase positive others were negative. Debruyne, (1998) indicated that, the prevalence of staphylococci in any food is an indication of contamination from the skin, nose, arms, throat, hands, fingers, hair, face and eyes of personnel working with the cure or meat samples. These bacteria are associated with mucous membrane and skin. Inadequately cleaned equipment, like knives and axes, may also be sources of staphylococci, (Thatcher and Clark, 1975) as well as the curing ingredients (salt and sugar) which were purchased from the open market for this project.

Kayang, (1987) reported that, the presence of large numbers of staphylococci generally indicate that, sanitation and temperature control have somewhere been inadequate. This means that, storage of the cured products under ambient conditions, provided the necessary atmosphere for the survival of mesophilic bacteria (of which staphylococci are part) in the cure and cured hams and bacons. The ability of staphylococci to survive in salt medium is due to their high tolerance to salt under warm temperatures, (Gradwohl, 1973). Some of the toxigenic cocci are very salt tolerant and grow in sodium chloride solutions that approach saturation, while some species also

tolerate nitrite fairly well and therefore can grow in curing solutions and cured meat if other environmental conditions are favourable, (Frazier, 1958). They are fairly tolerant to dissolved sugars. Though the staphylococci isolated was not speciated, it must be noted that, there are grave implications if any of the isolated species are among the pathogenic types. This is because, they contribute to the stability and safety of the meat product, (Hechelmann and Kaksprowiak, 1992). Pathogenic staphylococci produce heat stable enterotoxins that can cause food-poisoning with diarrhoea, nausea, vomiting, severe abdominal cramps and weakness, (Jay, 1978). He stated further that, the pathogen, *Staphylococcus aureus*, also produces boils and carbuncles in man while their enterotoxin cause gastroenteritis or inflammation of the lining of the stomach and intestines. To avoid any such illnesses, cured hams and bacons should be adequately and properly cooked before eating. Unfortunately the toxin of *S. aureus* is heat stable; so cooking will only eliminate the vegetative cells but not the toxin.

Streptococcus sp. has wide temperature range of growth (10 – 45°C) with some degree of salt tolerance. The presence of this species still goes to emphasize the probable lack of proper hygiene on handling of carcasses during slaughter and processing. Some species are associated with the upper respiratory tract infection of man and other animals. The presence of this bacteria may therefore not be due only to the cure but also the carcasses. Ingestion of meat with this contaminant may cause diseases such as scarlet fever and septic sore throat. They are also associated with mastitis.

Proteus sp. may be found in the intestinal tract of man and animals and the presence of some species in foods in large numbers, may indicate faecal contamination. This raises a big question about the hygiene standards of the persons who were selling the common salt and sugar used as cure on the open market. Education about personal hygiene and some basic teaching of food microbiology is strongly recommended for those who deal in food items. There was a low frequency of occurrence of *Proteus sp.* in the cured hams and bacons after curing and smoking. This means that, certain factors in the cured hams and bacons might have discouraged their growth. This may be due to their low degree of tolerance to salt and the effect of smoking which decreased with the water activity of the cured products. The presence of *Proteus sp.* signifies contamination of the carcass with the gut content and offals during evisceration perhaps by inexperienced or careless butchers in the slaughtered house, (Gracey, 1981). Animals for the present experiment were slaughtered on a slaughter slab. Cross-contamination may have taken place from the slab to the carcass or the spilling of the gut content with the meat. This could have been the probable source of *Proteus sp.* to the meat. Another source of this bacteria may be from a decaying material of plant or animal origin which may have found its way onto the meat or salt used. (Jay, 1978). Inefficient disinfection after slaughtering and deboning, results in pieces of left over animal tissue to become the sites of multiplication for *Proteus sp.* Another probable reason, though quite remote with respect to the present experiment, is the issue of "miss-cured" hams. Hams that are not properly cured, may sometimes be the suitable substrates for *Proteus sp.* (Gracey, 1981). Others are of the opinion, that, the presence of *Proteus sp.* may be an indication of a probable wound infection, pus from abscesses, urinary tract infection, otitis, dysentery and diarrhoea before slaughter.

Although starter cultures are used in curing meat in the meat industry, care needs to be taken to use desirable microorganisms that impart desirable flavours during curing. All cure before application should be sterilized before the desired microbes are introduced into the cure for the curing process. With reference to the three proteolytic bacteria (*Staphylococcus sp.*, *Streptococcus sp.* and *Proteus sp.*) isolated in the cure before curing, sufficient heat must be applied to destroy them before cure application as dry or pickle cure is made.

5.5.2 FUNGAL GROWTH DURING ROOM TEMPERATURE CURING

The results showed no fungal growth during coldroom temperature curing. *Monilia sp.* and sometimes its perfect stage of growth, *Neurospora sp.* was found growing on dry cured room temperature hams and bacons. Their growth was much prominent on dry cured room temperature bacons. No fungal growth was however observed on 2 days DCRT products. Smith, (1969) reported that *Monilia sp.* is able to grow on the products because it is osmophilic and therefore can tolerate high salt temperatures on the surface of the products. It can also grow between warm temperatures and even very low temperature as -40°C . Growth was more profuse on bacon because *Monilia sp.* possesses lipase enzyme and is a saprophyte. It is easy for the spores of the fungus to fall on the flat broad bacon surface whenever it was exposed during room temperature curing. The presence of *Monilia sp.* indicates that, the curing room and surrounding areas have a high concentration of the organisms which may initiate spoilage of meat products cured and prepared in the vicinity. Its presence is also indicative that bacterial growth is taking place in the product and is far advanced. This inference is made because the growth of bacteria, paves the way to a sharp lowering of the pH of meat to limits unsuitable for the growth of many other bacteria except for

some fungi like *Monilia sp.*. *Monilia sp.* was absent on 2 days DCRT products probably because the pH, was relatively too high to allow for its growth.

The absence of *Monilia sp.* on pickle cured products at room temperature was due to the fact that, products were submerged in the pickle cure. It therefore had no organic substrate to grow on.

5.5.3 MICROBIAL CONTAMINANTS IN STORED CURED HAMS AND BACONS

5.5.3.1 BACTERIA

Apart from *Staphylococcus sp*, *Streptococcus sp*, and *Proteus sp*. which were found in the majority in the hams and bacons prior to smoking, other bacteria were isolated from the products during storage in the smoke house after smoking. These were *Bacillus sp*. *Escherichia coli*, *Streptococcus sp*; *Enterococcus sp*. *Serratia sp*. *Citrobacter sp*; *Klebsiella sp*, *Pseudomonas sp* and Arizona sp.

The presence of these microorganisms indicate the type of handling and contamination that might have taken place after curing of the hams and bacons. The presence of these microbes were also similar to microorganisms normally found on cured meat as indicated by Jay, (1978), Hechelmann and Kasprowiak, (1992), Muller and Fehlhaber, (1995) and Schuppel, Salchert and Schippel, (1996). The source of many microbes is the soil, (Jay, 1978): Spores of bacilli may be easily conveyed in the air from the soil on to cured hams and bacons after the opening of the smokehouse door for the taking of samples or go through the chimney of the smokehouse. It is not easy controlling bacilli because there are many varieties ranging from aerobic to facultative types and can hydrolyze meat proteins, (Muller and Fehlhaber, 1995). The

same authors reported that, *Bacillus sp.* are saprophytic and belong to the group of microbes with the highest proteolytic activity. In some cases, enzyme activities of *Bacillus sp.* are still detectable even after the longest heat exposure times of 20 minutes at 71°C and 10 minutes at 75°C. These residual exoprotease activities can survive a wide range of heat treatments used in the food processing industry and can, in certain milieus lead to undesirable changes in the stored products, (Muller and Fehlhaber, 1995). Deterioration of the cured hams and bacons and the effect on sensory properties are partly attributed to these exoprotease activities. During the experiment, smoking temperature was below 63°C and therefore implies that, the bacilli exoproteases present were not inactivated. In order to minimize the incidence of bacilli, it would be expedient to keep the cured hams and bacons in an environment that has a minimum access to air blowing from outside.

The incidence of *Escherichia coli* is a clear indication of exposure of the meat to animal and human gut contents or faecal matter. The presence of the other gram negative species *Serratia sp.*, *Pseudomonas sp.*, *Proteus sp.* and other members of the group *Enterobacteriaceae*, is also strongly indicative of cross-contamination of the meat with gut contents of mammals and birds. In the present experiment this may have been during exsanguination or "sticking" of the animal with an unclean knife which had been used earlier to eviscerate an animal. Gram negative bacteria were in the majority of the microorganisms isolated Board, (1968), reported that, the predominance of gram-negative rods in products, is an indication of abundance of supply, of relatively simple nutrients in the product. Many of the gram negative bacteria have a relatively simple nutritional need. Autolysis or preliminary hydrolysis of proteins by the meat enzymes undoubtedly helps microorganisms start growing and cause spoilage with off-odour in

the meat by furnishing the simple nitrogen compounds needed by many microorganisms that cannot attack complete and complex native protein. After the use of simpler nutrients, only microbes with advanced form of nutrition can survive, (Frazier, 1958). According to Gracey, (1981) some of the organisms with advanced form of nutrition included *E. coli*, *Proteus sp.* and *Pseudomonas sp.* as one group and the second group are those with simple form of nutrition including *Serratia sp.* and *Klebsiella sp.* These two groups of organisms were encountered on the cured products between the first and fourth and eighth days of storage. Sapong, (1990) reported that, *Serratia sp.* and *Klebsiella sp.* represent possible aerobic contaminants, and may have found their way into the cured meat whenever they were occasionally blown unto the meat from the ground or during washing of the carcasses with contaminated water. The variable mode of cross-contamination may explain why the bacteria occurrence on the various cured meat products was without pattern.

The incidence of *Arizona sp.* a closely related species of *Salmonella sp.* and causal agent of bacillary dysentery, typhoid and enteric fevers, (Gradwohl, 1963), indicated cross-contamination of the products, (Table 16) by the personnel working with the meat, and insects such as flies and cockroaches. Some houseflies (*Musca domestica*) were occasionally observed around the smokehouse. Some of the flies may have entered the smokehouse through the chimney and transmitted bacteria and even possibly, *Streptococcus faecalis* from a gut source or contaminated matter to the products in storage. The chances of this type of contamination occurring more frequently was lessened by placing a wire mesh around the chimney during storage in the smokehouse. The low frequency of occurrence of these two pathogens over the duration of the whole experiment goes to indicate the little role *Musca domestica* played

as a vector during spoilage. *Streptococcus faecalis*, is known to be very resistant to curing and is only destroyed at temperatures above 72°C. This probably assured their survival since the maximum temperature during smoking was 62°C.

Pseudomonas sp. frequency of occurrence was lower than normally expected on meat products. Most of the species that occur in this genus, are cold storage microflora and do not thrive well in warm conditions. Few have the ability to thrive successfully under a wide range of temperatures ranging from room temperatures to temperatures lower than room temperature, (Saviour and Board, 1972). *Pseudomonas sp.* is able to utilize extensively the native proteins of the cured hams and bacons for satisfying its nutritional requirement, (Board, 1965) and is highly suspected as one of the species contributing to the foul odour produced during the experiment, (Gracey, 1981).

5.5.3.2 MOULDS

The contamination of a food product by an agent of food-borne diseases constitute a hazard to the health of consumers, (Saudi and Mansour, 1990). The presence of the five fungi isolated is in agreement with authors like Jay, (1978), Kiermier, (1981), Leistner and Eckardt, (1981), Orth, (1981), Gracey (1981) and Mansour, (1986).

Most of the moulds isolated from the cured hams and bacons are very important, (table 17). They included *Aspergillus sp* and *Penicillium sp.* which can produce mycotoxins in meat and milk products, Kiermeir, (1981) and Leistner and Eckardt, (1981) or even carcinogenic mycotoxins by *Aspergillus sp*, (Jay, 1978); *Cladosporium sp.* which is responsible for the development of black spots on preserved meat at low

temperatures (-6°C) or temperatures just below freezing point, Smith, (1969) and Mansour, (1986). Some strains of *Penicillium sp.* which produce toxins at low temperatures, (Orth, 1981). The rest of the fungi, isolated in the experiment, were *Monilia sp.* and *Rhizopus sp.* which are noted largely for food spoilage, (Jay, 1978).

Gracey, (1981) reported that, *Aspergillus sp.* *Penicillium sp.* and *Rhizopus sp.* have a wide limit of growth conditions and are also saprophytic and mesophilic. This accounts for their ability to grow on the cured hams and bacons. They grow at temperatures ranging from 20-30°C with water activity ranging from 0.88 to 0.80,

Gracey (1981). Possession of such wide growth capabilities accounts for the development of their spores on any cured ham or bacon. Their capabilities favour moist osmophilic conditions similar to those that existed (especially) on pickle cured products and dry cured ham. These cured products had high moisture and salt levels. The existing relative humidities and temperatures, ranging from 46.6 – 80.1% and 25.5 – 35.1°C respectively, provided adequate growth conditions for these mesophilic fungi. The moisture level in the cured hams (especially room temperature cured products), decreased with increase in days of storage in the smokehouse. This affected the water activity of the cured products by decreasing it and thus favoured fungal growth, (Frazier, 1958). Such factors account for the almost frequent presence of *Rhizopus sp.* and *Monilia sp.* on the cured hams and bacons.

5.5.3.3 YEASTS

No yeast cells were found in any of the cures but only in a few of the cured

products, (table 18). Their source is likely to be through spores floating in the atmosphere or picked from the curing table. Organisms may also have contaminated the cured hams and bacons during storage, as well as the slicing of the products for samples. Taking of samples for microbiological examination involved cutting a cross-section of the smoked hams or bacons, which exposed a new fresh muscle surface. A floating spore could therefore, easily settle on it from the atmosphere. Smoking of cut meat products surfaces after slicing is therefore highly recommended.

The growth of the isolated yeast cells (*Saccharomycetaceae*) were in only five products out of the twenty-eight products employed in the experiment. This low frequency of occurrence is in accordance with literature. Infact, studies carried out on yeast developed inside cured meat products are few and this probably may be due to the fact that some authors like Francisco et al, (1981) Jociles et al, (1983), Hugas *et al*, (1987), had not detected their presence or because, as in other cases, Giolitti *et al* (1971) and Carrascosa *et al*, (1988), had recorded very low levels. They may thus not play an important role in the curing process, (Molina, Silla and Flores, 1990). From the results, the yeasts isolated thrived well on pickle cured products, irrespective of the temperature of curing. This implies that, coldroom or room temperature curing was not capable of preventing the yeast from utilizing the available nutrients in the cured products. Yeasts are normally found on or in food products with a low water activity. However, it is still in accordance with Smith, (1969), Mansour, (1986) and Saudi and Mansour, (1990) that, they grew well in moist filled pickle cured products. The yeast isolated belong to the group of yeasts with physiological characteristics that permit generalizations fitting many habitats. Firstly *Saccharomyceteae* grow best with plentiful

supply of available moisture, (Frazier, 1958). He further indicated that, they are osmophilic, where they grow well in high concentration of sugar as was used in the pickle. In any case, osmophilic yeasts have a very limited growth, if at all with a water activity of 0.78 in both brine and sugar syrup. Possibly growth of yeasts in the cured products was not realised because of temperature variations which might have influenced the movement and distribution of cure solutes in a manner that could not support yeast growth.

It must be noted that the water activity values of the cured products vary with the nitrite properties, pH, temperature, availability of oxygen and presence of inhibitory substances, (Frazier, 1958). Very little is however, known about the effect they may exert on meat products when they develop in the interior of ham, (Molina, Silas and Flores, 1990). Certainly, their development on the surface of cured products have an evident influence on their sensory quality. This is because they improve the organoleptic characteristics on flavour.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

No type of cure (dry or pickle) was strong enough to prevent microbial growth under room temperature curing. Increase in days of room temperature curing, resulted in a corresponding off-odour particularly noticeable after 2 days curing with corresponding changes in muscle structure and colour in all cured hams and bacons. The maximum number of days of curing for both dry cured hams and bacons was 6 days. It was 5 days for pickle cured hams and bacons at room temperature. The corresponding coldroom curing did not cause any noticeable off-odour with increase in days of curing. Increase in days of coldroom temperature curing resulted in decreased TVCs after storage in the smokehouse for a day.

Storage of all dry pickle and cured products under ambient conditions in the smokehouse saw a progressive deterioration with increase in days of storage. The deterioration was particularly greater and more acute for room temperature cured hams and bacons than their corresponding controls.

Randomly sampling of all fresh hams and bacons usually showed no microbes in deep muscle tissues. Bacteria isolated from the cure (dry and pickle), were the same in the deep muscle tissues of cured hams and bacons. These included *Staphylococcus sp.* and *Proteus sp.* The source from which the cure was purchased was highly suspicious and brought into question the level of hygiene that is maintained by some

open air market sellers. Other contaminants during storage of the cured pork products included pathogenic and non-pathogenic bacteria of various implications. Gram negative enteric bacteria were in the majority and comprised *Arizona sp.*, *Pseudomonas sp.*, *Klebsiella sp.*, *Serratia sp.* and *E. coli*. Others were the spore forming *Bacillus sp.* and the problematic *Streptococcus faecalis*, which is noted for its extreme resistance to extermination in salt cure even at high temperatures (70°C) during curing.

Fungi isolated on the hams and bacons during room temperature curing and storage belonged to the genera *Penicillium sp.*, *Cladosporium sp.* and *Monilia sp.* Yeast present belonged to the family *Saccharomycetaceae*.

Due to decreased TVCs, and less likelihood of the growth of pathogens under cold room temperature, it is recommended that curing should be done under low temperatures rather than high temperatures.

Comparison of the TVCs of room temperature hams and bacons to their respective controls did not usually show very large differences in magnitude during storage. Hams that were dry cured or pickle cured under room temperature or coldroom temperature respectively had higher TVCs than bacons cured under similar conditions. Bacons are thus less likely to carry bacteria load high enough to cause diseases.

Curing under room temperature conditions is recommended only for bacons. The recommended or safest length of time for room temperature pickle curing is two days and that for room temperature dry curing is four days. Hams should not be dry or pickle cured under room temperature conditions due to high possibility of the growth of pathogens which can lead to illness.

There was not specific association of any microorganism to any particular cured product. The source of contaminants were from the cure and the environment. Cross-contamination was evident. The presence of *Staphylococcus sp.* implies contaminations from humans (working personnel) or animals. It indicated the possible presence of heat stable and enterotoxins which can be the source of gastro intestinal diseases. *Eschericia coli* implies faecal contamination. *Arizona sp.*, *Klebsiella sp.*, *Proteus sp.* and *Enterococcus sp.* have public health significance.

It is highly recommended that food handlers and dealers on the education on the handling of food and the maintenance of proper hygiene. Legislation should be enforced to insist that these personalities have a regular health check up. It is highly recommended that all cure be heat treated before application.

Knives and surfaces should be cleaned well before use and some amount of smoking should be done after the slicing off of a piece of the cured pork hams and bacons for use. Cooking of meat to be eaten must be adequately cooked before serving.

As much as possible, smokehouses used should be made from material (such as clay) to allow effective cooling. This would ensure low storage temperatures. The smokehouse should not be in the direct path of sun rays or near a heat emitting source, strong wind paths and must be as far as possible from unhygienic sources like a rubbish dump. Its door must be tight fitting to prevent entry of pests like cockroaches, flies and rodents.

Animals should also be thoroughly examined or inspected by qualified personnel and slaughtered under hygienic conditions. Proper disinfection of slaughtered houses and equipment should be a must.

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FIGURE a

Temperature & Relative Humidity variation during storage under ambient conditions.
(2 days dry cure ham and bacon- Experiment 1)

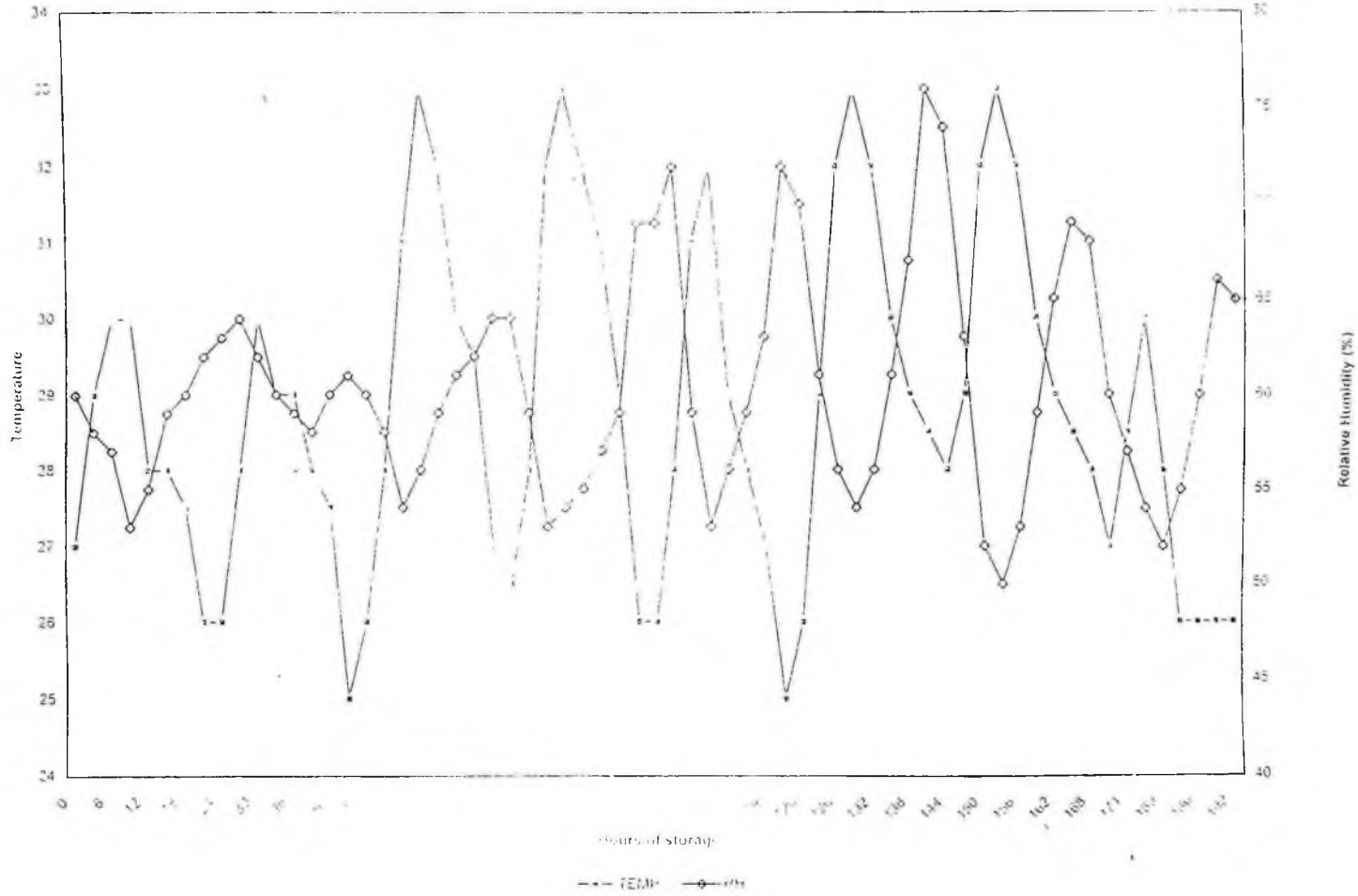


FIGURE b

Temperature & Relative Humidity variation during storage under ambient conditions.
(4 days dry cure ham and bacon- Experiment 2)

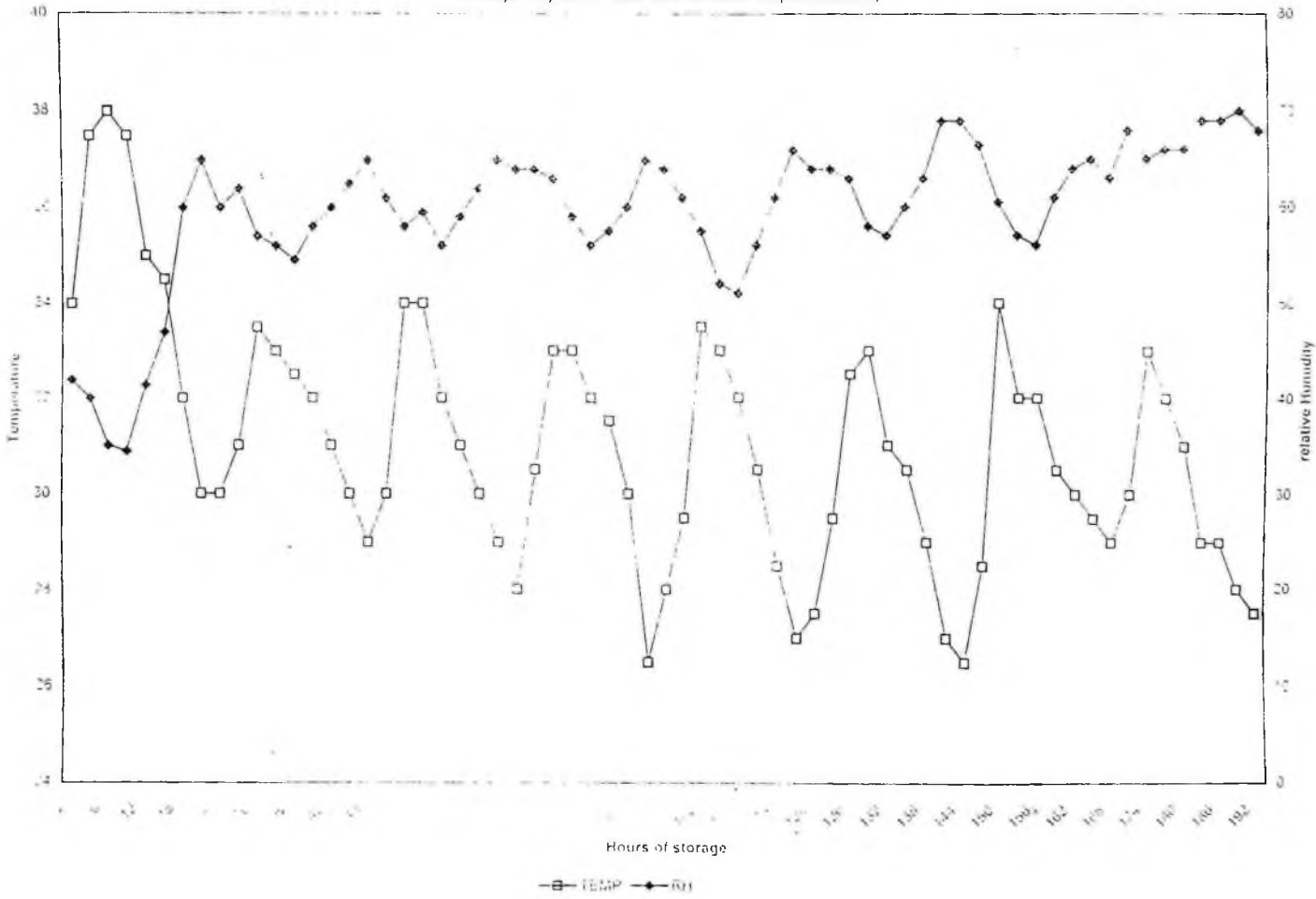


FIGURE c

Temperature and relative humidity variation during storage under ambient conditions.
(5 days dry cure ham and bacon- Experiment 4)

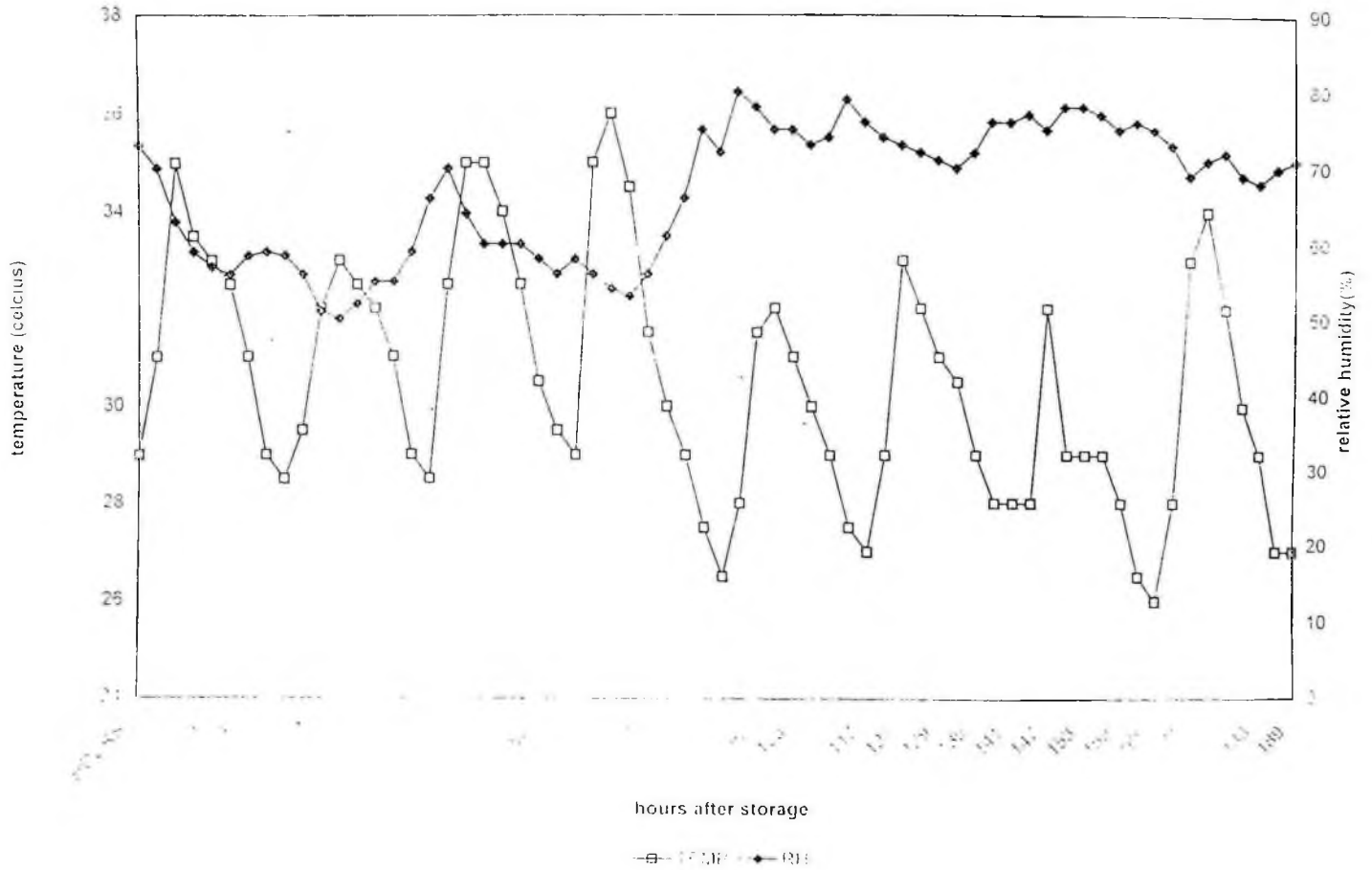
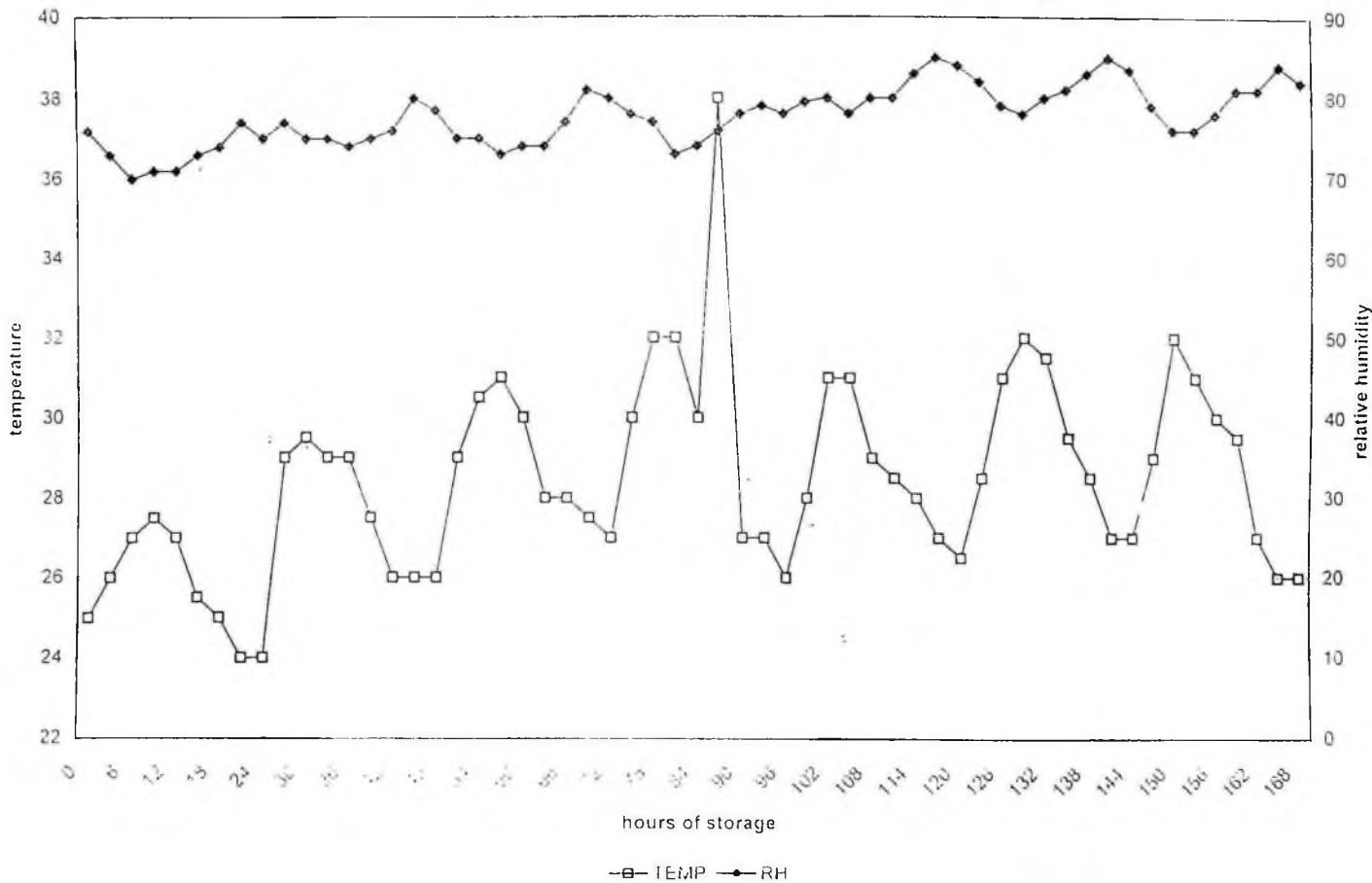
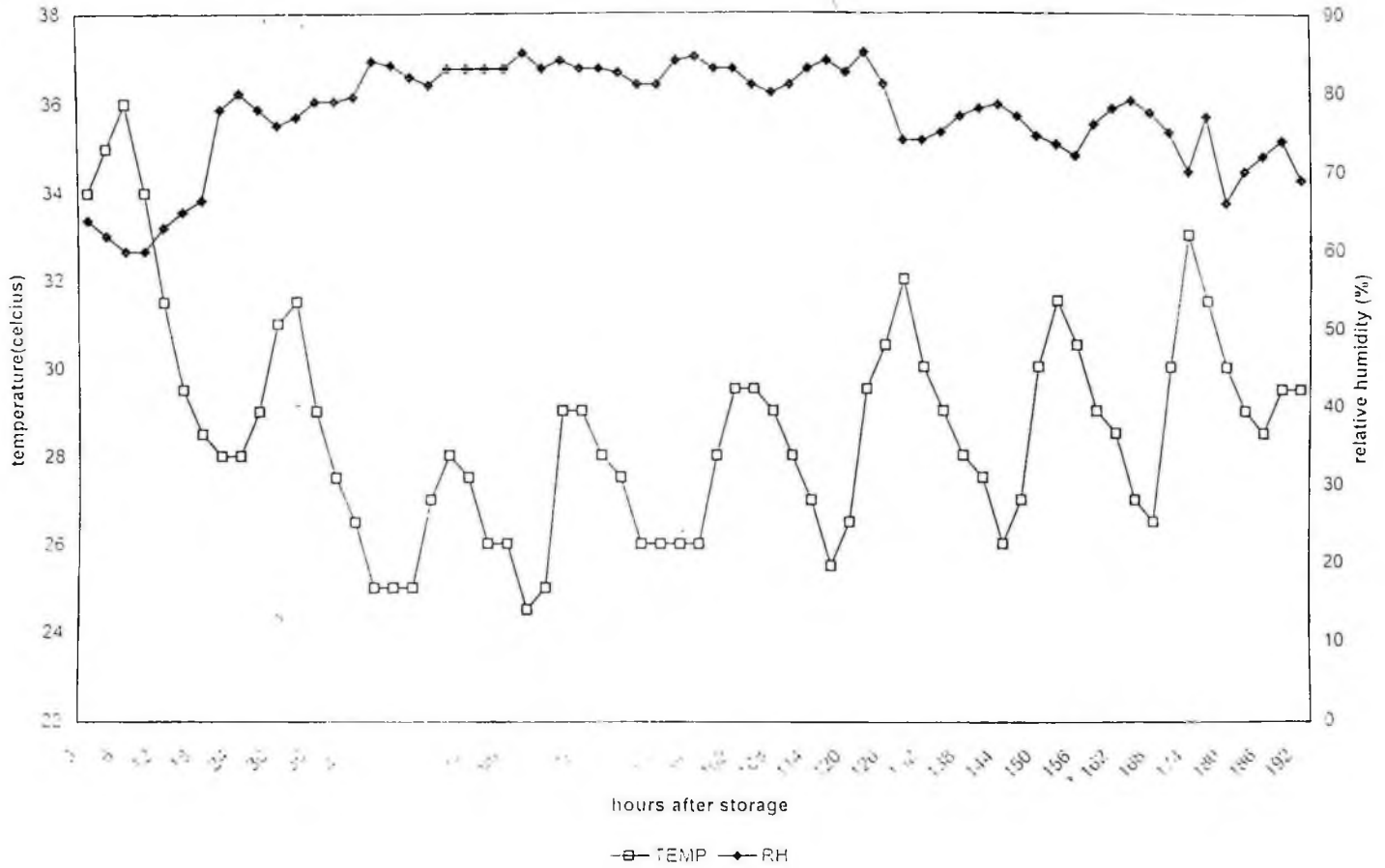


FIGURE d

temperature and relative humidity variation during storage under ambient conditions
(6 days dry cure ham and bacon- Experiment 3)



Temperature and relative humidity variation during storage under ambient conditions.
(2 days pickle cure ham and bacon- Experiment 5)



Temperature and relative humidity variation during storage under ambient conditions.
(4 days pickle cure ham and bacon- Experiment 6)

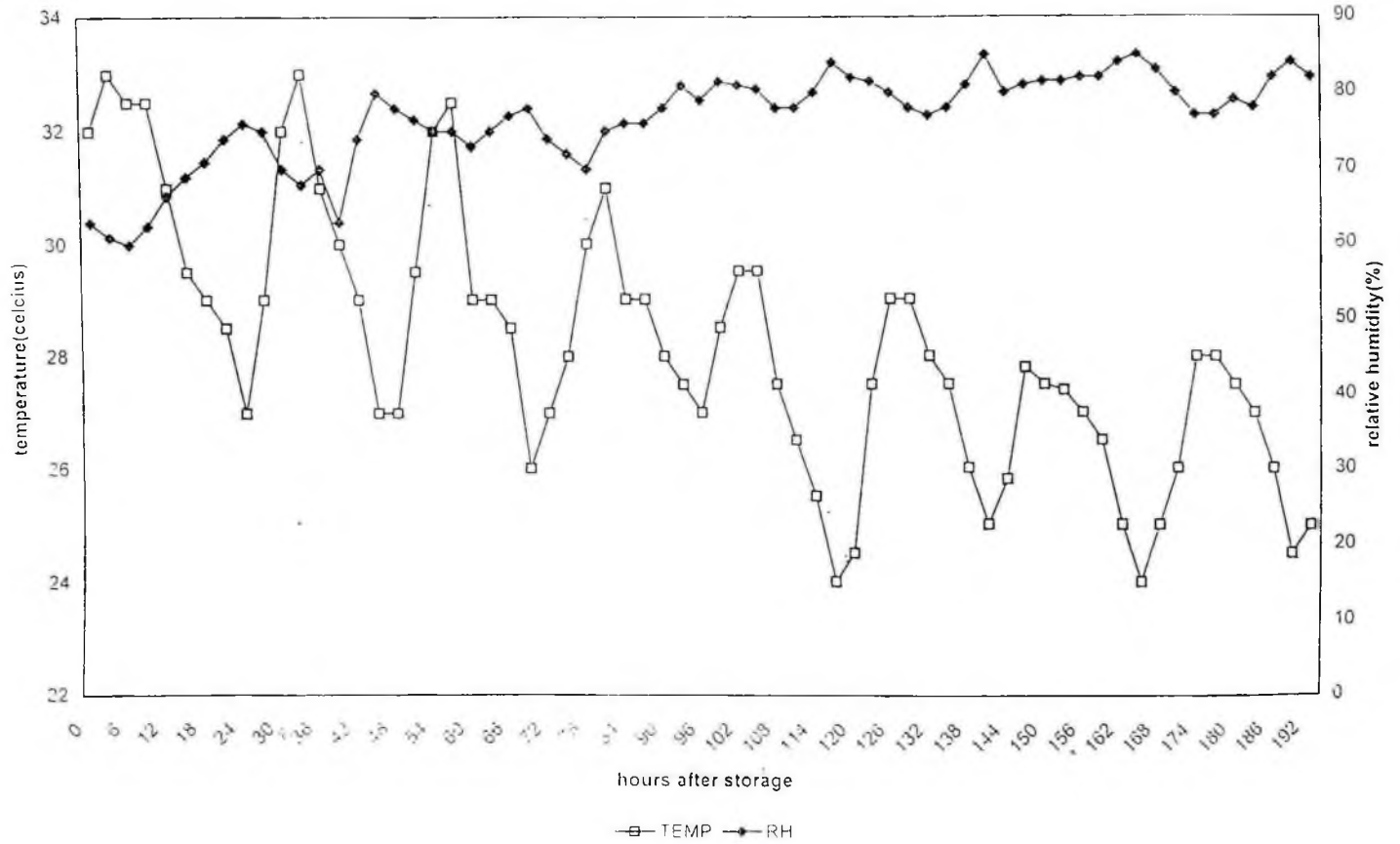


FIGURE 8.3

Temperature and relative humidity variation during storage under ambient conditions.
(5 days pickle cure ham and bacon- Experiment 7)

