FURTHER STUDIES ON THE KEEPING QUALITY OF CURED AND/OR SMOKED CHICKEN DURING STORAGE

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CERTIFICATE

This is to certify that the entire research work and results presented in this thesis entitled "Further studies on the keeping quality of Cured and/or Smoked chicken during storage," have been carried out by Shri Ramjee Prasad Singh, Research Fellow (I.C.A.R.), under my guidance and supervision.

(T.D. Mahadevan)

Dated, 30th May, 1975.

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DEDICATED

TO

MY PARENTS

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C O N T E N T S

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CHAPTER I

Introduction

INTRODUCTION

Nearly two-third of the world's population live in countries which are technologically less advanced than the nations of Europe and N. America. Because of the large population in the less advanced countries, the per capita consumption of food is much below the recommended standards by the nutritional committees of W.H.O. Where the congregation of the population is greater than the land could bear, agricultural production is low as reported by U.N.O. (1963). This statement is best suited for our country. Since the land mass cannot be stretched and as the human population is growing at a very high and fast rate, the essential nutrient requirements like proteins fats, minerals and vitamins etc., are not adequately met from agricultural produce, thus causing malnutrition. This finally leads to diseases. Of these the most important is protein deficiency and especially animal proteins. Hence, we have to find out ways and means to produce proteins of animal origin for all human population in our country. It is here that poultry can play an important role. Poultry could be grown in areas where optimum production of cereals cannot be achieved due to various reasons like irrigation, land, fertility of soil, etc. and again poultry can thrive well in extreme climates. Since poultry is a prolific producer with a short reproductive cycle, it can multiply rapidly. Thus, the protein deficiency can overcome effectively.

Poultry meat and eggs are sources of protein which are superior to many animal proteins. It has also been established that for healthy servival of man, he requires certain amount of animal protein and no other animal protein can outclass poultry meat and eggs. Due to certain religous prejudices,meats of some of the large animals are not used whereas poultry meat can be eaten by all as there is no bar. It is possible to produce more eggs from hens and more meat from broilers bringing better economic returns to the producer. But this achievement would be of little help, if the advantages due to breeding and feeding are not effectively utilised at the time of processing, preservation, transportation and marketing. Thus, if the large numbers of eggs produced by hens and meat produced by poultry could be property processed and preserved and much of the hunger for protein could be got over.

Poultry meat, like any other meat and meat products, is highly perishable because of its high biological value. There has been a significant and substantial increase in poultry production in India in the last decade (Panda, 1966). This calls for better processing,proper storage, quick transportation and efficient marketing so that the consumers as well as producers reap the benifit of advanced technological knowledge.

Unfortunately, India is situated in the tropics where ambient temperature during most part of the year is ideal for bacterial multiplication thus causing spoilage to meat and meat

products. There is not adequate refrigeration facilities in India to preserve, transport and market meat and meat products as is practiced in the advanced countries of the world. Hence, methods have to be evolved to preserve meat at room temperature so that its nutritive value is not lost and at the same time the palatability is not adversely affected. Bacterial flora on the meat increases soon after slaughter if precautions are not taken to keep them as low as possible.

Methods have been developed to suppress, if not destroy, most bacteria that grow on meat and meat products. Some of them like drying, storing at low temperature, canning, curing and smoking, irradiation and use of antibiotics have been tried in western countries for preservation of poultry meat. But due to various factors like cost, eating habits, etc., some of the best methods cannot be put to practice in our country. Added to it, the purchasing power of the people is low. Hence cheap, simple at the same time efficient methods have to be evolved to preserve poultry meat at room temperature for sufficiently long periods of time.

Curing of meat is a very old method. Some work has been done on curing and smoking of poultry meat in U.S.A., Beasley and Marsden (1941). But this method requires further studies as our conditions of killing, handling, processing and storage are different from those used in western countries. Realising the importance of this method of preservation of meat, Chatterjee et al. (1971) reported some preliminary studies on

curing and smoking of poultry meat. Sharma et al. (1972) have further enlightened on methods of curing and smoking. But more substantial work is required to improve on the above findings.

Cured meat is the product obtained by subjecting meat to the process of salting along with the use of one or more of the following ingredients-sodium nitrate, sodium nitrite, sugar, monosodium glutamate, sodium polyphosphate, ascorbic acid, citric acid, etc. Salt is used either in dry form or as brine or a combination of both. From consumer's point of view, cured meat has probably the most important property of a characteristic pleasant flavour. After curing meat is subjected to the process of smoking in an environment of smoke. Smoking, in fact, acts as a complement to the curing process.

Various chemicals and antibiotics have been tried for preservation of poultry meat by many research workers. But harmful result produced due to prolonged inclusion of antibiotics in the diet limit their use. Moreover, wide spread use of antibiotics in the feed may encourage the appearance of antibiotic-resistant strains of bacteria in the intestinal flora of the consumer.

All the possibilities and potentials of radiation preservation have not been fully explored. This method has been tried as a supplement to, rather than replacement of, other methods of preservation. In spite of its advantages in

food preservation which include effective inactivation of bacteria, less chemical changes in the final product, this method has not got wide acceptance because it is costly. At the same time it causes significant loss of nutrients as also colour, taste changes are noticed in the finished product. Small doses of irradiation is not sufficient to kill the micro-organisms present in meat. Energy level of irradiation exceeding 9 MeV (million electrovlots) induced radioactivity in the finished product.

Storage at low temperature is undoubtedly, the best method of preservation of poultry meat. Since refrigeration facilities are not easily available in India, this method is not of much use under our present conditions.

There are methods which can be very easily used by the processor and at the same time not harmful to the consumer. One such method is curing and smoking and can be used under Indian conditions because of handicaps in other methods mentioned above. And since, all the aspects of curing and smoking have not been tackled, further studies have been undertaken to find the effectiveness of curing and smoking for preservation of the cut-up poultry at room temperature without much loss to its original quality and thus facilitating easy handling and transportation of meat from centre of production to the point of consumption.

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CHAPTER II

Review of Literature

REVIEW OF LITERATURE

Salt and Sugar

Salt is more important in food preservation than is generally understood. The empirical observation that salting would preserve meat without refrigeration was made several thousands years ago. By 1000 B.C. salted and smoked meats were used by the people, Jensen (1949). Between 3000 B.C. and 1200 B.C., the Jews employed salt from the dead sea for the preservation of various feeds. Around this time Chinese and Greeks used salted fish in their diets. Later Romans included pickled meat in their diets. Since long salt was used as a preservative for meat and meat products.

Rockwell and Eberts (1924) studied the preservative action of sodium chloride. They concluded that the preservative action of salt may be due to five important factors like(1) dehydration action (ii) direct effects of chloride ions on feed (iii) removal of oxygen (iv) sensitization of co_2 and lastly interference with the proteolytic enzymatic activity in the meat.

Curing is usually carried out in an insulated and refrigerated room. Most curing operations are carried out at temperatures in the neighborhood of 2.2 to 3.3°C (Jacob 1954). McCutcheon and Lucke (1928) studied the action of sodium chloride to find out its preservative action and compared the efficiency of sodium chloride with magnesium sulphate. They concluded that although magnesium sulphate has a greater

dehydrating power on proteins, their experiments with bacteria demonstrated that magnesium sulphate is not as efficient in preventing growth of bacteria as sodium chloride. The same authors in 1927 reported that sodium chloride and potassium chloride tended to increase the permeability of cell to water while calcium chloride and magnesium chloride tended to decrease it. Further more, the cells are more permeable to water when the osmotic pressure of medium was high than when it was low. This was explained by assuming that water diffused through pores in a partially hydrated gel constituting the cell membrane.

Hamm (1957), Hamm and Grau (1958) observed the effect of salt on ground meat. They found that pH of meat decreases as salt concentration is increased and addition of 5-6% salt had a large effect on water-binding capacity of muscle. They reported that these changes were due to the changes in the electrostatic charges of meat proteins. The binding of water brings about a disruption of peptide chains resulting in swelling of muscle proteins. Jensen (1949) reported that salt pentration is inversely proportional to the thickness of meat. But so far no references are available to say whether different curing solutions should be used for sliced and whole portions of meat. Higher temperatures help in faster curing and maturation process but this increases the danger of spoilage by growth of undesirable bacteria and moulds (Kassai and Karpati, 1963).

Wistreich et al. (1959) found that increase in temperature results in increased rate of salt-pentration but they found no linear correlation between temperature and pentration when the temperature is high. Chatterjee et al. (1971) reported, cured-smoked chicken kept at higher temperature was having higher salt content in both dark and white meat than similarly treated birds stored at low temperature. Their findings are in agreement with observations of Moulton and Lewis (1940) that salt diffuses rapidly at higher temperature. Sharma . (1972) also concurred with the above findings.

Callow (1932) observed that pentration of salt into meat is through diffusion due to changed osmotic pressure of the systems and reaction of salt with muscle proteins forming protein-salt complex which has higher osmotic pressure than brine alone. As a result of this, the initial outward flow of water and soluble proteins from muscle to brine is eventually reversed. He also reported that the diffusion of sodium chloride into the muscle is rapid, equilibirum being established in about 48 hours in 25% brine. Pantaleen (1959) as cited by Grau (1961) also reported similar findings.

Wistreich et al. (1959) in their studies reported that accumulation of salt in the meat is linear with the salt concentration of brine. Basley and Marsden (1941) while working with curing of turkey meat, reported that in tendon removed leg the pentration of salt into drum-stick muscle was increased. They also supported the findings of Wistreich et al.

(1959), that pentration of salt into muscle depends on concentration of brine. Further, they observed that keeping time of muscle under curing pickle also influences salt-pentration in the meat. Chatterjee (1968), Borys, et al. (1969) and Starszeski et al. (1969) observed that salt pentration depends on nature and anatomical characteristics of the tissue.

Bulgarian workers as cited by Grau (1961) have reported that the presence of metallic ions in brine reduces pentration of salt but other group of workers from the same country contradict this. In the latter's opinion, hard water increases better absorption of salt whereas soft and distilled water retards it (cited by Grau, 1961). Smith et al. (1951) found little difference in pentration of salt between coarse and fine grained salt. Grau and Bohm (1958) made similar observations.

Callow (1939) reported that freezing affects the structure of muscular tissues. He observed that pentration of salt into pork which has been frozen and thawed is about 20 percent greater than in fresh meat. He further added that salt has an accelerating effect on the oxidation of fat. As a result cured meats are more liable to spoilage than fresh meat through oxidative rancidity of fat. Vacuum-packaging and use of antioxidants help to prevent oxidation of fat in cured meat. Wierbicki et al. (1957) studied the water binding capacity of meat heated to 70°C and reported

that increased water binding capacity of cured meat was effective. But Hamm and Itawa (1962) were of opinion that heating the meat in presence of sodium chloride temporarily increased the water-binding capacity which gradually decreased. But they found no change at 30°C. Wismer and Pendersen (1960) observed that as a result of glycolysis in meat, the meat becomes more watery. It is thought that a watery meat can absorb more salt. But this was not found true because there were simultaneous drastic changes in the muscle-proteins leading to reduced water-holding capacity. Hence watery meat cannot hold more water.

Osmajones (1949) reported that there was no immediate growth of micro-organisms in salt concentration exceeding 13 percent in the meat. But halophilic bacteria showed growth even in meat treated with saturated (26.5 percent salt solution) brine solution. Cavett (1962) found that both temperature and salt concentration have synergistic effect on the spoilage flora of vacuum-packed meat. Pivnic and Thacher (1968) showed that meat having 6.2 percent salt and containing 10^6 spores/gram remained non-toxic for 18 months at 30°C but became toxid in presence of less salt. They also found that sodium chloride and sodium nitrite have a synergistic effect in inhibition of toxin producing bacteria.

Nunheimer and Fabian's (1958) showed that addition of acids (acetic, citric, lactic) reduced by 50% the amount of sodium chloride necessary for germicidal action. Mclean et al. (1968) observed that salt concentration more than 2 to 3 percent inhibits production of entrotoxin B. But Genigeorgis et al. (1966) reported

that strain S^6 produced entrotoxin in cured ham under anaerobic conditions with brine containing 9.2% Nacl. Kendereski (1971) reported that aerobic spore-forming bacteria, clostridia were more sensitive to inhibitory effect of cooking salt than cocci.

Mrak and Bonar (1939) while working on the growth of yeast on pickled ham showed the presence of only three genera viz. Debaryomyces, Pichia and Mycoderma. They found Debaryomyces to be high salt tolerant and to predominate on brines containing 15% or more salt, while the other two genera were found in brine containing four to fifteen percent salt. Ingram and Dainty (1971) reported that Nacl at 5% level inhibited partly and at 7% level inhibited completely the growth of bacteria. They further added that the inhibitory action of salt on growth of pseudomonads may be either due to inhibition of the proteolytic power of pseudomonas or interference with the metabolism of amino acids which leads to the production of evil-smelling compounds or both.

Gelman and Filatova (1971) observed weight changes when salted fatty herrings were stored at temperature below freezing point. The maximum weight increment was 6.92 ± 0.28 percent. The lean fish tended to loose weight at about 2% above the initial level, while fatty fish maintained the weight increment. They concluded that the weight increment depends on factorslike (1) the fat and salt content in the fish (2) on temperature (3) the time of storage and (4) on the ratio of herring weight to salt. Delvalle, et al. (1973) studied the bacterial count and

estimated rancidity of stored quick salted fish and reported that fish with high salt content tended to have low moisture content and low bacterial count and vice versa. To find out the halophilic bacterial count, they used nutrient agar media with 10% sodium chloride and incubated at 37°C.

Grau (1955) observed that small amount of sugar in curing solution increases the flavour, tenderness and colour change, etc. Over and above, sugar contributes to the maintenance of optimum pH. Mihalyi (1969) reported that ham treated with starch syrup or with saccharin had the best taste. Greenwood et al. (1940) reported that sugar contributes in setting up of a reducing condition during curing process. Horowitz-Wlassowa (1941) observed that bacteria of subtilis group form acetylemethyl carbinal from sugar. This compound protects nitrite from oxidation as it is easily oxidised.

NITRATE AND NITRITE

Curing of meat by utilising sodium nitrite as an essential ingredient is an ancient practice which persists to the present day. The colour of natural meat is due to presence of a substance in meat called myoglobin. Its structure is more or less similar to haemoglobin. The role of nitrite originated primarily as an agent for fixing colour in the meat but considerable evidences have since been developed supporting a preservative function as well. As early as 1891, Polenski demonstrated that nitrate in the cure was reduced to nitrite as a result of bacterial action. The characteristic red colour of meat and meat products was considered peculiar to cured meats and it was formed in the presence of nitrite as observed by Kisskalt (1899). (Cited by Wasserman and Tally, 1972).

Beasley and Marsden (1941), Malcolm et al. (1957) and Hinner and Marsden (1961) have described the use of salt peter in curing of turkey meat. Jensen and Hess (1941) reported that in bacon-cure, the nitrate added in curing solution was reduced to nitrite and nitrite was further reduced to hydroxylamine. Tarr (1944) showed that 1.07% $NaNO_2$ was bactericidal to S.aureus and other facultative anaerobes at a pH of 5.6 but was not bactericidal at pH 7.2. He further added that in cured flesh stored for three days at 10°C, sodium nitrate was reduced to nitrite and nitrite was further reduced. Characteristic colour of cured meat is due to nitrosomyoglobin, a complex

formed by combination of nitric oxide with reduced muscle pigment. Eddy and Ingram (1956) observed that Bacillus species of bacteria produced nitrous oxide and nitrogen from nitrate added in curing of bacon. Castellani and Niven (1955) demonstrated that S.aureus was capable of growing in the presence of significantly high concentrations of $NaNO_2$ than when cultures were grown in an aneorobic environment.

Niinivaara and Pohja (1957) observed that red colour of cured meat was due to reduction of nitrate to nitrite by various species of Micrococci, Bacilli, Sarcina, Alkaligens and Aerobacter. Eddy (1958) and Patterson (1963) found the most micrococci developing on the cured meat where responsible for reduction of nitrate to nitrite. They further observed that undesirably high concentration of nitrite production was found when the number of micrococci became large. Walter and Tayler (1963) reported that nitrite and not nitrate can be metabolised anerobically to nitric oxide by enzymes present in post-morten muscle tissue without dependence on bacterial action. The same authors in 1967 confirmed that under anaerobic conditions skeletal muscle enzyme system brought about reduction of both metmyoglobin to myoblobin and of nitrite to nitric oxide. Bacterial flora of the muscle though active in the utilization of nitrate and nitrite, were not capable in reducing the nitrite to nitric axide. They further added that the tissue enzyme cytochrome-oxidase, to be responsible for production of nitric oxide and production of cured meat colour. Brooks (1937) observed

that in absence of O_2 nitrite reacts with myoglobin to form equimolar quantities of metmyoglobin and nitrosomyoglobin, if substances capable of reducing (metmyoglobin) and nitrite are present.

Lawrie (1952[b]) reported that succinic dehydrogenase enzyme of muscle was responsible for reduction of metmyoglobin to myoglobin. Fox (1962) and Fox and Thomson (1963) have shown that nitric oxide react directly with metmyoglobin and that the complex can then be reduced to nitrosomyoglobin. They also added that the rate of formation of nitrosomyoglobin is much faster than the rate of reduction of metmyoglobin the latter need not therefore be an essential step in the process.

Richardson (1970) working on bacterioidal property of nitrite reported that nitrite may inactivate bacterial enzyme by combining with amino groups of certain enzymes. Urbain and Jensen (1940) reported that at elevated pH, the oxidation of nitrosomyoglobin to metmyoglobin is retarded. Benheim (1943) suggested that nitrous acid produced from nitrite and having bactericidal effect combines with amino groups of certain enzymes. Castellani and Niven (1955) found that antibacterial activity of nitrite increases as pH is lowered within an acid range. They further added that microbial inhibition is reversed by by addition of sulphydryl compounds such as thioglycolic acid and cystine. This observation reveals that nitrite or one of its breakdown products interfere with the sulphur nutrition of susceptible microorganisms.

Pivnic et al. (1970) reported that shelf-life of cured meat is increased by inhibition of bacterial spores by the nitrite. Pivnic and Bird (1965) observed that moderate increase of sodium chloride with addition of 62 ppm of sodium nitrite prevents the toxigenesis by Clostridium botulinum type A, B and E to a remarkable extent in cooked, vacuum-packed meat in plastic pouches. Dyer and Castell (1949) observed that there was no significant reduction of nitrite on storage. Wasserman and Talley (1971) reported that frankfurters prepared without sodium nitrite, in the cure, and cooked but not smoked had an unpleasant grey colour. When the same, without nitrite, was smoked and cooked had brown surface. But a desirable pink colour was obtained when $NaNO_2$ was added in the cure. This finding shows that $NaNo_2$ imparts good colour to the finished product.

Eddy et al. (1960) observed a substantial loss of nitrate from maturing bacon without any corresponding accumulations of nitrite. They were of opinion that some quantity of nitrite may disappear through purely chemical reaction with meat constituents i.e. ham products and amino compounds. Ingram and Panty (1971) reported that yeasts which are commonly seen in the latter stage of storage may be responsible for the fall in nitrite concentration.

Pivnic et al. (1967) confirmed that sodium nitrite is an unstable alkali salt and its level in cured product is affected by temperature and storage conditions. They observed 2 to 3 fold loss in nitrate concentration at 30°C than at 20°C.

Nordin (1969) observed that rate of nitrite loss was linear to its concentration and increased with temperature of lowering of pH. There was rapid loss of nitrite at room temperature because of rapid bacterial multiplication and subsequent utilisation of nitrite by these flora. Takaji et al. (1970) observed that concentration of nitrite in cured meat was affected by the factors like curing periods, period of storage, type of raw meat, pH of meat, cooking time and cooking temperature.

Buchann and Bloberg (1972) reported that S.aureus metabolizes nitrite when cultured aerobically. Duncan and Foster (1968[a]) demonstrated that nitrite at commercial levels (/_ 200 ppm) was inhibitory for spores and vegetative cells of various species of the spore forming genera viz. Clostridium and Bacillus. Roberts and Smart (1974) found that sodium nitrite heated in the lab medium was more inhibitory to spores of Clostridia species than nitrite added as a filtered sterilised solution to the same medium. They further reported that the inhibitory activity of heated nitrite medium was not stable indefinitely as growth sometimes occured on re-inoculation with vegetative cells. Lijinsky and Epstein (1970) reported that nitrite combines with secondary amines and forms carcinogenic nitrosamine. According to report given by Medical World News (1975) it is evident now that nitrosamine is carcinogenic in nature. It has also been reported that the occurance of stomach cancer had decreased in the country where adequate refrigeration facilities are available. All the same it is felt that the danger of carcinogenesis from nitrite cured meat is extremely small.

SMOKING PROCESS

Smoking of meat has traditionally been a method of extending the shelf-life of meat. Smoke helps to preserve meat by acting as an antioxidant, a bactericidal agent, a bacteriostatic agent and by providing a protective film on the product's surface. It gives a pleasant smoky flavour to the finished product.

Callow (1927[a]) noted that smoke from hard woods and produced by slow combustion of sawdust inhibits microbial growth, retards fat oxidation and imparts flavour to the cured meat. He further added that bactericidal action of smoke is partially due to formaldehyde.

Bugge (1927) reported that on heating wood a wide range of compounds are generated depending on the type of wood, the temperature of smoke chamber etc. Cutting (1952) has reported that partial combustion of wood produces smoke containing organic compounds such as aldehydes, acetone, lower alcohals, formic, acetic and higher fatty acids, phenols, tar, ketones. The absorption of these components are more effective than superficial drying action of smoke. The pioneer work carried out by Pettet and Lane (1940) have shown that oak wood smoke contained 0.12% formaldhyde, 0.57% higher aldehydes, 0.67% ketones, 0.38% formic acid, 1.71% acitic and higher acids, 0.96% methyl alcohol, 4.81% tar, 0.07% phenol and 4.21% resins. (Cited by Draudt, 1963)

Draught (1963) reported that colour development on the surface of meat depends on the component of wood resin. Smoke provides a desirable colour and flavour of smoked products. Lea (1933) found that smoking gave substantial protection against rancidity development on the surface of bacon. This was due to antioxidant property of smoke constituents.

In unsmoked bacon stored for 98 days at -10°C, the peroxide value was very high on the surface and reached a negligible value at about 1/2" from the surface. For smoked and similarly stored bacon the peroxide value was low on the surface itself, and very low at about 3/4" from the surface and declined still further in the interior of meat. These observations indicate that smoke components are found largely at the surface of the meat. Shewan (1949) found that cold smoking at 28-30°C produced 25% reduction in viable bacterial count and that bactericidal stability was a function of phenolic compounds present in smoke and smoking time. Gibbons et al. (1954) demonstrated that smoke constituents were cheifly responsible for the well known bactericidal effect of the combined smoking, heating and drying process. Hanley et al. (1955) made a study on the comparative usefulness of electrostatically smoked and conventionally smoked foods. They found a slight difference in flavour of the bacon smoked by these two methods.

Kitchell and Ingram (1966) observed that bacon after curing and smoking had perceptible difference both in the number

and types of bacteria. They found that with greater intensity of smoke treatment there was greater reduction in bacterial numbers and thus longer shelf-life. Husaini and Cooper (1957) found that steam-distillate fraction of smoke contained most of the flavouring materials found in smoke particularly phenols, acids and carbonyls. The non-steam distillate fraction was largely made up of water insoluble tar and water soluble wood resins. They further reported that phenolic compounds are of great importance in smoke flavour and others have secondary role. Foster and Simpson (1961) reported that various components of wood smoke consists of two phases (1) a disperse liquid phase containing smoke particles and (2) a dispersing phase or gas phase. The disperse particle size are of the order of 0.196 to 0.346 u in diameter. These particles are liquid droplets formed by condensation. Smoke particles contain a relatively low portion of volatile compounds and a high proportion of non volatile smoke components. They further added that smoking of meat might evolve vapour absorption by surface and interstitial water is much more important than direct deposition of smoke particles on meat surface. The rate of deposition of phenols was nearly 20 times more for wet meat surface than to dry surface.

Kurko (1959[b]) reported that phenols were effective antioxidant whereas other components including neutral materials (alcohal and carbonyl) organic bases and organic acids were ineffective as antioxidants. Phenols with higher boiling point were slightly more effective than those having lower boiling

points. Tilgner (1957) found that fish smoked at 65-75°C had most of the psychrophilic bacteria destroyed and only the most resistant mesophiles were expected to remain alive. Ziemba (1969) found that typical smoked colour formation was due mainly to carbonyl-amino reactions. The acid constituents of smoke might intensify the colouration by hydrolysis of proteins and increasing the concentration of reactive amino groups. Krylova et al. (1962) reported that the intensity of flavour in smoked products would depend on the reaction between the components of smoke and functional groups of meat proteins. Thus, phenols and polyphenols react with SH groups and carbonyls with amino groups.

Brummendorf (1970) described the use of smoke producing tablets containing mixture of sawdust, spices and other flavouring agents. The moisture content of such tablets was 7-9% and compressed to form the shape of tablet. Randal and Bretzler (1970) reported that reaction between smoke constituents and functional group of meat proteins produced a decrease in pH. This was thought to be due to pentration of organic acids of smoke constituents into the meat.

MICROBIAL SPOILAGE OF POULTRY

The precise beginning of man's awareness of the presence and role of micro-organisms in food is uncertain. It is presumed that man first encountered problems of food spoilage about 8000 years ago. With the advent of prepared foods, the problem of disease transmission by foods and faster spoilage due to inefficient storage and outbreaks of food poisoning made their appearance. The awareness of spoilage of prepared foods apparently dates from around 6000 B.C. Although man was aware of quality attributes in meats by the turn of 13th century, it is doubtful if he had any knowledge of the possible role and relationship between meat quality and micro-organisms. According to Jay (1970), 'Kircher'- a monk who as early as 1658 was perhaps the first man to suggest the role of micro-organisms in spoilage of food. He referred to bacteria as 'worms' invisible to the naked eye. L.Pasteur (1837) for the first time appreciated and understood the role of micro-organisms in spoilage of food and in 1860 he employed for the first time the use of heat to destroy undesirable organism in wine and beer.

Ayers et al. (1950) reported that spoilage of poultry meat was mainly due to the presence of micro-organisms on the surface of the meat and especially Pseudomonas at lower temperature. Nagel et al. (1960) concurred with the above findings. Ayers et al. (1950) also reported that log 4.40 (per centimeter square) microbes were present on chicken meat surface. May et al. (1961) reported the presence of log 5.02 bacteria on the broiler skin

surface. The same authors in 1962 showed that whole poultry meat tended to have a lower microbial count than cut-up poultry. Since most of the organisms were present on surface hence surface count was more valid than counts taken from deep tissues.

Gunderson et al. (1954) after extensive work for two years on poultry and poultry-products identified the following species of bacteria - Achromobacter, Aerobacter, Alcaligenes, Escherichia, Bacillus, Flavobacterim, Micrococcus, Paracolobactrum, Proteus, Staphylococcus, Corynebacterium and Salmonella. Woodburn (1964) investigated the incidence of salmonellae in dressed broiler-fryer chickens. He found that 72 of 264 i.e. 27% harboured salmonellae representing 13 serotypes. S. infantis, S. reading and S. blockley were the most common serotypes. Kraft et al. (1966) stated that microbial flora of fresh poultry consists of pseudomonas and other gram negative organisms as well as Corynebacterium , Arthrobacter, Microbacterium and other organisms.

Walker and Ayres (1956) reported the presence of 600 to 8100 organisms per square centimeter on the skin, prior to processing but after processing it increased to 11,000 to 93,000 per sq. cm. Woodburn (1966) found no significant difference in the number of viable organisms present on cut-up and whole carcasses. But Ayres (1950) observed that microbial load on cut-up poultry was higher than whole carcasses. Galton and Arnstein (1960) considered poultry to be the largest single vehicle in the dessemination of out breaks of food borne infections.

Elliott and Michener (1961) reported that off-flavour from poultry carcasses appeared when log of the bacterial number reached from 6.5 to 8.0 per sq cm. They further added that at log concentration of 7.5 to 9.0, slime formation occured. Psychrotolerant Pseudomonads and Achromobacter grow faster on skin than on other muscle tissue as per the findings of Clark (1970). According to Gibbons (1958), black or red discolouration in cured meat could be formed by certain halophilic Pseudomonads.

Nagel et al. (1958) observed that scald tank water and faecal material from the vent were the chief source of contamination of poultry meat during processing procedures.

Jay (1969) has shown that at low temperature the source of nutrients for psychrophilds consists mostly of soluble non-protein substances of which 3.5 percent, especially the carbohydrates in the form of lactic acid, glucose, free amino acids and related compounds like nucleotides are present in the skin. He also noted that foul odour generally associated with meats getting spoiled probably owe their origin to free amino acids and due to production of H_2S from sulphur containing acids, NH_4 from many amino acids and related compounds present in meat and indole from tryptophan. Lerke et al. (1963) reported that primary proteins of meat are probably not attacked until the supply of the simpler constituents are exhausted.

Hansen (1960) and Cavetta (1962) observed that packaging the cured meat in oxygen impermeable wrapper, the bacteria utilized the oxygen that is present in between the wrapper and meat for their respiration. If the O_2 atmosphere of the wrapper is replaced by carbon-dioxide, the type of bacteria also change; enterococci succeed in developing initially but ultimately lactic acid bacteria predominate. Kraft and Ayres (1952), Halleck et al. (1958), were of opinion that spoilage of meat at 20°C and above is not the same as that observed at low temperature. The aerobic spore formers are conspicuous at higher temperatures. These mesophiles and thermophiles possess enzymes that are more proteolytic than those possessed by psychorphiles.

Roberts and Ingram (1966)reported that clostridia were not seen in adequately cured meat and stored in warm temperature. They are not able to give any reason for this.

Barnese and Thornley (1956) observed a much greater proportion of facultative anaerobic species (Enterobacteriaceae) at hi her temperature. This might be due to the fact that at higher temperature the respiration of the meat tissue is much greater, so there is likely to be less available oxygen in the tissue near the surface on which bacteria grow. Jay (1970) observed that smoked cured meat became relatively less susceptible to spoilage by bacteria. The most common form of spoilage in cured-smoked meat caused by moulds which may be aspergillus, mucor, botrytis, penicillum and others.

Patterson and Gibbs (1973) studied the microbiological activities of cooked chicken. They observed that by cooking the chicken at 85°C for 50 minutes the residual flora consisted largely of spore forming bacteria. The predominant residual species were Bacillus subtilis and Clostridium bifermentans. Non-spore forming bacteria were not detected either after cooking or after storage at 1-3°C for upto 7 days. But storage at 16°C for 3 days markedly increased the number of non-spore forming organisms, although off-flavour typical of spoilage were not apparant. Hobbs and Spooner (1966) had also obtained very similar results with commercially cooked birds. Mead (1969) also supported the ideas of Hobbs and Spooner but reported that *storing the cooked chicken (85°C for 50 minutes) at temperature* of higher than 16°C, the danger of potential food poisoning organism multiplying was greater.

Varadarajulu and Narasimbarao (1975) reported that microbial load on live poultry was found to be 1.03×10^4 organisms/cm^2. After dressing, evisceration and washing, the microbial load on the carcass ranged from 3.2×10^3 to $9.6 \times 10^5/cm^2$ with mean value of $1.17 \times 10^5/cm^2$. They further added that dressed eviscerated, chilled and packaged chicken could be preserved at $4.0 \pm 1°C$ for 108 hours with no loss in quality. The shelf-life could be extended if the initial contamination on dressed chicken is reduced and limited to log $3/cm^2$.

OTHER ADDITIVES (MONOSODIUM GLUTAMATE, ASCORBIC ACID TRIPOLYPHOSPHATE, etc.)

Ikadak in the course of his poineering investigations on the protein constituents of seaweed (Laminaria japonica) isolated this compound, monosodium glutamate, in 1909. He also mentioned that this compound does not have taste when taken alone but if ingested alongwith proteins its natural flavour is enhanced, (Barry, 1949), Neukam (1956) reported that monosodium glutamate is practically tasteless but intensifies the flavour of certain foods. Dove (1948) observed that monosodium glutamate (MSG) accentuates and blends the natural flavours of foods such as meat, poultry, seafood, certain vegetables etc. Merory (1968) reported a unique characteristic shown by monosodium glutamate in that it increased salivation and thus better appreciation of the delicate aroma components of food while eating. The nature of action bringing about this increase in taste is not clear.

Fingl (1970) reported that monosodium glutamate was found to induce a feeling of sickness in some people referred to as "Chinese restaurant syndrome". The characteristics symptoms observed were dullness, nervousness and iching in the shoulder region. But Morselli and Garattinis (1970) contradict the above observation and reported that monosodium glutamate even when injected in high doses had no effect on the occurance of such sickness. Annon (1970) found that glutamic acid orally

ingested improved mental function but cause brain damage when injected in high doses.

Chatterjee et al. (1971) have also reported the use of monosodium glutamate in chicken meat curing. Araujo et al. (1973) reported that some people experience a great thirst after meals containing monosodium glutamate. It has been suggested that monosodium glutamate may have a direct thirst-producing effect on the central nervous system. But the authors noticed failure of glutamate salts to produce any thirst inducing effect since potassium glutamate did not produce an increase in thirst when given ad. lib., in experiments. Olney (1969) has reported brain lesions caused by injection of monosodium glutamate.

Ascorbic acid

Grau (1969) Mohler and Raible (1959) and Jacquest et al. (1969) observed that there was increased colour development when ascorbic acid was added in the pickling solution. They further reported that it helps in colour retention and a reduced free nitrite concentration in the finished product. Henrickson et al. (1956) reported that brine containing monosodium glutamate and ascorbic acid gave a grey pink coloured product which is a desirable characteristic for ham.

Polyphosphates

Elliott et al. (1964) reported that there was inhibition of growth of non-fluorescent strains of bacteria in synthetic media by addition of pure polyphosphate in the media. They further added that inhibitory action may be due to chelating property of

polyphosphate with metal ions such as magnesium (Mg). Chatterjee et al. (1971) used trisodiumpolyphosphate in the curing of chicken meat and observed that addition of this compound in the cure reduced cooking losses and increased water-binding capacity of meat. Shutts and Wierbicki (1973) studied the effects of phosphate concentration on meat shrinkage, on swelling and on pH of meat. They found that trisodiumphosphate showed a less shrinkage when the concentration of trisodiumphosphate was increased from 0.5 to 1.0%. Trisodiumpolyphosphate also increased the pH of meat and increased the water-holding capacity thus reducing shrinkage.

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CHAPTER III

Material and Methods

MATERIALS AND METHODS

Curing solution was prepared by dissolving the following ingredients in weighed quantity in measured volume of boiled water. Water used was boiled for half an hour so that the dissolved metal ions in tap water may not interfere with the action of the curing agents at the time of curing. In all the trials only wet curing was adopted.

Preparation of curing solution

The following ingredients were used for preparation of curing solution.

Commercial table salt (TATA)	5.44 kg
Cane sugar	2.72 kg
Sodium nitrate (A.R)	65.00 gms
Sodium nitrite (A.R)	85.00 gms
Mohosodium glutanate (A.R)	20. 00 gms

The above ingredients were dissolved in 40 litres of mineral free water giving a salinometer reading of 60° at $20^{\circ}C$. This pickle solution contained approximately 13.6% salt, 6.8% sugar, 0.16% sodium nitrate and 0.21 percent sodium nitrite and 0.05% monosodium glutamate.

Proximate Analysis

Aliquote samples were taken from fresh as well as cured and cured-smoked birds at 4th and 8th days of storage and moisture, protein, fat, ash, chloride and nitrite were estimated to find out the effect of curing and curing and smoking on shelf-life and palatability. Emphasis was given

to chloride and nitrite contents since this has direct bearing on preservation and food and druglaws. Seperate analysis for both dark and white muscles was done. For all chemical analysis A.O.A.C. method was followed.

Moisture and Ash

About 10 grams of well minced meat was accurately weighed in a chemical balance in a dry, clean moisture cup and kept in air oven at 100°C for 16-18 hrs. The moisture cup was removed from oven, cooled in dessicator and weighed. Weight lost was taken as moisture content of the sample and percentage moisture was calculated as follows.

Calculation

$\frac{C-A}{B-A} \times 100$ where A= weight of moisture cup

B= weight of moisture cup + wet sample

C =weight of moisture cup + dried sample

Estimation of Ash

Dry sample obtained after moisture estimation was taken in a dry clean silica basin and was heated until sample was well carbonized and then with the help of tongs the silica basin was transferred to a muffle-furnace maintained at a temperature of 550°C. Silica basin was removed alongwith White ash and after cooling in a dessicator final weight was taken.

Calculation

$\frac{C-D}{E-D} \times 100$ where C = wt. of silica basin + white ash
D = wt. of silica basin
E = wt. of silica basin + dried sample

Protein estimation

Principle - All the nitrogen compound of meat are converted to ammonium sulphate by boiling with concentrated H_2SO_4. Subsequently the ammonium sulphate is hydrolysed by strong alkali. By steam distillation, the liberated ammonia is collected in boric acid solution containing Toshiro's indicator (King and Wooton, 1956). Ammonia with boric acid forms ammonium borate which is then titrated against standard N/70 H_2SO_4.

Preparation of Toshiro's Indicator

Exactly 80mgs of methyl red and 20 mgs of bromocresol green were dissolved in 100 ml of methyl alcohol. 10 ml of this indicator were added to 1 litre of 2% boric acid solution.

Preparation of catalyst (Digestive mixture)

A mixture of copper sulphate and sodium sulphate in the proportion of 5:95 respectively was prepared. This digestive mixture was used to hasten the process of digestion.

Estimation

Micro-Kjeldahl method was followed for the estimation of total nitrogen. 2 grams of well minced meat was digested with 20-25 ml of commercial H_2SO_4 in a Kjeldahl flask.

A spoon of catalyst, mentioned above, was added to hasten the process of digestion. The flask was then kept on digestion bench and heated until the sample turned green and it was made sure that no trace of black praticle remained in the sample. After cooling, the digested sample was quantitatively transferred into 250 ml volumetric flask and volume was made upto the mark. Aliquote sample of 10 ml was taken into micro-Kjeldahl's distillation set for distillation. The liberated ammonia, upon additionof 40% sodium hydroxide was absorbed by boric acid solution containing Toshiro's indicator and titrated against N/70 H_2SO_4.

Calculation

A x 0.0002 x 25 x 50 x 6.25 = gram protein/100 grams of meat sample

where "A" represents burette reading of N/70 H_2SO_4.

Ether extract

About 3 grams of minced meat was taken into a thimble. Thimble was kept in a beaker and dried in the oven for about 6 hours at 100°C. Then the thimble was transferred to the Soxhlet extraction apparatus. Petroleum ether (60-80°C) was taken in a dried weighed extraction flask of the extraction apparatus and was fitted into the stem of the extraction apparatus. The apparatus was allowed to run for about 4-6 hours. The flask was removed after recovering the ether of the flask and the last traces of solvent evaporated on a water bath. After drying, the extraction flask was kept in drying air oven at 100°C for 30 minutes. Then it was transferred to a dessicator

and weighed after the flask had cooled to room temperature.

Calculation

$$\frac{A_2 - A_1}{A} \times 100 = \% \text{ fat}$$

whereas A = wt. of meat sample

A_1 = weight of empty extraction flask

A_2 = final wt. of flask with fat

Chloride estimation

Principle - More than enough 0.2 N silver nitrate ($AgNO_3$) was added to the sample to precipitate all chloride as silver chloride and the remaining excess silver nitrate was titrated with 0.2 N ammonium thiocynate (NH_4SCN) solution.

Reagents required

1. 0.2 N silver nitrate solution
2. 0.2 N Ammonium thiocynate solution
3. Ferric Alum indicator (saturatedsolution of ferric ammonium sulphate) ($FeNH_4SO_4$) 2, $12H_2O$.

Estimation

About 3.0 grams of minced meat was taken in a conical flask. It was then moistened with 10 to 20 ml of 0.2 N $AgNO_3$ solution. 15 ml of conc. HNO_3 was also added and boiled for 10 minutes, care was taken to dissolve all meat in flask. Conc. $KMnO_4$ solution was added in small quantity and boiled again. Addition of $KMnO_4$ was continued till the colour of

$KMnO_4$ completely disappeared. After that 25 ml of H_2O was added and boiled and diluted to approximately 150 ml. The flask was allowed to cool at room temperature for about 5-10 minutes and then 25 ml of ether was added and flask was thoroughly shaken. After wards, 5ml of ferric alum indicator was added and titrated against 0.2 N NH_4 SCN solution until colour changed to permanent light brown.

Calculation

Ml of 0.2 N NH_4 SCN solution used was substracted from ml of 0.2 N $AgNO_3$ solution added and was calculated on the basis of 10 grams sample.

1 ml of 0.1 N $AgNO_3$ = 0.058% NaCl.

Nitrite estimation

Principle - The quantitative determination of nitrite is based on spectrophotometric measurement of reddish purple diazo dye produced at pH 2.0 - 2.5 when sulphanilic acid is diazotised by the nitrite to be determined and coupled with α-naphthylamine hydrochloride. The advantage of this method is that coupling product has an intense red colour so that the spectrophotometric measurements can be made with relatively high accuracy.

Reagent - Modified Griess reagent

(1) 45 ml of glacial acetic acid was taken in a measuring cylinder and the volume was made upto 300 ml with double distilled water. 0.5 gram of sulphanilic acid and 0.128 gram α-naphthylamine hydrochloride were taken separately in two beakers. They were dissolved in above prepared solution with slightly heating to

dissolve them. Then both were mixed together and kept in amber glass bottle.

2) Nitrite Standard Solution

1.1 gram of silver nitrite was dissolved in approximately 150 ml of double distilled water in one litre volumetric flask. Concentrated NaCl solution was added to it till precipitation was completed. Volumetric flask was kept in dark for about an hour to settle the precipitate. Then volume was made upto one litre. 100 ml of supernatant fluid from this flask was transferred to another 1 litre flask and volume was made upto one litre. 10 ml from second flask was transferred to third 1 litre volumetric flask and volume was made upto the mark.

1 ml of final solution = 0.0001 mg N.

3) Saturated solution of Mecuric chloride

Preparation of standard curve

Suitable volumes (series of 10 ml, 20 ml, ...50 ml) of standard nitrite solution was taken in 50 ml volumetric flask and volume was accurately made upto mark in all cases. 2 ml of modified Griess reagent was added in all five flasks. After one hour of colour development readings were taken in spectrophotometer at wave length of 520 mμ, setting the instrument to zero absorbance with blank of 50 ml double distilled water plus 2 ml of reagent and a curve was drawn from with known quantities of nitrite. It was found that there was a 5 μg nitrite nitrogen in final solution which was later multiplied by the factor 3.29 to get ppm of nitrite.

Estimation

5 grams minced meat was taken in 100 ml beaker and approximately 40 ml H_2O was added to it and heated to 80°C and mixed with a glass rod to break up all the lumps of meat. It was then transferred to a 500 ml volumetric flask. Approximately 250 ml hot water was added and flask was transferred to steam bath for about 2 hours. Flask was removed and 5 ml of saturated $HgCl_2$ solution was added and mixed. It was cooled to room temperature and volume was made upto 500 ml with distilled water. It was filtered and suitable amount was taken in 50 ml flask and volume was correctly made up. 2 ml of reagent was added and kept for about 1 hour for colour development. In the calorimetric tube a suitable quantity was taken and reading was taken at 520 mμ setting instrument to zero absorbance with blank. Concentration of nitrite was calculated from standard curve.

Calculation

$$\text{PPM Nitrite N} = \frac{\text{µg nitrogen nitrite from graph x dilution}}{\text{ml of sample taken x wt. of sample taken}}$$

PPM nitrite = PPM nitrite N x 3.29.

Bacteriological studies

Swab method for bacteriological examination was used throughout the experiment as described by McVicker et al. (1959) and Kutula (1966). Absorbent cotton was wrapped firmly on a wooden 3/4" long applicator to form a swab about 1/16" diameter. Each swab were moistened in sterile saline (0.85%) after sterilizing in hot air oven at 160°C for 1 hour. This was pressed

to make free of excess moisture. The skin surface (approx. 6 cm sq.) at different places i.e. breast, back and thigh, under wings were swabbed three times in each direction, rotating the swab in the process. The applicator stick was broken aseptically about 1/2" above the swab and the swab was placed in a test tube containing 10 ml of sterile saline. The tube was vigrously shaken by rotating it inbetween the palms to disperse the cotton. Serial dilutions were made from diluent and 1 ml was poured into a petridish and plated. All petridishes test tubes and pipettes were sterilised in dry heat. Nutrient agar media was poured in each inoculated plate near the flame and plates were incubated at 37°C for 24, 48 and 72 hours. The composition of nutrient agar used was as follows.

Peptone (Oxoid)	10 grams
Tryptone (Difco)	10 grams
Sodium chloride	5 grams
Meat extract	10 grams
Agar	20 grams

All the above mentioned ingredients were dissolved in 1000 ml of distilled water and pH was adjusted to 7.4 with sodium hydroxide (1 N). Then media was then autoclaved at 15 lbs for 20 minutes. The prepared nutrient agar was plated to see whether it was sterile or not before using for bacteriological estimation.

Organoleptic Evaluation

The birds after one week of preservation were steam cooked at 10 lbs pressure for 15 minutes. Fresh birds for each organoleptic evaluation were also cooked under identical conditions and were also kept in plates before a panel of judges for evaluation. The identity of plates were not disclosed to the judges but they were told about thetype of experiment. They were requested to make their own opinions of the quality of the meat in the performa shown on next page. All the organoleptic tests were performed in the afternoon between 4 to 5 P.M. No spices were added at the time of cooking or during eating so that the natural meat taste was evaluated. A total of 9-12 judges participated in each organoleptic evaluation. The judges were not specially trained for organoleptic evaluation. Every effort was made to have the same team of judges. But sometimes due to certain difficulties the judges had to be changed.

Statistical Methods

Mean and SE were calculated by standard statistical methods. Analysis of variance of three way classification with equal number of observations per subclass was performed as per Snedecor (1968), where treatment effects were found significant the Duncan multiple range test was applied for pairwise comparison of treatment means as Federer (1967).

MEAT QUALITY EVALUATION SHEET

1. Name of the taster (A panel at least 5 to 10 members)

2. Date

3. Time of evaluation (preferably half to one hour before or after meals).

4. Method of cooking- Steam cooking (10-15 lbs pressure for 10 to 15 minutes).

5. Age of the bird

6. Test for meat component:

(a) Meat is

Tender	Moist
dry	tough
stringy	fatty
	Any other, please specify

(b) Describe flavour, if any

Extremely poor	poor	fair
good	very good	excellent

(c) Colour poor fair good excellent

(d) rate of saltiness, if required.

Very much undesirable	much undesirable	Undesirable	Desirable

In above tastings, please mark box(es) and other points which nearly describe the component.

7. Acceptance score sheet

	Points
Like very much	7
Like moderately	6
Like slightly	5
Neither like nor dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike very much	1

Please circle the point at which you rate the quality (Use reverse side for additional remarks).

EXPERIMENTAL TECHNIQUE

Twenty eight birds were taken for each trial from the experimental poultry farm of the Institute. There was slight variation in age, breed and other nutritional and managemental conditions among birds from trial to trial as birds of same age-group and same breed were not available. The average age of birds in all the trials ranged from six to eight months of age.

The birds were killed by the improved Kosher method where the jugular vien and carotid artery were severed without damaging the trachea and gullet. They were allowed to bleed for about 2 to 2½ minutes and were scalded at a temperature of 55°C for about 1½ to 2 minutes. The birds were dressed by the feather picking machine. The birds were then eviserated and washed. Neck was trimmed off and the birds were devided longitudinally into two equal halves using a meat cutting machine. Giblets and neck were not used for the study. These halved chickens were cooled in cold water to a temperature 45°F for one hour to reduce the body heat. They were hung on draining rack for draining off the excess of water for about one hour. Weight of individual halved portion was then taken.

One whole bird was taken at random for proximate chemical analysis. Bacterial swabs were applied on eight halved chickens at random to find out initial bacterial load. Individual wing bands were applied to identify these birds. Two of them were kept as control, one at room temperature (25-26°C) and the

other at refrigeration temperature (5-6°C). Rest of the six halves (with individual identification marks) of known bacterial load were kept in curing solution along with other remaining fourty six halves.

Care was taken to keep at least 10-15 cms of curing solution above the topmost layer of birds. The barrels were kept in the cold storage room maintained at a temperature of 5-6°C and relative humidity of 80%. After every 24 hours of curing the birds were re-arranged in the pickle, so that the top birds went to the bottom and the bottom birds came to top so as to ensure uniform curing. At the end of 72 hours, the birds were withdrawn from the curing solution, throughly washed in running tap water to remove all the excess of salt present on the surface of the birds and hung on racks for 2 hours to remove all the surface moisture. Six halves, whose initial bacterial load was known were taken for estimation of bacterial load at the end of curing process. They were also washed and then hung in a racks for one hour to remove all the surface moisture. After draining and drying they were weighed individually to the nearest milligram.

Six halves of the chicken of known bacterial count were wrapped in polyethylene bags and two of them were kept at room temperature in a tray and other at refrigeration temperature. Samples from five halved chickens were analysed chemically for moisture, ash, protein, fat, chloride and nitrite content.

This gave the initial figure for all the parameters studied.

Remaining 41 halves were divided randomly into two groups of 20 and 21 cut-up chickens. All the twenty of the first group were packed in polyethylene bags and half of them was kept at room temperature and the remaining half at refrigeration temperature.

The other group of twenty one halved chickens were subjected to cold, hard wood smoke, in a closed chamber. The door of the chamber was opened at intervals so that supply of air was restricted and the wood did not burn. Birds were hung by their wings in such a way as to provide maximum exposure of skin surface as well as the visceral cavity to the smoke.

They were arranged in such a way as not to come into contact with each other. The temperature of smoke chamber was maintained between 40-45°C and the chickens were smoked for four hours. After removing the chickens from the smoke chamber they were individually weighed and two of them were used for proximate analysis. Samples from four smoked chickens were taken for estimation of microbial load and two of them were kept at room and the other two at refrigeration temperauture. Remaining 15 halves were packed in polyethylene bags. Seven of them were stored at room temperature and the remaining eight at refrigeration temperature. While packaging the halved chickens in polytethylene bags care was taken to exclude as much air as possible.

Total bacterial load from previously identified birds was estimated at an intervals of 48 hours till they became unfit for human consumption. Two cut-up chickens from each group were used on 4th and 8th day of storage for chemical analysis. On 8th day two halves of each group were cooked at 10 lbs pressure in pressure cooker for 15 minutes. Samples from cooked birds were taken for chemical analysis and were also used for organoleptic evaluation. No attempt was made at any time to identify the various genra of bacteria present in the birds. The whole experiment was repeated thrice and the average value is presented in the thesis.

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CHAPTER IV

Result

RESULT

The following parameters were studied to find out the effectiveness of curing and curing-smoking for preservation of poultry meat.

Weight changes

It was observed that there was a gain of 7.13% in weight in cured chicken calculated on dressed weight at the end of curing process. But when the cured birds were smoked at 40-45°C for four hours there was a shrinkage of 6.87% on basis of fresh cut-up weight and 13.41% on cured weight.

Chemical analysis

Under chemical analysis, means and standard error for moisture, protein, fat, ash, chloride and nitrite content of meat subjected to different treatments, temperatures and storage period were estimated and the values are presented in Table 1. For statistical analysis, the data was divided into three parts i.e. 0 to 4 days, 4 to 8 days and 8 days to the time cooking, based on period of storage and temperature.

Moisture

The moisture content of fresh chilled chicken was found to 73.87 percent. There was significant increase in the moisture content of cured meat by 1.94 percent (Table 1) but no significant moisture loss was observed in cured chicken stored at room temperature (25-26°C) for four days (Table 5). On the other hand, cured meat stored at refrigeration temperature

(5-6°C) for four days lost about 0.45 percent moisture which was not significant. Cured meat after one week of storage at refrigeration temperature lost 3.32 percent moisture (Table 1) which was significant.

Cured poultry meat subjected to smoking lost 5.60 percent moisture as compared to fresh cut-up meat and 7.34 percent over cured meat. Cured-smoked meat stored for four days at room temperature showed no significant loss of moisture (Table 5). But cured-smoked chicken stored at refrigeration temperature for four days lost 1.94 percent moisture and 3.01 percent after one week of storage. From Table 7 it is evident that both in the dark and white meats there was no significant loss in moisture in cured-smoked chicken kept at room temperature for eight days. On perusal of data presented in Table 1, it is observed that moisture loss from cured meat at refrigeration temperature was more than cured smoked chicken stored under identical condition.

Cooking the meat on 8th day at 10 lb pressure for 15 minutes, it was observed that there was a significant loss of moisture from all treatment as shown in Table 8. After cooking, there was 13.20 percent loss of moisture from cured chicken kept at refrigeration temperature. Moisture loss in cured-smoked chicken stored at room temperature and at refrigeration temperature was found to be 8.67 and 10.56 percent respectively. The moisture contents of dark and white meat samples in any treatment were not significantly different as presented in Table 2, 4,6 and 8.

Protein and fat

The protein content of fresh meat was found to be 20.66 percent. There was a significant decrease in protein content of cured meat when analysed at the end of curing treatment. After smoking the cured chickens and on subsequent storage the protein content was found to be significantly less which is correlated to the moisture content of meat (Table 2, 4, 6). Cooked meat had also a higher protein content (Table 8).

There was no significant change on protein content of meat subjected to different storage conditions. Though the protein content of dark and white meat at any level of treatment or period of storage were not significantly different as can be seen from Anova Table 2, 4, 6 and 8 but dark meat had slightly higher protein content as compared to white (Table 1).

Fat

From data on Table 1, it is found that the breast muscle was having a little higher fat content than dark meat but when data was statistically analysed, non-significant difference was observed. There was significant decrease in fat content of meat analysed at the end of 72 hours of curing treatment (Table 2) Table 3 shows that fresh chicken had higher fat content and was significantly different from cured and cured-smoked chicken but fat contents of cured and cured-smoked meat were not significantly different from each other.

There was no significant difference in the fat content of chicken meat subjected to different treatments and difference storage conditions upto 8 days (Table 4, 6). On perusal of Table 8 it is found that there is a significant decrease in fat content of cooked chicken after 8th days of storage.

Ash and Chloride

There was significant increase in ash content of cured and cured-smoked meat immediately after curing and smoking. There was also increased chloride content in cured and cured-smoked meat from that of fresh meat soon after curing and smoking. Cured and cured-smoked chicken were having higher chloride content when they were kept at room temperature immediately after curing and smoking while chickens stored at refrigeration temperature irrespective of treatment were having lower chloride content (Table 1 and 2) than similarly treated chicken kept at room temperature.

There was significant difference in chloride content of dark and white meat samples in all treatments, Table 2, 4 6 and 8. On perusal of Table 1 it is clear that dark meat contains higher level of ash and chloride content than white meat irrespective of treatments and storage periods. Chloride contents increased with ash content and this seems to be correlated in both cured and cured-smoked chicken.

Nitrite

Fresh meat had no nitrite in it. Cured chicken showed a significant increase upto 212 ppm. On subsequent smoking this level came down to 168 ppm. When cured and cured-smoked chickens were stored both at room as well as at refrigeration temperature for four days there was significant increase in nitrite level. Highest level of nitrite was found in cured chicken stored at refrigeration temperature for four days and the increase in nitrite content over fresh cured meat was as high as 40 percent. There was higher level of nitrite in meat stored at refrigeration temperature than at room temperature on 4th day of storage in all treatments.

On 8th day of storage there was significant decrease in nitrite level both at room as well as refrigeration temperature but the rate of loss of nitrite was higher at room temperature than at refrigeration temperature (Table 1). Nitrite level in both dark and white meat samples were significantly different in all treatments (Table 4, 6 and 8). Dark meat was always having higher content of nitrite than white meat in treated birds and this was observed even after cooking. Cured meat stored at room as well as refrigeration temperatures for 8 days and cooked at 10 lb pressure for 15 minutes showed a significant loss of nitrite (Table 1 and 8).

Cured meat stored at room temperature for four days showed an increase of 18 percent nitrite over that observed

immediately after curing. When cured-smoked chicken were stored at room temperature for four days the nitrite level increased to 7.2 percent over that immediately after curing and smoking. But on storage for 8 days at the same temperature (25-26°C) the nitrite content of cured-smoked meat had dropped by 32.15 percent than that obtained at the end of 4 days of storage.

Similar observations were made with cured meat stored at refrigeration temperature. It was found that nitrite content of cured meat stored for 4 days at refrigeration temperature had increased by 40 percent over the nitrite level immediately after curing. There was an increase of nitrite level by 23.75 percent when the cured-smoked chicken was kept at refrigeration temperature for 4 days as compared to the figure immediately after curing and smoking but continuing the storage for 8 days the nitrite level dropped by 36 percent over that observed after 4 days of storage.

Bacteriological studies

Values in respect of bacteriological studies made in trial 1st, 2nd and 3rd are presented in Table 10, 11 and 12 respectively. Only total count was done and no species of bacteria was identified. This experiment revealed the bacterial load on the (1) skin surface of fresh cut-up chicken (2) effect of curing and (3) curing and smoking treatments and the influence of storage temperature on the growth of bacteria.

The untreated control showed enormous bacterial growth within 24 hours at room temperature and started smelling badly. Such meat became unfit for human consumption with 24 hours of storage at room temperature. Significant decrease in the bacterial count in meat was observed when subjected to the process of curing. Further to curing, smoking for 4 hours at 40-45°C showed that bacterial load had still gone down (Table 10, 11 and 12).

Fresh cut-up chicken had a load of log 4.28/sq. cm. immediately after curing. When the cured meat were stored at refrigeration temperature the rate of bacterial multiplication slowed down and thus length of preservation period was proportionally increased upto 18th day whereas untreated control chickens could be kept at such temperature for only 10 days. From Table 10, 11, 12 it can be observed that smoking gave complementary effect to the curing treatment because there was significant reduction of bacterial multiplication than the cured meat itself. By smoking the cut-up chicken there was not only reduction in bacterial load than the cured meat itself but also the rate of bacterial multiplication was slowed down. This effect was seen both at room as well as refrigeration temperatures.

Keeping the cured and cured smoked chicken at refrigeration temperature the rate of bacterial growth was still lower than similarly treated birds stored at room temperature. The

rate of bacterial multiplication in cured smoked chicken at refrigeration temperature upto 22nd day was slow, after which a rapid growth was noticed. (Table 10, 11, 12).

Fungus growth was noticed on the cured smoked chickens stored at room as well as refrigeration temperature on 14th and 34th day of storage respectively. The fungal colonies were visible to the naked eye on the skin surface particularly inside wing and a few colonies were also present on breast muscle.

Organoleptic evaluation

Mean and standard error for acceptance score are presented in Table 13. Analysis of variance for acceptance score showed highly significant difference between treatments, between judges, treatment and judges interaction and between trials. Judges preferred cured-smoked birds stored at refrigeration temperature followed by cured refrigerated meat. Though there was no significant difference between cured-smoked and cured chicken kept at refrigeration temperature, the judges gave a little more rating to the cured smoked birds stored at refrigeration temperature. Fresh meat significantly differed from cured and cured smoked chicken kept both at room and refrigeration temperatures (Table 14). During evaluation the judges were of opinion that cured refrigerated and cured-smoked chicken stored at room temperature were a little more salty but they did not observe any undesirable salty taste. Judges preferred the "Smoky flavour" of meat which was quite different from cured and fresh meats.

*****

Treatment Code No.	Details of treatments
1	- Fresh chilled cut-up- chicken- 0-day
2	- Cured cut-up chicken (after 72 hours of curing treatment at 5°C)
3	- Cured and smoked cut-up chicken (just after smoking at 48C for 4 hours).
4	- Cured cut-up chicken room (25-26°C) on 4th day of storage.
5	- Cured cut-up chicken Refrigerated (5-6°C) on 4th day of storage.
6	- Cured and smoked cut-up chicken at room (25-26°C) on 4th day of storage.
7	- Cured-smoked cut-up chicken at refrigerated (5-6°C) on 4th day of storage.
8	- Cured meat (cut-up) refrigerated (5-6°C) on 8th day of storage.
9	- Cured and smoked cut-up chicken at room (25-26°C) on 8th day of storage.
10	- Cured and smoked cut-up chicken refrigeration (5-6°C) on 8th day of storage.
11	- Cured cut-up chicken refrigerated (5-6°C) when cooked on 8th day at 10 lb pressure for 15 minutes.
12	- Cured and smoked cut-up chicken room (25-26°C) when cooked on 8th day at 10 lb pressure for 15 minutes.
13	- Cured and smoked cut-up chicken refrigerated (5-6°C) when cooked on 8th day at 10 lb pressure for 15 minutes.

(Since the treatments are large in numbers and the full name will occupy too much space, the treatments are coded as shown above and are referred to by the code number in the text).

TABLE I

Means ($\bar{X}$) and standard errors (S.E.) for moisture, protein, fat, ash, chloride and nitrite content of meat subjected to different types of treatments, temperature and storage

Storage time and temperature	Sample	Moisture %	Protein %	Fat %	Ash %	Chloride %	Nitrite p.p.m.
T_1	Dark	73.65±0.32	20.83±0.12	3.55±0.28	1.10±0.07	0.71±0.03	0.00±0.00
	White	74.09±0.22	20.50±0.20	3.68±0.16	1.06±0.07	0.67±0.02	0.00±0.00
T_2	Dark	75.59±0.25	17.14±0.12	3.10±0.07	4.16±0.08	4.17±0.12	215±6.00
	White	75.63±0.34	17.02±0.14	3.16±0.12	4.23±0.09	3.78±0.14	209±15.58
T_3	Dark	68.20±0.13	23.79±0.12	3.27±0.04	4.75±0.12	4.22±0.16	170±18.40
	White	68.34±0.15	23.69±0.08	3.12±0.08	4.70±0.10	3.97±0.15	167±12.70
T_4	Dark	75.06±0.16	17.10±0.14	3.09±0.13	4.71±0.13	4.44±0.09	265±14.43
	White	75.01±0.[illegible]	17.12±0.14	3.20±0.23	4.71±0.10	4.12±0.14	240±5.85
T_5	Dark	72.32±0.24	18.99±0.25	3.23±0.11	4.49±0.07	4.15±0.17	302±18.60
	White	72.34±0.27	19.14±0.19	3.29±0.13	4.37±0.14	3.85±0.07	294±12.70
T_6	Dark	67.77±0.22	24.03±0.12	3.27±0.15	4.84±0.11	4.29±0.13	182±13.22
	White	68.21±0.35	23.70±0.29	3.41±0.23	4.63±0.20	3.98±0.16	180±12.25
T_7	Dark	66.34±0.31	25.87±0.19	3.03±0.11	4.89±0.21	4.25±0.07	227±11.05
	White	66.32±0.33	25.79±0.30	3.15±0.18	4.74±0.15	4.22±0.18	219±5.78

Contd...

Table I (Contd...)

Storage time and temperature	Sample	Moisture %	Protein %	Fat %	Ash %	Chloride %	Nitrite p.p.m.
T_8	Dark	71.43±0.38	20.99±0.21	3.23±0.10	4.47±0.27	4.31±0.15	198±9.81
	White	71.68±0.53	20.58±0.52	3.25±0.13	4.46±0.13	4.13±0.08	176±6.96
T_9	Dark	67.74±0.37	24.00±0.33	3.28±0.24	4.92±0.19	4.47±0.14	116±3.75
	White	68.19±0.14	23.69±0.18	3.33±0.14	4.77±0.10	4.19±0.12	113±5.23
T_{10}	Dark	65.05±0.16	26.80±0.18	3.24±0.16	4.80±0.18	4.23±0.11	145±8.09
	White	65.47±0.55	26.59±0.29	3.27±0.16	4.87±0.16	4.12±0.15	142±5.19
T_{11}	Dark	62.32±0.22	31.55±0.16	2.45±0.19	3.80±0.15	3.21±0.12	89±6.64
	White	62.51±0.30	31.49±0.23	2.56±0.13	3.67±0.19	2.92±0.06	83±8.08
T_{12}	Dark	59.50±0.35	34.23±0.23	2.18±0.13	3.90±0.29	3.37±0.12	15±2.02
	White	59.69±0.28	34.18±0.35	2.23±0.14	3.88±0.10	3.11±0.08	13±1.45
T_{13}	Dark	57.74±0.20	36.58±0.18	2.01±0.07	3.95±0.10	3.17±0.16	24±3.84
	White	57.69±0.41	36.53±0.19	2.09±0.12	3.72±0.15	3.04±0.10	22±2.30

Table II

Analysis of variance(Chemical analysis of fresh, cured(after 72 hours of curing treatment) and cured-smoked cut-up chicken just after smoking).

Source of variation	Degree of freedom.	Mean Square					
		Moisture	Protein	Fat	Ash	Chloride	Nitrite
Between treatments	2	88.30**	68.56**	0.41**	23.27**	22.36**	-
Between samples	1	0.19	0.06	0.001	0.00	0.23*	
Treatment x samples	2	0.06	0.06	0.03	0.00	0.16*	
Between trials	2	0.45	0.08	0.13	0.00	0.03	
Error	10	0.13	0.04	0.05	0.02	0.02	

* $P \leq 0.05$ (Significant)

** $P \leq 0.01$ (Highly significant)

Table III

Duncan multiple range test for pairwise comparison of treatment means of fresh, cured (at the end of curing) and cured-smoked (immediately after smoking) cut-up chicken.

MEAN IN DESCENDING ORDER

Moisture				
	Treatment	T_2	T_1	T_3
	Means	75.61	73.87	68.27
Protein				
	Treatment	T_3	T_1	T_2
	Means	23.84	20.67	17.08
Fat				
	Treatment	T_1	T_3	T_2
	Means	3.61	3.19	3.13
Ash				
	Treatment	T_3	T_2	T_1
	Means	4.72	4.00	1.08
Chloride				
	Treatment	T_3	T_2	T_1
	Means	4.09	3.69	0.69

Note: Means under the same bar are not significantly different.

Table IV

Analysis of Variance

Chemical analysis of cured and/or smoked cut-up chickens stored at different temperatures from 0 to 4th day of storage

Source of variation	Degree of freedom	Mean Square					
		Moisture	Protein	Fat	Ash	Chloride	Nitrite
Between treatments	6	87.78**	73.43**	0.19*	10.91**	10.15**	53839.65**
Between samples	1	0 .22	0.04	0.05	0.05	0.58*	594 .38*
Treatment x samples	6	0.06	0.05	0.01	0.01	0.03	107 .54
Between trials	2	1.22*	0.43*	0.31*	0.006	0.009	1851.88*
Error	26	0.13	0.07	0.06	0.05	0.05	338.23

* P≤ 0.05 (significant)

** P≤ 0.01 (Highly significant)

Table V

Duncan multiple range test for pairwise comparison of treatment means from 0 - 4th day of storage

MEANS IN DECENDING ORDER

Moisture

Treatment	T_2	T_4	T_1	T_5	T_3	T_6	T_7
Means	75.61	75.03	73.87	73.33	68.27	67.99	66.33

Protein

Treatment	T_7	T_6	T_3	T_1	T_5	T_4	T_2
Means	25.78	23.87	23.84	20.67	19.06	17.11	17.08

Fat

Treatment	T_1	T_6	T_3	T_5	T_4	T_2	T_7
Means	3.61	3.34	3.19	3.16	3.14	3.13	3.09

Ash

Treatment	T_7	T_6	T_3	T_4	T_5	T_2	T_1
Means	4.81	4.73	4.72	4.71	4.43	4.20	1.08

Chloride

Treatment	T_4	T_7	T_6	T_3	T_5	T_2	T_1
Means	4.28	4.24	4.13	4.09	4.00	3.97	0.69

Nitrite

Treatment	T_5	T_4	T_7	T_2	T_6	T_3	T_1
Means	298.17	252.67	223.33	212.00	181.33	168.83	0.00

Note: Means under the same bar are not significantly different.

Table VI

Analysis of Variance

Chemical analysis of cured and cured-smoked cut-up chickens stored at different temperatures from 4 - 8th day of storage

Source of variation	Degree of freedom	Mean Square					
		Moisture	Protein	Fat	Ash	Chloride	Nitrite
Between treatments	6	81.83**	75.80**	0.04	0.18	0.06	23843.10**
Between samples	1	0.48	0.24	0.006	0.06	0.49*	1070.09*
Treatmentx sample	6	0.07	0.07	0.03	0.01	0.02	134 .81
Between trials	2	1.78**	1.11*	0.25*	0.005	0.02	683 .59*
Error	26	0.16	0.13	0.07	0.08	0.05	295 .95

* P $\leq$ 0.05 (Significant)

** P $\leq$ 0.01 (Highly significant)

Table VII

Duncan multiple range test for pairwise comparison of treatment means from 4th to 8th day of storage

MEAN IN DESCENDING ORDER

Moisture

Treatment	T_4	T_5	T_8	T_6	T_9	T_7	T_{10}
Means	75.03	73.33	71.55	67.99	67.97	66.33	65.26

Protein

Treatment	T_{10}	T_7	T_6	T_9	T_8	T_5	T_4
Means	26.69	25.78	23.87	23.84	20.78	19.06	17.11

Fat

Treatment	T_6	T_9	T_8	T_{10}	T_5	T_4	T_7
Means	3.34	3.27	3.24	3.21	3.16	3.14	3.09

Ash

Treatment	T_9	T_{10}	T_7	T_6	T_4	T_8	T_5
Means	4.84	4.83	4.81	4.74	4.71	4.46	4.43

Chloride

Treatment	T_9	T_4	T_7	T_8	T_{10}	T_6	T_5
Means	4.33	4.28	4.24	4.22	4.17	4.13	4.00

Nitrite

Treatment	T_5	T_4	T_7	T_8	T_6	T_{10}	T_9
Means	298.17	252.67	223.33	187.33	181.32	143.83	115.00

Note: Means under the same bar are not significantly different.

Table VIII

Analysis of Variance

Chemical analysis of cured and cured-smoked cut-up chicken stored at different temperatures upto 8th day of storage and cooked under 10 lb pressure for 15 minutes

Source of variation	Degree of freedom	Mean square					
		Moisture	Protein	Fat	Ash	Chloride	Nitrite
Between treatments	5	163.02**	228.88**	2.01**	1.55	2.23**	27566.31**
Between samples	1	0.52	0.05	0.02	0.05	0.39*	342.25*
Treatment x samples	5	0.08	0.15	0.02	0.01	0.00	86.00
Between trials	2	0.41	0.52	0.06	0.03	0.02	16.33
Error	22	0.29	0.19	0.07	0.72	0.04	111.60

* P/_ 0.05 (Significant)

** P/_ 0.01 (Highly significant)

Table IX

Duncan multiple range test for pairwise comparison of treatment means on 8th day of storage and cooked at 10 lb pressure for 15 minutes

MEAN IN DESCENDING ORDER

Moisture

Treatment	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}
Means	71.55	67.97	65.26	62.42	59.59	57.71

Protein

Treatment	T_{13}	T_{12}	T_{11}	T_{10}	T_9	T_8
Means	36.63	34.21	31.48	26.70	23.85	20.79

Fat

Treatment	T_9	T_8	T_{10}	T_{11}	T_{12}	T_{13}
Means	3.27	3.24	3.21	2.40	2.16	2.05

Ash

Treatment	T_9	T_{10}	T_8	T_{12}	T_{13}	T_{11}
Means	4.84	4.83	4.46	3.89	3.84	3.73

Chloride

Treatment	T_9	T_8	T_{10}	T_{12}	T_{13}	T_{11}
Means	4.33	4.22	4.17	3.24	3.10	3.06

Nitrite

Treatment	T_8	T_{10}	T_9	T_{11}	T_{13}	T_{12}
Means	187.33	143.83	115.00	86.33	23.17	14.83

Note: Means under the same bar are not significantly different.

Table X

Trial I

Bacterial load in log unit per sq. cm.

Days of storage	Control		Cured		Cured-smoked	
	Room (25-26°C)	Refgn. (5-6°C)	Room (25-26°C)	Refgn. (5-6°C)	Room (25-26°C)	Refgn. (5-6°C)
0 day	4.52	4.68	4.42	4.46	4.69	4.59
After 72 hrs of curing treatment	-	-	4.08	4.19	4.28	4.31
Just after smoking	-	-	-	-	3.52	3.61
2nd day	++	4.80	5.97	4.37	3.94	3.57
4th day		5.35	6.98	4.84	4.57	3.64
6th day		6.20	++	5.09	5.27	3.98
8th day		6.45		5.61	5.86	4.07
10th day		6.88		5.92	6.40	4.21
12th day		++		6.41	6.89	4.35
14th day				6.70	xx	4.76
16th day				6.85		4.92
18th day				6.92		5.28
20th day				++		5.39
22nd day						5.51
24th day						5.67
26th day						5.82
28th day						6.14
30th day						6.57
32nd day						7.12
34th day						xx

++ Spoiled (started smelling) and hence discarded. Further bacterial load not estimated.

xx Spoiled but not smelling. Fungus growth was visible hence further bacterial count was not done.

Table XI

Trial II

Bacterial load in log unit per sq. cm.

Days of storage	Control		Cured		Cured-smoked	
	Room (25-26°C)	Refgn. (5-6°C)	Room (25-26°C)	Refgn. (5-6°C)	Room (25-26°C)	Refgn. (5-6°C)
0 day	4.82	4.69	4.70	4.78	4.64	4.55
After 72 hrs of curing treatment	-	-	4.20	4.29	4.38	4.30
Just after smoking	-	-	-	-	3.51	3.43
2nd day	++	4.85	6.18	4.32	3.98	3.59
4th day		5.40	7.12	4.65	4.55	3.68
6th day		6.22	++	5.10	4.98	4.00
8th day		6.43		5.56	5.94	4.18
10th day		6.91		6.15	6.44	4.23
12th day		++		6.34	7.02	4.35
14th day				6.48	xx	4.85
16th day				6.78		4.98
18th day				++		5.20
20th day						5.38
22nd day						5.47
24th day						5.63
26th day						5.90
28th day						6.24
30th day						6.44
32nd day						6.98
34th day						xx

++ Spoiled (started smelling) and hence discarded. Further bacterial load not estimated.

xx Spoiled but not smelling. Fungus growth was visible hence further bacterial count was not done.

Table XII

Trial III

Bacterial load in log unit per sq. cm.

Days of storage	Control		Cured		Cured-smoked	
	Room (25-26°C)	Refgn. (5-6°C)	Room (25-26°C)	Refgn. (5-6°C)	Room (25-26°C)	Refgn. (5-6°C)
0 day	4.72	4.64	4.58	4.55	4.50	4.67
After 72 hrs of curing treatment	-	-	4.24	4.27	4.21	4.32
Just after smoking	-	-	-	-	3.58	3.50
2nd day	++	4.80	6.19	4.35	3.92	3.58
4th day		5.37	7.14	4.61	4.55	3.67
6th day		6.25	++	5.08	4.90	4.12
8th day		6.48		5.56	5.87	4.16
10th day		6.87		6.19	6.47	4.27
12th day		++		6.30	6.98	4.39
14th day				6.51	xx	4.86
16th day				6.84		4.90
18th day				++		5.08
20th day						5.42
22nd day						5.49
24th day						5.59
26th day						6.02
28th day						6.18
30th day						6.30
32nd day						6.15
34th day						xx

++ Spoiled (started smelling) and hence discarded. Further bacterial load not estimated.

xx Spoiled but not smelling. Fungus growth was visible hence further bacterial count was not done.

Table XIII

Mean & Standard Error (S.E.) for acceptance score of cured and cured-smoked cooked cut-up chicken stored at different temperatures

Judges	Cured Refrigerated (5-6°C) T_{11}	Cured-smoked refrigerated (5-6°C) T_{13}	Cured-smoked room (25-26°C) T_{12}	Fresh chicken T_1
1	5.33 ± 0.89	6.66 ± 0.39	5.33 ± 0.35	5.00 ± 0.00
2	5.00 ± 0.57	6.33 ± 0.36	6.00 ± 0.57	6.33 ± 0.68
3	6.00 ± 0.57	5.66 ± 0.38	5.66 ± 0.38	4.66 ± 0.40
4	5.66 ± 0.38	6.66 ± 0.39	5.66 ± 0.90	4.00 ± 0.57
5	6.66 ± 0.39	6.66 ± 0.39	4.66 ± 0.89	5.33 ± 1.15
6	5.00 ± 0.57	6.00 ± 0.57	4.00 ± 0.57	4.00 ± 0.57
7	6.33 ± 0.68	5.66 ± 0.90	5.33 ± 0.89	4.33 ± 0.35
8	5.66 ± 0.69	6.33 ± 0.70	6.33 ± 0.68	4.66 ± 0.37

Table XIV

Analysis of Variance

Acceptance score of cured and cured-smoked cooked cut-up chicken stored under different conditions.

Source of variation	Degree of Freedom	M.S
Between treatments	3	9.45**
Between judges	7	1.56*
Treatment x judges	21	1.82*
Between trials	2	22.79**
Error	62	0.20

Note: * P< 0.05(Significant)
** P< 0.01(Highly significant)

Duncan multiple range test for pairwise comparison of treatment means

MEAN IN DESCENDING ORDER

Treatment	T_{13}	T_{11}	T_{12}	T_{1}
Means	6.25	5.70	5.37	4.75

Note: Means under the same bar are not significantly different.

CHAPTER V

Discussion

DISCUSSION

Moisture loss and weight changes

A significant increase in the weight of cured meat was observed. The increase was of 7.13% of dressed weight. But this significant gain in weight cannot be attributed to increase in moisture content of dark and white muscle alone which was found to be only 1.74%, far below the increased weight of cured meat. Thus, this might also be due to increased absorption of sodium chloride and nitrite alongwith water which also added upto the weight of cured meat. Beasley and Marsden (1941) also reported similar findings. It is also assumed that besides dark and white meat, water might have also been absorbed by other loose tissues and bones of carcass, which were not analysed in the present study. Sharma, et al. (1973) have reported lower gain in weight of cured meat. They studied whole chicken carcasses whereas in the present study halved chicken were used. This increase in weight might be due to the fact that the chickens were halved before immersion in curing solution. There was more exposure of surface area which facilitated increased absorption of chloride ions by the muscles. Jensen (1949) also observed increased absorption of salt in sliced bacon as compared to whole bacon treated and stored under similar conditions.

On smoking the cured cut-up chicken, there was a shrinkage of 6.87 percent and 13.41 percent based on dressed and cured weight respectively. However, the shrinkage observed

in this experiment is more than that reported by Chatterjee et al. (1971) and much more than that obtained by Sharma, et al. (1973). The possible reasons might be that the skin surface and viseral cavity of chickens were more exposed than the whole carcass used by Sharma et al. (1973) in their study. Chatterjee et al. (1971) used polyphosphate in the chicken cure. Analysis of moisture content of dark and white muscle showed that there was 5.60 percent loss compared to fresh meat and 7.30 percent when compared to cured meat. This means there was loss of moisture from other parts also. It might also due to loss of water from bones which are hollow. Beasly and Marsden (1941) have also reported that the portion of the meat nearer the head, during smoking process, looses more water than the other parts.

There was more loss of moisture at refrigeration temperature than at room temperature in all treatments. In both cases birds were packed in polyethylene bags. Since the polyethylene bag is only semi-permiable to air, water from birds stored at room temperature escaped but formed an atmosphere having high moisture content. But when stored at refrigeration temperature, the moisture forms into small droplets and get condenced in the inside surface of the bag thus more water is lost at refrigeration temperature. This finding is in agreement with the findings of Chatterjee et al. (1971) and Sharma et al. (1973).

Cured meat stored at refrigeration temperature had increased moisture loss than cured-smoked chicken stored under identical condition of storage. Thus the moisture loss was found to be directly proportional to the moisture content of both cured and cured-smoked meat. The other possible reason as reported by Draught(1963) may be also true. He is of opinion that smoking tends to cause surface dehydration and formation of a thin protective film of smoke on the surface of meat which might have prevented surface evaporation. But no significant loss was noticed in cured and cured-smoked meat stored at room temperature which is similar to the findings of Chatterjee et al. (1971).

Protein and Fat

There was slight decrease in both protein and fat content just after curing. This may be due to the fact that during curing process there is also diffusion of soluble nitrogen and fat from meat to the brine solution. Fields and Dunker (1952) have also reported loss of small amounts of nitrogen and fat during wet curing process. The increase in protein content of cured-smoked chicken kept at refrigeration temperature and that of cooked meat was proportional to the decrease in moisture content. Sharma et al.(1973) also reported similar findings.

After the initial loss of fat during curing process, there was no further loss of fat in chickens irrespective of treatments and storage conditions. This might be due to

fact that smoke-constituents might have prevented fat undergoing oxidation, acting as antioxidants. Rockwell and Eberzt (1924) also reported similar findings. But there was loss of fat content in meat samples cooked at 10 lb for 15 minutes and fat-globules were also seen in the left-over liquid in the pressure-cooker. Fat gets melted during cooking and hence this loss.

Chloride and Ash

Cured chickens kept at room temperature was having a significant increase in both chloride and ash content as compared to similarly treated birds kept at refrigeration temperature. This might be due to the fact that at higher temperature, there is a rapid diffusion of salt. Similar findings have been reported by Moulton and Lewis (1940), Chatterjee et al. (1971) and Sharma et al. (1973). In cured-smoked halves there was higher salt content than the salt content of cured chicken. This may be due to the fact that there was higher temperature in smoke chamber which might have increased salt-pentration. There was a significant difference in the salt concentration of both dark and white muscle irrespective of treatment and storage conditions. This may be due to anatomical difference of the tissue. Chatterjee (1968), Borys et al. (1969) and Starszaski et al. (1969) also observed that salt-pentration depends on nature and anatomical characteristic of the tissue. The other reason might be relatively less amount of fat in

dark meat than that of white meat. Borys et al. (1969) has also found that lean bacon, having less fat, took 7-11 hours to reach four percent salt concentration at 50°C while back fat took hundred and twenty eight hours to attain similar concentration under identical condition of storage. Cooked meat had low salt and ash content in both cured and cured-smoked meat. This might be due to fact that salt is excuded during cooking and the juice present in the pressure cooker was also salty. Sharma et al. (1973) also reported similar findings.

Nitrite

There was significant increase in nitrite content of cured meat due to diffusion of nitrite into the meat during curing treatment. When cured chickens were smoked, there was significant loss of nitrite in smoked meat. This might be due to the higher temperature of smoke chamber. Pivnick et al. (1967) have reported 2 to 35 times more loss of nitrite at 30°C than at 20°C. Nitrite content, in cured and cured-smoked chicken, was higher when they were stored at refrigeration temperature than similarly treated chicken kept at room temperature. This finding was in agreement with the findings of Osmajones (1949) and Sharma et al. (1973) but rate of nitrite loss in cured-smoked chicken was higher than reported by Sharma et al. (1973). This may be due to the exposure of more body surfaces and visceral cavity of carcasses to smoke-heat.

Nitrite content increased significantly in cured and cured-smoked chicken upto 4th day of storage. This might be due

to the fact that absorbed nitrate might have been reduced to nitrite by the nitrate-reducing bacteria and thus simultaneous increase in nitrite level. The increased level of nitrite was observed in chickens stored both at room as well as refrigeration temperatures. This reductionof residual nitrate into nitrite by nitrate-reducing bacteria has been reported by Takagi et al. (1970); Eddy et al. (1960) and Patterson (1963).

There was significant reduction in nitrite content of cured and cured-smoked meat from 4th to 8th day of storage. This might be due to the fact that during storage there was multiplication of bacterial population and which might have started utilising nitrite since nitrate in meat might have been exhausted by then. Eddy et al. (1960) also reported the breakdown of nitrate to nitrite and utilisation of nitrite and its subsequent breakdown end-products by a large number of bacteria. The possible reason might also be that on longer storage yeast might have also utilised nitrite as reported by Ingram and Dainty (1971).

There was significantly decrease in nitrite level in chickens stored at room temperature upto 8th days than at refrigeration temperature for a similar period. This might be due to higher temperature of room than that of refrigeration temperature and rapid bacterial multiplication at room temperature. Nordin (1969) observed that loss of nitrite during later period of storage may be due to increased temperature of room and rapid bacterial growth.

Cooked chickens had significantly lost nitrite. Pivnick et al. (1967) and Takagi et al. (1970) reported that cooking cured and cured-smoked meat at 70°C there is one third to one half reductionof nitrite content in the cooked product.

The average nitrite content in cured meat was 212 ppm at the end of curing treatment and at end of 8 days of storage at refrigeration temperature it was 181 ppm. In the cured-smoked chicken the average nitrite content was 169 ppm immediately after smoking whereas after storing for 8 days at refrigeration temperature it was only 143 ppm. After cooking, the nitrite content was 86 ppm in cured birds stored for 8 days at refrigeration temperature and in cured-smoked birds stored for 8 days at refrigeration temperature it was only 23 ppm. Since meat is consumed only after cooking and as the value is only 23 ppm, cured and smoked meat could be safely consumed. There is no chance of nitrite poisoning.

Bacteriological studies

Cured meat had significantly reduced bacterial count than fresh meat. 13.6 percent of salt in the curing solution might be a causative factor for inhibition of proteolytic bacteria. The reduction of bacterial population might be also attributed to low pH due to addition of sodium chloride. The pH of brine solution in the present study was 5.8. Bem et al. (1969) and Ingram and Dainty (1971). Sharma et al. (1973) also observed similar findings. The initial load of bacteria after dressing

and making the cut-up chickens was relatively more than that reported by Sharma et al. (1973) who used whole chicken. May et al. (1962) have also reported that cut-up poultry contains relatively more microbes on skin surface than the whole chicken. This is but natural since more area is exposed during the cutting-up process and more chance for bacteria to multiply.

The bacterial growth was not much upto 4th day of storage at room temperature and upto 20th day at refrigeration temperature in cured-smoked chickens. This might be due to the combined action of sodium chloride and nitrite. Pivnick and Thatcher (1968) have also reported the synergistic inhibitory action of salt and nitrite on bacterial multiplication.

Significant reduction in bacterial population was observed on smoking the cured chickens. This reduction in bacterial population might due to superficial dehydration leading to non availability of water to bacteria or due to formation of a thin film of smoke on the surface of meat or combination of both . Tilgner (1957), Ketchell and Ingram (1966) and Sharma et al. (1973) have also reported significant reduction of bacteria following smoking operations. The other reason may be that cured-smoked meat had low moisture content thus preventing bacterial multiplication. Randall (1969) found a decrease in pH of meat after smoking which he thought to be due to absorption of acid constituents of smoke. Bacterial inhibitory property of smoke-constituents have been reported

by Draudt (1963). Chickens stored at refrigeration temperature in all the treatments had shown lower bacterial count than similarly treated chickens kept at room temperature. This might be due to complemetary action of refrigeration temperature to curing and smoking process as reducing the bacterial multiplication

Cured-smoked chickens kept at room temperature as well as refrigeration temperature showed mold growth on 14th and 32nd day of storage respectively. There was no reduction in bacterial population following use of mycostatin because it is affective only against fungus.

Organoleptic evaluation

There was significant difference between treatments, between judges, between trials and judges and treatment interaction. The reasons for these component of variations may be attributed to the facts that birds of all the three trials were not of same age, breed and nutritional status. Significant difference between judges may be due to the fact that judges of the taste panel were not trained to evaluate the quality of meat. The other reason in support of this may be that meat cooked in the present study were quite different from the type of cooking to which the judges were accustomed to.

Judges preferred cured-smoked chicken followed by cured refrigerated and fresh meat. They liked the characteristic flavour of smoked meat. They also appreciated the characteristic appearance of smoked chicken and pink colour of cured refrigerated chicken.

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CHAPTER VI

Summary and Conclusion

SUMMARY AND CONCLUSION

Meat has been the food of man from times immemorial. Proteins of meat have high biological value and is required for the growth and well being of human beings. Because of its high nutritive and biological value, meat proteins are broken down by bacteria producing dangerous end-products within a short time. Unless effective methods are taken to preserve meat and meat products, consumption of meat might become dangerous.

Of the many methods followed for preservation of meat, curing is one. It is a simple and easy process. Curing, as a method of preservation, is not practiced in western countries due to the availability of refrigeration facilities. But in our country, this method might become very useful since low temperature for preservation of meat and meat products is not easily available. Hence, an investigation was undertaken to study the effect of curing and/or smoking on the preservation of dressed, cut-up poultry at various temperatures.

Curing solution was prepared by dissolving 5.44 kg salt, 2.72 kg sugar, 65 gm sodium nitrate, 85 gm sodium nitrite and 20 gm monosodium glutamate in 40 litre of mineral free water giving a salometer reading of 60° at 20°C. Birds were dressed, cut-up longitudinally and immersed in above solution for 72 hours and stored at a temperature of 5-6°C. Care was taken to see that the birds were uniformly cured by changing their position every twenty-four hours. Birds, after curing were withdrawn from solution, washed in running water and were hung in the draining rack to remove excess of water. They were divided into 2 groups, one group was used as it is and the other

was smoked in hard wood smoke at 40-45°C for 4 hours. Birds were then divided into following groups for the experiment.

1. Untreated control kept at room temperature (25-26°C).
2. Untreated control kept at refrigeration temperature (5-6°C).
3. Cured birds stored at room temperature.
4. Cured birds stored at refrigeration temperature.
5. Cured and smoked birds kept at room temperature.
6. Cured and smoked birds kept at refrigeration temperature.

The birds were then analysed at the end of 4th day and 8th day of preservation using following parameters to assess the effect of curing/or smoking on the quality of birds. Parameters used in this study were moisture, protein, fat, ash, chloride, nitrite, microbial estimation and organoleptic evaluation.

As a result of study, following conclusions were drawn.

(1) Fresh untreated birds cannot be stored at room temperature for more than 24 hours whereas fresh untreated birds can be stored at refrigeration temperature for 10 days.

(2) Cured birds had a shelf-life of 4 days at room temperature and 18 days at refrigeration temperature.

(3) Cured-smoked birds can be kept at room temperature for 12 days withbut any adverse effect whereas similarly treated birds can remain edible upto 32 days at refrigeration temperature.

(4) The moisture content of cured birds increased during curing process but decreased during smoking.

(5) The protein content of meat decreased after curing process but increased after smoking which was correlated with the moisture content of meat.

(6) The fat content of birds decreased after curing. There was not much change after this initial decrease in all types of treatments and storage conditions. But cooked meat showed low fat content.

(7) The chloride content of meat increased significantly after curing. But chloride content of meat stored at room temperature was significantly more than that kept at refrigeration temperature. The chloride content of meat was directly proportional to the ash content of cured and cured-smoked birds. But during cooking, the chloride ions were exouded and the birds were not excessively salty when tasted by organoleptic tests.

(8) The nitrite content of cured and cured-smoked birds increased during first 4 days of storage irrespective of temperature of storage. But the rate of increase was lower at room temperature than at refrigeration temperature. By 8th day of storage, the nitrite level had significantly reduced both at room as well as refrigeration temperatures, but the rate of reduction was more at room than at refrigeration temperature.

(9) The cured chicken had lower bacterial count initially as compared to fresh bird. The bacterial multiplication was slow up to 4th day at room temperature after which the load became so high that meat was not edible. Similarly treated birds kept at refrigeration temperature had slow rate of bacterial growth and remained edible for 18 days.

(10) Cured-smoked chickens were having low bacterial load than cured chickens and rate of bacterial multiplication in birds stored at refrigeration temperature was slow than kept at room temperature. This lower multiplication may be a cumulative effect of lower temperature as well as presence of chloride and nitrite ions.

Mycostatin added at 10 ppm to the curing solution prevented fungal growth in cured-smoked chicken stored both at room as well as refrigeration temperatures. Smoking reduced the moisture content of birds resulting in lower shrinkage during preservation.

Taste panel judges preferred cured-smoked refrigerated birds followed by cured-refrigerated. Judges liked the characteristic "Smoky flavour" of cured-smoked meat. The pink colour of cured birds was also liked by them.

This study indicated that cured birds could be stored at room temperature (25-26°C) for 4 days and for 18 days at refrigeration temperature (5-6°C). Cured-smoked birds can be safely stored for 12th day at room temperature (25-26°C) and for as long as 32 days at refrigeration temperature (5-6°C). Since this method is simple to apply and is effective for short period of preservation, curing and smoking could be applied for transportation of meat from point of production to the point of consumption, without any adverse effect on meat quality.

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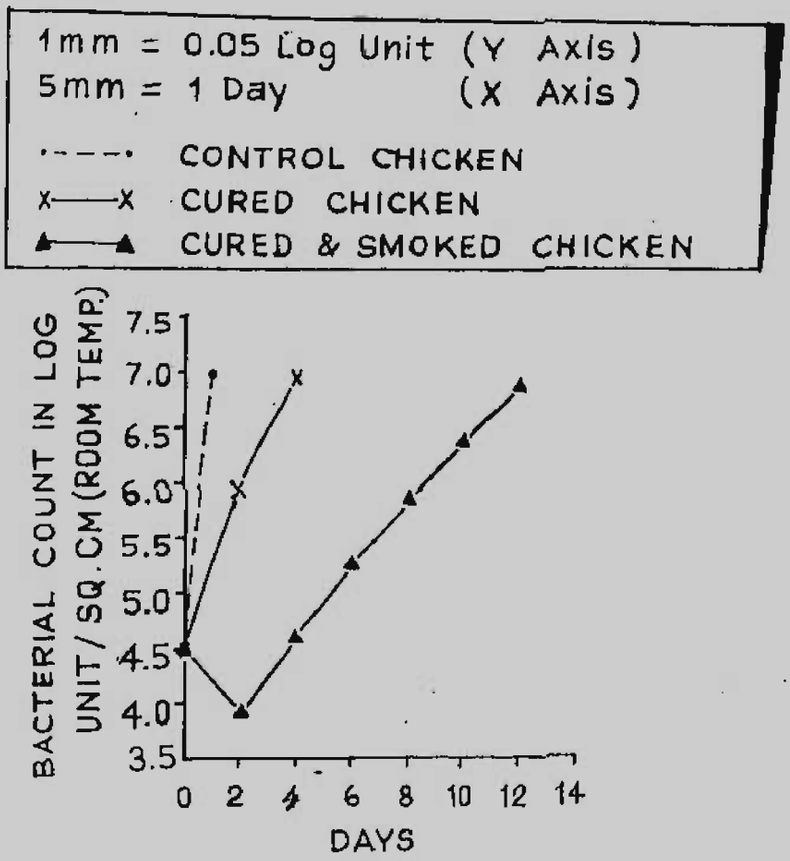

FIG.1_ BACTERIAL LOAD ON SKIN SURFACE OF CHICKEN KEPT AT ROOM TEMPERATURE (25-26°C) UNDER DIFFERENT TREATMENT

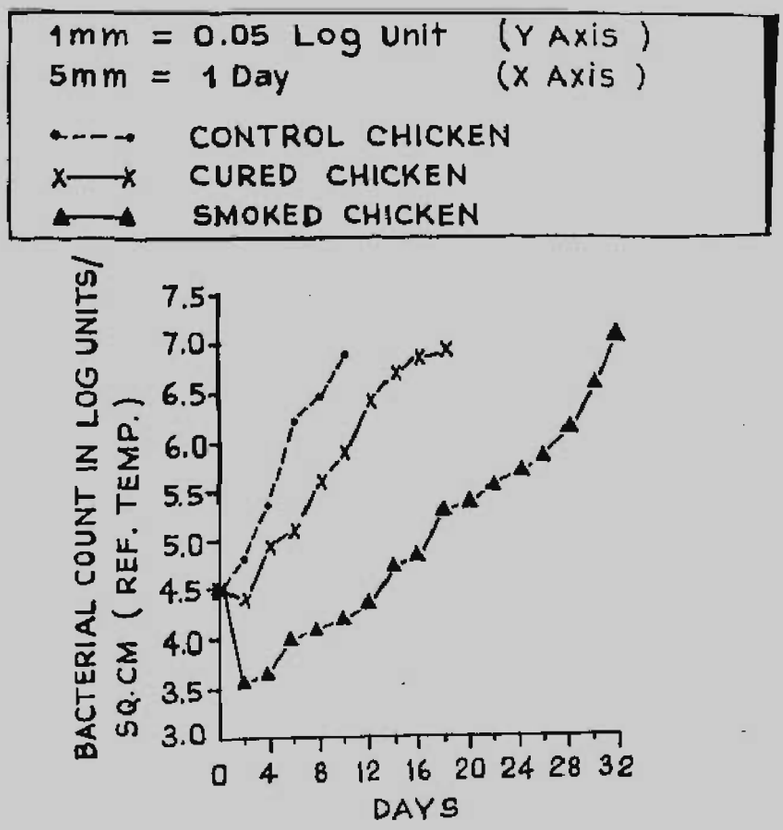

FIG.2 - BACTERIAL LOAD ON SKIN SURFACE OF CHICKEN KEPT AT REFRIGERATION TEMPERATURE (5-6°C) UNDER DIFFERENT TREATMENTS

1. FRESH CHICKEN
2. CURED CHICKEN
3. CURED-SMOKED CHICKEN
4. CURED CHICKEN KEPT AT ROOM TEMPERATURE
5. CURED CHICKEN KEPT AT REFRI.TEMP.
6. CURED- SMOKED CHICKEN KEPT AT ROOM TEMP.
7. CURED- SMOKED CHICKEN KEPT AT REFRI.TEMP.
8. CURED CHICKEN KEPT AT REFRI.TEMP.
9. CURED- SMOKED CHICKEN KEPT AT ROOM TEMP.
10. CURED- SMOKED CHICKEN KEPT AT REFRI.TEMP.
11. COOKED CURED CHICKEN AT REFRI.TEMP.
12. COOKED CURED - SMOKED CHICKEN AT ROOM TEMP.
13. COOKED CURED- SMOKED CHICKEN AT REFRI.TEMP.

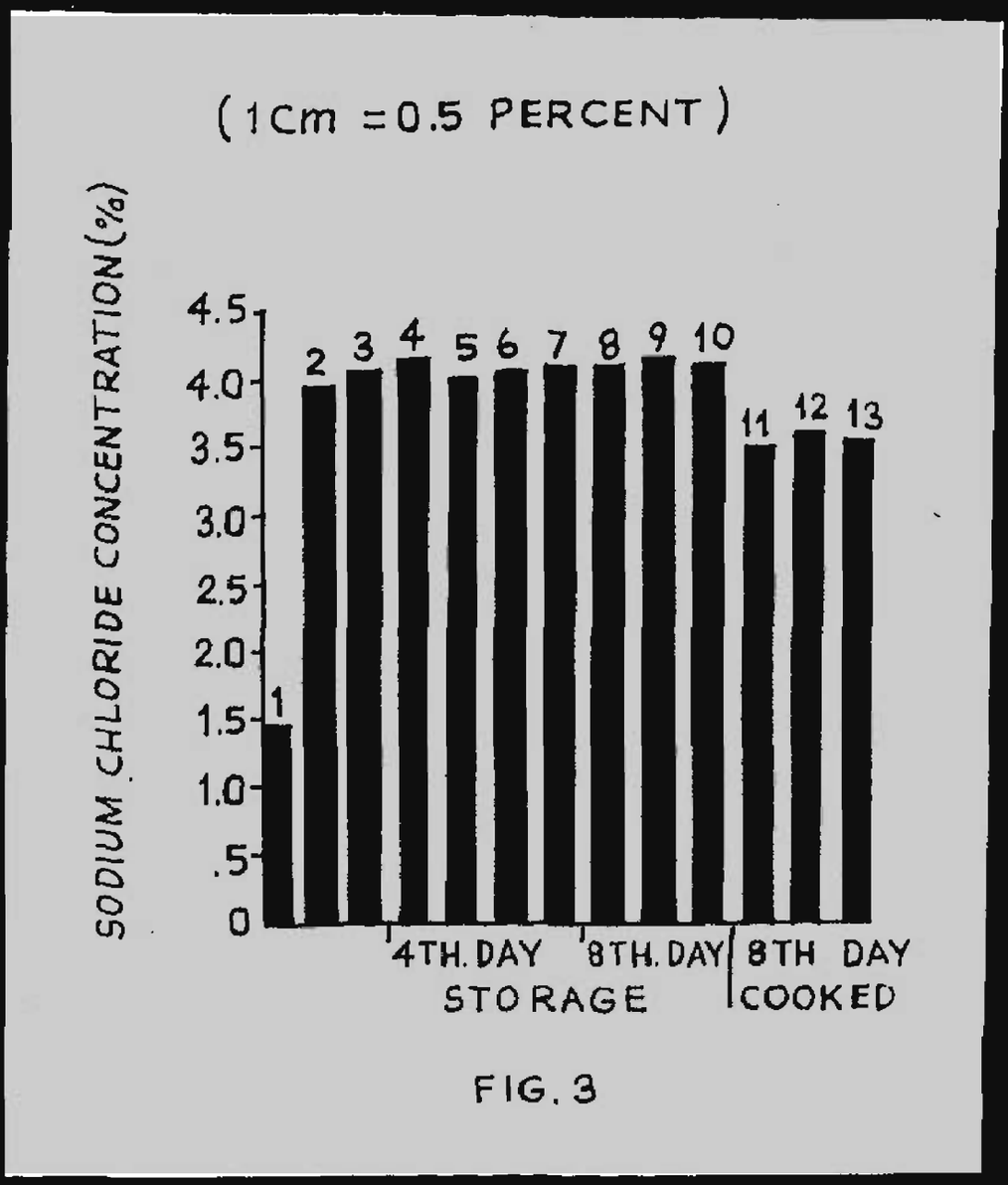

FIG. 3

FIG-3 SHOWING CHLORIDE CONCENTRATION

1. FRESH CHICKEN
2. CURED CHICKEN
3. CURED -SMOKED CHICKEN
4. CURED CHICKEN KEPT AT ROOM TEMPERATURE
5. CURED CHICKEN KEPT AT REFRI. TEMP.
6. CURED-SMOKED CHICKEN KEPT AT ROOM TEMP.
7. CURED-SMOKED CHICKEN KEPT AT REFRI.TEMP.
8. CURED CHICKEN KEPT AT REFRI.TEMP.
9. CURED-SMOKED CHICKEN KEPT AT ROOM TEMP.
10. CURED- SMOKED CHICKEN KEPT AT REFRI.TEMP.
11. COOKED CURED CHICKEN AT REFRI.TEMP.
12. COOKED CURED- SMOKED CHICKEN AT ROOM TEMP.
13. COOKED CURED- SMOKED CHICKEN AT REFRI.TEMP.

(1 Cm = 30 PARTS PER MILLION)

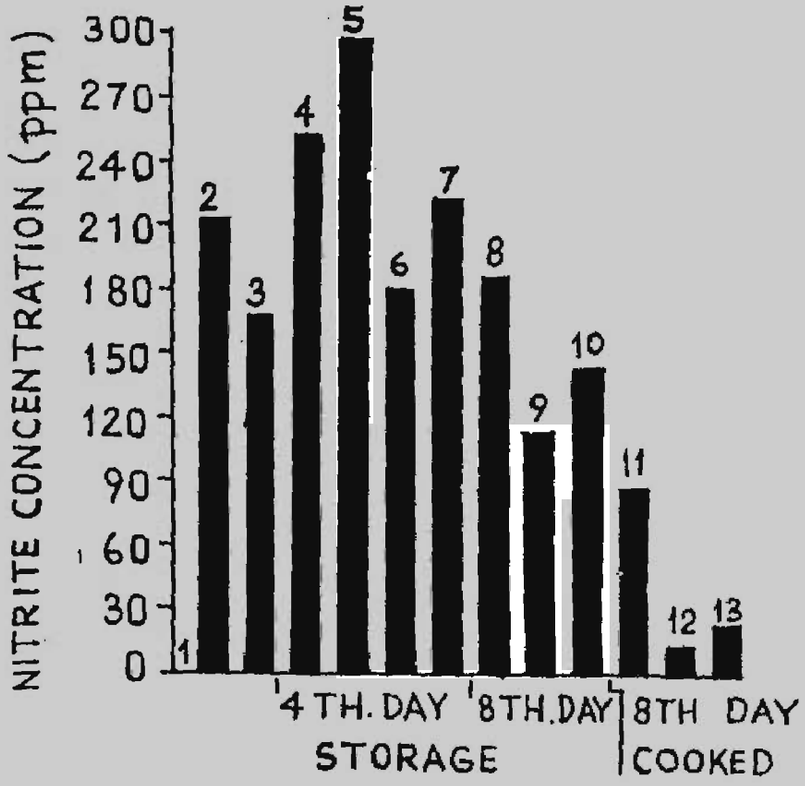

FIG. 4

FIG-4 SHOWING NITRITE CONCENTRATION