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I am submitting herewith a thesis written by Harriet Jo Gant entitled "Microbial Growth and Spoilage of Vacuum Packaged Country Ham Slices as Affected By Preservation Treatment." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

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MICROBIAL GROWTH AND SPOILAGE OF VACUUM PACKAGED COUNTRY HAM SLICES AS AFFECTED BY PRESERVATION TREATMENT

A Thesis

Presented for the

Master of Science

Degree

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ABSTRACT

In recent years there has been a trend to reduce the amount of salt and nitrate/nitrite used to produce country cured hams to yield a more moist and mild product. It was thought that this practice has caused problems in maintaining ambient temperature shelf life of vacuum packaged country ham slices. Twelve country hams were collected from a Tennessee country ham producer at a plant experiencing chronic problems in maintaining shelf life of vacuum packaged country ham slices. Hams collected were in the range of 3.78 to 6.04% (mean) salt. Six center cut slices were cut from each ham and vacuum packaged at the plant site using the exact manner used for hams going to retail sales. Slices were transported to the University of Tennessee laboratories where they were incubated at 28^{o}C for 1, 2, 3, 4, and 6 weeks. Chemical and microbiological test were preformed on the ham slices to determine of there was a relationship between spoilage characteristics and the level of preservatives present.

Chemical analysis revealed that the preservatives levels present at the range examined would not prevent spoilage of product. Microbial loads of hams were high initially and increased rapidly in the first week. It was found that hams above 4.5% salt content did not show

spoilage characteristics such as distention of package and strong odors until after the second week of incubation. Hams having less than 4.5% salt, began to have off-odors and swelling of packages within one week. Nitrate had the greatest decrease in levels between day 1 and week 1, with a subsequent increase during the second to sixth week storage periods. One reason for this may be that there was generally a decrease in moisture content over the storage period, thus reducing the weight of the sample and concentrating nitrate. Concurrently as nitrate decreased, nitrite increased. This was possibly due to the reduction of nitrate to nitrite by bacteria present in the ham. Ambient temperature storage of ham slices having less than 4.5% salt will not give a satisfactory product for more than one week.

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CHAPTER I

INTRODUCTION

In many Southeastern states the production of country hams constitutes a significant part of the pork industry (Eakes et al., 1975). The practice of curing meat goes back to antiquity. Ancient people discovered that meat, if properly salted and dried would have a long shelf life. This allowed stock piling during warm weather for a reserve food supply and for transportation (Kemp et al., 1982). With the successful advent of refrigeration and it's availability in the home, curing discontinued to be designed solely as a means of preservation (Kramlich et al., 1973). Today, there are still some consumers who value this old tradition as a refreshing breeze from the roots of history. Although involving a great deal of work and expense, country ham has a flavor and general aura of enjoyment not found among today's hastily prepared mass produced meat products (Puolanne, 1982).

The production of country ham has increased rapidly during the last few years. However, an increasing percentage of the total hams being produced is sold in vacuum packages as slices rather than as whole hams. Many retail outlets display vacuum packaged country ham slices in non-refrigerated areas to attract the attention of consumers

and encourage impulse sales. Storing hams at ambient temperatures accelerates growth of microorganisms (Kemp et al.,1975), oftentimes leading to distention of packaging. Most merchandisers feel that appearance is the most important factor in boosting impulse sales. Meat retailers must make sure that the featured items have good eye appeal and maintain a desirable appearance (Harwell et al., 1975). If consumers find branded packages that are distended or discolored they will not purchase the product. Continuous observance of packages with these characteristics by a branded product may cause the consumer to avoid the brand all together. Because of merchandising techniques used for vacuum packaged country ham slices it is essential that they maintain a ambient temperature shelf life.

In recent years there has been a tendency to use low amounts of salt and a minimal curing time in order to obtain a moist mild product. Due to this practice, some producers are experiencing significant product losses (Figure 1). Water activity (A_w) may not be sufficiently lowered and nitrate and nitrite may be depleted. If low salt meats are vacuum packaged in impermeable films and held at room temperature, there may be a danger of Clostridium botulinum growth (Ayres, 1975; Ayres et al., 1982)

This study was undertaken to determine the minimum amount of salt necessary to preserve vacuum packaged sliced country ham for a minimum of 14 days at 28°C.

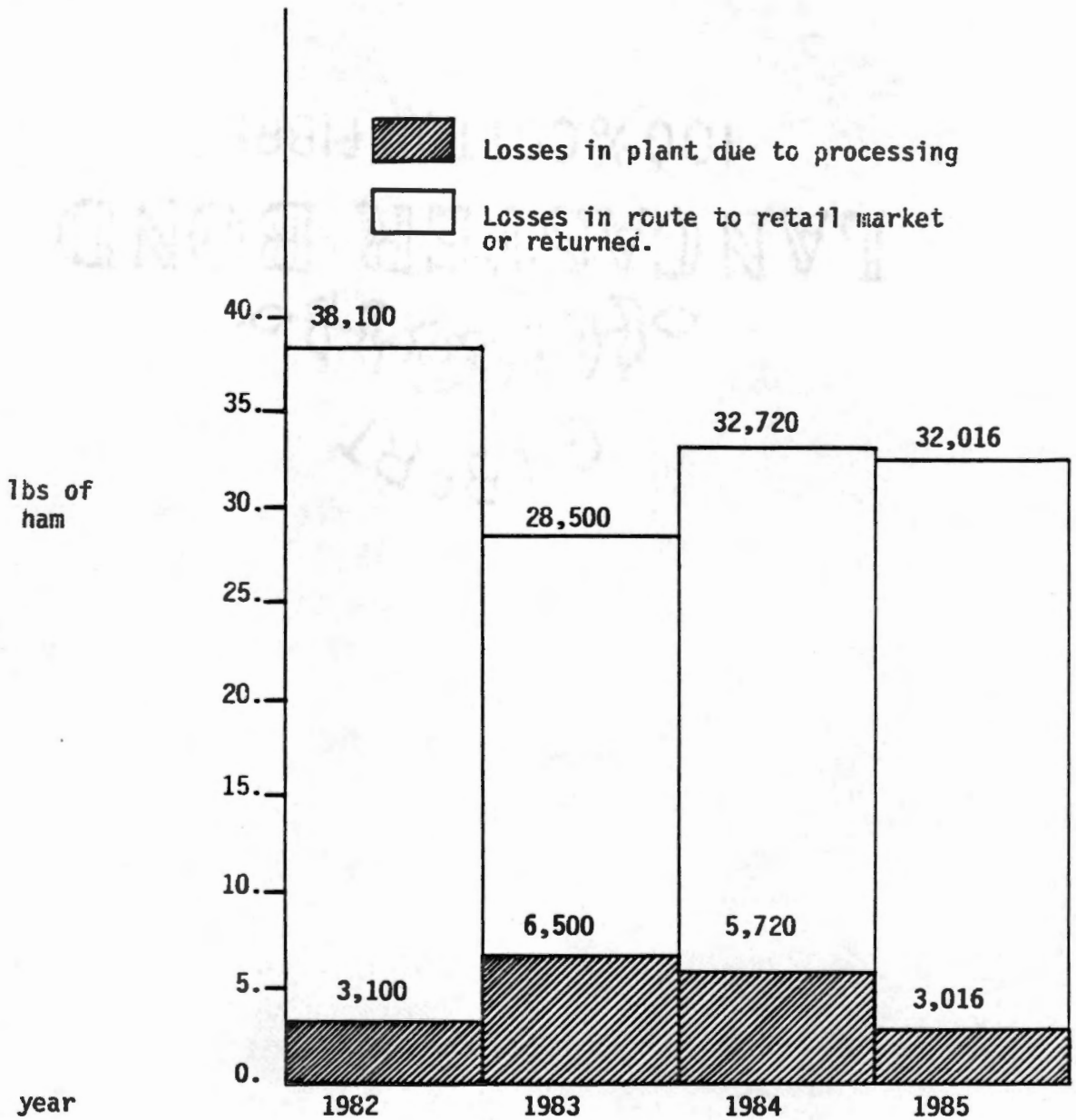


Figure 1. Losses of country hams in one Tennessee processing plant.

CHAPTER II

LITERATURE REVIEW

In September 1975, the Federal Register proposed that the Federal Meat Inspection Act be amended to establish standards of composition for "Country Ham," "Country Style Ham," and "Dry Cured Ham." As a result of this notice many processors and consumers responded. The standards of identity set forth for country ham are shown below:

> All hams must be produced from a single piece of meat.
>
> They may not be injected with curing solutions nor placed in curing solutions.
>
> The entire exterior of the ham shall be coated by the dry application of salt or salt combined with other permitted ingredients.
>
> When nitrate and/or nitrite are added, the application of salt shall be in sufficient quantity to insure that the finished product has an internal salt content of at least 4%.
>
> When no nitrate and/or nitrite are added the finished product must have a brine concentration of not less than 10% or a water activity of not more than 0.92.
>
> To be labeled "Country Style" or "Country," the combined period for curing and salt equalization shall not be less than 45 days. The total time for curing, salt equalization and drying shall not be less than 70 days.
>
> The weight of the finished hams shall be at least 18% less than the fresh uncured weight of the ham.
>
> Optional ingredients for products are nutritive sweeteners, spices, seasonings and flavorings, sodium or potassium nitrate and/or nitrite (Mussman, 1977).

Although a variety of compounds can be used in curing meats, the basic curing ingredients are salt, sugar or some other sweetener, and nitrite and/or nitrate. In addition other compounds such as spices for flavoring may be used. (Kramlich et al., 1982; Varney, 1967; Christian, 1982; Melton, 1982)

Salt is basic to all curing mixtures and is the only ingredient necessary for curing. Salt acts by dehydration and altering of the osmotic pressure so that it inhibits bacterial growth and subsequent spoilage. Use of salt alone, however gives a harsh, dry, salty product that is not very palatable. Additionally, salt alone produces a dark, undesirable colored lean that is unattractive and objectionable to consumers (Kramlich et al., 1982).

Sugar aids in giving a more desirable color and keeps the ham from becoming too hard by preventing some of the moisture removal during aging. Sugar also interacts with the amino groups of the protein and upon cooking forms browning products which enhances the flavor of cured meat. (Kramkich et al., 1975). However, too much sugar can cause excessive slime formation during the curing period (Christian, 1975).

Nitrite performs four main functions in cured meats. It is responsible for the development of cured meat color, through it's reduction to nitric oxide and reaction with the meat pigment myoglobin. Secondly, nitrites have been shown

to contribute to cured flavor in some processed meats. Thirdly, the germination outgrowth and toxin production of C. botulinum is inhibited by nitrite. (Eakes et al., 1975) Fourthly, nitrite retards development of rancidity. Nitrate serves primarily as a source of nitrite (Kramlich et al., 1982).

Spices or other surface rubs may be added as a flavor enhancer. However, these rubs should not be added to hams until they are to be sold. Black pepper, red pepper and other spices are normally high in mold spores and will add to the mold problem during aging if rubbed on the surface (Christian, 1975).

When curing meats the curing mixture should be applied in multiple applications of 2 or 3 times taking care to use the proper amount of cure per pound of meat (Christian, 1975; Melton, 1982; Varney, 1965).

In recent years there has been a shift in the meat processing industry. Centralized slaughter, cutting and processing procedures have increased the problem of bacteriological contamination. The meat products are transferred from slaughterhouses to processing plants and then to the market. The most critical problems are time and temperature. Strict systems of control are needed to prevent contamination, extend shelf life, reduce shrinkage and trim losses and to maintain appearance and acceptability of products (Menzie, 1975).

In the commercial ham business, many thousands of dollars are lost each year due to improper refrigeration Rapid chilling of the hog carcass is a must to prevent spoilage in curing hams. If hams do not thoroughly chill, they may sour before being cured or during the curing period. Carcasses should be below 40°F internally. Ham souring bacteria may be present in the fresh ham and will multiply at temperatures above 40°F (Christian, 1975).

When purchasing from a meat packer, the hams should be specified to be between 36 to 40°F, have a fresh appearance and not be coated with salt before delivery. Hams showing signs of bruising should not be cured because they will likely spoil during the curing process. The curing process should begin within 24 hours after slaughter (Christian, 1975).

Salt penetration in pork is fairly slow. The cure mixture should be placed on the hams taking care that all lean meat surfaces are rubbed thoroughly. Hams should be left in cure for at least 40 days. Temperature is very critical during this period. The hams should be stored between 36 to 40°F. Below 36°F the salt penetration is decreased and above 40°F the ham souring bacteria multiply and cause spoilage (Christian, 1975). At the end of the cure time hams should be brushed with a stiff brush to remove excessive salt deposits that may contribute to over cure areas near the surface. When following recommended

cure times, it isn't necessary to wash or soak hams in water (Melton, 1982).

Hams should be put back under refrigeration at 50°F and hung on a rack in the aging room for salt equalization. During the curing schedule most of the salt is near the surface and very little has penetrated through the skin side (Christian, 1975). The rate of cure penetration is approximately seven days per inch of thickness. During cure and equalization, green hams will shrink approximately seven percent. At the end of equalization, hams can then be hung on a rack in the aging room. Special care should be taken that hams do not touch each other as mold will likely develop at these points (Christian, 1982).

The temperature of aging should be no more than 100°F. The internal temperature of a ham is usually 5°F below the room temperature . Higher temperatures allow a product to be aged in a shorter period of time. However, the lower temperature/longer time cure will produce a ham with more tangy country ham flavor. It is generally recommended that hams age 40 days at 85°F. During aging, hams will shrink another 10 to 12%. There are many time-temperature combinations for curing country hams, however, federal regulations require that country hams be treated for Trichinella spiralis at a minimum of 83°F, a period of 18-22 days. The additional time for a total of forty days is needed to develop the aged flavor (Christian, 1982). The internal

temperature of ham in aging should not exceed 95°F or many of the enzymes that cause aged flavors may be destroyed (Christian, 1975; Christian, 1982).

Vacuum packaging and curing agents, particularly sodium chloride and nitrite, change the balance of microflora from 30% Lactobacill, 30% Streptococcus and 40% Micrococcus to 70% lactic acid bacteria (Ayres et al.,1980). Since the atmosphere within a vacuum package is moist, growth of bacteria is accelerated. By excluding oxygen from the package, growth of aerobic bacteria is suppressed. Vacuum packaging helps to establish anaerobic conditions, thus encouraging the growth of anaerobes, microaerophilic organisms and facultative anaerobes. A study on sliced cured meat on the Swedish market discovered no differance in total bacterial count between vacuum packed and non-vacuum packed products after storage (Sacharaw, 1970).

Kemp et al. (1982) studied vacuum packed ham slices stored at 1°C and 24°C over a 30 day period and found that organoleptic scores favored the groups sampled within a week at 24°C and up to a month at 1°C. After a month at 24°C, all organoleptic scores had decreased, showing that it is essential to keep sliced products refrigerated even when nitrate or nitrite is used. After storage at 24°C for a month, hams cured with nitrate had higher lactobacilli counts, indicating that nitrate selectively enhanced microbial growth.

Since 1929, the use of sodium or potassium nitrite has been approved by the U.S. Meat Inspection Division of USDA. The amount is limited so that the finished products contain no than 200 ppm of nitrite (Cho et al, 1970; CFR, 1985). Nitrite has been found to have significant bacterialcidal properties if the pH is in the range of 4.5 to 5.5 provided the bacterial population is not abnormally high (Hill et al., 1973). Hill et al. (1973) found that nitrite in souse loaf increased from no detectable residual nitrite initially to 13 ppm after a 10 day storage study. He also found that the nitrite content was highly variable during a 30 day storage period study. However, the only significant increase was between the initial and tenth day of storage. It is postulated that all of the available nitrite was depleted during curing and processing with a subsequent conversion of sodium nitrate to nitrite during storage. Eakes et al, (1975) found a rise in residual sodium nitrate from 133 ppm to 142 ppm. This occurred in a cure that used potassium nitrate and sodium nitrite. Low levels of nitrate were recovered from hams to which only nitrite was initially added. This occurrence has been observed in other processed meats and may be partially resulting from the conversion of nitrite to nitrate with the autocatalytic oxidation of the iron in meat pigments. Studies also have revealed that the formation of nitrite was correlated with the concentration of pigment, but it was independent of experimental

conditions and amount of nitrate added. During storage the accumulation of nitrate may also be attributed to the disassociation of nitrous acid to nitric oxide and nitrate and from the oxidation of nitric oxide in aqueous systems to give nitrate and nitrite.

Eakes et al. (1975) studied the effect of temperature on the depletion rate of nitrate and nitrite using a ground pork model system. Nitrate and nitrite were depleted after 4 days storage at 29°C. Rapid depletion of nitrate after 2 days at 29°C and a corresponding increase in nitrite is indicative of microbial nitrate reduction. A gradual decrease in nitrate and nitrite concentration was observed in samples stored 12 days at 4°C.

Meat processors strive to prevent product deterioration for a reasonable length of time to allow for marketing of their products. The period of time during which products remain salable is commonly referred to as shelf life . A common spoilage situation in vacuum packaged products is the gassy package, in which gas producing organisms on the surface of the product evolve gas which in turn causes the package to become distended. The gas is carbon dioxide, which is odorless, colorless and tasteless. The carbon dioxide results from a fermentation process induced by the microaerophillic or anaerobic bacteria. Usually, but not always when gas is formed, acid is also present, as the

bacteria that cause gas formation usually form acid as well (Kramlich et al., 1982).

Spoilage of whole hams is usually not detected until a ham is sliced. A small wooden stick or a stainless steel probe can be used to detect spoilage before slicing or selling a ham. By probing the ham in the area of the aitch bone or shank area, then smelling the probe, off odors are easily detected (Christian, 1975).

A good country ham will have a moisture content in the range of 50 - 60% and a salt content of 4.5 to 6.5%. This is important as the salt content must be high enough to prevent bacterial action in the ham. Products with high enough salt content are often too dry for most microbial growth other than Staphylococcus aureus and aspergilli or penicillia (Ayres, 1975). When the moisture content of a ham is too high, there is likely to be a breakdown of protein causing an off flavor. In some cases, there is a complete spoilage and the ham will puff and blow (Christian, 1975).

With cured pork, water activity values range from 0.87 to 0.93. These levels are below those necessary for the growth of spore forming aerobic bacteria, pseudomonads, achromobacters, salmonellae and C. botulinum. If cured meats are normally salted, these organisms are eliminated (Ayres, 1975). Salt tolerant microorganisms predominate in

the resident microflora of country-style hams and apparently have little effect on ham quality (Bartholomew et al.,1977).

A study by Hunt et al. (1939) found that as the age of the ham increased the number of microorganisms decreased. Hams that didn't age properly or which were rancid or sour frequently showed very large numbers of proteolytic organisms. The number of organisms was low in hams which retained the fresh cured aroma and texture.

Certain types of lactic acid bacteria have been found to be antagonistic to bacteria of public health concern and provide a more acceptable microflora for human consumption. It may be possible to eliminate undesirable microorganisms by inoculating hams with approved concentrated starter cultures to produce hams free from bacteria of public health hazard and with superior flavor and quality characteristics (Bartholomew et al., 1977).

Outbreaks of C. botulinum in Germany have been associated with "home cured ham" Toxin can be formed in 11 days at 46°F. Puolanne (1982) suggest rapid post-mortem chilling of hams to 39°F or below, that initial pH should be 5.8 or less and a maximum level of 600 mg/kg of potassium nitrate be used in cures.

During the curing process, temperatures must be kept below 46°F until the salt content is high enough for the water activity to reach values below 0.96. C. botulinum is unlikely to grow in cured meats at normal refrigeration

temperatures. However, if the consumer stores the vacuum package at high temperatures, bacterial growth is encouraged. Stray pathogenic organisms cannot establish themselves in the growth pressure exerted by the indigenous anaerobic flora (Sacharow,1970). Kemp et al. (1982) inoculated ham slices cut from hams made with different curing mixtures after curing and aging with five strains of C. botulinum spores. No toxin developed. This indicated that the water activity, even in hams where no nitrate or nitrite was used where sufficiently low to prevent toxin formation.

Because hams are not cooked through out storage and the proteins are not heat denatured, the meat is less susceptible to staphylococcal attack, However, micrococci, lactobacilli and yeast proliferate. With further reduction of water activity molds become the predominate flora (Ayres et al., 1980).

The amount of mold growth varies considerably depending on storage conditions. The higher the relative humidity of the storage environment the more mold growth that is present. Draughon and coworkers (1981) found that a reduction in mold growth could be obtained if whole hams were coated in wax during the aging time.

Cleanliness is the first line defense against spoilage, so a good sanitation program must be followed consistently (Varney, 1967). However, extreme hygienic conditions have contributed to loss of traditional flavor and aroma in

products. This is probably true since the natural microflora is reduced via extreme hygiene. The use of nitrite, initially appears to further decrease growth of some desirable microflora associated with traditional flavor and aroma. Therefore, Puolanne (1982) has suggested that a starter culture may be used to produce a better product.

Cured pork valued at many thousands of dollars is lost each year due to improper curing and storage (Christian, 1975). A basic trouble shooting rule of thumb says that if more than one half of one percent spoilage is occurring, than the curing and aging procedures need some adjustments (Anonymous, 1982). As vacuum packaged country ham slices continue to grow in popularity and marketing techniques do not change it is extremely important for manufacturers to produce a product that is safe and acceptable to consumers. This research has attempted to identify some areas of concern and some possible solutions to problems that may already be occurring.

CHAPTER III

MATERIALS AND METHODS

1. SAMPLE PREPARATION

Ham samples were provided by a Tennessee country ham processor. All processing and packaging was done by the plant using the same method that is used for their hams going to retail distributors.

Samples were selected for the study based on salt analysis at the plant site. Analysis was performed on a slice of ham taken from a whole ham. Twenty-seven whole hams were sampled. Upon selection each ham was cut into six center cut slices and individually vacuum packaged. Ham slices were labeled with initial salt concentration and placed in the incubator upon arrival at the University of Tennessee. There was a one day time period between packaging and initial analysis because ham slices had to be transported to the university laboratories. Incubation times were for 1, 7, 14, 21, 28 and 42 days at 28^{o}C. At the incubation intervals, one ham slice from each ham was removed and analysed chemically, microbiologically, and visually.

2. PROCESSING PROCEDURES

Hams were received from one of three packing houses. Salt and nitrite/nitrate mixture was rubbed into the hams by hand. Hams were held for 3-5 days at 38-40°F and were resalted. Hams were stored at 38-40°F and allowed to set 42 days with cure mixture on them. At the end of this period excess salt was then washed from hams and then the hams were placed in the dehumidifier for 14 days for salt equalization at 58-60°F. Hams were placed into the smoker for 10 days at 97-103°F. Unsmoked hams were placed in a room at 97-103°F for 10 days to age. Hams were then placed in a 80% humidity room and held for 14-21 days at 58-60°F. Prior to packing, hams were deboned in a chilled room of 58-60°F. After deboning, hams were temper-frozen for 42 hours prior to slicing to facilitate slicing and to reduce Microbial flora.

A homogeneous sample of each slice of ham was aseptically prepared using a Waring commercial blender (7011 model 31BL92). Ham slices had excess fat and connective tissue removed and were cut into small cubes to facilitate grinding. Meat cubes were placed in a sterile blender cup and ground one minute. Eleven grams of meat were used for microbiological testing. The remainder of the samples were frozen and held until chemical analysis could be performed on them.

3. SALT ANALYSIS

A. VOLHARD METHOD

Salt analysis was performed on each ground slice of ham after the selected incubation period to determine the salt concentration of slices (AOAC: 24.010, 1984). Approximately 1.5 grams of ground ham sample was placed in a 250 ml Erlenmeyer flask. Five milliliters (ml) of standardized O.5N Silver Nitrate (Fisher, Fair Lawn, NJ) solution was added to the ham sample using a volumetric pipette. Fifteen milliliters of concentrated nitric acid (Fisher, Fair Lawn, NJ) was added. The flask was then placed on the hot plate and wet ashed. Potassium Permanganate (Baker, Phillipsburg, NJ) was added in small portions and boiled until the permanganate color disappeared or until the solution became colorless or nearly so. Twenty five milliliters of distilled water was added. The solution was boiled for five minutes. and removed from the hotplate. After cooling, 100 ml of distilled water, 5 ml of ferric indicator (Fisher, Fair Lawn, NJ) and 10 ml of methyl ether (Fisher, Fair Lawn, NJ) were added to the flask. The solution was titrated against 0.1N ammonium thiocyanate (Fisher, Fair Lawn, NJ)

until the solution became light salmon in color. Percent salt was calculated by the following formula:

$$\% \text{ salt} = \frac{\text{\# ml } 0.5N\ AgNO_3 - 0.2\ (\text{\# ml } 0.1N\ NH_4SCN) \times 2.92}{\text{grams of sample}}$$

B. SODIUM SENSITIVE ELECTRODE

A five point standardization curve was set up to determine the percent salt in the ham slices using the Fisher Accumet Model 825 MP pH meter equipped with the sodium responsive electrode #13-639-20 (Fisher, Fair Lawn NJ) and Calomel reference electrode #13-639-52 (Fisher, Fair Lawn, NJ). Ham samples were diluted at a 1:15 ratio with distilled water.

C. QUANTAB CHLORIDE TITRATOR STICKS

Quantab Chloride Titrator Sticks #1176, (Miles, Elkhart, IN) that measure 0.05-0.60% NaCl were used. A 1:15 dilution of the ground ham sample and distilled water was used. Meat and boiling water were mixed thoroughly to obtain a good extraction of the salt from the sample. Filter paper was folded into quarters and placed into the solution. The lower end of the titrator stick was placed into the solution. The test strip column was then allowed

to become saturated. The scale was read and percent salt determined using the calibrated table provided with the test strips.

4. NITRITE ANALYSIS

Nitrite analysis was preformed on ham samples using the Sulfanilic acid method (AOAC: 24.041,1984; Koniecko, 1980). An aqueous extract from ham was reacted with Greiss reagent containing sulfanilic acid (Fisher, Fair Lawn, NJ) and N-1-Naphthyl-ethylenediamine (Sigma, St. Louis, MO). Color developed was measured spectrophotometrically (Bausch and Lomb, Spec 20, Rochester, NY) and the nitrite concentration in parts per million (ppm) was determined by a standard curve prepared by measuring the color development of solutions containing known amounts of nitrite. Absorbance was measured at 540 nm.

5. NITRATE ANALYSIS

Nitrate analysis was done using the Xylenol method (AOAC: 24.041, 1984; Koniecko, 1980). Nitrates were extracted from the meat sample with hot water. Nitrates are oxidized to nitrites. 2,4-Dimethylphenol (Eastman Kodak, Rochester, NY) was added and the color complex was distilled into dilute alkali. The color was measured by

spectrophotometer and nitrate was determined by a standard curve prepared at the same time. Absorbance was measured at 450 nm. Total nitrate was equal to the nitrite measured at 450 nm minus nitrite measured at 540 nm.

6. MICROBIOLOGICAL ANALYSIS

Meat samples were pour plated on Standard Methods Agar (BBL, Cockeysville, MD) containing 1% NaCl (Sigma, St. Louis, MO), APT agar (BBL, Cockeysville, MD) and on Violet Red Bile agar (BBL, Cockeysville, MD). VRB and SMA plates were incubated at 32°C under atmospheric conditions. A duplicate set of SMA and APT plates were incubated in a CO_2 incubator (Napco, Portland, OR) under microaerophilic atmospheric conditions of 5% O_2: 10% CO_2 and 85% N_2. Decimal dilutions were prepared using 0.1% peptone water (Difco, Detroit, MI) containing 1.0% NaCl. Plates were counted and recorded using protocol as set forth in Standard Methods For the Examination of Dairy Products (APHA, 1984).

7. pH

The pH was measured using the Fisher Accumet pH Meter, model 600 which was standardized in the sample range. The ground meat samples were prepared using a 1:10 dilution. The meter was read to the nearest tenth.

8. MOISTURE

Moisture was run on each ham slice after incubation. Approximately 2 grams of sample was placed in a covered aluminum drying dish and heated in a vacuum oven (Precision, Chicago, IL) for 24 hours at 121°C. Samples were cooled in a desiccator and then weighed on an analytical balance (Mettler, AE 160; High Towns NJ).

9. SENSORY ANALYSIS

Ham slices were removed from the incubator at specified intervals and evaluated visually for distention of package and organoleptically for smell of product. Strong smells were identified as closely as possible as to type of odor present such as acid, ammonia or nutty. Samples showing extreme distention of packages and strong odor were considered spoiled.

10. STATISTICAL ANALYSIS

All experiments had two replicates. Analysis of Variance (ANOVA) was performed to determine interactions and differences among means. Differences when significant ($p<.05$) were separated by Duncan's Multiple Range Test (Steel and Torrie, 1960). Variables to be analysed

included: length of storage, salt concentrations, nitrate, nitrite, pH, and microbial loads. Regression analysis was preformed to compare the three different methods of salt analysis. All analyses were conducted using the Statistical Analysis System (SAS Institute, 1985) of the University of Tennessee Computer Center.

CHAPTER IV

RESULTS AND DISCUSSION

1. SALT ANALYSIS

The Volhard method (AOAC) of salt analysis was considered the standard for determining salt content. Salt analysis of hams showed that the mean salt content of samples ranged from 3.91 to 5.69% (Table 1). Hams of different salt levels were needed to study chemical and microbiological factors affecting shelflife of country hams. Separation of means by Duncan's Multiple Range Test showed that except for samples two and three all six salt contents were significantly different. There were no significant differences in salt levels over time for samples ($p<0.05$), indicating that salt content of country ham slices did not change significantly over the six week storage period. Slices from the same hams had similar salt contents (Table 2).

It has become important in the food industry to find simple, inexpensive and rapid methods for detecting salt. Two such methods were investigated in addition to the Volhard method for salt determination. The two methods were the Sodium Electrode method and the Quantab Chloride Titrator method (Table 1).

Table 1. Analysis of salt in vacuum packaged county hams slices by the Volhard method, Quantab Chloride Titrator method and the Sodium Sensitive Electrode method.

SAMPLE	VOLHARD		TITRATOR 1[2]		TITRATOR 2		ELECTRODE	
1	3.91	a[1]	3.47	a	3.33	a	4.39	a
2	4.33	b	3.90	ab	3.49	a	5.30	b
3	4.46	b	4.24	b	3.44	a	5.25	b
4	4.70	c	4.11	ab	3.91	a	5.72	b
5	5.05	d	4.49	b	3.74	ab	5.26	b
6	5.69	e	5.23	c	4.49	b	7.84	c

[1]Means in columns followed by different letters are significantly different.

[2]Titrator 1 and Titrator 2 were the Quantab Chloride Titrator method ran on consecutive days.

All means are averaged over time using twelve samples.

Table 2. Salt analysis of country ham slices as a function of time.

SAMPLE	MEAN % SALT	% NaCl WEEKS OF STORAGE 0	1	2	3	4	6
1A	3.8 a[1]	3.8	3.9	3.7	3.7	3.8	3.8
1B	4.1 a	4.0	4.3	3.9	4.0	4.1	4.0
2A	4.3 b	4.0	4.3	4.4	4.2	4.5	4.5
2B	4.3 b	4.3	4.4	4.4	4.3	4.3	4.3
3A	4.4 b	4.2	4.6	4.2	4.7	4.4	4.2
3B	4.5 b	4.4	4.5	4.7	4.2	4.8	4.7
4A	4.8 c	4.4	5.2	4.3	5.3	4.9	4.7
4B	4.6 b	4.7	4.2	5.0	4.6	4.3	4.8
5A	5.1 d	4.9	5.1	5.0	5.2	5.1	5.4
5B	5.0 d	4.8	5.2	4.8	5.1	5.1	4.8
6A	5.3 d	5.3	5.4	5.4	5.2	5.2	5.5
6B	6.0 e	5.6	6.1	5.8	6.1	6.5	6.2

[1]Means of % salt followed by the same letter in column are not significantly different ($p<0.05$).

All slices within a treatment were from the same ham.

Each replicate is shown separately.

Salt analysis by the electrode method was inconsistent and unpredictable. In some instances, values for salt by electrode were very similar to the Volhard method, however,the electrode measured as high as 1.2% excess NaCl in samples which had 5.7% NaCl. Therefore, the electrode method, as used in this study, does not give a satisfactory salt analysis for country style ham slices.

Quantab Chloride Titrator sticks (QCT) were run in duplicate on each sample to determine reproducibility and accuracy of this method of analysis. These data are presented in Figure 2. Day one and Day two data were from the same ground meat sample analyzed on successive days. Totally different regression lines were generated. Data generated on salt content of hams with the QCT differed significantly ($p<0.05$) on successive days. Another problem with the QCT was that the sticks could not detect differences in samples having higher or lower salt content with any degree of accuracy (Table 1). The QCT did not detect differences in salt content of samples 2, 3, 4, and 5 during the first analysis and they did not detect differences for 1, 2, 3, 4, or 5 for the second analysis (Table 1). The QCT could detect differences of > 1.5% NaCl in samples. Therefore, the variation from day to day and the lack of sensitivity of this method prohibit it's use as an analytical technique for salt analysis in country ham.

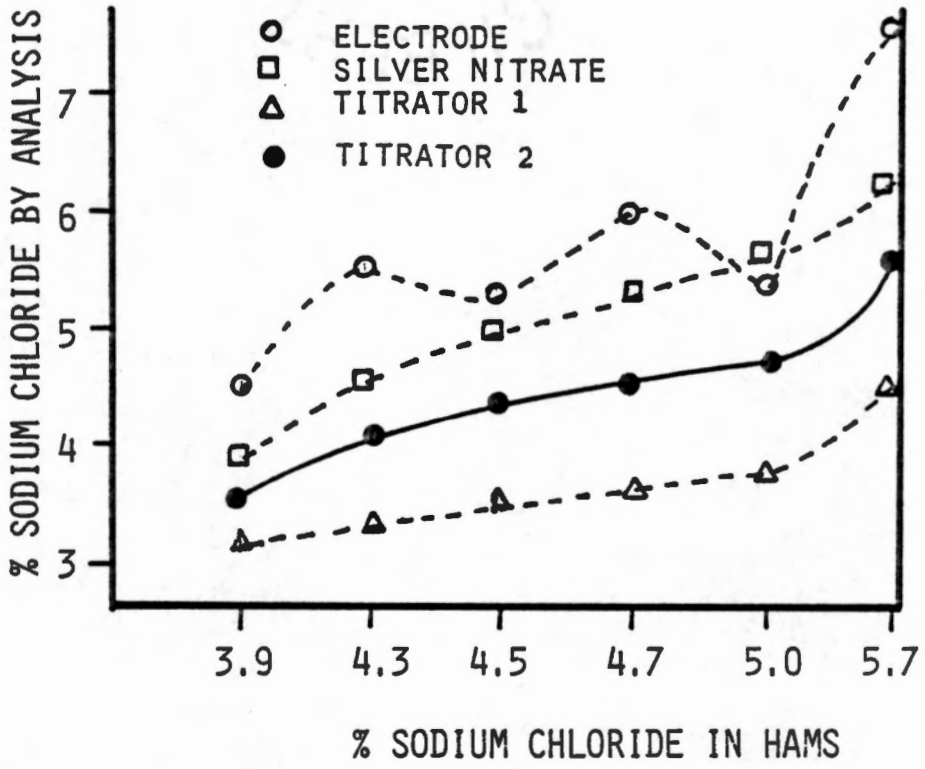


Figure 2. Comparison of the Sodium Sensitive Electrode and the Quantab Chloride Titrator sticks to the Volhard method of salt analysis in Country Style Ham.

All points are the mean of two replicates

The Volhard method did not detect differences over time; however, the QCT did indicate significant differences in NaCl over time. I am not sure if these differences are real or if they are artifacts due to the variability of this method. The QCT appeared to be responsive to other chemicals in addition to salt, and the by-products of microbial growth affect their salt readings.

Although it would have been desirable to use the faster methods for salt determination, the results of this research indicated that the rapid methods do not give reproducible or accurate results.

2. MOISTURE

As salt levels increased, the moisture content decreased significantly ($p>0.05$) (Table 3). The osmotic activity of salt and the subsequent moisture evaporation from hams would cause this change to occur (Kramlich et al., 1982). There was an interaction between sample and replication for moisture content ($p<.05$). This was probably due to the fact that values for moisture content were significantly different ($p<0.05$) for each replicate. There were observed changes in moisture content over time although, it did not change significantly ($p<0.05$) (Table 4). One possible reason for these changes over time could have been evaporation caused by routine handling of the

Table 3. Mean salt and moisture content of vacuum packaged country ham slices as a function of salt content.

SAMPLE	SALT (%)	MOISTURE (%)
1	3.91 a[1]	66.9 a
2	4.33 b	62.3 bc
3	4.46 b	61.8 bc
4	4.70 c	63.1 b
5	5.05 d	59.7 d
6	5.69 e	61.0 cd

[1]Means in columns followed by different letters are significantly different ($p<0.05$).

Table 4. Percent moisture analysis of country ham slices as a function of time.

SAMPLE	MEAN % MOISTURE	% MOISTURE WEEKS OF STORAGE 0	1	2	3	4	6
1A[1]	66.1	67.8	67.0	63.2	66.8	66.5	65.8
1B[2]	67.7	68.4	69.8	67.2	66.2	67.9	66.7
2A	63.5	63.7	64.5	64.9	64.8	60.1	62.8
2B	61.1	63.2	60.1	59.0	59.1	63.2	69.1
3A	59.9	57.2	60.1	60.0	61.8	61.4	58.9
3B	64.0	63.8	63.4	65.8	63.6	63.0	64.1
4A	58.1	59.0	57.2	59.3	59.9	55.4	58.0
4B	63.6	66.3	63.4	63.1	63.9	61.2	63.9
5A	62.3	64.5	62.2	60.2	61.2	63.7	61.8
5B	61.5	57.4	60.8	62.4	61.3	60.7	60.6
6A	58.3	60.1	59.1	54.4	59.8	58.2	58.9
6B	63.9	65.7	63.7	64.7	61.1	63.1	65.1

[1]All slices within a treatment were from the same ham.

[2]Each replicate is shown separately and is disignated A or B.

samples. Salt levels and moisture content are closely linked due to physical laws controlling water activity, any change in one affects the other.

3. NITRATE/NITRITE

Nitrate varied significantly over time ($p<0.01$) (Figure 3). There was a high nitrate content at Day one which significantly decreased during the first week and than gradually increased through week six. Nitrate levels were not predictable based on salt content of samples (Figure 4). No linear relationship existed between nitrate and salt content.

The nitrite levels were correlated with salt level, as higher salt content hams showed higher nitrite levels. Replicates showed no significant differences for nitrite level.

At the same time that the drop in nitrate was detected, a consequent increase was observed for nitrite (Figure 3). This phenomenon was expected as microorganisms have been shown to convert nitrate to nitrite (Hill et al.,1973; Eakes et al., 1975). As salt level increased it was expected that both initial nitrate and nitrite levels would also increase. This did not prove to be the true. Sample 1 had the lowest salt content and the highest nitrate/nitrite levels. This

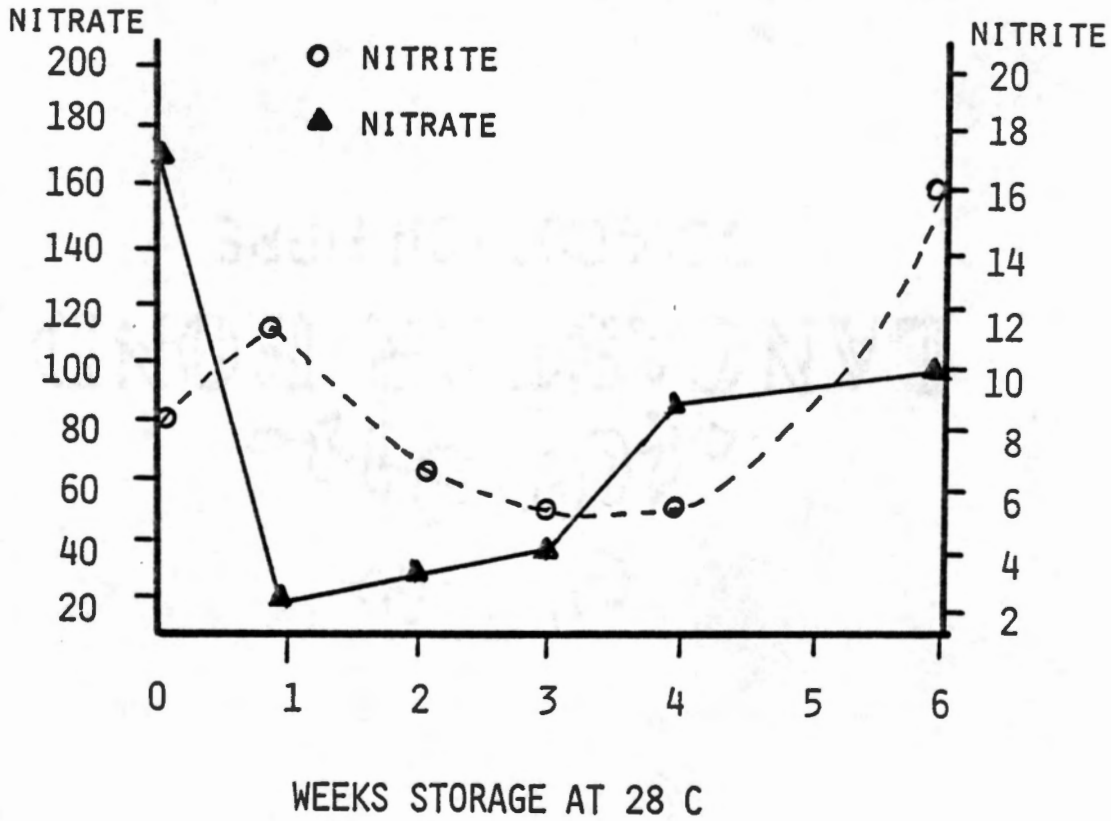


Figure 3. Average nitrate and nitrite content (ppm) of Country Style Ham averaged for each week of storage at 28°C.

All points are the mean of 12 samples.

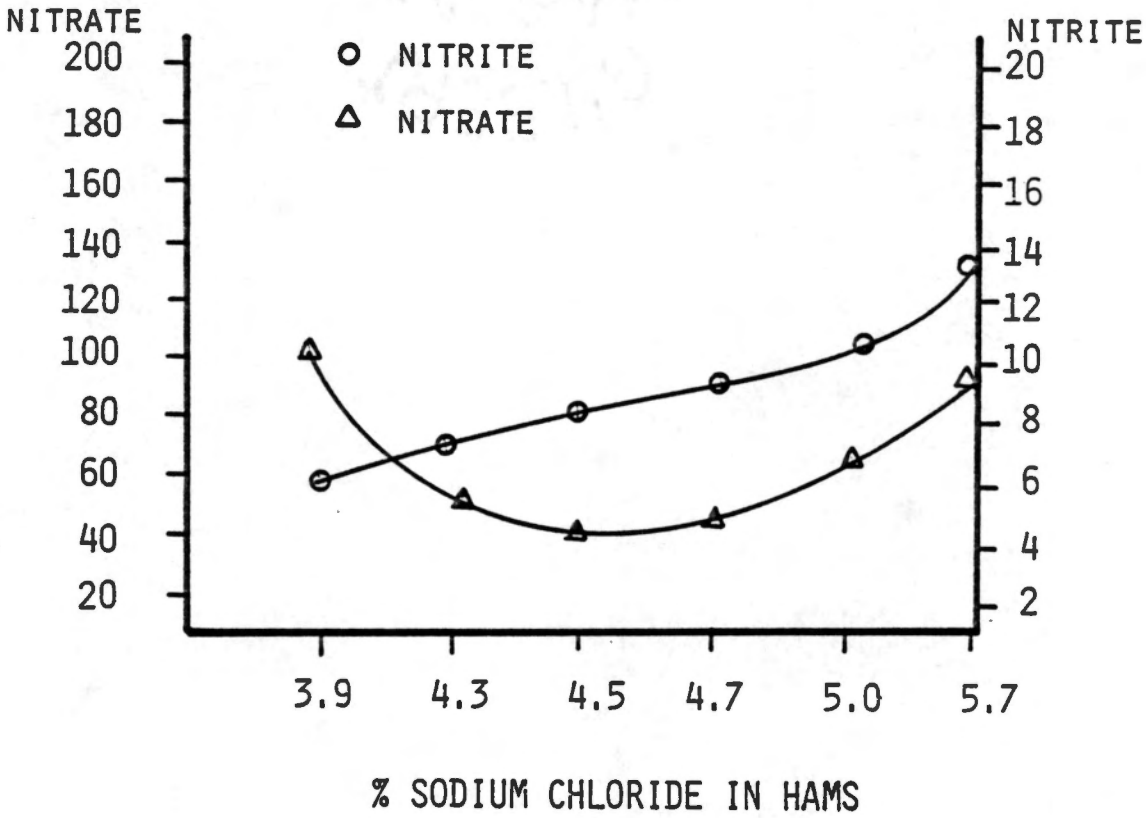


Figure 4. Average nitrite and nitrate content (ppm) of hams compared to the percent sodium chloride.

Points are the mean of 12 hams during the six week storage period.

could have been a mixing problem occurring during the preparation of the cure. The only variable that affected nitrate and nitrite levels significantly, was time. Eakes et al. (1975) also found that the depletion rate of nitrate was variable and did not appear to be affected by initial levels added or pH. The greatest effect on nitrite residuals was probably dependent upon the population of nitrate/nitrite reducing organisms within the product and the length of storage. Changes in the moisture content over the storage period (Table 4)may have contributed somewhat to the rise of nitrate/nitrite present because of the change in concentration in the ham samples.

4. pH

Samples having different salt contents had significantly different pH values ($p<0.05$). The pH of the sample was not predictable based on salt content (Table 5). However, there was a direct relationship between salt content and pH since the ham slices having the lowest salt content had the highest mean pH. Conversely, ham slices having the highest salt content had the lowest mean pH. Ham slices in the 4.3 to 5.0% salt range had mean pH values between those of the lowest and the highest salt level hams (Table 5). These differences were significant ($p<0.05$).

Table 5. Changes in pH over time for vacuum packaged country ham slices having different salt contents.

SAMPLE	%SALT	pH OVER WEEKS OF STORAGE						MEAN
		0	1	2	3	4	6	pH
1	3.9 a[1]	6.2	6.7	6.4	6.8	7.0	7.2	6.7 c
2	4.3 b	6.0	6.3	6.2	6.4	6.5	6.6	6.5 b
3	4.4 b	6.2	6.5	6.4	6.7	6.8	6.8	6.6 b
4	4.7 c	6.0	6.2	6.2	6.3	6.6	6.9	6.4 b
5	5.0 d	6.1	6.4	6.4	6.5	6.5	6.7	6.4 b
6	5.7 e	6.0	6.2	6.0	6.1	6.4	6.5	6.2 a

[1]Means in columns followed by different letters are significantly different.

The differences in pH of ham slices having lower and higher amounts of salt is due to microbial growth since initial pH values of ham slices in all treatments were approximately the same (Table 5). Within the first week of storage, pH values of ham slices having 3.9% salt increased 0.5 pH units. Whereas, pH values of ham slices having the highest amount of salt (5.7%) were virtually unchanged during the first three weeks of storage (Table 5).

Lactic acid bacteria tend to be salt tolerant and can also grow in vacuum packaged meats (Bartholomew et al., 1977; Sacharow, 1970; Kramlich et al., 1982). A sour odor was frequently the major spoilage odor, yet the pH values do not reflect major acid production. This could be due to the strong buffering capacity of meat or to the growth of other types of competitive microorganisms which use amino acids and raise the pH through ammonia production (Buchanan et al., 1974; Banwart, 1979).

5. MICROBIOLOGICAL ANALYSIS

Aerobic plate counts (APC) of vacuum packaged ham slices are presented in Table 6. Initial counts ranged from $\log_{10}$ APC 4.6 to 6.3/g and were higher than expected. We found there was no direct relationship between the amount of salt and count of microorganisms. Counts increased significantly during the first week (generally by as much as

Table 6. Aerobic plate counts of vacuum packaged country ham slices stored at 28°C for six weeks.

Sample	%Salt	Log10 CFU/g WEEKS OF STORAGE						Mean
		0	1	2	3	4	6	CFU
1	3.9 a[1]	5.5	7.6	7.9	7.4	7.2	7.6	7.2 a
2	4.3 b	5.1[2]	8.3	7.7	7.3	7.1	6.6	7.0 a
3	4.4 b	5.3	7.7	7.7	7.3	7.2	7.3	7.1 a
4	4.7 c	4.6	7.2	7.7	7.6	7.3	7.4	7.0 a
5	5.0 d	6.3	8.1	7.8	8.1	7.5	7.7	7.6 b
6	5.6 e	4.9	7.2	7.7	7.4	7.2	7.6	7.0 a
MEAN PER WEEK		5.0a[3]	7.5b	7.7b	7.5b	7.1b	7.2b	

[1]Means in columns followed by different letters are significantly different ($p<0.05$). All data are the mean of two replicates.

[2]Initial counts on the two samples were significantly different ($p<0.05$). Rep 1 APC was $\log_{10}$ 6.6 CFU/g, whereas Rep 2 CFU was only $\log_{10}$ 3.8.

3Means in this row followed by different letters are significantly different ($p<0.05$).

two log cycles). After the second week, APC's leveled off and in some treatments decreased slightly. Since the interior of hams is almost sterile before slicing, the high initial counts of the vacuum packaged slices indicates a serious bacterial contamination problem with the equipment and surfaces coming into contact with the ham slices. In addition, the rapid growth of these microorganisms at salt levels ranging from 3.9 to 5.6% indicated that the contaminants are haloduric. This rapid bacterial growth at high salt levels may indicate a chronic sanitation problem. It is possible that the contaminating bacteria have been selected over a period of time since the processing plant produces only country style hams and bacteria not able to grow or survive in a salty environment would have been destroyed.

Anaerobic plate counts were also very high initially in ham slices of all salt levels (Table 7). In all but treatment 4, anaerobic counts were in excess of $\log_{10}$ 7.0 CFU/g. Over the storage period, counts did not change significantly ($p<0.05$). However, there was evidence of bacterial action since packages swelled and developed off-odors. Anaerobic plate counts generally decreased slightly during the last two weeks of storage. This same phenomena was observed with the APC's and particularly with the APT counts (Table 8). The decrease in bacterial counts in late storage was probably due to an accumulation of toxic

Table 7. Anaerobic plate counts of vacuum packaged country ham slices stored at 28°C for six weeks.

Sample	%Salt	Log10 CFU/g WEEKS STORAGE 0	1	2	3	4	6	Mean
1	3.9 a[1]	7.8	7.7	7.6	7.3	7.3	6.7	7.4 ab
2	4.3 b	7.5	7.3	7.6	7.6	7.2	6.7	7.3 ab
3	4.4 b	7.5	7.0	7.7	6.9	7.8	7.5	7.4 ab
4	4.7 c	5.3	7.4	7.9	7.4	7.4	6.8	7.0 a
5	5.0 d	7.5	8.2	7.7	8.2	7.7	7.6	7.6 b
6	5.7 e	7.5	7.4	7.0	7.5	7.2	7.5	7.4 ab
MEAN PER WEEK		6.6b[2]	7.6a	7.5a	7.4a	7.4a	7.1ab	

[1]Means in columns followed by different letters are significantly different ($p<0.05$). All data are the mean of two replicates.

[2]Means in this row followed by different letter are significantly different ($p<0.05$).

Table 8. Microbial growth in vacuum packaged country ham slices as enumerated on APT agar.

Sample	%Salt	Log10 CFU/g WEEKS STORAGE 0	1	2	3	4	6	Mean
1	3.9 a[1]	7.8	7.9	7.6	7.7	7.9	6.7	7.6 ab
2	4.3 b	7.5	7.8	7.6	8.7	7.6	8.9	8.0 b
3	4.4 b	7.5	8.0	7.5	7.7	8.3	7.9	7.8 b
4	4.7 c	7.5	8.0	7.5	8.5	7.7	6.4	7.2 a
5	5.0 d	7.5	9.5	7.7	8.3	8.1	6.9	8.0 b
6	5.7 e	5.1	7.8	7.7	8.6	8.2	6.9	7.3 a
MEAN FOR WEEK		6.2c[2]	8.1a	7.6a	8.1a	7.8a	7.0b	

[1]Means in columns followed by different letters are significantly different ($p<0.05$). All data are the mean of two replicates.

2Means in rows followed by different letters are significantly different ($p<0.05$).

metabolic end products in the package since it was a closed system.

APT agar is frequently used in the enumeration of bacteria in meat products, particularly when they are cured and have been vacuum packaged. APT agar allows the growth of many types of bacteria, but also has special nutrients which encourages the growth of lactobacilli. Counts on APT (Table 8) agar seemed to closely parallel the anaerobic bacterial counts on ham slices having the highest salt content. They had $\log_{10}$ 4.9 CFU/g and $\log_{10}$ 5.1 CFU/g, but showed $\log_{10}$ 7.5 anaerobic plate count/g. These data suggest that the microaerophilic microflora of the higher salt content ham slices is slightly different and more oxygen tolerant than the lower salt content hams. The highest bacterial counts observed in our study were on APT agar in ham slices of the higher salt contents of 5.0 and 5.6% NaCl (Table 8). APT agar counts were also higher overall for all samples than APC's or anaerobic bacterial counts.

Coliform counts were not significantly different ($p<0.05$) for ham slices of different salt content (Table 9). Peak occurrence of coliforms was at week two. By the fourth week of storage, no coliforms were detected except in sample treatment three. This was possibly due to the inability of the coliforms to compete with the growth of the other indigenous bacteria in the vacuum package (Sacharow, 1970).

Table 9. Coliform counts of vacuum packaged country ham slices stored at 28°C for six weeks.

Sample	%Salt	Log10 CFU/g WEEKS STORAGE 0	1	2	3	4	6	Mean
1	3.9 a[1]	1.9	2.7	2.8	<1.0	<1.0	<1.0	1.7 a
2	4.3 b	<1.0	2.9	2.8	<1.0	<1.0	<1.0	1.6 a
3	4.4 b	<1.0	<1.0	3.3	<1.0	3.4	<1.0	1.8 a
4	4.7 c	<1.0	2.7	2.0	3.0	<1.0	<1.0	1.8 a
5	5.0 d	<1.0	<1.0	2.5	2.3	<1.0	<1.0	1.5 a
6	5.7 e	2.3	<1.0	3.7	<1.0	<1.0	<1.0	1.6 a

[1]Means followed by different letters are significantly different ($p<0.05$). All data are the mean of two replicates.

Because commercially produced hams have a milder cure and moister surface than dry cured hams produced in the past, slicing for packaging creates sources of contamination by producing more cut surfaces on which microorganisms can grow. Commercially produced hams allow the growth of micrococci, Microbacterium, various lactic acid bacteria and yeast. Spoilage of meat is due to the growth and metabolism of large numbers of microorganisms on the surface or interior. Most spoilage occurs on the surface. Both the numbers and types of organisms affect the spoilage characteristics of meat. Generally, spoilage of meat is evident when the number of organisms is 10^7 to 10^8 per cm^2 of meat surface. However, vacuum packaged cured or processed meats may have microbial levels over 10^8/g and be considered to be satisfactory for consumption (Banwart, 1979). It is not necessarily the number of microorganisms which causes spoilage in cured vacuum packaged meats so much as the type of microorganism present.

6. SENSORY EVALUATION

The most common indications of meat spoilage in country ham are 1) off-odors and slime, 2) fungal growth which is favored at A_w too low for bacterial growth, 3) bone-taint or deep spoilage due to anaerobic or facultative microorganisms and 4) discoloration primarily due to alterations of

myoglobin, the muscle pigment (Banwart, 1979). Sensory evaluation of the vacuum packaged samples over time tracked the major spoilage characteristics of hams (Table 10). Evaluations of samples indicated that samples of less than 4.5% NaCl could not give the two week shelf life at 28°C which was needed by the producer. Evaluation also indicated that even with the higher concentrations of salt the product may not last for the desired length of time. Possibly the major reason for this would be because of poor sanitation, indicated by the very high bacterial counts (Tables 6, 7, 8, and 9).

Table 10. Sensory evaluation of vacuum packaged country ham slices held at 28°C during the six week storage period.

SAMPLE	% SALT	WEEK[1] 2	WEEK 3	WEEK[2] 4
1.1	4.0	slight gas	spoiled	spoiled
1.2	3.8	moderate gas	spoiled	spoiled
2.1	4.3	slight gas	spoiled	spoiled
2.2	4.3	slight gas	spoiled	spoiled
3.1	4.4	moderate gas	spoiled	spoiled
3.2	4.5	good	spoiled	spoiled
4.1	4.8	slight gas	slight gas	spoiled
4.2	4.6	slight gas	slight gas	spoiled
5.1	5.1	good	good	good
5.2	5.0	good	good	nutty odor
6.1	5.3	slight gas	acid odor	spoiled
6.2	6.0	good	acid odor	spoiled

[1]No samples were spoiled at week 1.

[2]All samples were spoiled at week 6.

CHAPTER V

CONCLUSIONS

The first objective of this study was to determine the minimum level of salt concentration necessary in vacuum packaged country ham slices to obtain a two week shelf life at 28°C. The chemical analysis of the products indicated that microbial counts were not significantly affected by salt concentration over the levels tested. That was probably due to the high initial microbial loads, high pH, and high moisture content of the hams. Undesirable sensory characteristics, such as gassing of the package and smell of the product were less pronounced in ham slices containing at least 4.5% salt.

This study indicated that ham processing procedures at the processing facility need to be revaluated. The moisture content of the hams (>60%) was too high for a good quality country ham product. When the hams were being processed equalibrization times may have been insufficient. When the hams were sliced and vacuum packaged, heavy microbial contamination occurred due to faulty product handling. Slicers, belts, knives and employees hands should be cleaned and sanitized frequently to prevent cross contamination of product (Banwart, 1979). Some belts may need to be replaced entirely if cracks are observed.

It has become evident from results obtained by this research that vacuum packaged sliced country ham is an entirely different product from whole country hams. It may be necessary to develop different standards of identity for hams that are produced to be sliced and vacuum packaged. The indigenous environment that is created by the vacuum packaging process should be taken into consideration during processing. The vacuum package should only be considered as a customer convenience. In product having salt levels of 4.5 to 5.6%, the vacuum package does not prevent growth of microorganisms.

To obtain vacuum packaged products which are shelf stable for at least two weeks at 28°C, companies must improve sanitation practices and practice good processing procedures that lower the A_w to the proper level and allow the cure to equilibrate throughout the ham. Salt content of hams which are to be sliced and stored at 28°C (room temperatures) should be at least 4.5% if the initial microbial counts are high. If a longer shelflife for country ham slices is required, or a lower salt content is desired it appears that product must be refrigerated. One alternative to refrigerated storage of vacuum packaged country ham slices would be irradiation. Studies need to be undertaken to investigate the safety and acceptability of such products.

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