CHEMICAL COMPOUNDS ASSOCIATED

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Thomas Nelson Blumer

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CHEMICAL COMPOUNDS ASSOCIATED WITH AGED HAM FLAVOR

By

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INTRODUCTION

Previous work reported on aged flavor in country style hams has been mostly subjective in nature. A reliable objective test for aged flavor development in this type of ham during storage would be particularly useful in evaluating the influence of the kinds and amounts of curing agents, and the effect of temperature, humidity and airflow. Several papers have been published on the chemical and physical factors involved in the curing and aging; the most extensive of these is by Hunt, Supplee, Meade and Carmichael (1939). However, this work is concerned primarily with the chemical changes and secondarily in any association of such changes with aged flavor taste. More recently, Weir and Dunker (1953) compared certain chemical constituents of ham with organoleptic tests. This work was done with four different types of commercially cured hams so applies only indirectly with country style hams.

Country style hams are cured by essentially two methods. The first is the dry-cure, where salt, sugar and sodium or potassium nitrate are applied directly to the surface area of the ham. The second method is the brine-cure method which consists of use of the same ingredients except that they are added to water and the hams immersed in the brine. A survey of 1300 farmers by Dunker and Hankins (1951) has shown most farm meats are cured by the

dry-cure method. This survey also revealed that the curing agents used were: salt only; a home mixture containing various combinations of salt, sugar and pepper and saltpeter; and a commercially prepared mixture. The figures further showed that 80 per cent of the farmers dry-cure and 20 per cent brine-cure their meat; only 9 per cent pump their meat prior to curing. In addition to farmers, locker plant operators and meat packers in certain localities use the dry-cure method.

This investigation is concerned chiefly with the quantities of certain chemical components developed during the curing and storage of dry-cure country style hams. It is limited to the analysis of the water soluble non-protein compounds. Further, a comparison is made between the quantities of these chemical components at three different storage periods with taste panel ratings for aged flavor, tenderness and saltiness. Finally, the most reliable of these chemical measurements are used to formulate an equation that attempts to predict the degree of aged flavor in ham.

HISTORICAL REVIEW

This review is based on the literature dealing with the function and effect of curing ingredients used to cure country style hams and the evaluation of food flavors.

Function and Effect of Curing Ingredients

The curing agents listed as permissible in the regulations by the Bureau of Animal Industry (1925) other than spices are: salt, sugar, sodium nitrate, sodium nitrite and vinegar. Salt is the most important preservative ingredient used (Jones, 1937; Moulton and Lewis, 1940; Jacobs, 1944; Jensen, 1945; and Ziegler, 1948). Its chief function is to help prevent the growth of undesirable bacteria which commonly have access to meat.

According to Callow (1947) the process by which salt is absorbed in fatty tissue by way of its connective tissue is quite simple. In contrast, the principle of salt absorption in muscle tissue is rather complex. Immediately after death of an animal, the muscle fibers are filled with fluid, but very little fluid is between them at this time. This condition of the microstructure of muscle is termed by Callow (1937) as a "close" one. In this state, salt absorption is comparatively slow. Later, the muscle glycogen in the fibers is converted to lactic acid causing them to shrink and give off fluid. He refers to this condition as an "open" structure.

Both salt and sugar may penetrate into muscle with an open structure more rapidly than into that with a close structure. Callow (1936) stated that the change from close to an open structure is accomplished most completely at pH 5.7. In addition to hastening the penetration of salt, a low pH inhibits the growth of anaerobic bacteria. Ingram (1939) found that about 5 per cent salt can prevent the growth of anaerobic bacteria even when no acid is present.

Salt diffuses into the muscle tissue and water diffuses out during the curing process. This progresses at a slow rate with the dry-cure. In one respect, this slow rate of diffusion is advantageous if the theory of Callow (1947) is applied. A salt-protein complex is eventually formed where the osmotic pressure of the complex is greater than the surrounding solution. In brine-cure hams, this point may be reached comparatively soon; the water then flows back into the muscle fibers, and thus offers an explanation for the gain in weight of cured meat over fresh meat. In dry-cure hams, the removal of water and entrance of salt in muscle tissue continues for a much longer period making conditions less favorable for bacterial growth as the curing period progresses.

A disadvantage of dry-cure for hams is the length of time necessary for the absorbed salt to become equalized throughout the muscular tissue. In a study of the movement of salt after curing,

Miller and Ziegler (1936) and Ziegler and Miller (1938) showed that the outside inch of ham is at least ten times as great as in the center at the time they are removed from cure. According to Miller and Ziegler (1936), (1939); Besley and Hiner (1937) and Ziegler and Miller (1938), the length of time necessary for salt equalization in the ham is dependent upon the weight and thickness. The moisture-salt content and the amount and distribution of fat have been found by Blumer, Smith, Lucas and Tyler (1952) to be important factors in the rate of salt distribution. An increase in salt content will decrease the time necessary for any point in the ham to reach a "safe" salt concentration. A safe salt concentration with respect to spoilage was given by the Bureau of Animal Industry (1941) as 5 per cent. A high moisture content will allow a comparatively rapid distribution of salt. A high moisture content is usually associated with a low fat content in fresh hams; therefore, fat retards the distribution of curing ingredients.

From a flavor standpoint it is obvious that salt in an important flavor component in cured hams.

Sugar is ordinarily added to the curing mixture used for country style hams as cane or beet sugar in a crystalline form. It is added primarily to aid in the development of proper color as shown by Haldane (1901); Jensen (1935); Lewis (1936); Brooks (1937), and Greenwood, Lewis, Urbain, and Jensen (1940). It is also,

according to Lewis (1937), added for the purpose of reducing the hardening effect caused by the action of salt. Several workers, Tomhave (1925); Helser (1929); Moulton (1929); Warner (1938) and Ziegler and Miller (1938), have noted that a more desirable flavor develops when sugar is added to the curing mixture. It is possible that this difference may be due to texture. Lewis (1937) stated that sugar does not impart a sweet flavor to cured ham. Further evidence that sugar may not be a detectable flavor component was noted by Brady, Smith, Tucker and Blumer (1949). These observations do not preclude the possibility that sugars, when added, are included in the final flavor result but are masked by the presence of stronger flavor components.

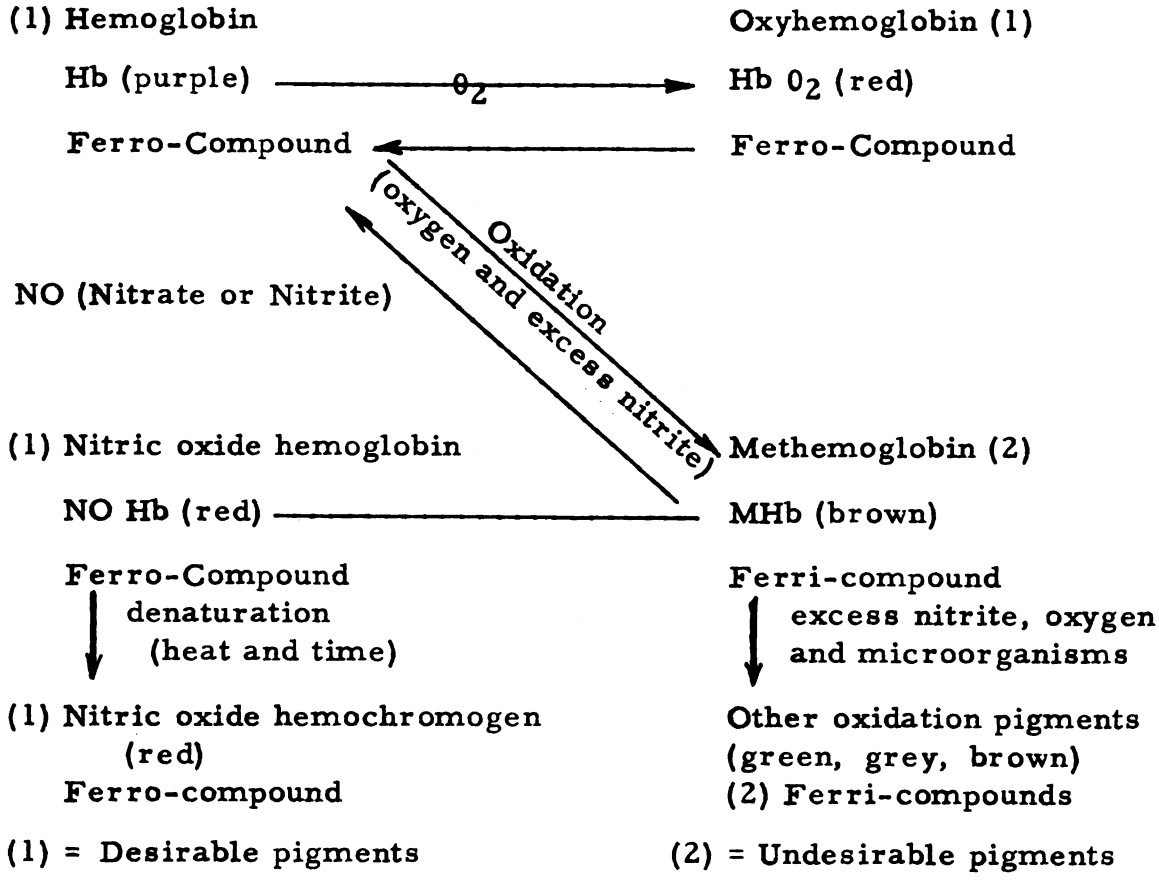
It should be mentioned that, like salt, sugar functions as a preservative. This is probably accomplished in curing hams by acting as a dehydrating agent. A protein containing food loses water to a sugar solution, but Callow (1932) reported that a sugar-complex is not formed as is the case with salt. Therefore, liquid will be removed and cannot be replaced. Probably the liquid is held between the muscle fibers and may account for the softer texture as compared to no added sugar.

The stable red color characteristic of cured meats is enhanced through the addition of nitrate or nitrite. The origin for this custom is unknown. The most probable explanation is that it was

an impurity in the salt used in early times. Its continued use is mostly for aesthetic reasons; however, any deviation from the normal color is considered to be a product of inferior quality. There is some evidence that nitrite has some preservative action, but this will be presented later.

A number of papers has been published on color and color formation. Reference is made here only to those having a relationship to this study and to those which add to the clarity of explaining the function of nitrates and nitrites.

A graphic presentation of the chemical compounds involved was given by Greenwood, Lewis, Urbain and Jensen (1940) beginning with the fresh meat and continuing through the cooking operation.



Sugars were mentioned previously as being beneficial to color formation. They help by assisting in the maintenance of conditions favorable for the growth of microorganisms that ferment sugar to acid. The reducing conditions thus established are essential for proper color formation. An excessive number of microorganisms if present in combination with appreciable quantities of reducing sugar will produce undesirable pigments. This principle of pH is essentially confirmed by Duisberg and Miller (1943), Urbain and Jensen (1940); Urbain and Greenwood (1940); White and Gibbons (1941); and Gibbons and Rose (1950). Nitrite destruction is caused due to the instability of nitrous acid which is formed in acid solution. These workers showed that proper color fixation is best accomplished between a pH range of 5 to 6. In cases where the pH is high and the temperature low, the rate of oxidation is slow. Brooks (1929), (1936) stated that the complete absence of oxygen tension within the meat permits the use of nitric oxide hemoglobin as a meat pigment.

Urbain and Jensen (1940) claimed that the pigments of cured meats are nitric oxide myo-hemoglobin and nitric oxide myo-hemochromogen. The chemical properties, however, were not unlike those reported by other workers. They noted that at 10° C (50° F.), a pH of 8.25 appreciably retarded the oxidation of nitric oxide hemoglobin; lower pH's increased the rate of oxidation. This

instability of nitrite makes it necessary to include nitrate in curing mixtures. Kleckner (1942) stated that this is true even though its production from nitrate depends upon many factors not easily standardized. Rose and Peterson (1953) described the limiting factor in the rate of nitrite reduction as an intermediary electron carrier rather than the overall reducing system.

Nitrite has been shown to have some preservative action. According to Tarr (1941), it is most effective in an acid medium. Therefore, the full benefit should be realized in cured ham. In comminuted pork seeded with spores of a Clostridium species, Bulman and Ayres (1952) found that nitrate had about the same preservative effect as sodium chloride, but nitrite had a preservative effect at levels as low as 0.04 to 0.08 per cent. In uninoculated meat these workers found that nitrite used in combination with sodium chloride or nitrate appeared to extend the storage life of pork.

It should be recognized that certain chemicals are added to cured meats during the smoking process. These are listed in the papers of Rideal (1903); Callow (1927); Hess (1928) and Pettet and Lane (1940). The most abundant of these are formaldehyde and higher aldehydes; formic, acetic and higher acids; phenols, ketones and resins. These chemicals also have a preservative action in

their property to stabilize fats as shown by Lea (1933); White, Gibbons, Woodcock and Cook (1942), and White (1944).

Smoke also has some preservative effect through its action on bacteria as shown by White, Gibbons, Woodcock and Cook (1942) and Jensen (1943). Jensen found low nitrite, good color and few bacteria at the end of an 18 hour smoking period. He also associated smoking with the production of desirable organoleptic properties. Flavor of woodsmoke is not discernible, however, from the overall flavor in aged country style hams.

The Evaluation of Food Flavor

Any food is evaluated by its conformity to a certain standard of perfection. Raw meat is evaluated by its appearance. This includes the proportion of fat, lean and bone, the color of fat, lean and bone, its texture and the intramuscular fat, commonly called marbling. The cooked meat is also evaluated by its conformity to an appearance standard, but in addition, to its conformity to a flavor standard. Since flavor ideals vary widely with different people, methods of accurately estimating and comparing flavor have presented a problem.

Most of the flavor tests conducted have been of a subjective nature; that is, they are based on the opinions of people. More recently, objective measurements have been used in an attempt to

evaluate food flavors. These are based on chemical, physical and microbiological properties. In the review of literature that follows the subjective method of food evaluation will first be considered.

Subjective Evaluation of Food

There are only four basic tastes; these are sweet, sour, salty and bitter (Fabian, 1940; Crocker, 1945; Remer, 1947; Maximow and Bloom, 1948). Taste is discerned chiefly by the taste buds. Maximow and Bloom stated that taste buds are found on the surface of the tongue, glassopalatine arch, soft palate, posterior surface of the epiglottis, and on the posterior wall of the pharnyx down to the level of the inferior edge of the cricord cartilage. Taste buds are shaped like a flask with a wide bottom and a short neck. Under low power of the microscope, they are seen in sections as pale, oval bodies in the darker stained epithelium. They are about 72 microns in length. They are in an upright position in the epithelial layer and extend through most of its entire thickness, from the basement membrane to the surface. A superficial layer of squamous epithelial cells over each taste bud is pierced by a small opening, termed the outer taste pore.

Two types of cells may usually be distinguished in a taste bud, the supporting cells and the neuroepithelial taste cells. The taste cells are distributed inside the bud, between the supporting cells.

They number from four to twenty in each taste bud. These authors have noted that the four fundamental flavors listed above give a varied response as to their taste when they are applied to individual fungiform papillae. Some of the papillae do not give any taste sensations while others give sensations of one or more taste qaulities. This is not due to structural differences in the various taste buds, as they appear entirely similar. Also, a general chemical sensitivity may be detected in regions of the mouth where there are no taste buds present.

The saliva secreted by the various salivary glands has a role in food flavor or, at least, acceptability. It contains water, mucin, proteins, mineral salts, and the enzyme ptyalin, which splits starch into water-soluble, less complex carbohydrates. The secretion of the glands is affected by the different kinds of food eaten, depending upon the nature of their stimuli.

Subjective tests used to evaluate food flavor may be divided into two main categories depending upon their purpose. Lowe and Stewart (1947), and Langwill (1949) classified them as preference or acceptance tests and difference or psychometric tests. In preference testing the degree of acceptance is obtained. The purpose of the difference tests is to determine quantitative differences by rating or scoring of food quality factors. Platt (1937) suggested that the difference test should be used where the standard of

perfection has been agreed upon; the preference test to determine how well the general public will like a given food.

Flavor difference tests. One of the most widely used systems for judging meat flavors has been used by Black, Semple and Lush (1934); Barbella, Hankins and Alexander (1936); Brady (1937); Griswold and Wharton (1941); Hardy and Noble (1945) and Noble and Hardy (1945). The Cooperative Meat Investigations Board adopted this chart for scoring meat flavors. It is a numerically graded chart having numbers from one to seven and increasing in intensity directly with the size of the number. A descriptive adjective accompanies each number and factor considered.

Other scoring systems have been used such as the zero to ten by Steinberg, Winter and Hustrulia (1949), and the two to fourteen system of scoring used by Ramsbottom, Strandine and Koonz (1945) for rating the tenderness of beef muscles. This is comparable to the one to seven system described above since an increase in tenderness is designated in units of two.

Selection of taste panel. The selection of a taste panel for evaluating difference in food is based initially on availability of personnel as emphasized by Black, Semple, and Lush (1934); Carl, Watts and Morgan (1944); White, Woodcock and Gibbons (1944); Fenton (1946); and Lowe, and Stewart (1947). Secondly, the members of the panel are selected on their ability to detect and evaluate

taste differences. It is best to use a trained or experienced panel as indicated by Alexander, Clark and Howe (1933).

If a trained panel is not available, the size of the panel desired is decided upon. There is quite a difference of opinion in the literature as to the number necessary. Although there are some extremes, the number usually used is between three and ten people. Platt (1931) was of the opinion that five to ten judges may be used depending upon the number of qualified judges available. If panel members selected are approximately equal in proficiency, Marcuse (1947) stated that a larger panel will permit more confidence in the average score. Peret (1949) stated that a panel of competent tasters is more reliable than a larger group, some of whom would probably be incompetent in their evaluation.

Sex appears to have no bearing on tasting ability. Snyder (1931) has indicated that taste deficiency is not sex-linked nor sex influenced. Taste deficiency is primarily due to a single recessive gene. Studies may be found in the literature which support the use of either male or female panels but it seems highly probable that no significant difference exists.

To select panel members from a group of people inexperienced in tasting a food, one of several means may be used. The two methods most frequently used with recent work are the triangular and two-sample taste-test methods. Advocates of the triangle

taste-tests such as, Roessler, Warren and Guymon (1948); Hening (1949); and Girardot, Peryam and Shapiro (1952) stated they are useful for selecting personnel for expert panels, wherein individual sensitivities to different taste factors are evaluated. In this test, three samples are tasted, two of which are alike. The judges are asked to select the odd sample. This test is also suggested by Roessler, Warren and Guymon (1948) for use with expert panels to note if differences are detectable between two different food samples. If a difference exists they may then be used to evaluate an inexperienced panel or a group of people to determine food preference trends. The results of this method of testing may be analyzed statistically.

The paired test method has been used by Cover (1936), (1937), (1940), (1941), (1943); Griswold and Wharton (1941) and Boggs and Hanson (1949). As the name implies, two samples are submitted to the judges. In testing with this method it is essential that a similar set of samples be scored at least two and preferably more times to avoid chance selection. If the second set is evaluated similarly by the judge it may not be necessary to repeat the test. One of the chief objections to this method of testing is the time necessary to evaluate a group of samples. Also, since the tests must be repeated, much labor is involved. Its use is somewhat

limited, but in cases where only two variables are present it is a convenient method. It worked quite well in this respect in the work of Cover (1937).

Byer and Abrams (1953) compared triangle and two sample taste methods using different concentrations of aqueous solutions of quinine and dextrose. The two-sample test showed results favoring this test over the triangular test in discriminatory value. The same was found true for quality judgments made within these experimental tests.

Another method of selecting judges is by use of the ranking test. In this test the judges rank the food with respect to the concentration of a certain flavor component, such as saltiness or sweetness. Ranking tests have been used by Crist and Seaton (1941); Carl, Watts and Morgan (1944); White, Woodcock and Gibbons (1944); Averman and Li (1948) and Crocker, Sjostrom and Tallman (1948). This method may be used most conveniently when only one or two flavor components are to be evaluated. Obviously the factor must be of a nature that will permit varying intensities to be prepared for tasting. Also, a suitable standard material must be available.

The dilution test may be used for some foods, but does not apply too well to meats with the exception of ground meats. It is only desirable for use with food that may be made homogeneous.

Dilution and ranking tests may be used in unison. The most frequent use of dilution tests has been made in evaluating individuals for primary tastes as done by Crocker (1937); King (1937); Knowles and Johnson (1941) and Fabian and Blum (1943).

Training the taste panel. Unless an experienced taste panel is available, the members of the panel must be trained. The ability to distinguish definite tastes can be improved with practice. This may require only one week or up to several weeks, according to Harding (1948), depending upon the sensitivity required and the number of factors involved. The judges' scores during the training period are noted for accuracy. If any judge deviates frequently from the mean, Hening (1949) claimed the judge should be replaced. This author gave a statistical formula suitable for testing significance of the differences in judges' scores. Deviation in judges' scores from the mean score of the panel has been used for checking the dependability of individual tasters by Peret (1949) and Terry, Bradley and Davis (1952). Harding (1948) stated that the taste testing should be held under conditions which are comfortable for the panel members. A well-lighted, preferably air-conditioned room is most suitable. If possible each panel member should work in a separate taste booth, where he may not observe the other panel members.

It is not advisable to judge foods in the same laboratory where the food is cooked (Cover, 1936; Dove, 1947; and Moser, Jaeger, Cowan and Dutton, 1947). Other conditions such as time allowed for tasting, water for mouth rinsing to remove "carry over" flavors, and the temperature of the samples are important. These must be regulated to a standard procedure.

Comparison of Objective and Subjective Flavor Scores

Lowe and Stewart (1947) noted that the reliability of any chemical or physical measurement used in the determination of flavor must be evaluated in terms of its agreement with subjective flavor scores. The authors stated that it is desirable to employ both subjective and objective tests in connection with research on the functional and organoleptic properties of food products. When a good objective test is established simultaneous organoleptic tests will sometimes prove helpful in explaining unexpected results for either test.

There has been some reported success in correlating tenderness in meat with taste panel scores and the mechanical shear test method as shown by Brady (1937); Griswold and Wharton (1941); Cover (1943) and Paul, Lowe and McClurg (1944). The Warner-Bratzler shear apparatus, reported by Bratzler (1932), measured the shear strengths fairly satisfactorily for duplicate samples in a

study by Deatherage and Reiman (1946) and Deatherage and Garnatz (1952), but they did not correlate too well with taste panel scores.

No significant decreases in palatability scores were noted by Steinberg, Winter and Hustrulid (1949) for stored beef with a 5 per cent moisture loss.

Ether-extract of the fat determinations in the lean of steer beef was shown by Branaman, Hankins and Alexander (1936) to be directly related to preference ratings by the judges.

Peroxide values of pork fat did not give good correlations with taste panel scores in studies by White, Woodcock and Gibbons (1944); Pearce (1945); Nauman, Brady, Palmer and Tucker (1951) and Hanley, Everson, Ashworth and Morse (1953). Schreiber, Vail, Conrad and Payne (1947) noted that the loss of flavor in frozen poultry paralleled increases in the peroxide determination value of the fat. The same trend was noted by Gortner, Fenton, Volz and Gleim (1948) with pork in frozen storage at comparatively high temperatures.

Press fluid determinations by Satorius and Child (1938) showed no direct correlation with flavor or aroma scores of a taste panel. However, Hardy and Noble (1945) found juiciness scores and taste panel scores were highly significant in roast pork loins. This is not a reliable test since the authors stated that the

difference is not great enough to predict judges' scores from the juciness scores.

In cured, smoked meats White, Woodcock and Gibbons (1944) found no correlation between aging period and flavor quality.

Crocker (1945) was of the opinion that volatile organic acids account for part of the flavor in fresh meat and that nitrogen compounds are contributing factors. Crocker (1948) stated that the flavor in raw meat is mostly in the juice and appears to be due to certain aspects of the blood. To some minor extent meat flavor may be due to the presence of creatinine and creatine. This author stated that there is a sweetness of taste in pork flesh which is more pronounced than in beef, a high volatile fatty acid content and additional bases among which are an earthy-potatoey flavor and a sulfury flavor somewhat similar to chicken. Red-meats were said to give flavor characteristics that were similar to fish and birds, while fish contained red meat characteristics.

Crocker (1935) reported that by searing a pot roast in hot fat at high temperature, a change in flavor is produced. Meat sugars are carmelized and nitrogenous bodies are said to undergo a partial carbonization.

Bouthilet (1951) in a series of studies on the volatile constituents of chicken concluded that the "meaty flavor" in chicken is due to a compound located in the meat fibers rather than in the fat.

Glutathione was the compound reported as having properties most similar to the flavor compound.

Chemical changes in cured meats. It has been indicated that meat may undergo many changes during the curing process due to its complex composition and that these changes influence the flavor. A National Livestock and Meat Board publication (1940) lists the major food constituents of meats as proteins, fats, inorganic constituents (minerals), carbohydrates, nitrogenous and non-nitrogenous extractives, pigments, enzymes, vitamins and water.

Cured hams aged at atmospheric temperatures by Hunt, Supplee, Meade and Carmichael (1939) showed changes in chemical composition. The following conditions were observed: Fat hydrolysis during the early months of aging was more rapid in the fat of the lean meat than in the fat of the adipose tissue; the amount of moisture present influenced the rate of fat hydrolysis; total nitrogen in the lean meat on a moisture and fat-free basis increased from approximately 2.3 per cent in the freshly cured hams to 3.5 per cent after aging one year; the sodium chloride concentration increased from approximately 6 per cent in dry-cured hams after aging one month to 8 to 10 per cent after aging one to two years; unsaturated acids were freed to a much greater extent than saturated acids during the early stages of aging; the iodine number of the free fatty acids decreased as hydrolysis continued. The authors stated that

the difference observed in the rates of hydrolysis of unsaturated and saturated acids is probably characteristic of enzymatic hydrolysis of fat under all conditions.

In cured and smoked meats the bacterial count, peroxide oxygen content of the fat and color of the lean changed directly with the length of the aging period as found by White, Woodcock and Gibbons (1944).

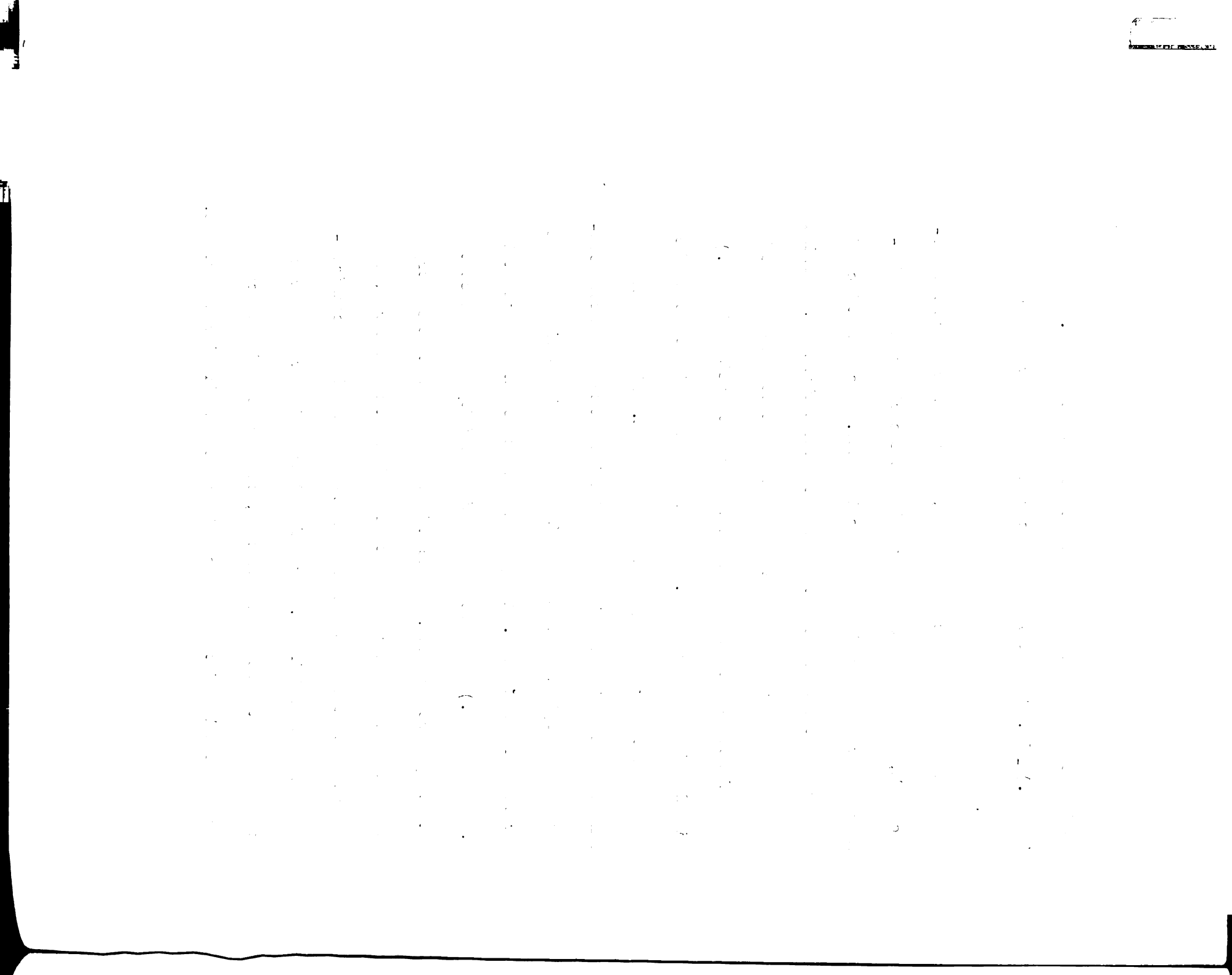
EXPERIMENTAL PROCEDURE

The hams used in this study were obtained from hogs of similar breeding, fed the same feed and grown under similar methods of management. The hogs were slaughtered when they reached a live weight of 200 to 240 pounds. They were not fed for a 24 hour period prior to slaughter, but had free access to water. After slaughter the carcasses were chilled for 48 hours at 0.56° to 1.67° C (33° to 35° F.), at which time they were fabricated into wholesale cuts. The hams were cut regular style and weighed from 13 to 16 pounds. A period of about six months was required to collect the 54 hams used in this study.

The hams were cured by the dry-cure method. The curing mixture was composed of eight pounds of kiln dried granulated sodium chloride, two pounds of table grade, white cane sugar and two ounces of sodium nitrate. The salt contained the following impurities: calcium sulphate 0.28 per cent, calcium chloride 0.03 per cent, and magnesium chloride 0.01 per cent. The curing mixture was applied to the hams at the rate of one ounce per pound of meat. The proper amount of curing mixture was weighed for each individual ham and divided into three equal parts. These parts were rubbed on the hams on the first, fourth and tenth days. During the

curing period the hams were held at a temperature of 4.44° C (40° F.) ± 1°. They remained in cure two days for each pound of ham.

At the end of the curing period, in order to remove the surface salt, the hams were removed from the curing room and immersed for two hours in a bath of cold water. A constant incoming supply of water kept the water bath fresh the entire time. The hams were then removed and hung to dry in a gas heated smokehouse in which the temperature was held at approximately 32° C (90° F.) with the aid of a thermostat. After two hours drying time smoke was generated by burning a hardwood sawdust. The smokehouse temperature during smoking was maintained at the same temperature as for drying. A continuous 24 hour smoking period was used for each group of hams. They were then stored at approximately 4.44° C (40° F.) at an average relative humidity of 45 per cent until they were sampled. While it is acknowledged that country style hams are usually stored at atmospheric temperatures, the lower temperature was used to keep the growth of surface microorganisms at a minimum. Even though the aging process proceeds at a slower rate at this temperature, the advantage in reducing environmental variations was considered to be of major importance.



Sampling Methods for Hams

The stored hams were sampled at the end of the first, sixth, and twelfth months. The identity of each ham was maintained throughout the duration of this study by tagging it with the number corresponding to the hog from which it was removed. When a group of hams was first placed in storage, one ham from each pair was randomly assigned to the group to be analyzed at the end of the first month. These hams composed the control group. When a ham was drawn for the control group, the other member of the pair was assigned to the sixth month group for the first half of the numbers drawn; the remaining hams were then placed in the twelfth month group. The use of this procedure, therefore, allotted twenty-seven, thirteen and fourteen hams to the first, sixth and twelfth month sampling periods, respectively.

The ham samples were obtained by removing three adjacent ham slices one-half inch in thickness, beginning at a point one quarter inch from the posterior end of the ischium. The first and third slices were used to determine the degree of aged flavor, tenderness and saltiness by means of a taste panel. The second slice was used for chemical analyses.

Selection of the Taste Panel

The taste panel was selected from a group of 71 persons. For convenience in evaluating the most proficient tasters, this group

was divided into three smaller groups. As test material two pairs of hams were obtained; these two pairs were judged to have a detectable difference in aged flavor, tenderness and salt content. These differences were not considered extremely great, so that some degree of skill was necessary in order to receive a good score.

The hams were sliced in such a way that the pair mates when tasted by the panel were from comparable slices as to location in the ham. Each group scored each pair of hams on two different days. The triangle test method was used, but in addition to selecting the odd ham the tasters were asked to score each ham as follows: Aged flavor - high or low; tenderness - high, medium or low; and saltiness - high, medium or low. The same pair of hams was tasted and scored twice on any given day, however, a different ham was used each time for the odd sample. Ten tasters, six men and four women, made no more than one error in judgment on any factor. These ten persons formed the panel used in this study.

Preparation of samples for taste testing. The first and third slices from each ham were broiled in the oven of an electric range. The slices from all first month hams were placed seven inches from the source of heat and broiled for 20 minutes at 176.67° C (350° F.). The sixth and twelfth month hams browned more quickly; therefore, they were broiled for only 15 minutes.

The cooked samples were trimmed free of fat and the semimembranous, abductor, semitendinosus and the biceps femoris muscles were cut into approximately one-half inch cubes. The cut samples were thoroughly mixed and served on plates to the members of the panel.

Tasting and scoring samples. The plates were identified by a number assigned to correspond with the ham number. Since no individual tasting booths were available, the test was conducted in a classroom where plenty of space could separate the individual tasters. A minimum of six tasters was used for any taste test and a maximum of eight.

Each panel member chose at random one sample of meat from each plate. Three factors, namely, aged flavor, tenderness and saltiness were scored on a sheet provided with three geometrical figures of similar design. Each figure was constructed in such a manner that ten equally spaced lines originating from a common straight line base traversed in an 180 degree semi-circle. Each figure was designated, "flavor", "tenderness" or "saltiness". The first, fifth, and tenth lines of each figure were heavier than the lines in between. These heavy lines were labeled: threshold, appeal and excess. This is a modification of the system used by Cartwright and Kelley (1951) and called a profile test. The word threshold, labeled as such on line one, was used to represent the

lowest possible score for any one factor. The word appeal, appearing on line five, was the most ideal rating for any one factor. Excess, was the designation given to line ten. The interpretation of the interspaced lines using the heavy lines as reference points was as follows: the lines between one and five were the divisions dividing the threshold interpretation from the ideal interpretation for any single factor. That is, any line below five was less than the proper amount. Similarly, any line above five was understood to be more than the desired amount. This scoring system was used for all hams tasted and for all three factors evaluated.

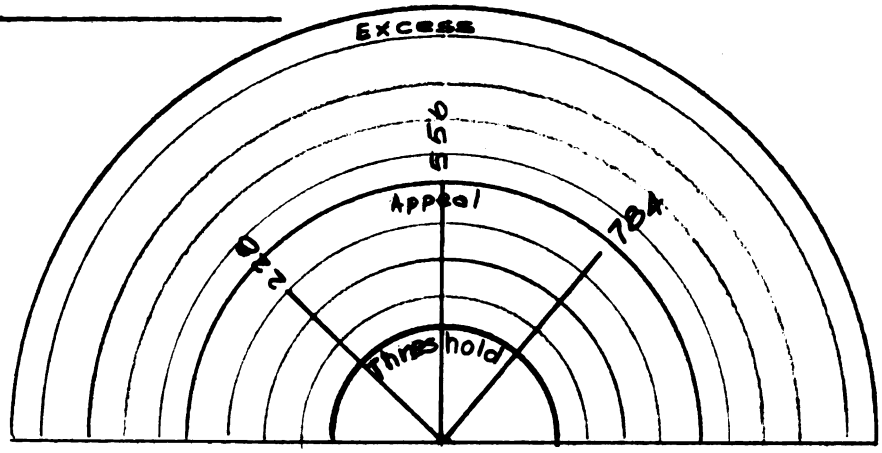
The panel had the opportunity to use the modified Cartwright and Kelly system of scoring on five different occasions prior to beginning this study. An effort was made to train the panel at these times by using hams considered to have "appeal" ratings for any or all of the factors to be tasted and comparing them with hams having more or less than an appeal amount. A sample score sheet is shown in Fig. 1. It may be observed that all hams for any one test period were recorded on the same sheet. This gave a graphic representation of all hams tasted at any time during a tasting period.

Preparation of Samples for Chemical Analyses

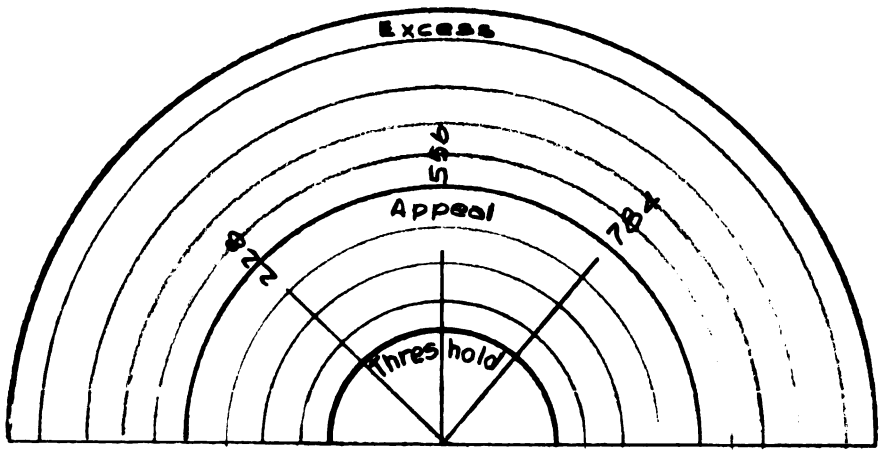
The four muscles used in the taste test were also removed from the second slice for the chemical determinations. The outer

Name _____

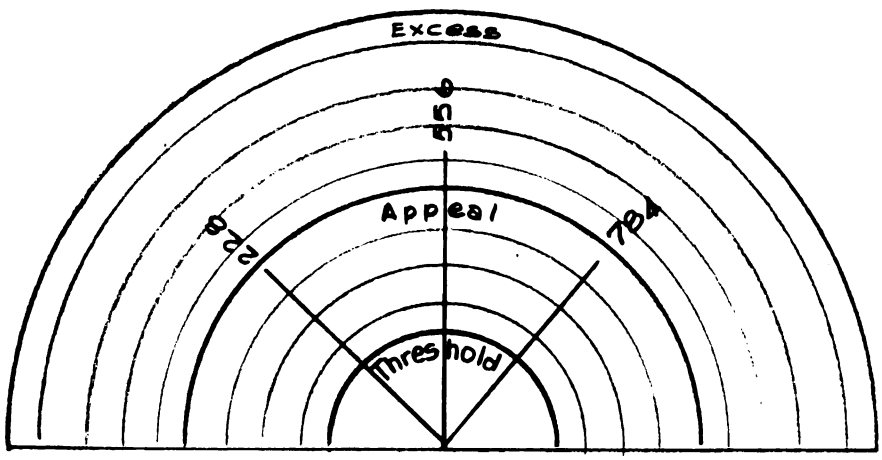
Date _____



FLAVOR



TENDERNESS



SALTINESS

Fig. 1. Sample Score Sheet - Taste Test Evaluation (12 Months)

fat was removed from the ham slice and used for determining the iodine number of the fat. The lean tissue was finely chopped with a knife and thoroughly mixed. Twenty-five grams of this tissue were weighed on a balance and diluted with 250 ml of distilled water. This mixture was then ground in a Waring Blendor for five minutes and filtered through fine filter paper and a Buchner funnel by mechanical suction.

The water extract was clarified by treating it with a 1 N solution of zinc sulphate at the proportion of ten to one. This mixture was then poured into a 250 ml capacity beaker and placed on a magnetic stirrer. The glass electrodes of a Beckman pH meter were then adjusted into the solution. A 1 N solution of sodium hydroxide was then added with continuous stirring until a pH of 7.8 had been reached. The neutralized mixture was poured into 50 ml glass centrifuge tubes and centrifuged for 15 minutes at 3000 revolutions per minute. The clarified liquid was then poured off and stored in 250 ml glass stoppered flasks in a refrigerator maintained at about 5° C. until used. The clarified solutions were used for the determination of the water - soluble compounds.

The remaining chopped muscle tissue was used as described under the section on analytical procedures.

ANALYTICAL PROCEDURES

Moisture. Five grams of the chopped lean meat were dried in an electric drying oven for 36 to 40 hours at 70° C. The meat was then dried for five additional hours in a vacuum oven at the same temperature, cooled in a desiccator and weighed.

Fat. The crude fat was determined on the moisture free sample by the method described in the Association of Official Agricultural Chemists (1945).

Salt. The salt content was determined by the electrometric titration method as described by Brady, Smith, Tucker and Blumer (1949).

pH. The pH was determined with a Beckman pH meter equipped with a glass electrode apparatus.

Iodine absorption number. The Hanus method was used to determine iodine numbers as given in the Association of Official Agricultural Chemists (1950).

Free fatty acids. The free fatty acids were determined by a slight modification of the method described in the Association of Official Agricultural Chemists (1950). Five grams of the ham fat were used in place of 7.05 grams. The sodium hydroxide for titration was standardized between 0.10 N to 0.20 N instead of exactly 0.25 N.

Total nitrogen. The Kjeldahl - Gunning - Arnold method was used as described in the Association of Official Agricultural Chemists (1945) with one exception: the sample was collected in 50 ml of boric acid according to the method of Scales and Harrison (1920).

Soluble nitrogen. The sample was prepared for the determination of soluble nitrogen by blending ten grams of the ham sample and 100 ml of water in the Waring Blendor for five minutes. The mixture was filtered by suction through sintered glass crucibles containing a small amount of Hyflow Supercell. Some supercell was also added to the solution before filtering. A 10 ml aliquot of the filtrate was then determined according to the Kjeldahl method described above.

Glucose. Five ml of the water extract were analyzed for glucose according to the method of the Association of Official Agricultural Chemists (1950).

Peroxide number. The method of Stansby (1941) was used to determine the peroxide content of the hams.

Unsaturated fatty acids. Fifty grams of each ham were ground with 75 ml of alcohol in a Waring Blendor. The mixture was then boiled for 15 minutes and the alcohol removed by filtration. The ham tissue was re-extracted twice, using 75 ml of boiling alcohol each time; then the residue was washed with 100 ml of ether and

dried in a vacuum oven at 50° C. The alcohol and ether extracts were combined and brought to dryness under reduced pressure in an atmosphere of nitrogen. The remaining lipids were dissolved in ether, dried with anhydrous sodium sulfate, and added to the previously dried ham tissue. This was extracted overnight, in a modified Pickel extractor, by anhydrous ether. The levels of the unsaturated fatty acids were determined spectrophotometrically after alkali-isomerization according to the general procedure of Mitchell, Kraybill, and Zschiel (1943) as modified by Brice, Swain, Herb, Nichols and Reimenschneider (1952).

Lactic acid. Lactic acid was determined by the method of Barker and Summerson (1941) on 1-5000 dilutions of the protein free extracts of the hams. The lithium lactate used in making the standard solutions was recrystallized from alcohol until duplicate values were obtained from two successive colorimetric readings.

Glycerol. The glycerol was determined according to the method of Lambert and Neish (1950).

Short chain fatty acids. Ten ml of the water extract was distilled by continuous ether extraction and prepared for chromatographic separation according to the method of Neish (1950). The separation technique of Marvel and Rands (1950) was used for separating the individual acids with the following exceptions: the solvent material was forced through the chromatogram tube with

nitrogen gas using a regulated pressure so that about 2 ml per minute were collected in 10 ml fractions in graduated cylinders; carbon dioxide free water in the quantity of 2 to 1 was added to the collected fractions before titration, and a stream of carbon dioxide free air was introduced into the titration flasks during the titration process.

Organic matter. The organic matter was determined on the clarified ham extract by the method of Johnson (1949).

RESULTS

The taste test scores and analytical values were analyzed by calculating the differences between paired hams in storage one month with those in storage six and twelve months. The difference between six months and twelve months was then determined. A test of significance was performed on each group difference (Snedecor, 1946) (See Tables IX and X).

Taste Tests
(See Table I)

Flavor. Inspection of the data shows that aged flavor increases progressively with time. The greatest increase was between six and twelve months. A significant increase in taste test scores is shown at twelve months (See Table IX). Flavor and taste test saltiness and chemical salt values are significantly correlated.

Tenderness. A slight increase trend in tenderness was found at six months; a somewhat smaller decrease for twelve months. The differences were not significant.

Saltiness. The taste test scores indicate that salt concentration increased progressively with time. A marked increase was recorded for both six and twelve months. The largest increase was between these sampling periods.

TABLE I

TASTE PANEL SCORES[1]

Ham No.	Code No.	1 Mo.	6 Mo.	Ham No.	Code No.	1 Mo.	12 Mo.
			Flavor				
53	1	2.88	3.17	228	1	5.12	4.00
250X	2	3.38	3.00	551	2	3.71	3.62
462	3	3.86	3.83	556	3	4.00	5.00
552	4	3.86	4.28	590	4	3.43	4.75
570	5	2.86	3.71	606	5	3.86	4.00
572	6	3.14	1.86	656	6	3.43	4.50
588	7	4.00	3.83	681	7	3.71	4.62
705	8	4.14	4.00	682	8	4.57	4.75
721	9	3.57	3.28	696	9	3.28	4.25
731	10	4.00	4.00	781	10	3.28	3.62
771	11	3.43	3.71	784	11	2.86	4.50
941	12	2.43	3.43	833	12	4.14	4.88
Average		3.46	3.51			3.78	4.37
Mean Difference			+0.05				+0.59
			Tenderness				
53	1	3.62	4.00	228	1	4.00	4.00
250X	2	3.88	4.00	551	2	4.28	3.25
462	3	4.43	4.83	556	3	4.14	3.25
552	4	4.43	4.28	590	4	3.71	4.12
570	5	3.86	3.28	606	5	4.00	3.12
572	6	4.00	4.00	656	6	4.57	4.75
588	7	4.57	4.33	681	7	3.71	3.75
705	8	4.43	4.57	682	8	4.43	4.12
721	9	4.43	5.14	696	9	4.43	4.38
731	10	4.14	4.28	781	10	4.14	4.00
771	11	4.28	3.86	784	11	4.28	4.25
941	12	2.71	4.14	833	12	2.71	4.38
Average		4.06	4.22			4.03	3.95
Mean Difference			+0.16				-0.08

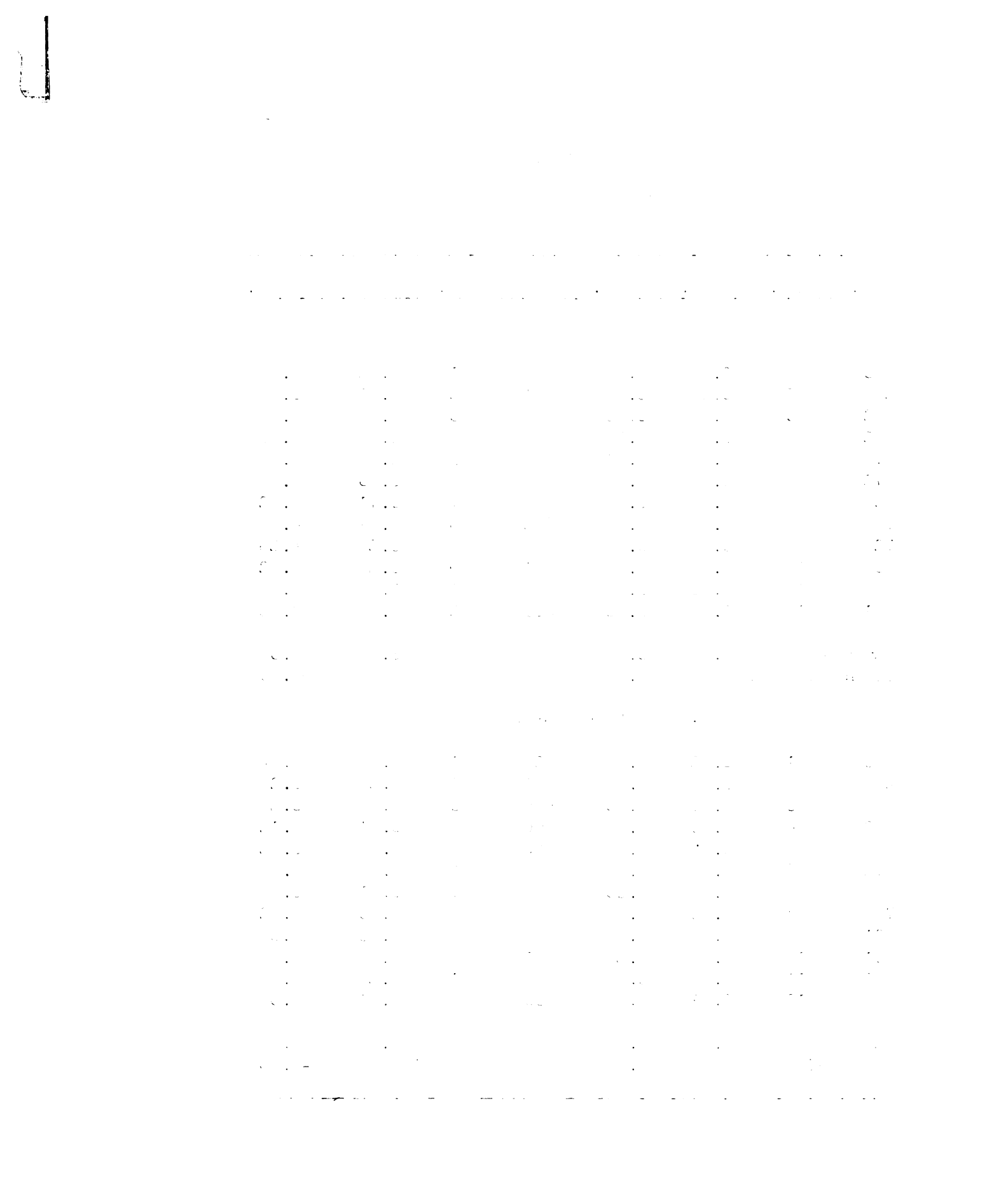


TABLE I CONTINUED

Ham No.	Code No.	1 Mo.	6 Mo.	Ham No.	Code No.	1 Mo.	12 Mo.
			Saltiness				
53	1	3.25	3.50	228	1	4.12	4.62
250X	2	4.25	3.33	551	2	3.57	4.62
462	3	3.57	3.67	556	3	3.57	6.25
552	4	3.57	4.28	590	4	4.28	5.25
570	5	2.86	4.14	606	5	3.28	3.88
572	6	3.00	3.14	656	6	4.43	6.50
588	7	4.28	5.00	681	7	4.28	7.14
705	8	3.71	4.14	682	8	4.43	6.12
721	9	3.86	3.86	696	9	3.28	4.75
731	10	4.14	4.28	781	10	4.00	4.71
771	11	3.28	3.86	784	11	2.86	4.62
941	12	3.86	4.14	833	12	3.57	4.57
Average		3.64	3.94			3.81	5.25
Mean Difference			+0.30				+1.44

[1] A 1 - 10 scoring system was used. A value of 5 is the best possible score. Values below 5 represent an insufficient property and over 5 more than sufficient.

Chemical Tests

All values reported here were obtained by calculating the average of duplicate samples.

Moisture. The average per cent reduction in moisture for the pairs at six months was 8.56; at twelve months, 13.47. The differences were significant at all sampling periods. They were also significant between six and twelve months. Moisture and saltiness gave a negative correlation as expected. However, this difference was not large enough to be statistically significant. The same is true between chemical salt values and moisture.

Fat. Variations in fat content between hams were larger than might be expected with paired hams. The greatest variation was found for the six months samples. Since the hams were paired it is a safe assumption that this difference is due to sampling error.

Salt (fresh basis). An increase in salt concentration was found for each successive sampling period. Per cent salt increase was greatest between six and twelve months. This is in agreement with the taste panel scores (See Table II).

Peroxide number. Higher peroxide values were obtained as the length of storage period increased. In order to further observe the effect of peroxide value on flavor response, several fat samples were obtained at weekly intervals from stored ham fats. The fat thus obtained was prepared for peroxide determination according to

TABLE II

MOISTURE, FAT AND SALT (FRESH BASIS) ANALYSIS

Ham No.	Code No.	Per Cent Moisture		Per Cent Fat		Per Cent Salt (Fresh Basis)	
		1 Mo.	6 Mo.	1 Mo.	6 Mo.	1 Mo.	6 Mo.
53	1	64.58	54.14	6.25	8.00	4.63	3.81
250X	2	65.78	57.15	3.26	6.76	4.30	5.00
462	3	64.92	61.46	6.23	3.46	5.02	6.82
552	4	66.78	56.40	4.58	9.34	4.47	4.19
570	5	64.28	47.59	5.86	17.12	3.83	4.18
572	6	63.16	54.26	11.36	13.99	2.59	3.44
588	7	64.88	59.30	2.70	4.04	6.14	7.46
705	8	65.84	45.73	5.34	18.56	2.68	5.24
721	9	64.39	56.46	5.29	5.15	3.38	4.91
731	10	65.44	61.41	4.42	7.70	4.07	4.41
771	11	68.15	58.33	3.38	4.92	3.22	4.18
941	12	56.34	59.18	11.44	9.20	4.53	5.50
Average		64.54	55.95	5.84	9.02	4.07	4.93
Mean Difference			- 8.56		+3.18		+0.86
			12 Mo.				12 Mo.
228	1	65.00	48.84	4.59	7.87	5.29	6.62
551	2	62.46	48.36	4.70	8.58	3.71	4.81
556	3	64.62	48.58	3.17	7.14	3.28	7.31
590	4	65.48	49.66	4.30	6.43	5.52	9.75
606	5	67.67	51.86	4.78	7.39	2.81	4.62
656	6	65.44	46.73	4.30	7.72	5.60	9.36
681	7	65.17	47.93	4.82	7.03	6.00	9.05
682	8	64.42	50.85	5.01	7.94	5.71	6.05
696	9	58.42	46.95	10.72	9.73	3.81	9.97
781	10	51.23	51.42	16.73	5.83	4.80	7.01
784	11	64.66	48.98	6.10	6.83	3.40	4.01
833	12	56.45	49.14	10.69	6.01	4.12	5.75
Average		62.58	49.11	6.66	7.38	4.50	7.02
Mean Difference			-13.47		+0.72		+2.52

the method previously cited. At the same time small portions of the melted fat were placed in the oven and heated for 10 minutes at 40° C, at which time they were removed and the odor and taste recorded by the taste panel. A change could be detected when the peroxide value reached about 40. This change was due to a rancid odor and flavor. Therefore, it may be concluded that the peroxide value is not a good indication of aged flavor in hams. This would apply in cases where the peroxide values are below 40; values in this experiment were below 20.

Free fatty acid. Increases in free fatty acid content were found. The greatest increase occurred between one month and six months. Peroxide number, iodine number or glycerol content did not appear to be correlated with free fatty acid.

Iodine number. A slight decrease in iodine number resulted after six months; an increase after twelve months. Similar trends were found for iodine numbers of outside fat and the fat extracted from the muscle tissue (See Tables III and VII).

Chlorides. The paired hams absorbed about the same amount of salt when calculated on a moisture-free - fat-free basis. In a few cases there were differences between pair mates, but by groups there were no appreciable differences.

TABLE III

PEROXIDES, FREE FATTY ACIDS AND IODINE NUMBER ANALYSIS

Ham No.	Code No.	Peroxides Milliequivalents Per kg of Fat		Per Cent Free Fatty Acids		Iodine Number	
		1 Mo.	6 Mo.	1 Mo.	6 Mo.	1 Mo.	6 Mo.
53	1	2.17	1.58	1.08	3.49	66.90	66.68
250X	2	2.77	5.81	1.79	2.96	68.10	57.20
462	3	7.21	5.94	1.52	3.24	68.80	68.06
552	4	4.25	6.15	2.37	4.83	63.76	64.18
570	5	4.50	7.21	2.72	5.58	62.50	61.90
572	6	3.92	11.96	2.45	5.36	62.32	62.52
588	7	4.28	12.68	2.07	4.20	58.96	60.58
705	8	3.84	10.31	2.87	4.26	64.62	62.56
721	9	6.62	5.46	4.01	5.59	64.29	63.67
731	10	3.85	8.58	3.72	4.88	61.85	66.49
771	11	4.61	14.74	2.20	5.32	64.95	66.35
941	12	6.56	16.38	3.56	4.86	61.47	63.42
Average		4.55	8.90	2.53	4.55	64.04	63.63
Mean Difference			+4.35		+2.02		- 0.41
			12 Mo.		12 Mo.		12 Mo.
228	1	6.20	6.23	1.96	3.40	65.69	73.44
551	2	5.60	5.03	2.04	5.25	61.18	62.32
556	3	6.31	12.84	2.03	6.25	63.76	64.74
590	4	7.71	7.81	2.22	5.76	61.51	64.19
606	5	5.15	7.28	2.81	7.72	63.01	63.69
656	6	6.74	6.89	2.08	5.41	60.12	61.33
681	7	5.19	10.90	2.24	5.48	61.01	62.77
682	8	5.64	8.01	2.12	8.38	63.40	70.64
696	9	3.77	10.26	2.76	5.51	68.48	66.39
781	10	5.64	8.05	2.71	5.80	65.12	67.71
784	11	6.40	8.97	2.33	8.97	62.84	64.78
833	12	4.62	6.40	3.38	6.95	63.38	66.24
Average		4.75	8.22	2.39	6.24	63.29	65.69
Mean Difference			+3.47		+3.85		+ 2.40

Soluble nitrogen. The greatest increase in soluble nitrogen occurred between six and twelve months. A significant increase was found for all sampling periods. This is in agreement with the work of Hunt, Supplee, Meade and Carmichael (1939).

Total nitrogen. The total nitrogen increased with length of storage period as was the case with soluble nitrogen (See Table IV).

pH. A very slight downward shift in pH was indicated. These differences were not appreciable, so essentially the pH remained the same.

Lactic acid. Although the lactic acid content tended to increase with time at six months, the average value for twelve months was slightly lower. These differences were not significant for the paired hams.

Glucose. The glucose content remained nearly the same for all sampling periods (See Table V).

Glycerol. The glycerol content showed very large increases after storage at six and twelve months. These values were appreciably different between sampling periods.

Organic matter. Values for organic matter markedly increased with time throughout the experiment (See Table VI).

Unsaturated fatty acids. Iodine absorption values were nearly similar between one month and six months, but higher at twelve months. Increased iodine values were found both in the outside fat

TABLE IV

CHLORIDE, SOLUBLE NITROGEN AND TOTAL NITROGEN ANALYSIS

Ham No.	Code No.	Per Cent Chloride (Fat and Moisture Free) 1 Mo.	Per Cent Chloride (Fat and Moisture Free) 6 Mo.	Per Cent Soluble Nitrogen 1 Mo.	Per Cent Soluble Nitrogen 6 Mo.	Per Cent Total Nitrogen 1 Mo.	Per Cent Total Nitrogen 6 Mo.
53	1	15.87	10.06	0.74	1.08	3.46	3.85
250X	2	13.89	13.86	0.99	1.20	3.66	4.54
462	3	17.40	19.44	1.02	1.80	4.35	4.37
552	4	14.94	12.22	0.81	1.32	4.38	4.77
570	5	12.84	11.86	0.85	1.19	3.82	4.66
572	6	10.18	10.82	0.78	1.06	3.36	4.66
588	7	18.94	20.36	1.21	1.24	3.91	5.42
705	8	9.26	14.66	1.18	1.12	4.12	4.78
721	9	11.16	12.79	1.06	1.60	4.59	4.99
731	10	13.51	14.27	1.31	1.27	4.11	4.61
771	11	11.32	11.38	1.00	1.18	4.84	4.81
941	12	14.05	17.38	0.86	1.16	4.27	3.98
Average		13.61	14.09	0.98	1.27	4.07	4.62
Mean Difference			+ 0.48		+0.29		+0.55
			12 Mo.		12 Mo.		12 Mo.
228	1	17.41	15.30	0.88	1.55	3.28	4.72
551	2	12.18	11.16	1.10	1.58	3.89	5.60
556	3	11.30	16.51	0.84	1.57	4.20	5.21
590	4	18.27	22.21	0.99	1.41	3.66	5.26
606	5	10.20	11.35	1.06	1.63	4.31	5.40
656	6	18.50	20.55	1.19	1.48	3.82	5.38
681	7	20.06	20.10	0.87	1.46	4.01	4.97
682	8	18.68	14.68	0.92	1.46	3.86	4.76
696	9	12.34	23.01	0.94	1.52	4.36	5.77
781	10	14.97	16.40	1.03	1.31	4.56	5.10
784	11	11.34	9.08	0.76	1.49	3.98	5.22
833	12	12.54	12.82	1.04	1.64	4.68	5.00
Average		14.82	16.10	0.97	1.51	4.05	5.20
Mean Difference			+ 1.28		+0.54		+1.15

TABLE V

pH, LACTIC ACID AND GLUCOSE ANALYSIS

Ham No.	Code No.	pH		Per Cent Lactic Acid[1]		Per Cent Glucose	
		1 Mo.	6 Mo.	1 Mo.	6 Mo.	1 Mo.	6 Mo.
53	1	5.35	5.57	0.95	0.68	0.48	0.58
250X	2	4.98	5.56	0.95	0.70	0.31	0.48
462	3	6.25	5.61	0.87	0.65	0.33	0.42
552	4	7.72	5.60	0.89	0.86	0.19	0.28
570	5	5.63	5.96	0.75	0.79	0.12	0.16
572	6	5.94	5.80	0.64	0.72	0.11	0.16
588	7	5.76	5.86	0.85	1.29	0.10	0.33
705	8	5.86	5.60	0.66	0.70	0.21	0.20
721	9	5.92	6.05	0.74	0.34	0.38	0.13
731	10	6.50	5.85	0.30	0.84	0.11	0.17
771	11	6.04	6.08	0.74	0.72	-	0.15
941	12	6.20	5.70	0.40	0.66	0.09	0.16
Average		6.01	5.77	0.73	0,74	0.22	0.27
Mean Difference			-0.24		+0.01		+0.05
			12 Mo.		12 Mo.		12 Mo.
228	1	5.80	5.85	1.11	1.33	0.46	0.61
551	2	6.25	6.19	0.66	0.71	0.12	0.80
556	3	5.62	5.86	0.90	0.65	0.39	0.63
590	4	5.76	5.88	0.85	0.62	0.18	0.23
606	5	5.62	6.16	0.84	0.58	0.24	0.29
656	6	5.57	5.90	0.62	0.36	0.11	0.25
681	7	5.38	5.77	0.68	0.52	0.26	0.36
682	8	5.60	6.12	0.72	0.36	0.46	0.36
696	9	6.15	5.70	0.59	0.63	0.31	0.27
781	10	6.02	5.77	0.62	0.72	0.46	0.31
784	11	5.63	5.85	0.94	0.71	0.29	0.29
833	12	6.00	5.82	0.68	0.60	0.56	0.38
Average		5.78	5.90	0.77	0.65	0.32	0.40
Mean Difference			-0.12		-0.08		+0.12

[1] Read at 570 Mu on model DU Beckman Spectrophotometer.

TABLE VI

GLYCEROL AND ORGANIC MATTER ANALYSIS

Ham No.	Code No.	Per Cent Glycerol[1]	Organic Matter[2]
		1 Mo.	
	1	0.15	3.75
	2	0.14	3.50
	3	0.10	3.08
	4	0.10	2.29
	5	0.13	2.50
Average		0.12	3.02
		6 Mo.	
53	1	0.27	4.38
250X	2	0.23	5.29
462	3	0.21	4.92
552	4	0.27	5.00
570	5	0.36	4.00
572	6	0.27	2.50
580	7	0.31	4.50
588	8	0.28	4.38
705	9	0.26	4.58
721	10	0.44	3.33
731	11	0.21	3.12
771	12	0.29	4.17
941	13	0.34	3.54
Average		0.29	4.13
Mean Difference		+0.17	+1.11

TABLE VI CONTINUED

Ham No.	Code No.	Per Cent Glycerol[1]	Organic Matter[2]
		12 Mo.	
228	1	0.33	6.79
551	2	0.50	4.38
556	3	0.42	6.96
580	4	0.53	4.79
590	5	0.44	4.96
606	6	0.62	5.96
656	7	0.51	4.75
681	8	0.33	5.83
682	9	0.37	4.38
696	10	0.45	5.62
781	11	0.37	5.29
784	12	0.36	5.88
833	13	0.40	6.38
905	14	0.51	5.33
Average		0.54	5.52
Mean Difference		+0.42	+2.50

[1] Values read on Beckman model DU Spectrophotometer at 525 Mu.
[2] Milliequivalents dichromate reduced per gram of ham. Values read at 650 Mu.

and the fat within the muscle. Linolenic acid values increased at six months and oleic acid decreased. However, the linolenic values were of such small magnitude that a slight difference could cause a significant change (See Table VII).

Short chain fatty acids. The total acid value for one month was low and the acid was separated only with the 75 per cent chloroform - 25 per cent butanol solvent mixture.

The separation was complete within this solvent mixture; the peak fraction occurring in the fifth fraction collected. This corresponded to the chromatographic separation of succinic acid as separated from a mixture of acids and as a single acid. A recovery of 91 - 94 per cent was obtained for a known quantity of succinic acid from the known acid mixture and for the single acid.

Chromatographic separation of acids from hams stored six months yielded a significant increase in total acid. The results obtained were quite erratic between hams. However, duplicate samples yielded essentially the same peak values. Extracts from five of the hams contained acid separated with 85 per cent chloroform - 15 per cent butanol solvent mixture. The most acid was extracted with the fourth fraction. Acetic acid in known quantities and chromatographically separated as described above for succinic acid gave similar results. A yield of 96 - 97 per cent was obtained. Values are shown in Table VIII for the 75 per cent chloroform - 25

TABLE VII

UNSATURATED FATTY ACIDS

Ham No.	Code No.	Iodine Number (Fat in Lean) 1 Mo.	Iodine Number (Fat in Lean) 6 Mo.	Per Cent Linoleic Acid 1 Mo.	Per Cent Linoleic Acid 6 Mo.	Per Cent Linolenic Acid 1 Mo.	Per Cent Linolenic Acid 6 Mo.
53	1	-	61.98	-	10.11	-	0.26
250X	2	-	59.59	-	10.56	-	0.33
462	3	65.59	63.48	7.71	7.86	0.70	0.70
552	4	64.07	62.38	7.77	7.75	0.54	0.51
570	5	60.14	61.22	6.02	4.30	0.19	0.05
572	6	60.09	62.58	4.18	7.20	0.00	0.06
588	7	59.34	60.88	4.95	7.63	0.04	0.30
705	8	65.76	64.78	7.15	8.26	0.34	0.53
721	9	62.38	67.29	6.58	9.96	0.38	0.67
731	10	64.34	62.08	6.89	7.49	0.17	0.59
771	11	67.27	62.43	11.00	10.10	0.28	0.71
941	12	61.18	63.32	5.89	6.81	0.00	0.35
Average		63.02	62.67	6.81	8.17	0.26	0.42
Mean Difference			- 0.35		+1.36		+0.16
			12 Mo.		12 Mo.		12 Mo.
228	1	-	73.02	-	11.30	-	0.23
551	2	64.19	63.59	6.46	7.60	0.27	0.00
556	3	64.07	71.78	5.52	6.58	0.12	0.00
590	4	60.02	64.67	5.14	6.97	0.04	0.08
606	5	64.94	62.86	4.00	5.64	0.09	0.00
656	6	59.85	61.32	4.73	6.06	0.06	0.00
681	7	61.75	62.67	4.42	7.76	0.08	0.23
682	8	62.55	63.60	7.86	7.46	0.37	0.13
696	9	66.20	70.33	9.54	9.21	0.44	0.18
781	10	63.00	63.13	7.26	8.38	0.24	0.00
784	11	64.91	65.23	6.35	8.14	0.16	0.00
833	12	68.17	69.94	6.87	10.45	0.16	0.22
Average		63.60	66.01	6.20	7.96	0.18	0.09
Mean Difference			+ 2.41		+1.76		-0.09

TABLE VII CONTINUED

Ham No.	Code No.	Per Cent Arachidonic Acid		Per Cent Oleic Acid		Per Cent Saturated Acids	
		1 Mo.	6 Mo.	1 Mo.	6 Mo.	1 Mo.	6 Mo.
53	1	-	0.85	-	46.64	-	44.13
250X	2	-	1.08	-	40.01	-	48.01
462	3	2.26	1.10	51.48	48.57	37.88	41.77
552	4	2.63	1.32	44.22	47.30	44.84	43.12
570	5	0.61	0.82	52.51	56.24	40.86	38.60
572	6	0.93	0.74	54.97	52.18	39.92	39.81
588	7	1.29	1.31	50.67	46.58	42.90	44.18
705	8	1.88	1.49	51.23	48.28	39.40	41.43
721	9	1.24	1.97	54.14	45.44	37.66	41.97
731	10	1.13	1.67	53.26	45.98	38.55	44.26
771	11	1.09	2.49	42.34	37.70	45.28	49.00
941	12	0.35	1.22	54.89	51.13	38.87	40.49
Average		1.34	1.34	50.97	47.17	40.62	43.06
Mean Difference			0.00		- 3.00		+ 2.44
			12 Mo.		12 Mo.		12 Mo.
228	1	-	2.23	-	49.49	-	36.76
551	2	1.62	0.93	51.56	51.98	40.09	39.49
556	3	1.49	0.99	51.89	62.92	40.98	29.50
590	4	1.05	1.38	52.39	52.53	41.38	39.04
606	5	1.44	1.33	58.56	53.63	35.90	39.40
656	6	0.72	0.94	54.19	52.52	40.30	40.49
681	7	0.74	1.28	56.80	48.63	37.96	42.09
682	8	1.75	1.20	46.13	50.90	43.89	40.32
696	9	1.75	1.81	47.78	52.42	40.49	36.38
781	10	1.11	0.81	52.73	50.34	38.66	40.47
784	11	1.75	0.88	47.78	52.90	40.49	38.08
833	12	0.98	1.60	57.87	50.15	34.12	37.58
Average		1.31	1.28	52.52	52.37	39.48	38.30
Mean Difference			+0.10		+ 0.15		- 1.18

TABLE VIII

MILLIEQUIVALENTS OF SHORT CHAIN FATTY ACIDS PER GRAM OF HAM[1]

Ham No.	Code No.	Total Acid	Solvent Mixtures[2]				
			B_{15}	B_{20}	B_{25}	B_{30}	B_{40}
	1	.0041	0	0	.0041	0	0
	2	.0087	0	0	.0087	0	0
	3	.0017	0	0	.0017	0	0
	4	.0087	0	0	.0087	0	0
	5	.0096	0	0	.0096	0	0
Average		.0066					
		6 Mo.					
53	1	.0640	0	0	.0310	.0330	0
250X	2	.0830	.0080	0	0	.0750	0
462	3	.0440	0	0	.0220	.0220	0
552	4	.0610	.0030	0	.0270	.0320	0
570	5	.0350	0	0	.0160	.0190	0
572	6	.0170	0	0	.0170	0	0
580	7	.0580	.0020	0	0	.0510	.0050
588	8	.0370	.0030	0	.0310	.0030	0
705	9	.0710	.0040	0	.0410	.0100	.0100
721	10	.0290	0	0	.0260	.0260	.0030
731	11	.0260	0	0	.0130	.0130	.0130
771	12	.0460	0	0	.0390	.0390	.0070
941	13	.0460	0	0	.0460	.0460	0
Average		.0475					
Mean Difference		+ 0.04					

TABLE VIII CONTINUED

Ham No.	Code No.	Total Acid 12 Mo.	Solvent Mixtures[2] B_{15}	B_{20}	B_{25}	B_{30}	B_{40}
228	1	.1000	.0050	0	.0300	.0400	.0250
551	2	.0900	.0100	.0100	0	.0600	.0100
556	3	.0650	.0150	0	.0100	.0400	0
580	4	.0435	.0025	0	0	.0410	0
590	5	.0850	.0100	.0150	.0250	.0200	.0150
606	6	.0440	.0040	0	.0400	0	0
656	7	.0450	.0200	0	.0150	.0050	.0050
681	8	.1300	.0200	.0650	.0250	.0200	0
682	9	.1340	.0050	0	.0650	.0550	.0090
696	10	.0600	.0150	0	.0200	.0250	0
781	11	.0400	.0050	0	.0350	0	0
784	12	.0650	0	0	.0450	.0150	0
833	13	.0450	.0050	0	.0250	.0100	.0050
905	14	.0450	.0050	0	.0150	.0150	.0100
Average		.0708					
Mean Difference		+0.064					

[1] Chromatographic separation short chain fatty acids.

[2] The B subscripts represent the per cent butanol in chloroform of the solvent mixture.

per cent butanol mixture as described for the one month hams. Eight hams contained acid extracted with 30 per cent butanol - chloroform mixture. This acid corresponded to lactic acid. Duplicate samples of lactic acid gave 80 to 85 per cent recovery with the same peak values as the ham extract. The peaks were not quite as sharp as with the other acids, but amount of recovery fell within the above range for a number of known samples. Duplicate samples on the ham extracts also gave about the same recovery of lactic acid. The acid extracted with 40 per cent butanol - chloroform mixture was also quite erratic. A known acid could not be found which corresponded to this acid. The difference between peak fractions of "lactic acid" and the unknown acid was constantly nine fractions. Also, when this acid was present the peaks were sharp, the quantity of acid, however, was usually small.

One acid was separated at twelve months that was not found at one or six months. This acid corresponded to glutaric acid. A recovery of 93 - 94 per cent was obtained with a good sharp peak.

The 15 per cent butanol - chloroform solvent liberated acid from every ham except one (Table VIII).

The statistical summary of the data is presented in Tables IX and X.

TABLE IX

STATISTICAL ANALYSIS OF THE DIFFERENCES OF TEST FACTORS AT PROGRESSIVE STORAGE PERIODS

Test Factor	"t" Values Obtained for the Differences From 1 Month[1]		Difference Between 6 and 12 Months
	6 Months	12 Months	
Flavor	0.2647	2.7444*	1.3976
Tenderness	1.0347	0.7270	0.0391
Saltiness	2.9431*	6.3731**	3.4282**
Moisture	-5.1432**	-8.9344**	2.1025*
Fat	2.1413	0.3419	1.2426
Salt (Fresh Basis)	3.2574**	4.9708**	3.1380**
Chloride (Dry)	0.5810	1.1324	1.3044
Soluble Nitrogen	3.9804**	12.5290**	2.1678
Total Nitrogen	3.5942**	9.2605**	2.1535
Peroxide	3.0093*	2.9847*	2.3033*
Free Fatty Acid	9.6855**	9.0909**	3.8832**
Iodine No. (Fat)	-0.3270	3.6704**	1.5212
pH	-1.0258	-1.4006	0.3333
Lactic Acid	0.4684	1.2541	0.1727
Glucose	1.0717	1.7686	0.6502
Iodine No. (Lean)	-0.3461	2.5863*	0.8234
Linoleic Acid	1.7337	1.6630	0.0465
Linolenic Acid	3.3938**	-2.3777*	2.8640*
Arachidonic Acid	0.4022	0.7831	0.0198
Oleic Acid	-2.4236*	0.1294	1.0640
Saturated Acid	1.0986	-0.4727	1.8064
Glycerol	92.7222**	138.5217**	3.6466**
Organic Matter	6.3581**	11.2574**	3.1625**
Separable Acids	8.8364**	8.2619**	1.4444

[1] Student's "t" test of significance.
* = significance at .05 level; ** = significance at .01 level.

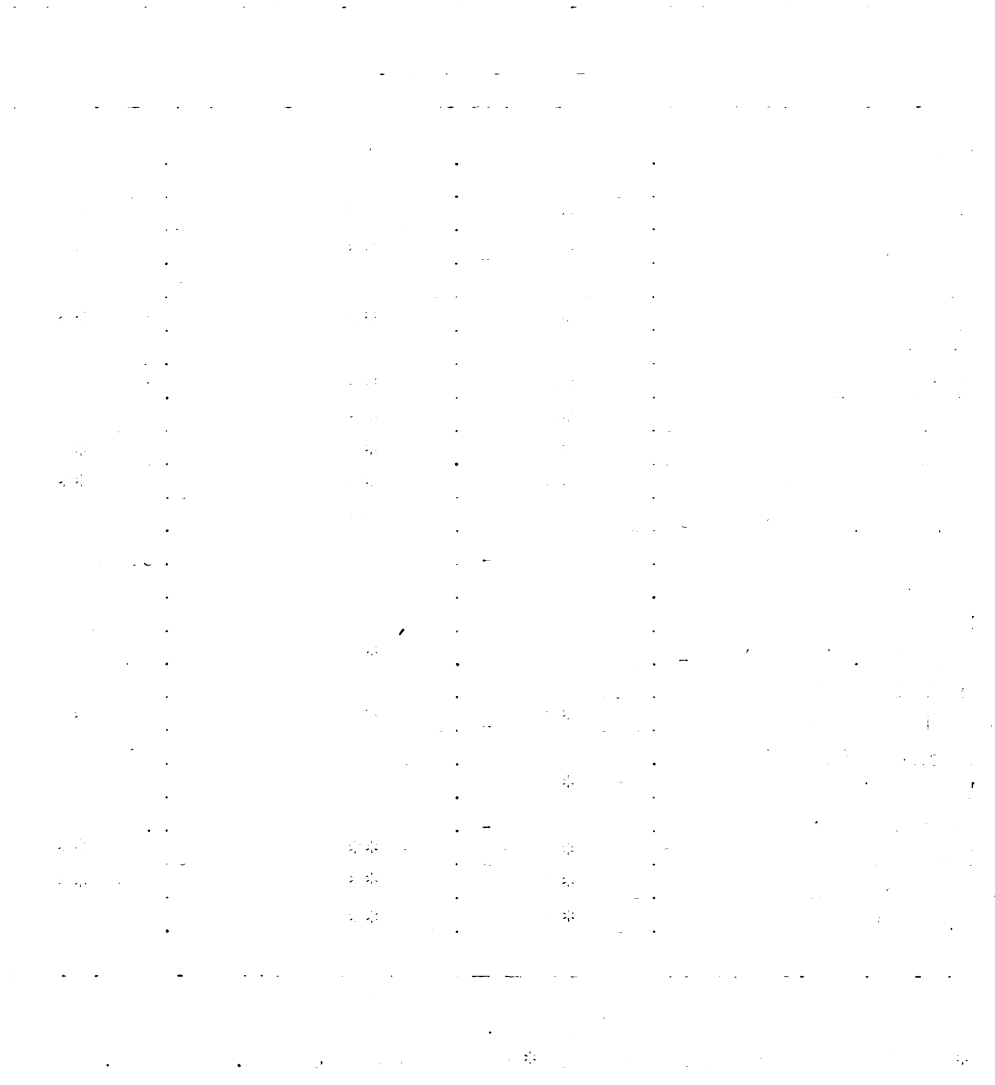
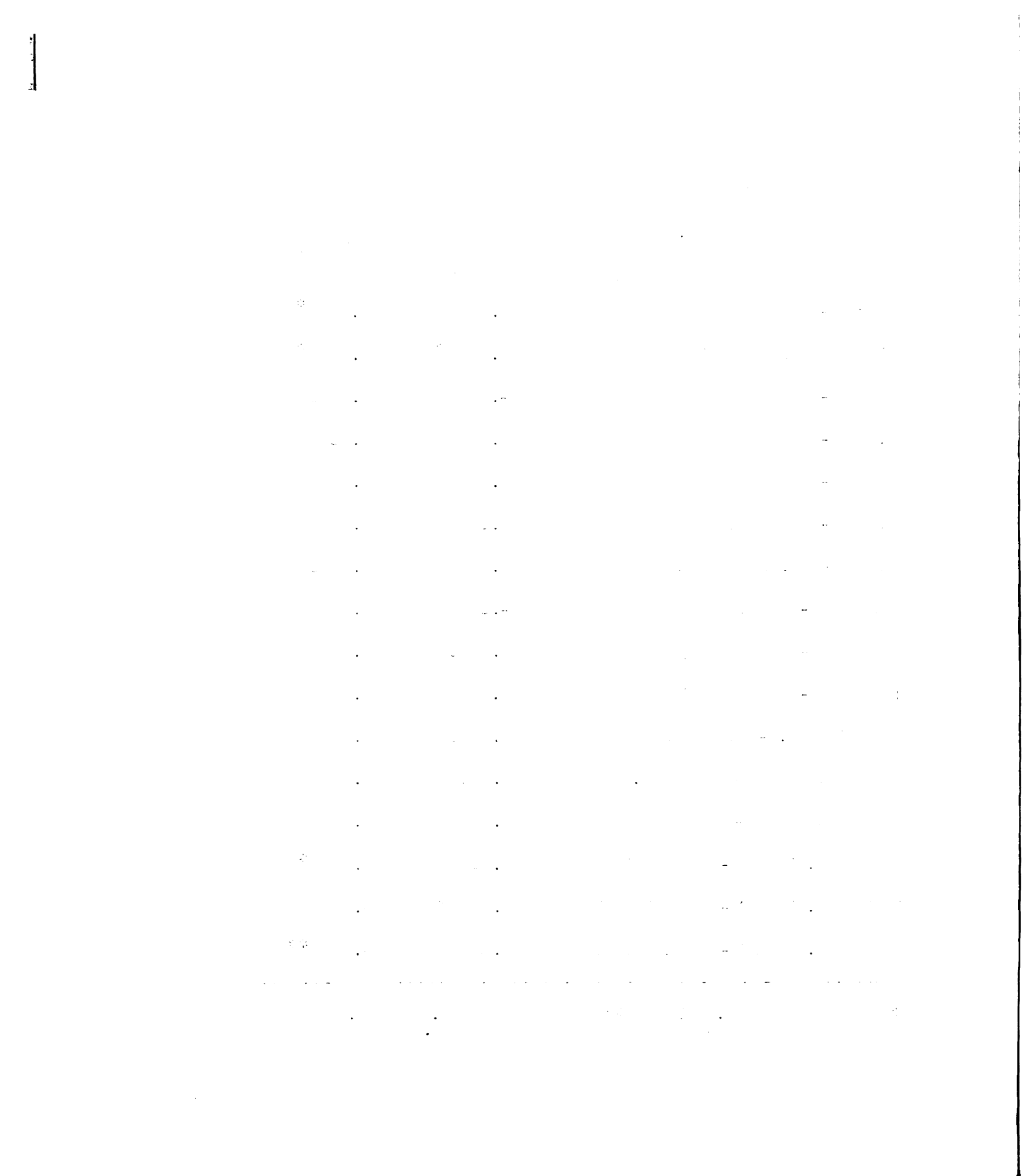


TABLE X

CORRELATION COEFFICIENTS FOR TEST FACTORS

	Correlation Coefficients	
	6 Months	12 Months
Flavor - Saltiness	+.5071	+.5475*
Flavor - Salt (Fresh)	+.6422*	+.6570*
Flavor - Soluble Nitrogen	-.1594	-.0163
Flavor - Free Fatty Acid	-.0019	+.4347
Flavor - Glycerol	+.2559	+.0237
Flavor - Organic Matter	+.3574	-.0861
Flavor - Total Separable Acid	+.3571	-.2063
Saltiness - Moisture	-.3503	-.2199
Saltiness - Salt (Fresh)	-.1193	+.2960
Moisture - Salt (Fresh)	-.0814	-.0918
Peroxide No. - Free Fatty Acid	+.1513	+.1177
Free Fatty Acid - Iodine No.	+.2230	+.0403
Free Fatty Acid - Glycerol	+.1705	+.0740
Iodine No. (Lean) - Oleic Acid	-.1388	+.6985*
Iodine No. (Lean) - Linoleic Acid	+.6520*	-.1565
Iodine No. (Lean) - Linolenic Acid	+.2251	-.9045**

* = significance at .05 level; ** = significance at .01 level.



DISCUSSION OF RESULTS

The validity of the results for the taste panel scores is based on the agreement of the tasters in the evaluation of aged flavor, tenderness and saltiness. Objective values obtained by chemical analysis are real values. Any attempts of correlation between subjective scores and objective values, however, must assume that the subjective values are also real values. This is true since the quality value of a food product is weighed heavily by its acceptability in terms of a preferred standard.

The taste panel scores within any sampling period did not vary appreciably. To varify this conclusion, the taste test scores were adjusted comparable to values obtained had the same tasters been used for a complete sampling period. That is, for one month, six months or twelve months. The formula used was as follows:

$$\overline{Sa} = \overline{S} \pm \frac{\text{missing J's} - (\text{no. of missing J's} \times (\overline{J})}{\text{no. of J's present}}$$

$\overline{Sa}$ = adjusted value; $\overline{S}$ = mean of the observed values and $\overline{J}$ = judge mean reference point. The values thus obtained as correction factors were between ± .02 to .03 of the observed values. Therefore, no adjustment of scores was necessary. The fact that the same judges were not available for every taste period did not appreciably affect the evaluation score. The selection and training

of the panel was probably the reason for this close agreement in scoring between panel members.

Although no difference was found between tasters, inspection of the data indicates that high flavor ratings were given for some one month old hams. The reasons for this may be due to a tendency to evaluate hams relatively within any one taste session. If some difference in flavor is detected between succeeding hams there is probably a tendency to score high or low depending on the relative difference.

Of the factors studied, salt had the most influence on flavor evaluation. This is reasonable since the salt concentration was fairly high. Insufficient salt greatly influenced aged flavor scores even though a separate rating was given for each factor. For example, ham number six, Table I, has a flavor score of 1.86, the smallest saltiness score for that group, 3.14 and the least salt as determined chemically, 3.44 per cent. It should be mentioned that both ham pair mates were low in salt content. The range in scores for saltiness was somewhat greater than for the other two factors considered but this was mostly offset by a corresponding high or low value.

Tenderness scores were erratic only for a few hams. This may have been due to the difference in samples rather than opinion differences. An attempt was made to trim the outside edge of the

cooked meat sample, but some difference in moisture content of "bite" samples may have caused the variation. The differences between groups of hams, however, indicated little difference in tenderness.

Since there was an appreciable moisture decrease it is logical to assume that the concentration of stable compounds should increase on a fresh weight basis. This is the case with salt. The concentration increased on a fresh basis, but did not change when calculated on a dry basis. From a flavor standpoint only the former need be considered.

Soluble nitrogen, total nitrogen and free fatty acids increased with storage time. This is in agreement with the work of Hunt, Supplee, Meade and Carmichael (1939). While these compounds increased quantitatively in an upward direction as did flavor score, only free fatty acids were close to being significantly correlated with flavor scores. A larger sample is necessary in order to definitely establish if aged flavor is correlated with free fatty acid.

Peroxide content in the quantities found in these hams is definitely not a factor that may be used to indicate aged flavor.

Lactic acid increases or decreases to a certain extent with pH, but the differences were too small in this experiment to be statistically significant.

Glucose values remained nearly the same during the entire storage period. The color appeared normal in every ham, therefore, there were no measurable affects from glucose. In several hams these values were higher than might be expected. The work of Lewis (1937) and Brady, Smith, Tucker and Blumer (1949) listed lower values. However, lower storage temperatures were used here than for the above studies.

A study by Corman (1948) indicated that in samples containing nitrite some interference in the idometric determination of sugar may result. To determine if nitrite caused erroneous readings in this study, the entire group of twelve months samples were treated with urea to eliminate nitrites as recommended by Corman. The values thus obtained were nearly identical with those without treatment. Therefore, the nitrite content was not sufficient to cause interference. When nitrites were added to samples in the amounts used by Corman, interference was appreciable. The nitrite content was quite low in all hams. The values were only 10 to 40 parts per million with little change throughout the entire storage period.

The method used for glycerol determination is dependent upon the formation of formaldehyde. A consistant increase of "glycerol" occurred with length of storage period. Since glycerol may be liberated with the hydrolysis of triglycerides a correlation analysis was conducted. These values were positive, but too small to be

significant. It may thus be assumed that all of the glycerol does not come from the result of fat hydrolysis.

Inspection of the data presented in Table VI indicates that the glycerol values fall quite closely within certain limits as follows: below .20 per cent, 1 month; .20 - .35 per cent, six months and .35 - .60, twelve months. Aged flavor scores may not be arranged in this manner, therefore, glycerol is a more predictable tool for use in estimating storage time. It should be noted however, that the differences between one month and six month hams were not obtained by paired difference. The differences obtained are relative between groups. Aged flavor scores are only associated with glycerol values in the respect that both show average increases with time.

The iodine number of both the outside fat and the muscular fat increased at the twelve months sampling period. Anaerobic conditions were approximated in each case, since the skin covered the outside fat during storage, and the sample was removed near the center of the ham. Jensen (1945) quotes Bach and Sierp (1924) in a statement to the effect that under certain conditions of anaerobiosis, carbon dioxide is split off and the fatty acids are changed into hydrocarbons resulting in a lower sponification and higher iodine number than the original fat. The saturated acids here, however, showed very little change from one month to twelve months.

The peroxide increase was higher at six than at twelve months. This is in agreement with the iodine values. Pork fat exposed to aerobic conditions normally increases in peroxide content during the induction period. The results of this study are, therefore, somewhat analogous to those quoted by Jensen (1945).

It has been previously mentioned that linolenic acid increased, but the quantity of this acid was so small that a slight change would be significant. It may have happened that the structure of this acid was altered in such a way that it gives a spectral reading similar to oleic acid. The quantity of oleic acid is determined by the magnitude of the iodine number and the quantity of other unsaturated acids as they are determined spectrophotometrically. It is obvious that the accuracy of the oleic acid determination and any interpretation concerning this acid is dependent upon the accuracy of the other determinations.

Other studies have indicated that the spectral characteristics of methyl linoleate and methyl linolenate are changed following alkali - isomerization (Swain and Brice, 1949; Privett, 1951 and Privett and Lundberg, 1951). Arachidonic and linoleic acid characteristics were also affected. No indication of a similar reaction seems likely in this study. The trends in fatty acid content do not indicate their association with aged flavor in the present study.

Chromatographic separation of short chain fatty acids indicated one specific trend. The acid separated with the 85 per cent chloroform - 15 per cent butanol was not present in one month old hams, only occasionally in six month hams and in all hams except one at twelve months. This seems to be a fairly good criteria on length of storage period. The other acids did not appear consistently enough to draw valid conclusions.

From the data it appears that aged flavor scores are best reflected by the salt content of the hams. An equation formulated to predict flavor scores should, therefore, have a salt factor included.

From the data presented the following equation applies quite well for the selection of hams on the basis of aged flavor scores:

Aged flavor = values $>$ 4% salt + values $>$ 4% free fatty acid + values $>$ .35% glycerol

This equation eliminates all one month old hams, all except two six months old hams and most of the lowest flavor scored hams in the twelve month group. A less critical equation would contain the following:

Aged flavor = values $>$ 4% salt + values $>$ 4% free fatty acids

Most of the lowest scored hams in the six months and twelve months group are eliminated by the use of this equation. If a 5 per cent salt value is substituted for 4 per cent salt in the above

equations, results are just as satisfactory and somewhat more discriminating. Since one or two high scoring hams are eliminated at this salt level, the 4 per cent level may be more practical. A larger number of hams would probably facilitate the use of more definite limits on the factors used in the equations. An upper salt limit should be included as well as the lower limit. There is some indication from the flavor scores that 10 per cent salt should be the upper limit. The moisture content and possibly the fat content will influence the limits under a given set of conditions. The most uniform moisture and fat content was found for the twelve months old hams in this study. This is no doubt one reason for the more uniform flavor scores.

The extent of aged flavor development is indicated comparable to the amount developed by hams aged about five months at 21.11° C (70° F.) and 70 per cent relative humidity.

For future work, the test factors included in the formula presented should be determined on a large number of hams. The equation may then be applied to these values and by this means select the hams with probable high aged flavor. The entire group of hams would be scored by the taste panel as described in this study. Hams given high flavor scores by the taste panel could be compared with those selected by the prediction equation. The reliability of the prediction equation could thus be established.

SUMMARY

Fifty-four hams were dry cured and stored at approximately 4.4° C (40° F.) until they were sampled chemically and by taste testing. Twenty-seven hams were sampled after storage for 1 month, thirteen of their pair mates at 6 months and the remaining fourteen at 12 months. The identity of the pair mates was maintained throughout the study in order that chemical values and taste test scores could be recorded for each specific ham. The differences between one month and six months and twelve months were calculated; the differences were analyzed statistically and tests of significance applied. Correlation studies were conducted on taste test scores and some of the chemical compounds.

Taste tests. Flavor increases with each sampling period, but was significant only at twelve months.

Tenderness did not change to any appreciable extent.

Saltiness increased to a marked extent at each sampling period, but there was a marked variation between samples.

Chemical tests. Salt, peroxides, free fatty acids, soluble nitrogen, total nitrogen, glycerol, water soluble organic matter and total water soluble chromatographically separable acid increased with each storage period. Moisture was found to decrease with length of time in storage.

Saturated acids increased at six months but not at twelve months.

Iodine absorption number decreased for external adipose tissue and muscular fat at six months, but increased at twelve months.

Oleic acid decreased at six months but increased slightly at twelve months.

Linolenic acid increased at six months but decreased slightly at twelve months.

Flavor was positively correlated with saltiness taste scores and chemically determined salt values. Free fatty acid values were positively correlated with flavor scores at twelve months but the values were slightly low to be significant at the 5 per cent level.

A prediction equation suggested for evaluating aged flavor in hams is as follows:

Aged flavor = values $>$ 4% salt + values $>$ 4% free fatty acids + values $>$ .35% glycerol

When this equation is applied to the hams in this study, most of the hams aged for twelve months are acceptable; all but two of the six months hams would be excluded and none of the one month old hams would be retained.

An equation that will eliminate the hams lowest on the flavor scale for six and twelve months is as follows:

Aged flavor = values > 4% salt + values > 4% free fatty acids

A sharper selection results when 5 per cent salt is substituted for 4 per cent, but in application 4 per cent may be the most satisfactory factor to use.

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