EFFECT OF PARAFFIN WAX COATING ON FATTY ACIDS IN COUNTRY CURED HAMS

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ABSTRACT

The left and right hams from twenty hogs were cured in a similar manner. All left side hams were covered with paraffin wax, while all right side hams were unwaxed. These pairs were aged for twelve months. Raw muscle samples were collected from another thirty pairs of hams prior to curing and aging. The left hams were again covered with wax while the right hams were unwaxed following equalization. The thirty pairs were cured and aged for six months. At the end of the aging period, center slice samples from ten pairs (left and right) of the twelve months aged hams, twenty-two pairs of the six months aged hams and corresponding twenty-two raw samples were extracted for short-chain (volatile) fatty acids. Extracted acids were analyzed by GLC and amounts expressed in mg/100 gram sample as received. Samples from ten pairs of twelve months aged hams, ten pairs of the six months aged hams and seven of the raw hams were extracted for total lipid. Ester derivatives of acids (C_{12} - C_{20} in lipid) were made and analyzed by GLC. Values were expressed as percentage total long-chain fatty acids.

The means and standard deviations of all fatty acids in the analyzed hams and their analysis of variance were computed to determine the effect of wax covering, and time of aging on individual fatty acids. In six months aged hams, waxed covered hams had less ($P<0.05$) butyric and isovaleric acids than unwaxed hams. Wax covering increased the relative levels of palmitoleic and linolenic acids. There was no significant wax effect on the other fatty acids of six months aged ham or any of the fatty acids of the twelve months aged hams. Aging hams increased ($P<0.05$) acetic, isovaleric and isobutyric acids but had no effect on propionic

and butyric acids. Aging six months decreased the relative amount of myristic, stearic, linoleic and linolenic acids while palmitoleic and oleic acids were increased. Acetic, isobutyric, isovaleric, myristic and palmitic acids increased with time of aging ($P<0.05$). Aging time had no significant effect on propionic, butyric, stearic and oleic acids.

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CHAPTER I

INTRODUCTION

Country cured hams have become popular nation-wide rather than just in Southeastern United States (Langlois and Kemp, 1974). Since this type of ham has a reasonably long shelf life, it promises a future supply of tasty protein for the developing countries where the population cannot preserve its meat supply due to a lack of refrigeration systems or canning industries. Cheddar cheese is coated acceptably with paraffin wax (Sweet, 1953), and Melton and Maxedon (1979) demonstrated that country hams can be coated also with wax during aging. The popularity of country cured ham is enhanced by its highly acceptable aroma (Kemp et al., 1974). The study of this aroma has implicated fatty acids among other substances as contributing to the aroma (Lillard and Ayres, 1969a).

Several studies have been conducted on the fatty acids of country hams. Some of these studies were concerned with relating the volatile fraction of fatty acids to the aroma of cured hams. The first known work regarding the separation and identification of volatile chemical compounds in aged country cured hams was conducted by Ockerman et al. (1964). Other studies were concerned with only the long-chain fatty acids. Most data on fatty acids of hams were reported as percentage of total analyzed fatty acids (Craig et al., 1964; Kock et al., 1968). Tsai et al. (1974) and deLumen et al. (1974) studied the specific amount of only long-chain fatty acids in hams. Up to this time the absolute amounts of the identified volatile fatty acids in fresh and in cured hams are lacking.

This study was designed to determine the specific (absolute) amounts of the volatile fatty acids and the percentage of each detected long chain fatty acid in fresh, cured but unwaxed, and cured and waxed country hams. The data obtained will show the effect of wax coating after equalization and aging on the analyzed fatty acids in country cured hams. This study will help in determining the acceptable range of these acids in 6 and 12 month aged hams. Such range in turn can be utilized in the quality control process of cross-checking the claimed age of cured ham products.

CHAPTER II

REVIEW OF LITERATURE

I. The Effect of Aging on the Fatty Acids in Ham

Previous studies indicate that aging influences the chemical properties of country ham. Hadden et al. (1975) separated by gas chromatography four major compounds in the headspace vapors of comminuted pork processed with or without sodium nitrite. All four peaks were present in all treatments examined. Since two peaks of their experimental chromatograph were much less predominant in the samples containing nitrite than in samples without nitrite, they concluded that nitrite was reducing the amount of some major volatile compounds. In their work the presence of nitrite had an effect ($P<0.01$) on the amount of each compound existing in the headspace vapors above the pork. In the same studies these workers found no apparent difference in rancid odor between the products containing only nitrite and the products containing nitrite and salt.

Ockerman et al. (1964) identified formic, acetic, propionic, butyric and isocaproic acids in ham. They reported that the number and quantity of acids isolated increased with the length of the aging period except for the quantity of formic acid. The percentage of formic acid decreased with storage time, but the relative decrease was believed to be due to an increase of longer-chain acids rather than a decrease in amount of formic acid. Kingsley and Graham (1978) reported that the level of myristic acid increased ($P<0.05$) in the aged hams. The samples were lower ($P<0.05$) in palmitic acid at the end of the aging period when compared to fresh ham values. Frozen hams were significantly lower in palmitic acid after

curing than their unfrozen counterparts. Thus, freezing appears to influence changes in fatty acid concentrations. Stearic acid values were significantly higher than fresh ham values at the end of aging for both unfrozen and frozen hams. In the frozen hams aging did not affect the levels of oleic acid. Also, aging had no effect ($P<0.05$) on the level of linoleic acid when fresh hams were compared with frozen aged samples. Thus, Kingsley and Graham (1978) concluded that all hams were significantly higher in stearic acid and lower in palmitic acid at the end of aging.

Cross and Ziegler (1965) observed that chromatograms of the volatile carbonyl compounds from cured hams had lower amounts of valeraldehyde and hexanal than chromatograms from uncured hams. The difference in contents observed between cured and uncured ham volatiles was less pronounced in butyraldehyde, propionaldehyde and acetaldehyde. They noted that these aldehydes seemed to be more prevalent in the uncured ham. The branched-chain aldehydes such as isobutyraldehyde and isovaleraldehyde occurred to the same extent in both treatments. This observation is noteworthy since fatty acids are believed to be the precursors of the aldehydes, the latter derived by oxidative cleavage of unsaturated fatty acid residues (Cross and Ziegler, 1965).

Parks (1965) found that oxygen attacks the methylene group of unsaturated hydroperoxides. Subsequent degradation of hydroperoxides through the creation of free radicals forms carbonyl compounds which contribute to flavor (Sulzbacher et al., 1963). Cross and Ziegler (1965) concluded that nitrite interfered with the oxidation of unsaturated liquids. Watts (1954) clarified this conclusion by stating that lipid oxidation is considerably delayed in cured meats. The thio-barbituric acid values of products stored under frozen conditions were reduced ($P<0.01$)

by nitrite and increased ($P<0.01$) by time (Hadden et al., 1975). Free fatty acids exhibited a steady increase ($P<0.01$) with each period of aging (Kemp et al., 1961). During aging, the relative percent of myristic, palmitric, stearic, and oleic acids decreased in all hams but increased approximately two-fold in palmitoleic, linoleic and linolenic acids (Craig et al., 1964). Blumer (1954) and Ockerman (1961) also noted an increase in linoleic acid with aging time. Ockerman (1961) reported an initial decrease and then an increase in percentage of linoleic acid. It is clear from all these points that while all investigators agree on the fate of linoleic acid, Kingsley and Graham (1978) and Craig et al. (1964) do not agree on the effect of aging on myristic acid in aged hams.

II. The Effect of Smoking and Heat Processing on the Fatty Acids in Country Ham

The influence of heat on the fatty acids of various animal tissue has been studied. Some workers reported that unsaturated fatty acids changed very little during heat processing (Willard et al., 1954; Siedler et al., 1964; and Giam and Dugan, 1965). Other workers reported that the concentration of unsaturated fatty acids decreased during cooking (Chang et al., 1952; Chang and Watts, 1952; and Filer et al., 1945).

De Lumen et al. (1974) reported that myristic acid calculated as percentage total fatty acid ester was higher ($P<0.05$) in cooked porcine tissues than in uncooked tissues. Palmitic, stearic, and linoleic acids were more concentrated in cooked tissues. Palmitoleic and linoleic acids expressed as percentage wet tissue were higher ($P<0.05$) in raw intermuscular fat. The loss of moisture during cooking was suggested as explanation for the higher values since expression of results on a dry tissue basis

had little effect on the significances of the differences cited. De Lumen et al. (1974) examined the mean values of triglyceride fatty acids in raw and cooked fatty tissues from ham. They reported that oleic acid (C18:1) was higher ($P<0.05$) while all other acids were only slightly higher in raw hams as percentage wet tissue. When expressed as percentage ether extract, myristic acid (C14:0) was slightly higher in raw samples than in cooked samples. When the data were calculated as percentage total esters, palmitic (C16:0), myristic, stearic (C18:0), and linoleic (C18:2) acids were slightly higher in cooked ham tissues while palmitoleic and oleic acids were slightly lower than in raw samples. Witte (1971) reported that cooking and curing decreased ham and picnic fatty acid contents.

Kemp et al. (1961) found that free fatty acids in cured hams increased with aging, but the rate of the increase decreased as smoking temperatures were increased. Thus, the temperature of smoking influenced free fatty acid development, and unsmoked hams developed a higher amount of free fatty acids. Increased penetration of smoke in skinned hams retarded rancidity development. In Kemp's study, free fatty acid content decreased ($P<0.01$) with increases in temperature and exhibited a significant ($P<0.01$) interaction between aging period and temperature.

III. Fatty Acids as Antibacterial Agents

Solutions of acetic and propionic acids (60:40, w/w) are used as fungicides on cereal grain in storage. Chung and Geopfert (1970) reported that acetic and propionic acids were the most effective of thirteen fatty acids tested in inhibiting the growth of Salmonellae in tryptic soy broth. Reynolds and Carpenter (1974) reported that the use of propionic acid with acetic acid resulted in a reduction of microorganisms at high pH than

when only acetic acid was used. Treatment with a solution of 1.5M acetic: propionic acid (60:40, w/w, pH 2.3) produced a reduction of two log cycles in total numbers of microorganisms with no apparent detrimental effects on the pork carcass. Biemuller et al. (1973) found reductions of one log number at pH 2.5 and two log numbers at pH 2.0 of total number of bacteria by treating with only acetic acid. Schultz and Leudecke (1977), who investigated the influence of neutral fats and fatty acids on aflatoxin production by Aspergillus flavus in a glucose-salts-amino acids medium, found that both 15% linoleic acid and 15% stearic acid fortification of the medium repressed aflaxtoxin B_1 abd G_1 synthesis. Caprylic acid reportedly has fungistatic properties in the pH 2.0 - 5.0 range (Hoffman et al., 1939). Ciegler et al. (1966) demonstrated that peroxidized methyl esters of soybean oil partially degraded aflatoxin B_1 in an aqueous system. According to Hoffman et al. (1939), at a given pH the antimicrobial activity of fatty acids increased with increasing chain length. They re-demonstrated that propionic acid was significantly more inhibitory to mold growth than acetic acid. Buchanan and Ayres (1976) examined the aflatoxin production per unit growth and reported that the decrease in aflatoxin production at higher pH levels of sodium acetate was not due primarily to inhibition of growth; but was due instead to the direct effect of acetate on aflatoxin production. These findings are encouraging to the production of aged hams since the safety and shelf life of the product are considered to be very important, and both acetic and propionic acids are natural compounds in aged hams.

IV. Effect of Wax Covering on Ham

Bray and Hanning (1949) investigated the use of a wax covering as a mold controlling device in stored cured pork. They reported that

proprietary wax No. 1 offered good protection against mold until breaks appeared in the wax coating. At the end of the experiment, about one-fourth of the hams and shoulders had been adequately protected from mold. In the same study proprietary wax No. 2 offered excellent protection to the hams and no mold growth was evident on the cuts covered with the wax. These investigators explained that proprietary wax No. 2 had the property of remaining soft and pliable at the ham storage temperature as opposed to proprietary wax No. 1 which became brittle. Their finding was striking in that the covering with wax No. 2 gave a significant improvement in flavour--what the investigators called "the most favorable results."

V. The Effect of Diet and Fresh Ham Quality on the Fatty Acid Composition of Pork Fat

Koch et al. (1968) investigated the effect of diet on the fatty acid composition of pork fat. They reported that back fat from the hogs fed 10% safflower oil diet (barley-soybean meal) contained significantly more linoleic acid and significantly less oleic acid than fat from the control hogs fed corn-soybean meal. They found that feeding of tallow tended to restore the levels of oleic and linoleic acids to those found in control hogs. A comparison of the levels of linoleic acid in the feeds with that of the resulting depot fat led them to the belief that dietary linoleic acid was deposited preferentially. This was consistent with the findings of Dahl and Perrson (1965) and Shorland and Mare (1945). Kock and co-worker's (1968) data suggested that the preferential deposition of linoleic acid lowered the level of total saturated fatty acids in the depot fat. This finding not-withstanding, Kock et al. (1968) and Blumer et al.

(1957) shared the view that changes in the oleic acid content of depot fat could not be explained by the dietary levels of oleic acid alone. Palmitoleic acid levels in depot fat appeared to be influenced in much the same manner as oleic acid. Based on the finding of Koch's et al. (1968), the fluctuations were much less drastic than those found in the backfat or leaf fat, although the fatty acid pattern of the intramuscular fat was affected by diet. The fatty acid composition of the intramuscular fat from the Longissimus dorsi muscle was affected much less by diet. Koch's et al. (1968) data suggested further that the inner layer of backfat exhibited a more extensive turnover of fatty acids than the outer layer. There was no difference in the fatty acid composition of the intramuscular fat between barrows and gilts. Kock and co-workers (1968) agreed with Greer et al. (1965) that the saturated fatty acid contents of the intramuscular fat appeared to remain quite constant. This finding is encouraging since the intramuscular fat is usually the one consumed in hams or pork products and consumers need not worry about unnecessary increase of saturated fatty acids caused in their diet by animal feeds.

Kemp et al. (1968a) studied the effect of fresh ham quality on aged ham quality and reported that fat from low quality ham was slightly, though not significantly, more unsaturated as denoted by higher iodine number than fat from high quality ham. According to their study intermuscular fat had lower free fatty acid values than subcutaneous fat, and free fatty acid values were higher in the high quality of hams.

There was a negative correlation of free fatty acid values with backfat and ham fat in aged hams (Kemp et al., 1964). The result of this study suggested that fat from leaner hams, which is normally less saturated, developed free fatty acids at a faster rate. There was no

significant correlation between sex and free fatty acid development. Craig et al. (1964) reported that aged country-style hams from hogs fed a ration containing 10% peanut oil, had lower free fatty acid values, more chemical fat, more oleic, linoleic, and linolenic acids, and received higher taste panel scores for aged flavor than hams from hogs which had no peanut oil. Different studies have supported the belief that diet affects the composition of swine depot fat (Hankins et al., 1928; Ellis, 1933). Feed high in unsaturated fatty acids will result in high levels of unsaturated fatty acids in carcass fat. It may be mentioned at this point that the lipids of an animal are not uniform in composition throughout the carcass. It has been demonstrated that the inner fat of pigs contains a higher proportion of saturated fatty acids than found in the outer fats (Ostrander and Dugan, 1961).

VI. The Relationship of Fatty Acids to the Flavor of Country Hams

Many studies have been conducted to elucidate the components of cured ham flavor. At this time there is no conclusion that any specific component or components of cured ham possesses the characteristic known as "cured aroma." Some investigators have reported that cured ham flavor is found in the volatiles obtained by vacuum distillation (Cross and Ziegler, 1965; Lillard and Ayres, 1969a; Ockerman et al., 1964). Other investigators have reported that many of the volatile components so far identified in cured ham have been found also in uncured ham as well as other types of meat (Landmann and Batzer, 1966). According to the results of Lillard et al. (1969b), the flavor compounds and flavor precursors of country-cured hams are not water soluble. On the contrary,

Hornstein et al. (1961) found that cold water extracts of lean pork contain desirable flavour precursors. In agreement with Hornstein's results are those of Piotrowsky et al. (1970) which state that aqueous extracts of cured and uncured hams could be identified by heating and observing the aromas produced. Piotrowski et al. (1970) explained further that although the aqueous extracts of cured hams yielded cured aromas, these odors were more intense in the chloroform-methanol-soluble fraction. This indicates that cured odor components or precursors were essentially in the lipid phase. Their results indicate that precursors of the basic meaty aroma are water soluble, whereas precursors of the cured ham flavor are associated with the lipid phase.

Wong et al. (1975) reported that the characteristic mutton aroma was primarily associated with the volatile acidic components of the meat. These investigators explained that 80 to 90% of these acids were even-numbered normal fatty acids containing between 6 and 12 carbons. They stated that the remaining fatty acids were branched or unsaturated in nature. Their finding about mutton might be true of cured ham. Kemp et al. (1968b), investigating the effect of alternating aging temperatures on the quality and acceptability of aged hams, found a relationship between improvement in flavor and higher free fatty acid values. This finding agrees with that of Blumer (1954) who used free fatty acid values as one criterion for evaluation ham flavor.

Day (1967) reported that fatty acids, among many other compounds, contribute to the flavor of other foods. It is reasonable to assume that they contribute to the flavor of country cured hams either as flavor compounds or flavor precursors. Lillard and Ayres (1969a) reported that the degradation products of ham lipids were important flavor compounds. They

proposed that these degradation products included precursors (among which are fatty acids) that are converted to flavor compounds when heated. In other words, the carbonyl compounds produced by autoxidation of the lipids contribute to the flavor of the hams.

Craig et al. (1964) studied the effect of ration on the quality of aged hams and indicated that ration might effect the development of aged flavor. In their study the relationship between aged flavor and free fatty acid was not appreciable ($r = 0.31$). At this point one can state that while fatty acids cannot be regarded conclusively as being the cured aroma compounds, they are somehow implicated as precursors of aroma compounds, contributing in a way yet to be elucidated to the flavor of hams.

CHAPTER III

MATERIALS AND METHODS

Hams, from which samples were obtained for this study, were cured with a mixture containing 8 pounds of salt, 3 pounds of sugar and 1 ounce of sodium nitrite per 100 pounds of hams. This curing mixture was applied for a period corresponding to two days per pound of individual ham at 40°F.

After the salting phase, the hams were washed and hung in stockinettes for a total of 56 days in an equalization room maintained at 50±2°F and approximately 65% relative humidity. Following equalization, the hams were aged at 75-77°F until they had shrunk 18% of their original weight. The ham from the left side of the hog was then coated with a paraffin wax heated to 180°F. Each ham was dipped into the wax twice to assure a complete coating. The ham from the right side of the hog was not coated and served as a control treatment. One hundred hams from 50 hogs were cured but only 64 hams from 32 of these hogs were used in this study. These 100 hams (50 pairs of left and right sides) were divided into 2 groups. The first group consisting of 30 pairs of hams (right and left sides) were aged for 6 months and the other group consisting of 20 pairs were aged for 12 months.

Fresh samples were removed from the butt face of each ham included in the six month group. A ham slice, including all muscles, was trimmed of external and seam fat, frozen in liquid nitrogen and powdered for extraction of volatile and long chain fatty acids.

A one-half inch slice was removed from the 6 and 12 month old hams 1 inch distal to the aitch bone, trimmed free of fat, frozen and

powdered in liquid nitrogen, and volatile and long chain fatty acids extracted.

I. Volatile Fatty Acids

For the extraction of volatile fatty acids, 50 grams of the uncured ham sample and 25 grams of the cured ham sample were used per ham. Twenty-two pairs of hams were studied for the uncured and 6 months aged hams and 10 pairs for the 12 months aged hams. Each sample was thoroughly suspended in 150 ml of distilled water. The proteins were precipitated with 25 ml of 1 N sulfuric acid and 40 ml of 20% phosphotungstic acid. Twenty-five ml of distilled water were added at this point to make a total of 240 ml of mixture. The resulting mixture was shaken vigoroughly and filtered through a 24-cm rapid flow filter paper. From this point the procedure recommended in AOAC (1970) was followed. One hundred fifty ml of the filtrate were steam distilled under constant volumne conditions (Figure 1). Two hundred ml of distillate were collected in a volumetric flask, transferred to a 500 ml flat bottom, 24/40 groundneck flask and were neutralized with 0.1 N sodium hydroximide solution using phenolphthalein indicator. An excess (1.0 ml) of sodium hydroxide was added. The resulting pink solution was evaporated to dryness on a vacuum rotary evaporator at 50°C. The volatile fatty acids present were liberated from the dry crystals of their sodium salts using 1.1 ml of 0.5 N dichloracetic acid in acetone. One ml of standard methyl heptyl ketone solution in acetone was added as an internal standard and the solution made up to 3.1 ml with pure acetone. The solution was collected in an aluminum foil-lined screw cap vial and stored at 40°F until GLC analysis.

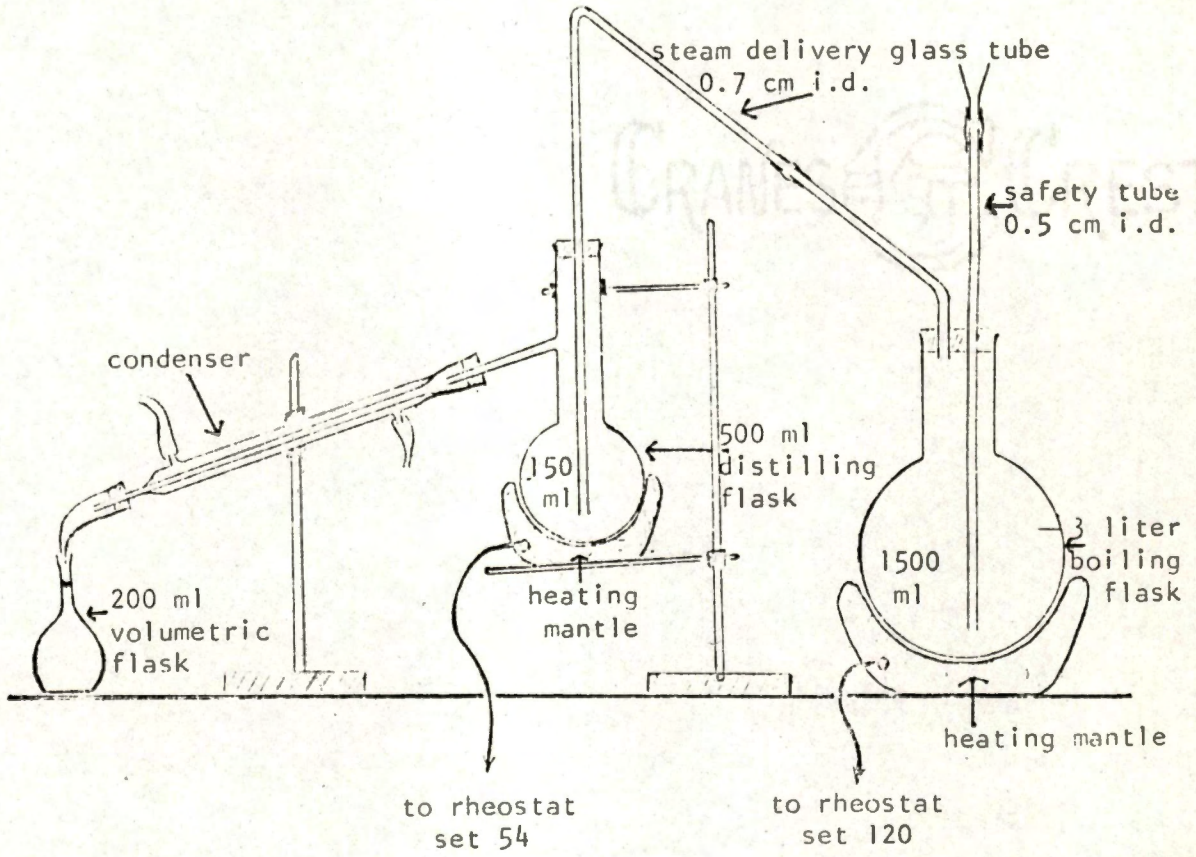

Fig. 1. Constant volume steam distillation apparatus.

The fatty acids present were identified by comparing the retention time and relative retention time (Appendix 1) of the chromatographic peaks of the sample with that of a standard containing 0.1668 mg of each expected volatile fatty acid--namely acetic, propionic, isobutyric, butyric, valeric, isovaleric, and hexanoic acids--in 3.1 ml of acetone solution.

Analysis was done on a Bendix Gas Liquid Chromatograph, Model 2600, equipped with a flame ionization detector, Dohrman recorder and an electronic integrator (Shimadzu Chromatopac E-1A). The column was 1.83 m x 6.35 mm glass packed with 10% FFAP, 80/100 Gas Chrom Q. The instrument was set at a detector temperature of 160^{o}C, injector temperature of 160^{o}C, isothermal oven temperature of 135^{o}C, and at an attenuation of 200. Nitrogen (carrier gas), hydrogen and air flow rates were 30 ml/minute, 40 ml/minute and 400 ml/minute, respectively. Two and a half microliters of the standard solution were injected and the peaks of the chromatogram identified. Two more interjections were made and the retention time and response factor of each acid were obtained from the electronic integrator (Appendix 2). Two and one half microliters of each sample solution were injected in turn and the peaks identified and quantitated as mg fatty acid per 100 g wet sample weight of ham (Appendix 2). Corrections for distillation loss and portion of extract distilled (Appendix 3) were made on the values read from the integrator to obtain actual experimental values.

II. Long-Chain Fatty Acids

In the analysis of long chain fatty acids in the hams, a modified procedure of Ostrander and Dugan (1961) was used to extract total lipids.

Then, methyl esters of the acids were prepared by the recommended official method Ce2-66 (AOCS 1973). Seven hams for the uncured, 10 pairs of hams for the 6 months aged, and 10 pairs of hams for the 12 months aged hams were studied. A 15 g powdered sample of each ham was blended in a Waring blender with 130 ml of methanol for five minutes at low speed. Sixty-five ml of chloroform were added and the mixture was blended for another five minutes. An additional 65 ml of chloroform was added and blended for 20 seconds. Then 1.5 g of zinc acetate, dissolved in 65 ml of deionized water was added to the mixture and blended for ten seconds. This mixture was filtered through a Whatman No. 1 filter paper in a Buchner funnel under suction. The residue and used filter paper were put into the blender. The funnel was wiped with half Kleenex tissue and the tissue was added to blender contents. All blender contents were blended with 100 ml chloroform for 2.5 minutes. This mixture was filtered through another Whatman No. 1 filter paper in a Buchner funnel under suction. The blender jar was rinsed with 75 ml chloroform which also was filtered as before. All filtrates were combined in a 500 ml graduated cylinder. The suction flask was rinsed in turn with 25 ml of methanol and chloroform, and the rinse added to the filtrate. The filtrate was stored under nitrogen at $4^{o}C$ until methanol and chloroform layers were distinctly separated. The volume of the chloroform layer was recorded. All layers were transferred to a 500 ml separatory flask and the distinct layers re-established overnight. The chloroform layer (bottom) was drained into an Erlenmeyer flash. Ten ml of the chloroform extract were pipetted into each of 2 dry, tared 50 ml beakers. The contents of these beakers was evaporated overnight under the hood and dried at $70^{o}C$ overnight in a vacuum oven. The beakers were cooled and re-weighed within two hours. The average weight of lipid in the

particular sample was calculated from the data obtained (Appendix 4). The remainder of the chloroform layer was poured into a 24/40 ground neck, 500 ml boiling flask and dried on a rotary evaporator at 40°C. The oil residue left in the flask was made up to 25 ml in a volumetric flask with a 20:1 chloroform:methanol solution, and transferred to a 25 ml Erlenmeyer flask. This was flushed with nitrogen and stored at 0°C (AOCS, 1978).

In preparing the methyl esters, the amount of the extract giving 250 mg (Appendix 5) was pipetted into a 125 ml 24/40 ground neck Erlenmeyer flask. Four ml of 0.5 N sodium hydroxide in methanol and four glass boiling beads were added. The flask was then connected to a condenser and the contents were boiled for five to ten minutes. Five ml of 10% borontrifluoride in methanol were added through the condenser and the mixture was boiled for an additional five minutes. Five ml of heptane were next added and the mixture boiled for one minute. The flask was removed from heat, cooled and a saturated sodium chloride solution added to bring the mixture to the neck of the flask. About two ml of the prepared fatty acid esters were drawn off into a dry test tube. A small amount of anhydrous sodium sulfate was added to the tube to dry the esters.

The fatty acid esters were analyzed within 24 hours of preparation on the same Bendix GLC Modell 2600 used for the volatile acids, except the column was 1.9 m x 4 mm i.d. stainless steel packed with 15% DEGS (diethylene glycol succinate) on a 100/120 mesh of Gas-Chrom Q support. The instrument was set at a detector and injector temperature of 285°C, isothermal oven temperature of 205°C, and attenuation of five K. Nitrogen (carrier gas), hydrogen and air flow rates were 30 ml/minute, 40 ml/minute and 400 ml/minute, respectively. Two microliters of standard composed of

2.0% myristic, 30.0% palmitic, 3.0% palmitoelic, 14.0% stearic, 41.0% oleic, 7.0% linoleic and 3.0% linolenic acid esters were injected into the column and the peaks identified by comparing the obtained chromatogram with that supplied by the supplier of the standard (Applied Science Laboratory, State College, PA). More 2.0 microliter injections were made and the response factor of each fatty acid identified was obtained and used to correct for molecular weight differences and nonlinearity of instrument response. Two microliters of each prepared sample were injected and the fatty acids present identified and quantitated as percentage of the total fatty acid esters present.

The variance of all fatty acids with treatment was analyzed using the "Proc GLM" and "Duncan-mean" programs prepared by the Statistical Analysis System (SAS), Barr et al., 1979.

CHAPTER IV

RESULTS AND DISCUSSION

I. Short-Chain (Volatile) Fatty Acids

In this study, six short-chain fatty acids were identified in country hams. These acids were acetic, propionic, isobutyric, butyric, isovaleric, and hexanoic acids (Figs. 2, 3, and 4). Traces of hexanoic acid were observed in both fresh and cured hams but could not be quantitated. The analysis of treatment (fresh, six months aged - waxed and six months aged - unwaxed) and animal variation effects are presented in Table 1. Significant treatment effects were observed for the five fatty acids studied. The level of significance ranged from $P<0.05$ for propionic to $P<0.001$ for acetic, isobutyric, and isovaleric acids. Variation due to animal differences was observed only for butyric ($P < 0.001$).

The means and standard deviations for the short-chain fatty acids in the fresh compared to six months aged hams are shown in Table 2. These data indicate that higher levels of the five fatty acids were found in both waxed and unwaxed aged hams than in the fresh. This difference between aged and fresh was significant ($P < 0.05$) for all but propionic acid. Waxed hams had lower ($P < 0.05$) amounts of butyric and isovaleric acids than the unwaxed hams. These results suggest that the semi-anaerobic conditions imposed by wax slowed lipolysis in the waxed hams.

Fresh or raw samples were not collected for 12 months aged hams; therefore, only waxed vs. unwaxed treatments were compared for short-chain fatty acids (Table 3) in this group. Of the five acids, only isovaleric showed a treatment difference. Propionic, isobutyric and

Peak A = Acetone (solvent)
B = Methyl heptyl ketone (internal standard)
C = Acetic acid
D = Propionic acid
E = Butyric acid
F = Hexanoic acid

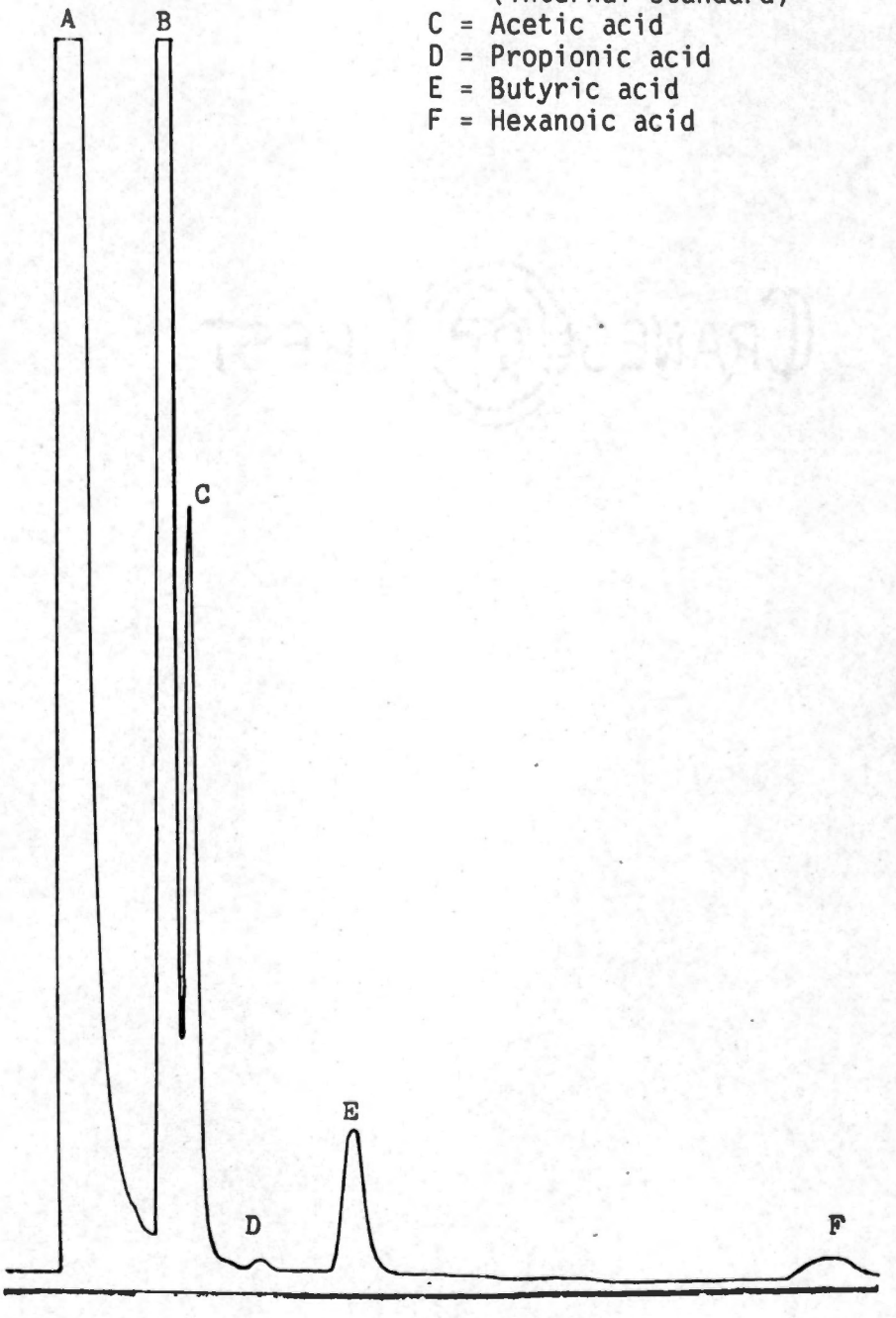

Figure 2. Chromatogram of short-chain (volatile) fatty acids in fresh ham.

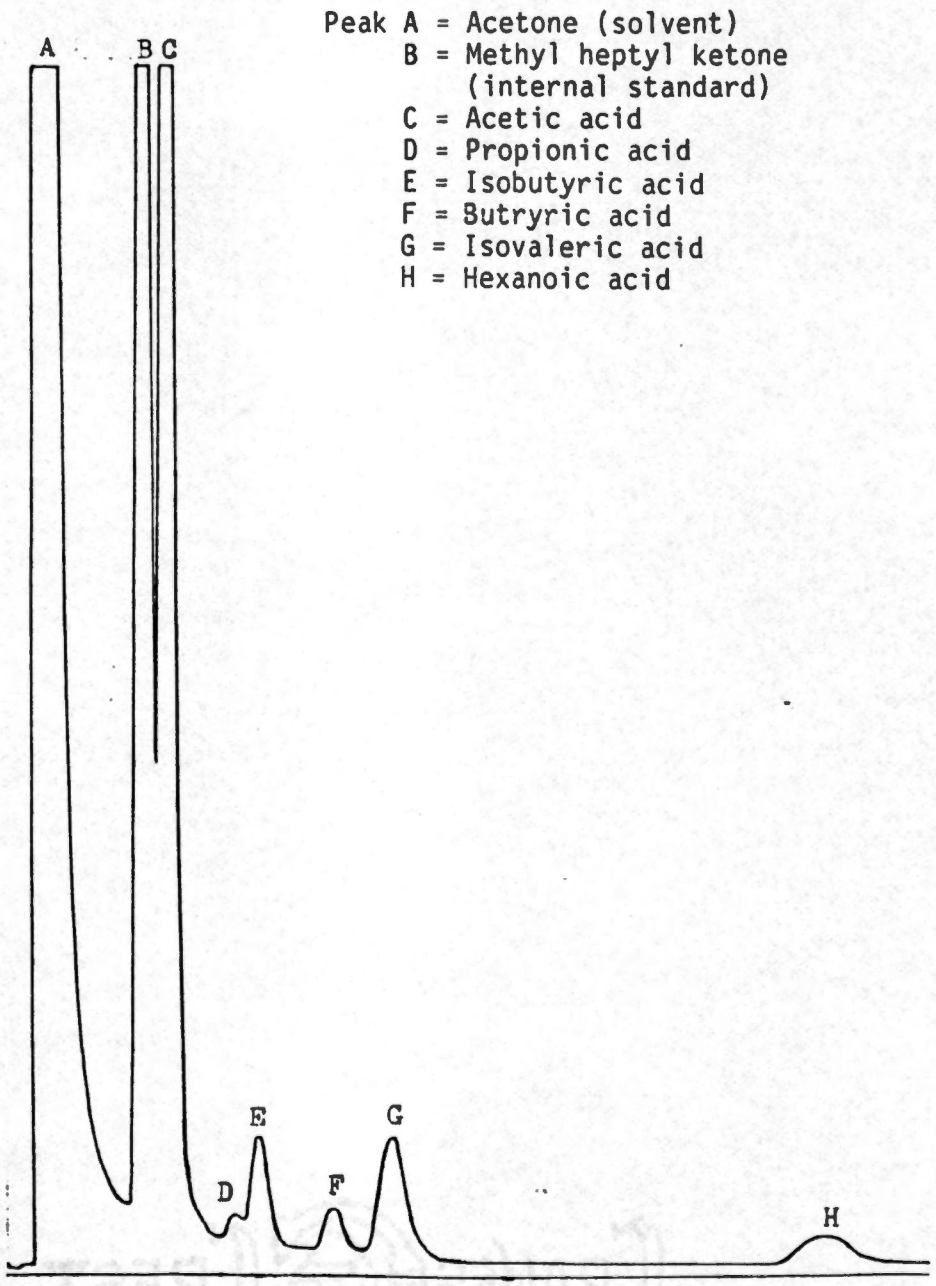

Figure 3. Chromatogram of short-chain (volatile) fatty acids in one year aged ham (waxed).

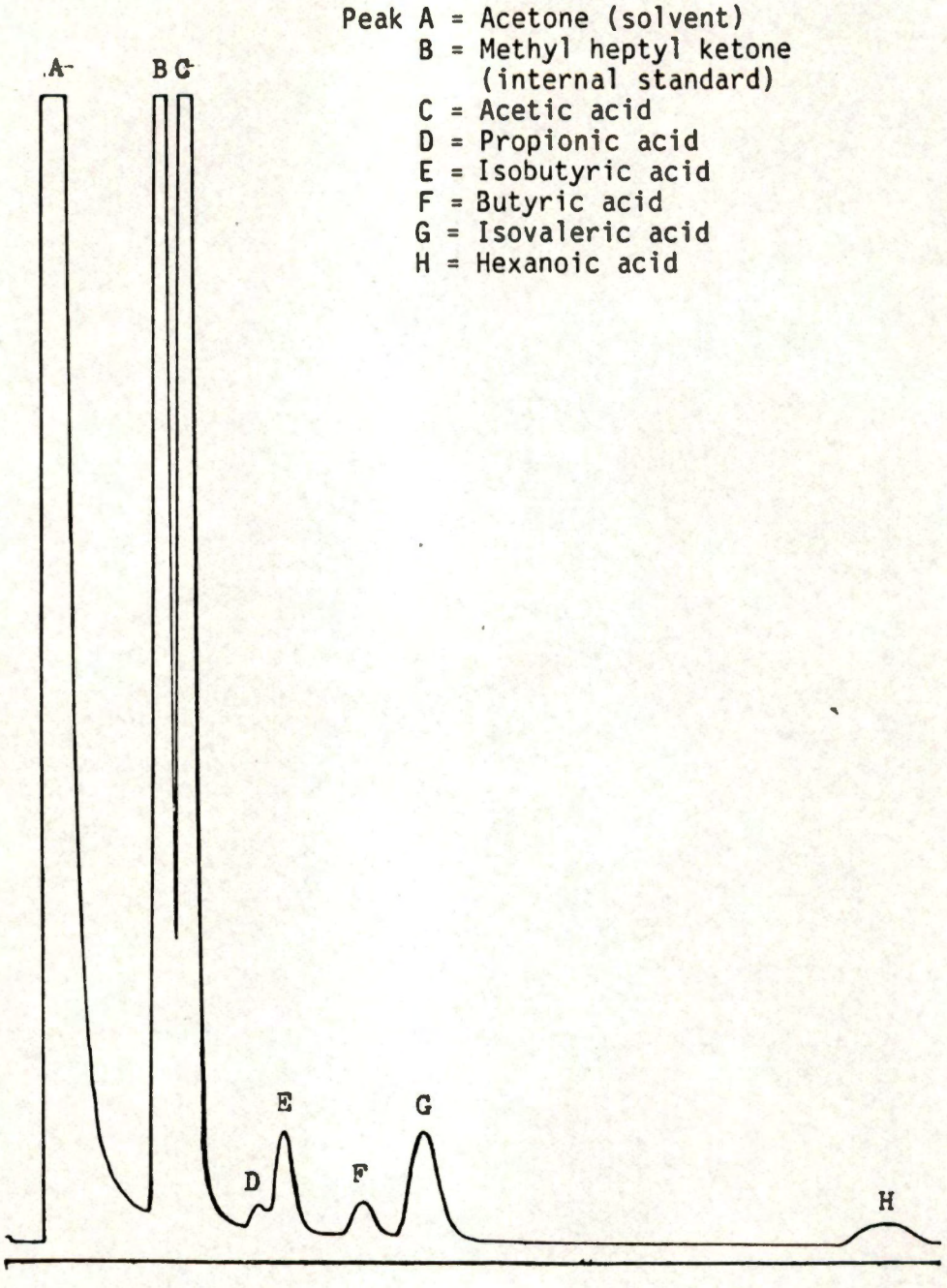

Figure 4. Chromatogram of short-chain (volatile) fatty acids in one year aged ham (unwaxed).

Table 1. Analysis of variance of short-chain (volatile) fatty acids in fresh and six months aged hams

Fatty acids	Source	df	Mean Square	Error mean square	R square
Acetic	Treatment[a]	2	1982.895***	23.661	0.839
	Animal	21	40.055		
Propionic	Treatment[a]	2	3.416*	1.069	0.382
	Animal	21	0.902		
Isobutyric	Treatment[a]	2	0.093***	0.006	0.630
	Animal	21	0.010		
Butyric	Treatment[a]	2	0.613**	0.082	0.732
	Animal	21	0.353***		
Isovaleric	Treatment[a]	2	0.783***	0.914	0.697
	Animal	21	0.026		

[a]Fresh hams and cured hams (waxed and unwaxed)

*P<0.05

**P<0.01

***P<0.001

Table 2. Treatment means and standard deviations of short-chain (volatile) fatty acids in fresh and six months aged hams

Fatty acid	Hams		
	Fresh[a]	Cured & Unwaxed[b]	Cured & Waxed[c]
	----------mg/100 g sample (wet basis)----------		
Acetic	7.91 ± 1.49[e]	25.38 ± 7.68[d]	24.46 ± 4.92[d]
Propionic	0.11 ± 0.07[e]	0.69 ± 0.59[d,e]	0.90 ± 1.63[d]
Isobutyric	0.00 ± 0.00[e]	0.13 ± 0.09[d]	0.09 ± 0.11[d]
Butyric	1.41 ± 0.39[e]	1.61 ± 0.49[d]	1.27 ± 0.36[e]
Isovaleric	0.00 ± 0.00[f]	0.38 ± 0.21[d]	0.25 ± 0.17[e]

[a]20 hams analyzed (n = 20)

[b]22 hams analyzed (n = 22)

[c]21 hams analyzed (n = 21)

[d,e,f]Mean in the same row bearing the same superscript letters are not significantly different at the $P<0.05$ level

Table 3. Analysis of variance of short-chain (volatile) fatty acids in waxed and unwaxed twelve months aged hams

Fatty acid	Source	df	Mean square	Error mean square	R square
Acetic	Treatment[a]	1	148.572	174.413	0.646
	Animal	9	301.341		
Propionic	Treatment[a]	1	0.000	0.017	0.827
	Animal	9	0.083*		
Isobutyric	Treatment[a]	1	0.026	0.063	0.879
	Animal	9	0.460**		
Butyric	Treatment[a]	1	0.065	0.070	0.624
	Animal	9	0.108		
Isovaleric	Treatment[a]	1	0.999*	0.253	0.831
	Animal	9	1.129*		

[a]Waxed and unwaxed hams

*P<0.05

**P<0.01

isovaleric acid levels varied significantly among animals from which the hams were removed.

Treatment means of short-chain fatty acids for the 12 months waxed and unwaxed hams (Table 4) show, in contrast to the 6 months hams (Table 2), no difference for any of the short-chain fatty acids. The relative amounts of the acids in the 12 months aged hams were higher than in the 6 months group for all except butyric acid. Butyric level did not change much from fresh to 12 months aging.

II. Long-Chain Fatty Acids

Seven long-chain fatty acids were identified in the same hams studied for short-chain fatty acids (Fig. 5). The analysis of variance for treatment and animal effects for these acids are presented in Table 5. Significant changes were observed in all of the long-chain fatty acids studied except palmitic as the hams were aged for six months. Also, these data showed that animal variation exists in the long-chain acids of hams.

The means and standard deviations of the long-chain fatty acids for fresh and six months waxed and unwaxed hams are shown in Table 6. A significant decrease in myristic, stearic, linolei and linolenic acids from the fresh to the six months aged state was observed. Since these values are relative, a decrease in one or more would result in an increase in another. This could explain the increases shown for oleic acid and lack of change in palmitic acid. The demonstrated increase in short-chain acids would be expected to result from a breakdown of the long-chain acids. No differences were observed between waxed and unwaxed six months ham for myristic, palmitic, stearic, oleic and linoleic. Higher relative amounts of linolenic and palmitoleic acids were shown by the waxed hams when compared with unwaxed hams.

Table 4. Treatment means and standard deviations of short-chain (volatile) fatty acids in waxed and unwaxed twelve months aged hams

Fatty acid	Hams: Cured & Unwaxed[a]	Hams: Cured & Waxed[a]
	----------mg/100 g sample (wet basis)----------	
Acetic acid	41.47 ± 19.45[b]	46.92 ± 9.87[b]
Propionic acid	0.59 ± 0.24[b]	0.59 ± 0.21[b]
Isobutyric acid	0.91 ± 0.49[b]	0.99 ± 0.53[b]
Butyric acid	1.21 ± 0.19[b]	1.33 ± 0.38[b]
Isovaleric acid	1.54 ± 0.75[b]	1.99 ± 0.90[b]

[a] 10 hams analyzed (n + 10)

[b] Means in the same row bearing the same superscript letters are not significantly different at the $P<0.05$ level

Table 5. Analysis of variance of long-chain fatty acids in fresh and six months aged hams

Fatty acid	Source	df	Mean square	Error mean Square	R Square
Myristic	Treatment[a]	2	0.035***	0.002	0.947
	Animal	9	0.062***		
Palmitic	Treatment[a]	2	1.080	0.445	0.773
	Animal	9	2.888**		
Palmitoleic	Treatment[a]	2	15.174***	0.996	0.878
	Animal	9	8.521***		
Stearic	Treatment[a]	2	1.286***	0.033	0.978
	Animal	9	2.129***		
Oleic	Treatment[a]	2	12.260***	0.505	0.949
	Animal	9	12.947***		
Linoleic	Treatment[a]	2	9.631**	0.919	0.916
	Animal	9	14.548***		
Linolenic	Treatment[a]	2	2.245***	0.194	0.796
	Animal	9	0.761**		

[a]Fresh hams and cured hams (waxed and unwaxed)

*P<0.05

**P<0.01

***P<0.001

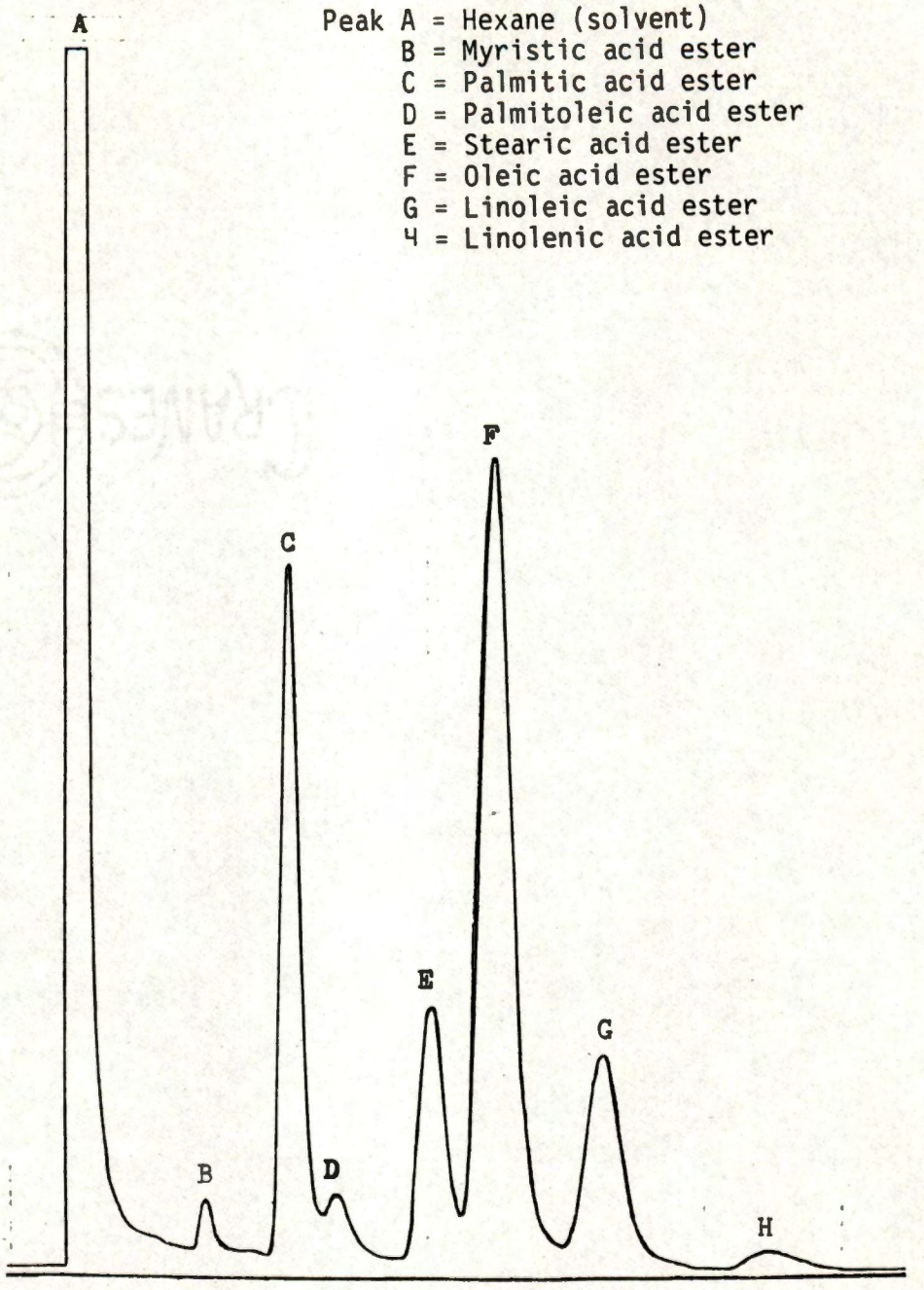

Figure 5. Chromatogram of long-chain fatty acids in ham.

Table 6. Treatment means and standard deviations of long-chain fatty acids in fresh and six months aged hams.

Fatty acid	Hams		
	Fresh[a]	Cured & Unwaxed[b]	Cured & Waxed[b]
	----------%----------		
Myristic	1.19 ± 0.12[c]	1.07 ± 0.18[d]	1.08 ± 0.16[d]
Palmitic	22.33 ± 0.97[c]	22.13 ± 1.01[c]	22.65 ± 1.18[c]
Palmitoleic	6.36 ± 2.65[e]	7.53 ± 1.94[d]	9.03 ± 1.33[c]
Stearic	11.18 ± 1.07[c]	10.55 ± 0.85[d]	10.42 ± 0.83[d]
Oleic	47.22 ± 2.20[d]	49.60 ± 2.39[c]	49.07 ± 2.21[c]
Linoleic	10.38 ± 2.69[c]	8.93 ± 2.45[d]	8.23 ± 2.30[d]
Linolenic	1.40 ± 0.44[c]	0.36 ± 0.83[3]	0.85 ± 0.50[d]

[a]7 hams analyzed (n = 7)

[b]10 hams analyzed (n = 10)

[cde]Means in the same row bearing the same superscript letters are not significantly different at $P<0.05$ level

The comparisons of waxed and unwaxed hams cured and aged for 12 months showed no differences for any of the long-chain fatty acids studied (Tables 7 and 8). Most of the variation observed in these hams was due to animal differences.

Table 7. Analysis of variance of long-chain fatty acids in unwaxed and waxed twelve months aged hams

Fatty acid	Source	df	Mean square	Error mean square	R square
Myristic	Treatment[a]	1	0.001	0.010	0.831
	Animal	9	0.049*		
Palmitic	Treatment[a]	1	0.139	0.491	0.872
	Animal	9	3.340**		
Palmitoleic	Treatment[a]	1	0.828	0.677	0.811
	Animal	9	2.815*		
Stearic	Treatment[a]	1	0.022	0.178	0.909
	Animal	9	1.768**		
Oleic	Treatment[a]	1	0.059	0.565	0.859
	Animal	9	3.435**		
Linoleic	Treatment[a]	1	0.156	0.214	0.930
	Animal	9	2.843***		
Linolenic	Treatment	1	0.003	0.074	0.813
	Animal	9	0.323*		

[a]Waxed and unwaxed hams

*P<0.05

**P<0.01

***P<0.001

Table 8. Treatment means and standard deviations of long-chain fatty acids in unwaxed and waxed twelve months aged hams

Fatty acid	Hams	
	Cured & Unwaxed[a]	Cured & Waxed[a]
	----------%----------	
Myristic	1.27 ± 0.11[b]	1.26 ± 0.21[b]
Palmitic	24.78 ± 1.18[b]	24.61 ± 1.56[b]
Palmitoleic	4.05 ± 1.08[b]	4.46 ± 1.52[b]
Stearic	11.44 ± 0.98[b]	11.51 ± 0.99[b]
Oleic	49.09 ± 1.33[b]	48.98 ± 1.50[b]
Linoleic	8.74 ± 1.12[b]	8.56 ± 1.34[b]

[a]10 hams analyzed (n = 10)

[b]Means in the same row bearing the same superscript letters are not significantly different at the $P<0.05$ level

CHAPTER V

SUMMARY

In this study, 20 pairs of hams were dry-cured. The left hams were covered with paraffin wax following equalization and all hams in this group aged for 12 months. Samples of another group of 30 pairs of hams were collected prior to curing and stored frozen until analyzed. The remaining portions of these 30 pairs were dry-cured. The left hams of this group were also covered with wax and all hams aged for six months. At the end of the aging periods, 10 pairs of the 12 months aged hams, 22 pairs of the 6 months aged hams and 22 of the stored uncured samples were extracted for short-chain (volatile) fatty acids. Extracted fatty acids were analyzed by GLC and amounts corrected for losses due to procedure techniques. Corrected values were expressed in mg/100 gram sample of ham as received. Fifteen gram samples from the same 10 pairs of the 6 months aged hams, and 7 uncured ham samples were extracted for total lipids. Ester derivatives (volatile) of the long-chain fatty acids present in the lipid were made and analyzed by GLC. Values were expressed as percentage total long-chain fatty acids.

The means, standard deviation, and the analysis of variance of all fatty acids quantitated were computed to determine the effects of wax covering, curing (aging) and time of aging on individual acids. The results indicated that in six months aged hams, wax covering decreased ($P<0.05$) the mean amount of butyric and isovaleric acids. In this same set of hams, wax covering enhanced the relative increase of palmitoleic and linolenic acids. There was no significant effect of the wax on the other analyzed fatty acids in six months aged hams. In the 12 months

aged hams there was no wax effect on any of the fatty acids at the 5% level of significance.

Aging increased ($P < 0.05$) the amount of acetic, isovaleric and isobutyric acids but had no significant effect on propionic and butyric acids. Aging slightly decreased the relative amount of myristic, stearic, linolenic and linoleic acids while palmitoleic and oleic acids were slightly increased. Acetic, isobutyric, isovaleric, myristic, and palmitic acids increased with the time of aging at $P<0.05$ level of significance. Time of aging had no significant effect on propionic, butyric, stearic, and oleic acids.

In 6 months aged hams, animal effect was observed only on butyric acid, but in 12 months aged hams animal effect was observed on propionic, isovaleric and isobutyric acids ($P<0.01$). This effect was observed on all long-chain fatty acids analyzed. Myristic, palmitoleic, stearic, oleic and linoleic acids showed effect among animals at $P<0.001$ level of significance while palmitic and linolenic acids showed effect at $P<0.01$ level of significance. Except for linoleic acid, animal effect on the long-chain fatty acids seemed to diminish as aging progressed to 12 months. Thus, this study indicated that the relative amount of a long-chain fatty acid in a ham sample is influenced by the animal producing the ham. An effect among animals is understandable since the diet on which an animal is fed affects the fatty acid composition of the pork fat (Koch et al., 1968). Since wax covering could protect aged hams from mold (Bray and Hanning, 1959), without affecting the dietary fatty acids adversely as this study has shown, it may serve as an excellent packaging material for long aged dry-cured hams.

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APPENDICES

APPENDIX 1

RELATIVE RETENTION TIME OF FATTY ACIDS

Peak identification

The chromatogram of a standard solution was obtained under the same conditions as before. The retention time of all components was recorded and the relative retention time of each component to that of internal standard calculated. Identification of the fatty acids was achieved by matching their retention times and relative retention times obtained for standard solution.

$$\text{Relative retention time to internal standard} = \frac{\text{Retention time of component}}{\text{Retention time of internal standard}}$$

Relative retention time of short-chain (volatile) fatty acids.

Component	Retention time (min)	Relative retention time to internal standard
Acetone (solvent)	0.28	
Methyl heptyl keptone (internal standard)	1.78	1.00
Acetic acid	2.12	1.19
Propionic acid	3.02	1.70
Isobutyric acid	3.37	1.89
Butyric acid	4.42	2.48
Isovaleric acid	5.23	2.94
Hexanoic acid	11.20	6.29

Relative retention time of long-chain fatty acids

Component	Retention time (min)	Rehative retention time to internal standard
Heptane (solvent)	1.30	0.715
Myristic acid ester	3.13	1.00
Palmitic acid ester (internal standard)	4.38	
Palmitoleic acid ester	4.98	1.14
Stearic acid ester	6.40	1.46
Oleic acid ester	7.35	1.68
Linoleic acid ester	8.85	2.02
Linolenic acid ester	11.18	2.55

APPENDIX 2

CALCULATING THE AMOUNT OF SHORT-CHAIN (VOLATILE) FATTY ACIDS IN SAMPLE

Response factor (correction factor)

Instrument response factor (RF) for each component is calculated using the data from the peak of the component and the peak of the internal standard in chromatogram of the standard solution.

$$RF = \frac{\dfrac{\text{peak area of component}}{\text{concentration of component}}}{\dfrac{\text{peak area of internal standard}}{\text{concentration of internal standard}}}$$

$$RF = \frac{(\text{peak area of component})\ (\text{concentration of internal standard})}{(\text{peak area of internal standard})\ (\text{concentration of component})}$$

Note: the electronic integrator used in this analysis gives the reciprocal of RF value.

Amount of component in sample

For short-chain (volatile) fatty acids.

$$\text{Mg component per 100 g sample} = \frac{(\text{peak area of component})\ (\text{concentration of internal standard})\ (100)}{(\text{peak area of internal standard}(\ (RF)\ (\text{wt. sample})}$$

Note: While the concentration of the standard in short-chain fatty acid analysis was in mg/ml, the concentration of the long-chain fatty acids was in % total long-chain fatty acid.

APPENDIX 3

DETERMINING THE CORRECTION FACTOR FOR DISTILLATION LOSS OF SHORT-CHAIN (VOLATILE) FATTY ACIDS

Five milliequivalents in 150 ml solution of each short-chain (volatile) fatty acid identified in the sample were separately distilled in the constant volume distilling apparatus (Fig. 1, p. 15). Two hundred ml of distillate were collected for each acid and titrated with 0.01 N sodium hydroxide solution. The amount of each acid recovered was determined in milliequivalents (meq) and the percentage recovery calculated.

meq of acid = normality of NaOH x volume of NaOH used

$$\% \text{ recovery} = \frac{\text{amount recovered}}{\text{amount distilled}} = \frac{\text{meq recovered}}{5 \text{ meq}}$$

$$\text{Correction factor} = \frac{100\%}{\% \text{ recovery}}$$

Component	Calculated distillation correction factor
Acetic acid	1.75
Propionic acid	1.24
Isobutric acid	1.02
Butyric acid	1.08
Isovaleric acid	1.02

Determining correction factor due to volume of extract distilled.

Added to ham sample during extraction:

Water		175 ml
20% phosphotungstic acid		40 ml
1 N sulfuric acid		25 ml
	Total	240 ml solution

Volume distilled = 150 ml

Correction factor due to volume used = $\frac{240 \text{ ml}}{150 \text{ ml}}$ = 1.6

This factor is applied to each short-chain fatty acid.

To get the actual experimental value of any short-chain (volatile) fatty acid, the amount obtained by GLC is multiplied by (1.6) (distillation correction factor for that acid).

APPENDIX 4

TOTAL LIPID IN HAM SAMPLE

A fifteen gram sample of ham was extracted with chloroform-methanol solvents. The total volume (V_t) of chloroform layer was measured. Two 10 ml portions of this layer were dried in a weighed beaker in a vacuum oven at 70° C and weight of lipid (X g) calculated. The average weight ($\bar{X}$ g) in 10 ml chloroform extract was found.

$$\text{Wt of lipid in 15 g sample} = \frac{(V_t \text{ ml})\ (\bar{X} \text{ g})}{10 \text{ ml}}$$

where V_t is total volume of chloroform extract

$\bar{X}$ g is average weight of lipid in 10 ml portion

The remaining portion of the chloroform layer was dried down to 25 ml solution in a vacuum rotary evaporator under a slow stream of N_2 gas.

In this 25 ml portion:

$$\text{gram lipid/ml} = \frac{\frac{(V_t \text{ ml})}{10 \text{ ml}}\ (\bar{X} \text{ g}) - 2\ \bar{X} \text{ g}}{25 \text{ ml}}$$

APPENDIX 5

DETERMINING THE VOLUME OF CHLOROFORM EXTRACT NEEDED TO PREPARE LONG-CHAIN FATTY ACID ESTERS

Sample size needed = 0.125 g lipid

$$\text{Volume of extract needed (in ml)} = \frac{0.125 \text{ g lipid}}{\text{g lipid/ml extract}}$$

VITA

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