STUDIES ON VOLATILE AND NON-VOLATILE COMPONENTS OF PORK ADIPOSE TISSUE

Thesis for the Degree of Ph. D.

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Leamon D. Williams

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THESIS

This is to certify that the

thesis entitled

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OF PORK ADIPOSE TISSUE

presented by

Leamon D. Williams

has been accepted towards fulfillment
of the requirements for

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J. M. Pearson
Major professor

Date November 14, 1963

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ABSTRACT

STUDIES ON VOLATILE AND NON-VOLATILE COMPONENTS

OF PORK ADIPOSE TISSUE

by Leamon D. Williams

The role of adipose tissue in the overall flavor of meat and meat products is not well understood. However, objectionable flavors and/or odors such as those associated with rancidity and sex odor in pork are known to be associated with the adipose tissue. This investigation was undertaken to identify some of the basic components in pork adipose tissue and study their involvement in the flavor problem.

The first objective of this study was to ascertain the incidence of sex odor in pigs of different sexes. Adipose samples taken from the flank area or ham facing were tested by heating. Utilizing a trained odor panel, the incidence of sex odor in boars was 64%, which was significantly higher than any other sex group. The difference in incidence between sows, barrows and gilts was not statistically significant. The incidence of sex odor was approximately 1%, 5% and 5% for sows, barrows and gilts, respectively.

The taste panel scores of cooked salami and braunschweiger, which contained boar meat exhibiting strong sex odor were essentially the same as the panel scores obtained from control preparations. This indicated that boar meat possessing strong sex odor can be incorporated into some comminuted meats without detection. This was found to be true only for comminuted meats that are consumed without heating or in those products containing liver. In the latter case, apparently the strong odor of the liver masked the sex odor.

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The second phase of this study deals with the separation, isolation and identification of the volatile and non-volatile components of pork adipose tissue. Particular emphasis was placed on the unsaponifiable fraction of pork fat, since it is this fraction that contains the component(s) responsible for sex odor.

The volatiles from heated fat were collected in traps containing 2% lead acetate or 2,4-dinitrophenylhydrazine. Carbonyls were indicated by the formation of a precipitate in the 2,4-dinitrophenylhydrazine traps. Hydrogen sulfide, varying from 44 to 54 ug per kg of fat, was found by the methylene blue colorimetric procedure. Statistical analysis of the hydrogen sulfide data indicated that there was no significant difference in the amount present in either boar or barrow fat. Fatty acids and ammonia were found to be present in the volatiles by titration and ammonia test paper, respectively.

The hydroxylamine test for esters showed that cold saponification of pork fat with sodium ethylate was as efficient as hot saponification, if the cold reaction was allowed to proceed for at least 36 hours at room temperature. For beef fat, cold saponification must be allowed to proceed for at least 48 hours in order to approach the efficiency of the hot method. Utilizing the hot saponification procedure, boar fat was found to contain 0.53 g of unsaponifiable material per 100 g of fat, while barrow fat contained 0.50 g. Beef fat was shown to contain 0.46 g of unsaponifiable material per 100 g of fat.

The unsaponifiable material was studied utilizing the Liebermann-Burchard test for -5 sterols, the nitrochromic acid test for alcohols,

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the hydroxylamine test for esters, the Le Rosen test for unsaturation and the 2,4-dinitrophenylhydrazine test for carbonyls. In addition, ammonia, nitrogen- and sulfur-containing compounds were qualitatively determined utilizing ammonia test paper, the Kjeldahl procedure and the sodium formate reduction method, respectively. The antimony trichloride test for vitamin A and a colorimetric procedure for cholesterol were employed for both qualitative and quantitative determinations.

Qualitative tests indicated that carbonyls, -5 sterols, ester linkages and unsaturated compounds were present in the total unsaponifiable material. However, tests for alcohols, fatty acids, sulfur- and nitrogen-containing compounds were negative.

Unsaponifiable material was separated into 9 fractions using silicic acid chromatography. The presence of vitamin A and cholesterol was further substantiated by thin-layer chromatography and qualitative tests. Squalene was identified by thin-layer chromatography. In addition, cholesterol esters, a 7-ketosterol and a triterpene alcohol were indicated.

Gas chromatographic analysis verified the presence of cholesterol, vitamin A and squalene in the unsaponifiable material. Sulfuric acid extraction and subsequent gas chromatography indicated that at least 4 saturated hydrocarbons were present. In addition, a carbonyl and possibly a free radical were indicated as a result of the autoxidation of squalene.

Infrared analysis further verified the presence of carbonyls and the absence of alcohols and fatty acids in the unsaponifiable fraction of pork fat.

Colorimetric quantitative determinations showed 8.71% of the total

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unsaponifiable material to be cholesterol. This is equivalent to 46.16 mg of cholesterol per 100 g of fat. No differences were observed between boar and barrow fat.

Pork fat was found to yield 1.45 to 1.98 units of vitamin A per gram. The content of vitamin A in boar and barrow fat was essentially the same.

STUDIES ON VOLATILE AND NON-VOLATILE COMPONENTS OF PORK ADIPOSE TISSUE

By

Leamon D. Williams

A THESIS

Submitted to
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1963

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To his wife, Joyce, the author is especially grateful for her understanding and encouragement throughout the course of this study.

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INTRODUCTION

Flavors and/or aromas are primary factors in consumer acceptance of food products. In recent years more sophisticated methods of processing and distribution have resulted in more uniformity of flavors and/or aromas associated with a particular food or food product. As a result there is considerable interest in the isolation and identification of flavor and/or aroma components present in various foodstuffs.

Meat is one of the food commodities that has been the subject of extensive flavor investigations. Studies on meat flavor have been primarily directed toward beef, poultry and more recently mutton. The major effort in flavor studies on beef has been undertaken in studying the compounds produced when beef is irradiated, whereas, poultry and mutton flavor studies have been centered around normal meat flavor. Studies on pork flavor have been few, and little is known concerning this complex system.

In the past, research emphasis has been directed toward studying the flavor components in the lean portion of meat. However, recent studies indicate that many of the characteristic flavors in meats are contained in the fatty tissue. Hornstein and Crowe (1960, 1963) reported that lean tissue of lamb, beef and pork contribute an identical meaty flavor and that the characteristic aroma of each of these species was obtained from the fatty tissue. The overall effect of fat on flavor and/or aroma enhancement is not well understood at this time. Flavor problems such as rancidity, mutton flavor and boar flavor appear to be associated with the fatty tissues.

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Boar flavor in pork, more commonly referred to as "sex odor" or "boar odor", has been of significant importance to the meat processor for many years. Because of this off odor many boars and stags are condemned under Federal Meat Inspection. Thus, many pork processing establishments avoid slaughtering boars, or processing boar carcasses.

The eventual solution of many flavor and/or aroma problems in meat and meat products lie in complete characterization of the basic chemical components contributing to meat flavor. Special flavor problems, such as boar flavor, mutton flavor or irradiation flavor can only be elucidated after a thorough knowledge of the chemistry of normal meat flavor is known. Since the chemistry of normal pork flavor is not well understood, the problem of characterizing the components responsible for boar flavor becomes more difficult. Craig et al., (1962) reported boar flavor components to be water-insoluble, ether-soluble and definitely associated with the fatty tissues, particularly the unsaponifiable matter, of boars.

Methods of analysis available to the researcher until recently have made it a tedious and difficult task to determine the chemical components responsible for flavors and/or aromas. Much depended on the keenness of the sense of smell of the individual investigator in interpreting odors. However, with the application of gas chromatography, mass spectroscopy and infrared spectroscopy to problems involving food flavors, new approaches are available for elucidation of the chemical components contributing to flavors and/or aromas.

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EXPERIMENTAL OBJECTIVES

The experimental objectives of this study were as follows:

1. To study the incidence of sex odor in pigs of different sexes.

2. To isolate and identify some volatile components of boar and barrow adipose tissue.

3. To determine the exact amount of unsaponifiable matter in boar and barrow adipose tissue.

4. To fractionate and study the composition of the unsaponifiable material in boar and barrow adipose tissue.

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REVIEW OF LITERATURE

Flavor and Aroma of Meats

Flavor was defined by Crocker (1937) as that property of a food or beverage that makes it excite the senses of taste and smell. He further stated that the aromas associated with a food may be defined as those volatile substances contributing to the overall flavor by exciting the sense of smell. Thus, a food might rate high in every aspect except aroma, but because of a poor aroma be hastily rejected by the consumer. Kramlich (1959) emphasized the importance of flavor and pointed out that little is known concerning the chemical nature or components contributing to food flavors.

Since flavors and aromas are so vitally important to the acceptability of foods, a number of workers have attempted to characterize the chemical components responsible for flavor. Meat is one of the food commodities that has been the subject of extensive flavor investigations.

Poultry

The chemical nature of chicken flavor has been studied more widely than the flavor of beef, lamb or pork. Considerable information has been compiled about the identity of components in the volatile fraction, which is responsible for the typical aroma of chicken broth. Bouthilet (1951) identified ammonia and hydrogen sulfide in the volatiles arising from heated chicken. Pippen and Eyring (1957) confirmed these results and showed that essentially all the volatile nitrogen occurs as ammonia. Pippen et al., (1958) isolated, separated and identified a number of carbonyl

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compounds, as their 2,4-dinitrophenylhydrazone derivatives, from chicken volatiles.

Research workers have also expended considerable effort to determine which anatomical location of the chicken contains the most flavor. Carcass components investigated by Bouthilet (1949, 1950, 1951), Pippen et al., (1954) and Peterson (1957) include skin, bone, fat and muscle. The findings of these investigators indicate that the flavor is largely concentrated in the muscle tissues. However, Pippen et al., (1954) obtained results indicating that fat contributes to the aroma of chicken broth.

Mutton

Ziegler (1958) and Kean (1959) pointed out that the characteristic flavor of mutton was objectionable to certain individuals. In view of this fact, research on the chemical composition of mutton flavor has been quite extensive in recent years. The characteristic flavor and aroma of mutton has been described by Jacobson et al., (1962) as being "fragrant", "oily", "sweet" and somewhat "musty". The volatility of lamb flavor was indicated by Hofstrand et al., (1960). These workers reported that lamb broths lost identifying aromas, and fats were changed in aroma by the application of heat. Jacobson et al., (1962) compared volatiles from cooked lamb with the odors of various chemicals, and found the odor to be similar to ethyl oleate, diacetyl and a number of sulfur compounds.

McInnes et al., (1956) reported the presence of steam volatile acids in mutton fat, and indicated that these acids could have an influence on mutton flavor. These authors found isobutyric, isovaleric, alpha methyl

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butyric, an antieso acid and a series of normal acids from formic (C_1) to capric (C_{10}) in the volatiles of lamb fat. Hornstein and Crowe (1963) reported the characteristic aroma of heated lamb to be due to the fatty tissue. They stated that a major portion of the odor obtained upon heating was contributed by carbonyl compounds. They further reported that the lean meat from mutton contributes a basic meaty flavor similar to that obtained from lean beef and pork.

Jacobson and Koehler (1963) studied lamb flavor in the volatile and water-soluble fractions. They found carbonyl compounds in the volatile materials of roasted lamb to be important contributors to aroma. Monocarbonyls present were identified as n-alkanals of 2 to 10 carbon atoms, 2-alkanones of 5 to 10 carbon atoms and possibly 2-methylcyclopentanone. These same authors also found glucose, fructose, inositol and 19 amino-containing components in the water-soluble fraction of raw and cooked lamb.

Beef

Considerable progress is being made toward characterizing the chemical components of beef flavor. Crocker (1948) reported the flavor of cooked beef to be quite complicated chemically, and to consist more of odor than taste. He indicated that hydrogen sulfide, amines of several kinds and possibly indoles were contributors.

Kramlich and Pearson (1958) studied the solubility characteristics of beef flavor and reported that the flavor components were largely water soluble in both the cooked and raw fractions. Comparisons of flavor thresholds with gross chemical analysis indicated that neither fat content

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nor fat-free dry matter were responsible for differences in the intensity of flavor. Hornstein et al., (1960) also reported cooked beef flavor to be water-soluble. These authors found small amounts of carbonyls, ammonia and hydrogen sulfide in the more volatile fraction. In addition, an oily, viscous, liquid mixture having a very low vapor pressure, but with a strong aroma, was also isolated.

Yueh and Strong (1960) also reported the presence of carbonyls, ammonia and hydrogen sulfide in cooked beef volatiles. Carbonyls definitely identified included acetaldehyde, acetone, and diacetyl. The presence of dimethyl sulfide, formic, acetic, propionic, isobutyric and butyric acids was indicated. Hornstein and Crowe (1960) identified 11 volatile carbonyls in beef fat heated at 100° centigrade. In this same study, flavor precursors in lean meat were described as being cold-water extractable, low molecular weight compounds.

Kramlich and Pearson (1960) collected beef flavor volatiles in a series of cold traps upon heating a meat-water slurry and using nitrogen as a carrier gas. Paper and gas chromatography coupled with qualitative tests indicated that carbon dioxide, methyl mercaptan, acetone and acetaldehyde were present in the volatile fraction from cooked beef. In addition, methyl sulfide and water were tentatively identified as being present.

Bender and Ballance (1961) examined the volatile fraction of beef extracts with gas chromatography. These authors also reported the presence of methyl mercaptan, acetaldehyde, ethyl mercaptan, dimethyl sulfide, acetone, ethyl methyl ketone, methanol and ethanol in beef flavor volatiles. Components not previously reported in meat extracts included,

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proprionaldehyde, isobutyraldehyde and isovaleraldehyde. Batzer et al., (1962) studying the precursors of beef flavor by isolation techniques, concluded that they were among the relatively simple, water-soluble components in the lean tissues. Inosinic acid or inosine was identified as being one of the components present. A glycoprotein, containing glucose as the sugar moiety, was also identified as a flavor precursor. Seven amino acids were identified from the protein moiety of the glycoprotein. It was also indicated that some of these amino acids were necessary precursors of meat flavor.

Pork

Essentially nothing is known about the chemical nature of normal pork flavor components. One of the very few studies that has been conducted concerning the chemistry of pork flavor was reported by Hornstein and Crowe (1960, 1963). These workers reported that the volatiles from lean pork were almost identical to those reported for lean beef and lamb. Acetone, acetaldehyde, formaldehyde, ammonia, hydrogen sulfide and carbon dioxide were identified in this fraction. Hornstein and Crowe (1960) also studied the volatiles of pork fat and reported the presence of various fatty acids along with 12 different carbonyl compounds. These researchers attempted to determine the contribution of the volatiles from fat to the overall flavor of pork. It was concluded that the characteristic flavor differences in pork and beef were contained in the fatty tissues. Thus, these authors hypothesized that fat may not only produce different flavor components in different ratios, but perhaps also act

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For many years it has been known that the chemical composition of pork fat could be altered by controlling the diet of the animal. In this respect, the belief, that lipid tissues of pork act as a storage depot for flavor components is not a new one. Reine et al., (1953) fed spoiled meat scraps and horse manure to growing pigs. Taste and odor tests of the meat showed that the meat scrap group had taken on repulsive flavors and odors. This was especially true of the lard from the pigs fed meat scraps, where the more volatile aldehydes, methyl ketones, secondary amines, trimethyl amine, skatole, primary amines and phenols were found as compared to the lard from control pigs.

Kemp et al., (1952) and Grummer et al., (1950) examined the flesh of hogs which had been treated with benzene hexachloride prior to slaughter and found that this compound produced off flavors and odors when applied immediately before death. The flavors of both fat and lean were reported to be affected.

Vacuum packed, stored, dehydrated pork developed an off-flavor and odor during storage at elevated temperatures at different intervals of time according to Burnett et al., (1955). These authors reported that the volatile constituents obtained on storage were basic in nature and exhibited reducing properties. Acetaldehyde was present in dehydrated pork samples stored at both 20°F. and 94°F, whereas ammonia was detected in samples stored at 100°F. and 160°F.

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Induced off-flavors, such as those previously discussed, can many times be eliminated by a more careful selection of rations for the animals. However, some pork exhibiting poor flavor is not the result of poor feeding and management, but is characteristic of certain animals due to unknown physiological reasons. The problem of boar flavor in pork has been recognized for many years. There is little information available as to the nature of the odor and the chemical agents responsible for its production. Lerche (1936) reported that as soon as the male hog was sexually mature and the testes became capable of functioning, a specific sexual odor appeared in the meat and fat of the animal. He described the odor as being "onion like or unpleasantly perspirative" and claimed that it occurred in all boars with normally developed testes. He also stated that it occurred in cryptorchids, unless the testes lying in the abdominal cavity were atrophied. He stated that the tissue must be heated and the vapors smelled in order to detect the odor. This was accomplished by placing small pieces of tissue in water in an Erlenmeyer flask and boiling. The vapors were then allowed to bathe the nostrils of the tester. Tissues were also tested by frying in a skillet. On cooling the heated tissue, no odor was evident. He also noted that after male hogs had been castrated, the sexual odor disappeared from the tissue. The tissue was found to be devoid of odor 57-68 days post-castration according to this study.

Howe and Barbella (1937) claimed that occasionally during cooking, meat from very old animals, such as bulls, cows or rams developed an extremely unpleasant odor, often characterized as ammoniacal. They also

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reported that sexual odors in tissues were usually associated with boars and stags that had been slaughtered soon after castration.

Gereke (1936) postulated that a connection must exist between the testes and the parotid salivary gland, which is located in the mouth near the ear. According to this author, cryptorchid boars may often be detected by an over production of saliva. The excess salivary secretion was also known to occur when boars are sexually excited. The parotid gland, according to him was a good tissue to detect the odor. He stated that the odor was not dependent upon the size of the testes, but more upon the condition of the animal. He noted sex odor to be especially evident in the case of small, thick-skinned animals, which were lacking in fat and retarded in development.

Kunze (1936) claimed that the tissues of cryptorchid boars, which had been condemned because of too strong a sexual odor could be freed of this odor by "pickling". He stated that after pickling for three weeks, the tissue was completely free from odor. He claimed that the odor producing material had been bound by the salt.

Miller (1958) outlined the procedure for killing boars and stags in Federally inspected plants. Stags and boars should be separated from the regular kill during antemortem inspection and identified for checking for sexual odor on post-mortem examination. The lean and fat are then tested for odor by heating. A carcass which exhibits sexual odor should be condemned as unfit for food. Miller (1958) grouped carcass odors into two categories: (a) those traceable to material ingested by the animal and (b) the odor known as sexual odor in swine. He stated that sex odor can

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usually be detected in carcasses of boars and recently castrated stags. He further pointed out that care should be exercised not to confuse this condition with odor which is imparted to a portion of the carcass by contamination with smegma from the prepuce.

Since the odor has been known to disappear several weeks post-castration, it would seem to be sex linked in some way. In view of this fact, the implication of androgenic hormones has been postulated. Bratzler et al., (1954) found that meat from boars was definitely inferior from a palatability standpoint. These investigators cooked pork chops from boars by braising and found a definite "off" or "boar" odor, which carried through to the cooked product. No objectionable odor was detected in the chops from 180 pound pigs castrated 34 days before slaughter. One group of pigs was castrated 118 days before slaughter and implanted with a 193 mg. pellet of testosterone propionate at the time of castration. Examination of the heated tissue from these pigs failed to show any evidence of sex odor. Johnston et al., (1957) fed two levels (9 or 15 mgs. per lb. of feed) of methyl testosterone to growing fattening pigs and found no objectionable odor in their flesh. Perry et al., (1956) obtained similar results when methyl testosterone was fed to pigs of similar weights and ages.

Christian and Turk (1958), in a study of sexual odor in the boar, designed an experiment using the following treatments: (1) untreated boars, (2) untreated barrows, (3) boars with the preputial diverticulum removed, (4) barrows injected subcutaneously with 20 mg of testosterone daily, (5) boars fed 10 mg of stilbesterol daily and (6) boars fed 50 mg of

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stilbesterol daily. Boar odor and flavor were determined by panel evaluation of the boneless roasted loin. Results indicated that the panel could detect the most odor in group one followed by groups three, five and four in that order. After freezing and storing samples from the above treatments for five months, they reported that the intensity of the odor was generally somewhat lower. This indicated that the odor had decreased during freezing and storage.

Plimpton et al., (1961) found that implanting 48 and 96 mgs. of stilbesterol at a live weight of 155 pounds significantly reduced "boar odor" in the longissimus dorsi muscle of boars slaughtered at 200 pounds. The odor was evaluated by a trained panel. These workers reported a highly significant correlation between the length of treatment period (days on stilbesterol) and reduction of "boar odor". The odor scores for the muscle of treated boars were not significantly different from those of barrows.

Self (1957) conducted one of the most extensive studies concerning the problem of sex odor in pork. His work was prompted by the Research Department of Oscar Mayer & Company. This company had received consumer complaints regarding "boar odor" in pork. The odor was noted in their kill line, although they do not slaughter boars. Tissue samples were taken from the diaphram muscle of 343 pigs as they passed down the kill line. Samples were wrapped in aluminum foil and heated to approximately 200°F. under infrared tubes. Testing was performed by breaking open the foil wrapper and allowing the volatile substances to bathe the nostrils of the tester. Odor intensity was classified as strong, medium or none. The incidence of the odor was found to be as high among female hogs as

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among castrated males. Another group of older female hogs in various stages of the reproductive cycle was examined for odor. Approximately 17% of the group total had sexual odor, but there was no clear cut group of offenders when classified on this basis. Progesterone administered to 22 gilts did not appear to alter the incidence of the sex odor in this group. In a group of 31 sexually mature boars, a total of eight, or 25.8% had some sex odor. A study of boars by breed failed to show a greater incidence of the odor among any particular genetic group. The author concluded on the basis of data presented that sex and breed had little influence on the overall incidence of sex odor or boar odor in pork.

Dutt et al., (1959) removed the preputial diverticulum from boars and found that the characteristic ranting odor was reduced, but at ten months of age no difference in boar odor was found between carcasses from operated and unoperated boars. Lean areas were free from the odor, but all fat areas sampled had boar odor when heated. The greatest concentration was found in the fatty tissue of the prepuce, where brownish orange areas were noted in the dorsal and lateral regions of the orfice. Histological examination showed this tissue to be modified subaceous glands containing a colloid-like material. Similar glands in barrows appeared to be non-functional, when examined histologically. Alcohol-ether extraction of the body tissues showed that the agent responsible for the odor is lipophilic and these authors postulated that it was a muscone. These workers surgically removed the glandular area in five immature boars. The animals were slaughtered at weights varying from 290 to 350 pounds.

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No odor was detected in any portion of the carcasses of two boars from which the preputial glands had been completely removed. Some odor was noted in carcasses of boars where post-slaughter examination revealed that not all of the glandular area had been removed. It was concluded that the preputial glands, which are dependent upon the male sex hormone for secretory activity, produced a fat-diffusible material, which was responsible for sexual odor in boar carcasses.

Probably one of the most extensive studies concerning the chemical composition of sex odor was carried out by Craig et al., (1961). These workers reported sex odor in pork was produced when lean containing fat, fat alone and most organs from a boar were heated in a skillet or in boiling water. Sex odor was not entirely absent in boar fat at body temperature, although volatilization appeared to occur to a much greater extent at approximately 100-108°C. These researchers also found the components responsible for sex odor to be water-insoluble, ether-soluble and definitely associated with the fatty tissues of boars. It was absent in rehydrated moisture-free, fat-free lean. Distillation methods proved unsatisfactory for collecting the components responsible for sex odor in recognizable form, since no consistent and reproducible differences could be detected between boar and barrow fat, when the collected volatile compounds were analyzed by the heat test and gas chromatography. Cold saponification of boar fat yielded a small quantity of unsaponifiable matter that produced a concentrated, permeating sex odor on exposure to heat. Thus, these authors assumed the agent(s) responsible for sex odor in pork was/were located in the unsaponifiable material. Cholesterol and

squalene were found in this fraction in both boar and barrow fat, but sex odor was not produced when these compounds were heated.

Irradiated Meat Flavor

New methods of food preservation have in some instances introduced new problems for the flavor and aroma chemist. Irradiation of meat and meat products is a good example of an undesirable flavor being produced during processing of foods. Considerable research has been conducted on the elucidation of irradiated meat flavor. Proctor et al., (1955) reported that high levels of cathode ray treatment may induce changes in food flavor, that make irradiated foods organoleptically unacceptable. Similar results were noted by Pratt and Eckland (1956). These authors studied the effect of irradiation on several meat items and vegetables. Groninger et al., (1956) evaluated the organoleptic properties of beef, pork, cured ham, salmon, tuna and halibut and found significant flavor changes in the irradiated product.

Pearson et al., (1959) stated that the most critical problem in connection with the acceptance of irradiated meats is the development of a characteristic flavor, commonly referred to as "irradiation flavor". Their study indicated that hydrogen sulfide, methyl mercaptan and carbonyls were responsible for a considerable part of the poor acceptability of irradiated beef, pork and veal. Similar results were reported by Hedin et al., (1960). They reported that the irradiated or "wet dog" odor was most prominent in the undialyzable meat slurry. When a sample was dialyzed, the most intense irradiated odor was produced in the non-dialyzable fraction.

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Since cysteine and methionine could no longer be detected in the protein after irradiation and the odor was quenched by sulfhydryl reagents, it was postulated that the odor was associated with sulfhydryl or closely related compounds.

Batzer et al., (1955) and Marbach et al., (1956) also implicated sulfur containing compounds as the source of some undesirable odors in irradiated meat. Marbach et al., (1956) further concluded that hydrogen sulfide is not present in gamma irradiated meat but is formed from some volatile sulfur-containing complex. These volatiles were trapped in a cadmium hydroxide solution. Wick et al., (1961) detected at least 12 substances in irradiated beef volatiles. Of the 12 substances detected, 3 (methylthio) propionaldehyde (methional) was a major component and contributed heavily to the unpleasant odor.

Batzer et al., (1957) reported that meats with high fat content did not develop off odors to the same extent as did leaner meats. Their results indicated that carbonyl compounds produced in irradiated ground beef muscle differed from those obtained from irradiated fat from both beef and pork. The amounts of these compounds present were shown to increase with increasing irradiation dosages. At the same irradiation dosage, Marbach and Doty (1956) found that less hydrogen sulfide was released from ground beef of high fat content (20%) than from beef containing less fat (< 10%).

Unsaponifiable Material

Components responsible for boar flavor in pork were reported to be located in the lipid tissues of the animal by Dutt et al., (1959) and

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Craig et al., (1962). The report by Craig et al., (1962) further indicated that the component(s) responsible for the undesirable flavor or odor in boars was/were specifically contained in the unsaponifiable portion of the fat.

According to the methods of analysis of the American Oil Chemists Society (1946), unsaponifiable matter is defined as, "substances frequently found dissolved in fats and oils which cannot be saponified by the caustic alkalies but which are soluble in ordinary fat solvents. Included in this fraction are higher aliphatic alcohols, sterols, pigments and hydrocarbons".

Plant Lipids

Thorbjarnarson and Drummond (1935) saponified olive oil and obtained a soft, yellow, waxy material with an iodine value of 255. Precipitation by digitonin removed 16.9% of the total unsaponifiable material, which contained essentially all of the sterols. When the unsaponifiable matter was chromatographed on an aluminum oxide column, these workers were able to show four colored zones. When these zones were submitted to a spectral analysis, it was found that they were absorbed in a range of 260 to 352 mu. A hydrocarbon fraction was eluted from the column before the first colored zone. The hydrocarbon fraction was compared to squalene, and the authors concluded that squalene was not present in the unsaponifiable portion of olive oil. Fitelson (1943) examined various natural plant fats and found that the squalene content ranged from none in cocoa butter to a maximum of 708 mg. per 100 g. of fat in one sample of olive oil. The squalene content of olive oil ranged from 136 to 708 mg. per 100 g. of

fat. The high squalene content of olive oil prompted Fitelson (1943) to devise a simple method for its determination, and makes it possible to detect olive oil in mixtures of other vegetable oils.

Shone (1962) separated the unsaponifiable matter in tung oil by thin-layer and column chromatography, utilizing a mixture of silica gel and plaster of paris for both types of chromatography. He found that the unsaponifiable fraction of tung oil contained 26% Δ^5-stigmasten-3B-ol, 9% α-tocopherol, 36% γ-tocopherol, 3% tocopherol artifact and 0.16% carotenoid pigment. No evidence was found to indicate the presence of a glyceryl ether or squalene in the amounts reported by former workers.

Bauer et al., (1958) investigated the composition of unsaponifiable material from tall oil distillates. Tests for sterols by the digitonin method were negative on unsaponifiables from volatile fatty acid and rosin acid fractions. However, digitonin precipitation did disclose both free and combined sterols in the pitch distillation residues. A very good yield of 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds was obtained from the volatile fraction of the unsaponifiable material. These authors concluded that the volatile unsaponifiable materials contained in tall oil distillates were predominantly hydrocarbons and decarboxylated fatty acids and rosin acid products, resulting from heat degradation during distillation. These workers also concluded that the sterols did not break down during heating and do not contribute to an increase in the unsaponifiable material of tall oil distillates.

Capella et al., (1960) made a very extensive study on the unsaponifiable material from olive oil, soybean oil, teaseed oil, rapeseed oil, lard and tallow. Utilizing silicic acid column chromatography, these authors

were able to separate unsaponifiable material into their major chemical classes. The chemical classes identified were: paraffins and olefins, polyene hydrocarbons, waxes, sterol esters, higher aliphatic alcohols, triterpenoid alcohols, sterols, and an unidentified fraction. Polyene hydrocarbons were eluted from the column with carbon disulfide and the infrared spectrum of this fraction was identical to the one reported for synthetic and natural squalene. The wax and sterol ester fractions were accounted for as products of incomplete saponification of the original oil. On the basis of the analytical data for the sterol fraction of the plant oil unsaponifiables, these authors concluded that B-sitosterol was the major component of this fraction. Three other sterols were known to be present, one of which gave an R_f value very close to that of cholesterol.

Animal Lipids

Early studies on the composition of unsaponifiable matter were carried out on the unsaponifiable matter of marine oils. Squalene was found in shark-liver oil by Tsujimoto (1916). This author investigated two Japanese shark-liver oils and confirmed that these oils contain very high proportions of unsaponifiable matter. The unsaponifiable matter consisted mainly of a new highly unsaturated hydrocarbon with a formula of C_{30} H_{50}. In a continuation of these studies Tsujimoto (1920) reported that the new hydrocarbon was present in a large number of fish liver oils. This author also proposed the name squalene for this compound.

Boynton (1948) separated the unsaponifiable fraction of dogfish liver oil on an aluminum oxide chromatographic column. After preliminary separation, this researcher was able to identify vitamin A, cholesterol and

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glyceryl ethers in this material. Two of the glyceryl ethers identified were chimyl and selachyl alcohol.

Mosher et al., (1958) in an extensive study of menhaden oil unsaponifiable material was able to fractionate this material into three groups or classes: (1) squalene, (2) cholesterol and other sterols, and (3) pigments. These three fractions comprised 75% of the total unsaponifiable material, which was about 2% of the total oil. The balance of the unsaponifiable material was in the form of oily substances that were not characterized. Squalene accounted for about 10% of the unsaponifiable fraction. After aluminum oxide chromatography, the crude squalene mixture was separated into three isomers by the formation of hydrochlorides. These isomers were identical to synthetic and natural squalene derivatives. The steroid fraction amounted to about 30% of the total unsaponifiable material, and further investigations on this fraction indicated that 90% of this was cholesterol. From the remaining portion of the unsaponifiable material, alpha and gamma carotene, zeoxanthin, violaxanthin, xanthophyll and two oxygenated carotenes of undetermined structure were tentatively identified. These authors also pointed out that the unsaponifiable material was quite high in unsaturation and, therefore, easily autoxidizable.

MacKenna et al., (1952) chromatographically analyzed the unsaponifiable matter of human sebum and found that this fraction contained 30-46% hydrocarbons, 14-19% cholesterol, 20% normal chain aliphatic alcohols and a number of unidentified substances, including oxidation products of squalene. The hydrocarbon fraction was found to contain 30-40%

squalene and the remainder was mainly normal paraffins. Boughton and Wheatly (1959) studied the sterol and wax alcohol fractions of the unsaponifiable matter of human forearm sebum. Utilizing gas chromatographic techniques these authors identified alcohols with up to 20 carbon atoms. These alcohols formed a homologous series similar to the fatty acid series (C_1-C_{20}). In the sterol fraction these authors reported about 0.003% 7-dehydrocholesterol, 0.07% isocholesterol and various unidentified ketosteroids. Alkane 1:2 diols, calculated as C_{20} diols, appeared to account for about 1.6% of the total unsaponifiable material.

Eisner et al., (1962) reported that the sterols of butter and margarine were isolated by saponification and chromatographic separation of the unsaponifiable matter on a Florisil column. Gas chromatography of the sterol fraction from six samples of butter indicated only one component, cholesterol. The sterols from six samples of margarine apparently consisted of three major components: B-sitosterol, γ-sitosterol, and stigmasterol.

Koch and Koch (1941) investigated the lipids in the leg skin of chickens and were able to crystallize from the unsaponifiable matter a compound with the properties of lanosterol. Cholesterol was also identified in this ether extractable fraction, and spectral analysis indicated the presence of ergosterol and 7-dehydrocholesterol.

Cholesterol is one of the most widely disseminated organic compounds in the animal kingdom. It is found in almost every species of animal (Kritchevsky, 1958). Winterstein and Schön (1936) reported that lard contained just over 100 mg. of cholesterol per 100 g. of lard. Lange (1950) tabulated the cholesterol content of various foods including lard and whole pig fat. Lard was reported to contain 108 to 122 mg. of cholesterol

per 100 g. of fat, whereas, whole pig fat contained 50 to 122 mg. of cholesterol per 100 g. The same author reported that beef tallow contained about 75 to 140 mg. of cholesterol per 100 g. of fat on a moist material basis. Craig et al., (1962) reported the presence of cholesterol and squalene in the unsaponifiable fraction of both boar and barrow fat.

Fitelson (1943) determined the squalene content of 23 natural fats, and the results ranged from none in cocoa butter to a maximum of 708 mg. per 100 g. in one sample of olive oil. The fats examined included 16 vegetable oils, 2 marine oils and 4 land animal fats. With the exception of cocoa butter, these fats were further examined and typical squalene hexahydrochloride crystals were obtained from all. The same author reported that pork lard contained about 3 mg. squalene per 100 g. of lard. About 10 mg. of squalene was present in 100 g. of beef tallow.

The presence of vitamin A in lard was indicated by Herb et al., (1953). Biological assays on molecular distillates from lard revealed that lard contained vitamin A activity equivalent to about 0.4 to 2.0 units per gram. Chromatographic fractionation of the unsaponifiables and molecular distillates from lard yielded eluates which gave a positive Carr-Price test and typical vitamin A spectral curves. These authors concluded that the biological vitamin A activity in lard is largely attributable to the presence of typical vitamin A. They also concluded that the so-called "sparing action" of lard on utilization of added vitamin A in diets was probably due to the presence in lard of hitherto unrecognized typical vitamin A. Ames and Harris (1954) also identified the "lard factor" as vitamin A. Vitamin A, per se, was found in amounts sufficient to account for the

vitamin A bioactivity in lard distillates. These authors analyzed various lard distillates colorimetrically and found a range of apparent vitamin A content of 5.5 to 28 units per gram, which corresponds to about 0.5 to 2.5 units per gram in the original lard sample.

Vitamin E and/or the tocopherols are/is another group of organic compounds that are very widespread throughout the animal kingdom. Kofler (1943) found tocopherols in blood serum, milk and animal and vegetable fats. This author concluded that pork lard contained about 1 mg. of tocopherol per 100 g. of lard. Data of Mahon and Chapman (1960) show the total tocopherol content of lard to be 6 ug. per g. of lard. This amounts to about 0.7% of the total unsaponifiable material. Harris et al., (1950) found animal fats, such as lard and butter, to be very poor sources of tocoperols. These workers reported 2.7 mg. of total tocopherols per 100 g. of pork lard and 2.4 mg. of tocopherols per 100 g. of butterfat.

Potter and Kummerow (1950) studied the spectral characteristics of selected fats. In this research, the unsaponifiable material obtained from lipids of rat, chicken or turkey liver, chicken gizzard and skin tissue exhibited an appreciable absorption in the ultraviolet region from 230 to 320 mu. These authors also reported that crystalline carotene and vitamin A concentrate exhibited considerable absorption in this region, but cholesterol, choline chloride, glycerol and phosphoric acid showed no absorption in this region.

Morton (1956) was able to separate the unsaponifiable fractions from liver and kidney tissue lipids, using alumina as an adsorbent and mixtures of light petroleum and diethyl ether as eluting solvents. Four groups of

unknown substances were isolated and studied. These groups were called SA, SB, SC and SE. Spectral analysis of these fractions revealed a range of maximum absorption from about 232 mu. to 330 mu. Fraction SE was identified as ∝-tocopherol and was present in very minute quantities.

Saponification of Fat

Standard Methods

In principle, the saponification of fat involves the addition of a caustic alkali and removal of the unsaponifiable matter by extraction with a solvent, such as ethyl or petroleum ether. Mehlenbacher (1960) reported that the most satisfactory methods are based on wet extraction, i.e., extraction of the unsaponifiable matter from an alcoholic or aqueous solution of the soap. The Bolton-Revis (Elsdon, 1926) procedure, sometimes referred to as a dry extraction method, is based on extraction of the unsaponifiable substances from a solid soap. In the latter method, the fat is saponified with sodium hydroxide in 95% alcohol, followed by evaporation of the alcohol until a pasty mass results. The soap is then mixed with sand and sodium bicarbonate, and the mixture is evaporated to dryness. The dried mass is then extracted with petroleum ether to remove the unsaponifiable matter.

Deuel (1951) suggested that fat could be saponified in the cold by the use of a concentrated alkali. In this procedure concentrated alkali is mixed with the fat and is allowed to stand for several days. This technique is especially useful where the application of heat would bring about changes in some component in the unsaponifiable residue, which is purified by removal of the triglyceride. Craig et al., (1962) applied

this saponification technique to a study of sex odor in pork fat. In this case, the authors were interested in the more volatile fraction of the unsaponifiable matter, therefore, cold saponification was necessary.

Mehlenbacher (1960) reviewed the present methods of saponification and concluded that the two best known methods for the estimation of unsaponifiable matter in fats and oils are those of the American Oil Chemist's Society (1946) and the British Society of Public Analysis (1958). The method of the former prescribes the use of petroleum ether as the extraction solvent, while the latter method specifies the use of ethyl ether. There are a few other differences in the two procedures, but they are not as significant as the solvents. The petroleum ether method is comparatively easy to use and quite satisfactory for small amounts of unsaponifiable matter, such as are normally present in tallows, greases and most vegetable oils. With high percentages of unsaponifiable matter, such as occurs in marine oils, petroleum ether extraction yields low results and the use of ethyl ether is preferable.

Bauer et al., (1958) compared the extraction efficiency of ethyl ether and petroleum ether in the removal of unsaponifiable matter from tall oil distillates. The authors concluded that both ethers could be used interchangeably for the extraction of unsaponifiable material without any loss of accuracy or repeatability. A sub-committee (1933) of the Society of Public Analysis, which was responsible for the development of the ethyl ether extraction method, concluded that the permissible error in the estimation of unsaponifiable matter should not exceed ± 0.1% with vegetable oils and ± 0.2% with fish oils on the basis of the original fat.

Kirsten (1946) reported that the agreement in results obtained in different laboratories on oils having a high content of unsaponifiable matter was better when the ethyl ether method was used than was the case when the petroleum ether extraction method was employed.

Methods prescribed by both the American and British organizations are tedious and time consuming. Wood and Roschen (1938) attempted to obviate these difficulties by the use of a continuous extraction procedure. Mehlenbacher (1960) reported that this method was less tedious than most of the others, but in its present form, it tends to yield inconsistent results in the hands of different analysis. Buerki and Holt (1954) also described a continuous extraction procedure for unsaponifiable matter.

Yield of Unsaponifiable Material in Fats and Oils

All fats contain unsaponifiable matter in different amounts, ranging from a few tenths of 1% up to several percent. Normally, it is found in the common animal and vegetable fats and oils in quantities of 1 to 2% or less, although certain marine oils and a few unusual vegetable oils contain considerably more. Mehlenbacher (1960) reported that lard contained less than 0.8% unsaponifiable matter, whereas shark liver fat contained as high as 13 to 20%. A definite value for percent unsaponifiable matter in pork fat or lard has not been reported. However, the American Oil Chemists Society (1946) also reported lard as having less than 0.8% unsaponifiable matter. This same group reports that beef tallow contains less than 1% unsaponifiable matter. While studying the occurrence of an unsaturated hydrocarbon in olive oil, Thorbjarnarson and Drummond (1935) reported that this oil yielded a total of 9.6 g. of unsaponifiable material per kg.

of oil. Bauer et al., (1958) reported a yield of unsaponifiable material in a range of 1.7 to 1.9% of the original tall oil sample.

EXPERIMENTAL PROCEDURE

The first part of this investigation was designed to determine the incidence of "sex odor" or "boar odor" in swine of various sex classes, and to study the effects of incorporating boar meat having sex odor into some comminuted meats. The second phase of this study deals with fractionation and identification of the volatile and non-volatile components in the fatty tissues of boars and barrows, with particular emphasis on the unsaponifiable fraction.

Incidence and Utilization Study

Source of Experimental Material

Fat samples were taken from the flank area or ham facing of 79 boars, 78 sows, 86 barrows and 96 gilts. The samples were procured over a period of one year as pigs representing the different sex groups became available. Although all boars, gilts and sows were assumed to be sexually mature, the exact age of all animals was not known. The boars and sows showed considerable variation in size, but weights were not available. The majority of the barrows and gilts were in the usual market weight range of approximately 180 to 240 lb. Samples were identified by code numbers, wrapped in aluminum foil, frozen and held at -20°F. until removed for testing. Lean trimmings from both boars and barrows were also frozen in Cry-o-vac bags and stored at -20°F.

Method of Evaluating Fat for Sex Odor

Evaluation for the intensity of sex odor was made by an organoleptic panel of three individuals selected for their ability to detect sex odor

in pork. Selection of the panel was made on the basis of their ability to detect traces of sex odor. Prior to evaluation, the coded samples were removed from the freezer at random, allowed to thaw and diced into small cubes for testing. Then 12 g. of the diced fat were heated in a 250 ml. Erlenmeyer flask to about 110°C. (Craig et al., 1962), or until the odor from the fat became apparent. After preparation, the samples were tested one at a time under a laboratory exhaust hood. Each sample was scored individually by each panel member for intensity of odor, as either none, slight odor or strong odor. A numerical value of 0, 1 or 2 was assigned to none, slight odor and strong odor, respectively. Samples were tested at several different sessions to prevent the possibility of panel fatigue.

Since analysis of variance between scores for individual panel members revealed no significant ($P < .01$) differences in the ability of the panel members to detect sex odor, an arithmetic mean was calculated from the three observations on each animal. The arithmetic mean was then used to calculate the incidence.

Preparation of Comminuted Meats

Boar meat having very strong sex odor, or lean pork containing no sex odor were incorporated into standard formulations of cooked salami and braunschweiger. The recipe used for cooked salami is listed in table 1. Two formulations of salami were prepared, one utilizing boar meat with strong sex odor and the other using regular fresh pork trimmings.

The beef trimmings were ground through a 1/2 inch plate and then through a 1/8 inch plate. The pork trimmings were ground through a 1 inch plate

and then through a 1/8 inch plate. The ground meat was placed in a mixer and mixed thoroughly, while adding the curing and seasoning ingredients. The mixture was removed and immediately stuffed into 2 1/4 inch diameter opaque fibrous casings. The stuffed salami was then placed into a 38°F. cooler for 48 to 72 hours. The salami was next removed from the cooler and allowed to stand at room temperature for 1 1/2 to 2 hours.

Table 1. Recipe for cooked salami

Ingredient	Amount
Fresh boneless beef	10.5 lb.
Pork trim	15.0 lb.
Salt	340 g.
Sugar	57 g.
Sodium nitrate	2 g.
Sodium nitrite	1 g.
Whole black pepper	42 g.
Cardoman	7 g.
Garlic powder	7 g.

The sausage was placed in the smokehouse under heavy smoke at about 110°F. for 5 to 6 hours. Next, the temperature of the smokehouse was raised very slowly to a high of 165-170°F., or until the internal temperature of the sausage reached 145-150°F. The salami was then removed from the smokehouse and showered with cold water until the temperature was reduced to about 100°F. Next, the sausage was showered with warm water to remove grease and improve color. After removing from the shower, it

was allowed to stand at room temperature until completely cooled and dried. The cooked salami was then stored in a 38°F. cooler until tested.

Braunschweiger was prepared by the recipe listed in table 2.

Table 2. Recipe for braunschweiger

Ingredient	Amount
Pork liver (unblanched)	15 lb.
Pork trim	8 lb.
Beef trim	2 lb.
Dried skim milk	1 lb.
Onion powder	50 g.
Salt	340 g.
Sugar	42 g.
White pepper	28 g.
Garlic powder	3 g.
Cardoman	7 g.
Mace	7 g.
Sodium nitrite	1 g.
Sodium nitrate	2 g.

Two formulations of braunschweiger were prepared. One recipe utilized boar lean trimmings containing strong sex odor while the other contained regular pork trimmings without any sex odor. The beef and pork trimmings were ground through a 3/8 inch plate and then through a 5/32 inch plate. The liver was diced and placed in a silent cutter and chopped

for 3 minutes, adding all seasoning and cure. Ice was used while chopping to keep the temperature below 50°F. After the 3 minutes chopping period, the dried skim milk was added to the cutter and mixed thoroughly with the liver. Next the ground beef and pork was added to the cutter and chopped until a fine emulsion was formed (usually about 5 minutes). The emulsion was then removed from the cutter and stuffed into 4 1/2 inch diameter opaque, fibrous casings. The stuffed braunschweiger was cooked in a steam kettle in 160°F. water to an internal temperature of 150°F. After cooking was complete, the hot water was removed and replaced with cold water, adding ice if necessary, until the internal temperature was reduced to about 90°F. The sausage was next removed from the water and allowed to stand at room temperature for 2 hours, before being placed in a 38°F. cooler for storage.

Methods of Evaluating Comminuted Meats

After processing, both the salami and braunschweiger were evaluated by a 17-member consumer-type taste panel, utilizing the 9-point hedonic scale (Peryam and Pilgrim, 1957). The samples were also scored both hot and cold by the trained odor panel.

Statistical Analysis

In order to determine if differences in the incidence of sex odor between samples from pigs of different sexes were significant, the data were analyzed by the Chi-square method of analysis. Flavor acceptance scores of the comminuted products prepared from pork containing sex odor and pork free from sex odor were subjected to analysis of variance.

Methods for Isolating and Identifying Pork Fat Volatiles

Sulfides and Carbonyls

Fat was removed from the freezer and allowed to thaw. It was placed in a Universal food grinder, ground through a quarter inch plate and divided into 200 g. samples. All 200 g. samples not used immediately were placed in Cry-o-vac bags and stored again at -20°F. The previously ground 200 g. samples of pork fat were placed in a 1 liter double-necked round bottom flask and 200 ml. of distilled water were added. Utilizing a Glas-Col heating mantle, the fat-distilled water mixture was heated at 100°C. for 3 1/2 hours. Nitrogen gas was used as a carrier to convey the volatiles through the system of traps.

The apparatus used for the collection of volatile sulfides and carbonyls consisted of a series of traps shown in figure 1. The first trap contained 300 ml. of a 2% lead acetate solution, while the second and third traps each contained 300 ml. of a solution of saturated 2,4-dinitrophenylhydrazine in 2N hydrochloric acid. The first trap containing 2% lead acetate was recharged with fresh solution each time the trapping solution became slightly turbid. At the end of 3 1/2 hours the turbid lead acetate solutions were combined and made up to a volume of 1 liter with fresh 2% lead acetate. Chemical tests, that are described subsequently, were then carried out on this solution. The 2,4-dinitrophenylhydrazine solutions were observed for precipitates.

The hydrogen sulfide content of the lead acetate solutions was determined by a modification of the methylene blue method described by Marbach and Doty (1956), in which a comparison is made with a standard curve.

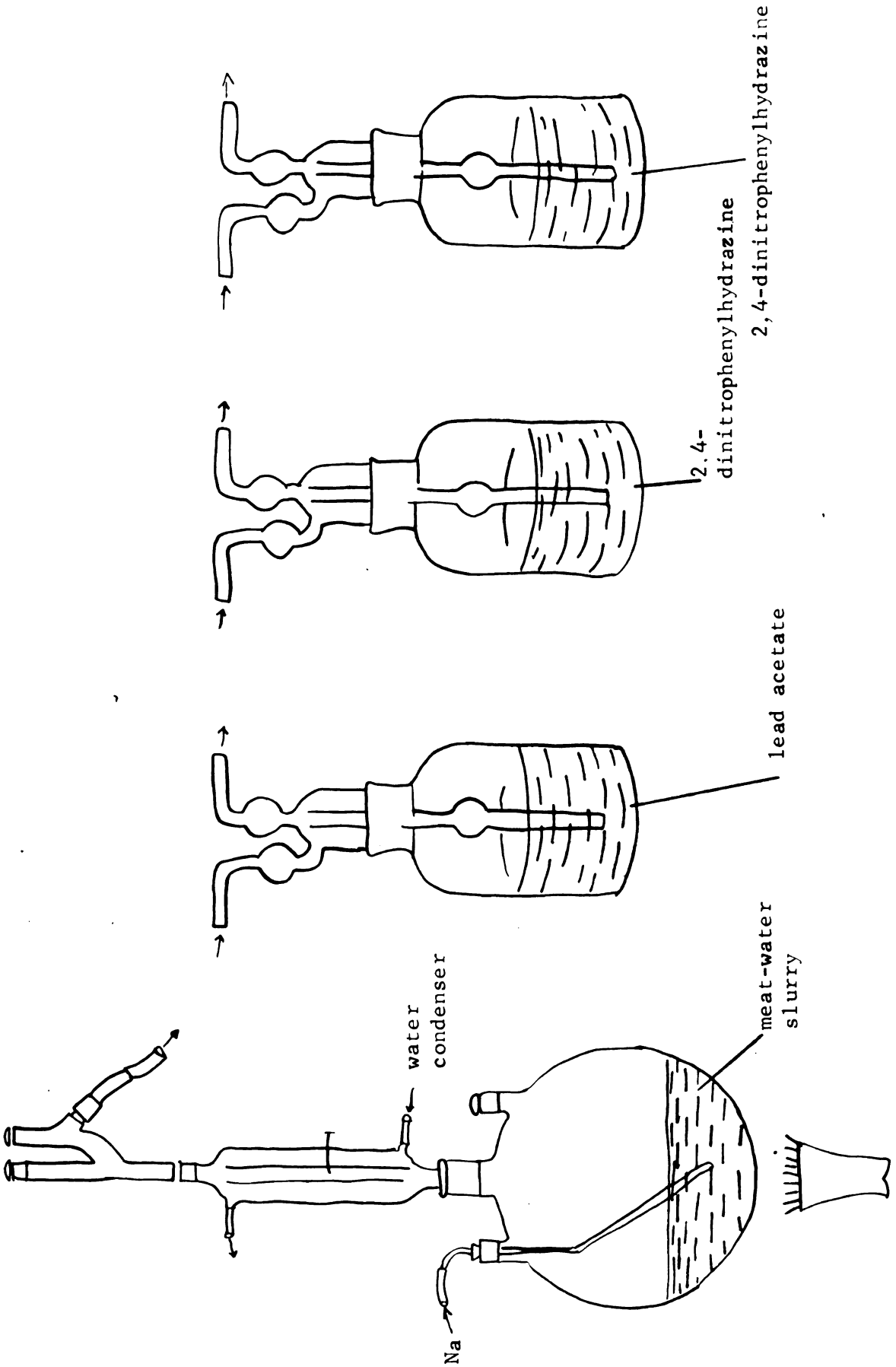

Figure 1. Diagram of collectio apparatus for volatile sulfide and carbonyl compounds.

Color development was initiated by the addition of 1.5 ml. of amine solution and 0.5 ml. of Reissner solution to 25 ml. of the lead acetate solution containing the hydrogen sulfide. The test tubes containing the solutions were quickly stoppered and agitated continuously for 10 minutes. After shaking, the solution was allowed to stand for 30 minutes at room temperature. A portion of the colored solution was transferred to a colorimeter tube and read at 665 mu in a Bausch and Lomb Spectronic 20 colorimeter. The absorbance for a blank reagent solution was deducted from the absorbance reading for the unknown. Using the corrected absorbance, the amount of hydrogen sulfide present was read from a standard curve prepared from data obtained by using solutions containing known amounts of sulfides.

The amine solution used above was prepared by dissolving 5.0 g. of N,N-dimethyl-p-phenylenediamine hydrochloride in 1 liter of concentrated hydrochloric acid. To prepare the Reissner solution 67.6 g. of ferric chloride hexahydrate was dissolved in distilled water and then made up to 500 ml. This was then added to 500 ml. of nitric acid solution, containing 72 ml. of boiled concentrated nitric acid (Sp. Gr. 1.42).

The hydrogen sulfide standard curve was prepared by washing a large crystal (about 1 g.) of sodium sulfide nonahydrate with distilled water until just the core (about 0.1 g.) remained. The core was then dissolved in distilled water and diluted to 1 liter. The solution was then standardized by the usual iodine-thiosulfate method using 100 ml. aliquots. Next the standard curve was developed as outlined in table 3. Color development was as described above, and absorbance values were read on the Bausch

and Lomb Spectronic 20 colorimeter at 665 mu. After subtracting the value for the reagent blank (tube 1, table 3), the hydrogen sulfide content was plotted against the corrected absorbance reading on rectilinear paper (figure 2).

Table 3. Scheme for developing hydrogen sulfide standard curve

Tube No.	1	2	3	4	5	6	7
Std. soln. (ml.)	0	0.5	1.0	1.5	2.0	2.5	3.0
Pb. acetate soln. (ml.)	25.0	24.5	24.0	23.5	23.0	22.5	22.0
Reissner soln. (ml.)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Amine soln. (ml.)	1.5	1.5	1.5	1.5	1.5	1.5	1.5
H_2S/ml. (mg x 10^{-6})	0.0	4.38	8.77	13.15	17.54	21.92	26.31
Absorbance (665 mu.)	0.002	0.021	0.060	0.100	0.145	0.180	0.229

Mercaptans were tested for by heating 25 ml. of the lead acetate trapping solution with 10 ml. of dilute hydrochloric acid. The vapors from the heated solution were trapped in a fresh solution of 2% lead acetate. The presence of mercaptans was detected in the fresh lead acetate solution by the appearance of a precipitate.

Volatile Fatty Acids and Ammonia

Pork fat for this determination was prepared as previously described for the hydrogen sulfide determinations. A ground fat sample of 1200 g.

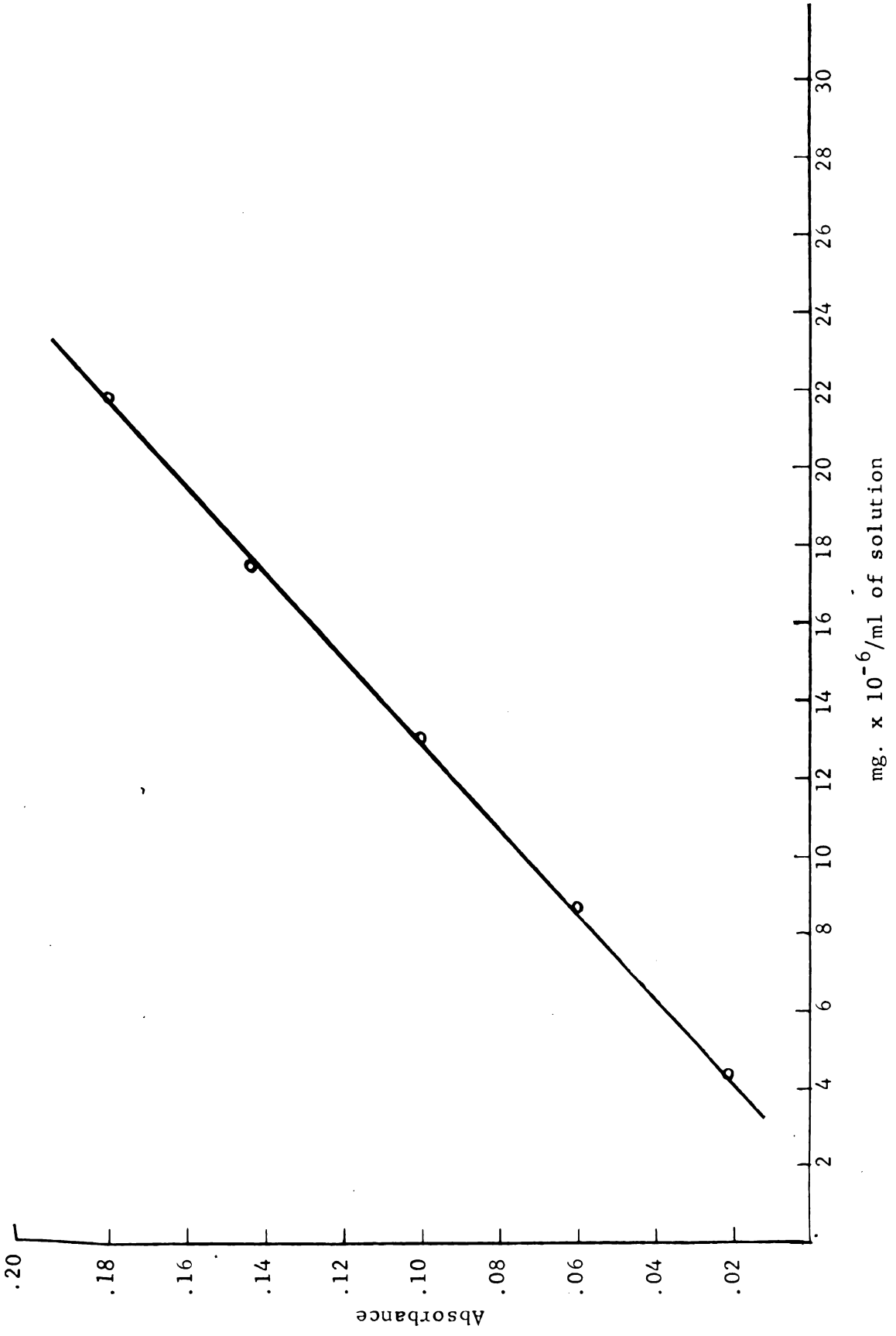

Figure 2. Hydrogen sulfide standard curve.

was placed in a 1 liter 2-necked round bottom flask, and 200 ml. of distilled water were added. The mixture was heated vigorously for 2 hr., utilizing a Glas-col heating mantle. The volatiles were collected in traps containing 300 ml. of chloroform with 100 ml. of distilled water layered on top of the chloroform. The traps were kept submerged in a wet-ice bath. Physically, the trapping system was identical to figure 1, except there were only two traps in this procedure and both of these contained a chloroform - water solution.

The acidity of the trapping reagents was determined using both Micro Essential Laboratory Hydrion Paper and titration of 50 ml. aliquots of both the chloroform and water layers of the trapping reagent. The titrating reagent was a 0.0272 N. methanolic sodium hydroxide solution, and n-cresol purple was used as the indicator (Corcoran, 1956).

The aqueous layer of the trapping reagent was tested for nitrogen containing compounds by utilizing 10% potassium hydroxide for hydrolysis. A 10 ml. aliquot of the aqueous trapping layer was then added to 5 ml. of the 10% potassium hydroxide solution and warmed gently with a Bunsen burner. Volatile ammonia was tested for by exposing litmus paper to the vapors arising from the hot solution. Ammonia test paper was also used to test for ammonia when the above solution was heated.

The ammonia test paper was prepared by mixing 10 ml. of 20% silver nitrate solution with five drops of 40% formalin and a few drops of dilute sodium hydroxide. This mixture was filtered and the filtrate immediately absorbed on strips of Whatman No. 1 filter paper. After drying, the paper was ready for use. The presence of ammonia was indicated, if the paper turned black or gray.

Methods of Saponification and Extraction

Both boar and barrow fat were saponified as an aid in their fractionation.

Cold Method

Deuel (1951) suggested that fat could be saponified in the cold by the use of a concentrated alkali. Craig et al., (1962) described a method for saponifying fat, utilizing a concentrated solution of sodium ethylate. The latter method was slightly modified for this study.

A 100 g. sample of ground fat was rendered by blending with 200 ml. of redistilled diethyl ether for 1 1/2 minutes in a Waring blender. The connective tissue was removed by filtering the ether extracted fat through four layers of cheese cloth. The filtered liquid was placed in a 2000 ml. Erlenmeyer flask and 400 ml. of redistilled ether were added. Sodium ethylate was prepared by dissolving 16 g. of kerosene-free metallic sodium in 200 ml. of 95% ethyl alcohol. Any alcohol that evaporated was replaced. When the sodium dissolved, the resulting sodium ethylate solution was added and stirred into the mixture of ether and fat. The flask was stoppered, shaken vigorously, and allowed to remain at room temperature for 24 hours or more.

After saponification was complete, the liquid was removed from the soap by filtering through 4 layers of cheesecloth. The soap was extracted with two 500 ml. aliquots of diethyl ether, filtered, and the ether extracts were combined with the original filtrate. The soap was then discarded. The combined ether extracts were next filtered through Whatman

No. 1 filter paper and added to a 2000 ml. separatory funnel containing 150 ml. of distilled water. After the layers separated, the water was drawn off. The washing procedure was repeated twice using 160 ml. of distilled water. Next the ether solution was washed 2 times with 150 ml. portions of 0.5 N aqueous potassium hydroxide. The ether filtrate was then washed repeatedly with 200 ml. portions of distilled water until the water extracts were no longer alkaline. If the ether and water layers were sluggish in separating, a small amount of ethyl alcohol greatly facilitated separation. If a complete emulsion formed, reagent grade sodium chloride was added to cause separation of the ether and water layers.

After washing, the ether extract was dried overnight with anhydrous sodium sulfate, filtered, and reduced to a volume of a few ml. under vacuum. After the volume was reduced to a few ml., the residue was transferred to a weighed flask by washing all residue into flask with diethyl ether. All ether was removed under vacuum at room temperature, sweeping the flask with dry nitrogen gas. The flask containing the unsaponifiable residue was weighed and the yield calculated. The unsaponifiable residue was stored in the refrigerator under nitrogen.

Hot Method, Diethyl Ether Extraction

A sample of ground fat weighing 100 g., 250 ml. of 12% alcoholic potassium hydroxide and 5 g. of pyrogallol were added to a 1000 ml. double-necked boiling flask. The flask was connected to a cold water condenser and the mixture was saponified for 1 1/2 hours under a stream of nitrogen. When saponification was complete the mixture was washed into a 2000 ml.

separatory funnel with 100 ml. of distilled water. The saponified solution was allowed to cool and then extracted with successive 600 ml., 100 ml. and 100 ml. aliquots of diethyl ether. The ether extracts were combined and washed as previously described for the cold saponification extract. The unsaponifiable residue was dried, and yield was determined in the manner previously detailed for cold saponification.

Saponification for Vitamin A Determination

A 50 g. sample of fat was weighed accurately into a 1000 ml. boiling flask fitted with a ground glass neck. A 250 ml. aliquot of 10% alcoholic potassium hydroxide was added to the flask, and the flask was connected to a reflux condenser. The solution and fat were then refluxed for 15 minutes. The mixture was allowed to cool and then transferred to a 2000 ml. separatory funnel with 750 ml. of distilled water. The water solution was then extracted successively with 625, 500 and 375 ml. portions of freshly redistilled diethyl ether. The ether extracts were combined and washed once with 500 ml. distilled water and then with 250 ml. of 0.5N potassium hydroxide. The ether extract was then washed with successive 500 ml. portions of distilled water until the wash solution was colorless to phenolphthalein. The ether solution was allowed to stand 15 minutes and the last droplets of water were drained off. Next the ether solution was transferred to a 2000 ml. Erlenmeyer flask and swirled with 15 to 25 g. of anhydrous sodium sulfate. The solution was next filtered through Whatman No. 1 filter paper to remove the sodium sulfate and then evaporated to dryness under vacuum. The residue was dissolved in chloroform and made

up to a volume of 25 ml. The chloroform solution containing the unsaponifiable residue was assayed by the antimony trichloride procedure.

Composition Studies on Pork Fat Unsaponifiable Matter

Qualitative Tests on Total and Fractionated Unsaponifiable Matter

Alcohols: This functional group was detected using the reagent solution of Walsh and Merritt (1960). The test solution was prepared by mixing 10 drops of 7.50 Nitric acid and 1 drop of 1% potassium dichromate in a small beaker. Then 1 or 2 drops of the unknown material was added. The solution turns from bright yellow to blue-gray if alcoholic groups are present. This test is good for primary and secondary alcohols.

Carbonyls: Most aldehydes and ketones can be readily detected with 2,4-dinitrophenylhydrazine as described by Walsh and Merritt (1960). The test solution is prepared by saturating a 2N hydrochloric acid solution with crystalline 2,4-dinitrophenylhydrazine. Ten drops of this solution were placed in a small beaker and 1 or 2 drops of total or fractionated unsaponifiable matter were added. The presence of carbonyls is indicated if a precipitate appears.

Aromatic nucleus and aliphatic unsaturation: Walsh and Merritt (1960) described a qualitative test for these functional groups. The test was carried out by adding 1 drop of 37% formaldehyde to 10 drops of concentrated sulfuric acid. To this solution 1 or 2 drops of the material to be tested was added. If the compounds present contain aliphatic unsaturation or an aromatic nucleus a bright wine color appears.

Liebermann-Burchard test for cholesterol: The Liebermann-Burchard test for cholesterol was carried out as described by Litwack (1960). A small

amount of unsaponifiable matter was dissolved in 2 ml. of chloroform. To this solution, 5 ml. of chloroform and 10 drops of acetic anhydride were added. The solution was thoroughly mixed, and 2 drops of concentrated sulfuric acidwere added. If cholesterol is present an orange color will appear and change to blue-green on standing.

Hydroxylamine test for esters: A 50 mg. sample of unsaponifiable material was dissolved in 5 ml. of diethyl ether, and 1.5 ml. of hydroxylamine reagent added. They were mixed in a test tube and allowed to stand for 30 minutes. Next 12.5 ml. of ferric perchlorate reagent were added and shaken. A color change from yellow to purple will occur if esters are present.

The hydroxylamine reagent was prepared by mixing equal volumes of 5% hydroxylamine hydrochloride in methanol and 12.5% sodium hydroxide in methanol. The sodium chloride precipitate was removed by filtration. This solution was stable for about 20 minutes.

The ferric perchlorate reagent was made by dissolving 0.4 g. of iron in 5 ml. of concentrated hydrochloric acid. Next, 5 ml. of 70% perchloric acid was added, and this mixture evaporated until it was almost dry. The residue was diluted to 100 ml. with distilled water. A 10 ml. aliquot of the aqueous solution was transferred to a 500 ml. flask. The 70% perchloric acid and ethanol were alternately added until 37.5 ml. of perchloric acid and 400 ml. of ethanol were added. While adding these reagents, the flask was cooled by holding it in running water. After the ethanol and perchloric acid were added, the solution was allowed to come to room temperature and then diluted to a total volume of 500 ml. with ethanol. The reagent was then ready for use.

Sulfur determination: Sulfur may be determined in organic mixtures by conversion to hydrogen sulfide through pyrolytic reduction (Feigl, 1960). This test was performed by evaporating a drop of unsaponifiable material with a drop of 20% sodium formate solution. After the mixture was evaporated to dryness in a micro-test tube, the mouth of the tube was covered with a disc of lead acetate paper. The bottom of the test tube was then heated with a microflame burner. If sulfur bearing materials are present the lead acetate paper develops a gray or black stain. The acetate paper was prepared by dipping Whatman No. 1 filter paper in a saturated solution of lead acetate.

Nitrogen determination: When wet neutral litmus paper strips were held in the stream of volatiles arising from heated pork fat, the color change of the litmus indicated that the compounds were basic. Thus, it was decided to run nitrogen determinations on the unsaponifiable material. These determinations were made employing the micro-Kjeldahl procedure outlined by The American Instrument Co. (1961).

Quantitative Determinations In Total Unsaponifiables

Cholesterol and triterpene alcohols: The procedure used for determining cholesterol and triterpene alcohols was described by Luddy et al., (1953). In this procedure, the sample to be analyzed was dissolved in chloroform and brought to a standard volume. For most samples, a concentration of 10 mg. in 100 ml. of chloroform solution was satisfactory. A 10 ml. aliquot of this solution was pipetted into a 25 ml. glass stoppered, graduated cylinder, and 5 ml. of reagent at 0°C. was added. The stoppered

glass cylinder was then inverted several times to mix the solutions thoroughly. The solution was then placed in a dark room at 20-25°C. for color development.

Color development reached its maximum after about 30 minutes and remained at about the same level for 10 minutes. During this 10 minute period, the samples were transferred to matched colorimeter tubes, and the optical density was measured at 550 mu for the triterpene alcohols and at 630 mu for cholesterol. A blank consisting of 10 ml. chloroform and 5 ml. reagent was used to zero the Bausch and Lomb Spectronic 20 colorimeter at both wavelengths.

The reagent was prepared by adding slowly while shaking, 1 volume of cold (0°) concentrated sulfuric acid to 4 volumes of acetic anhydride. While adding the reagents, the flask was immersed in an ice bath. The mixture was then diluted in a similar manner with 2.5 volumes of glacial acetic acid and 2.5 volumes of purified 1,4 dioxane. After mixing, the reagent was held at 0°C. until used. The reagent was relatively unstable and was used the same day it was prepared. If any color developed before using, it was also discarded.

The 1,4 dioxane was purified according to the method of Fieser (1941).

Calculation of the percent by weight of cholesterol and triterpene alcohol was made utilizing the following formulas:

$$\text{Absorptivity} = A = \frac{\text{absorbance}}{(\text{total conc. in g./l.})(\text{layer thickness in cm})}$$

$$\text{Cholesterol, wt. \%} = \frac{100\ (A_{630} - A_L')}{A_C - A_L'}$$

$$\text{Triterpene alcohol, wt. \%} = \frac{100\ (A_{550} - A_C')}{A_L - A_C'}$$

A_c=Absorptivity of pure cholesterol complex at 630 mu.

A_c'=Absorptivity of pure cholesterol complex at 550 mu.

A_L=Absorptivity of pure lanosterol complex at 550 mu.

A_L'=Absorptivity of pure lanosterol complex at 630 mu.

Vitamin A alcohol: The method described by Glick (1957) was used to determine vitamin A alcohol. Boar and barrow fat were ground and saponified as described previously. After 50 g. of fat were saponified, the unsaponifiable material was dried under vacuum at room temperature and made up to 25 ml. with chloroform. The sample solution was then assayed along with an internal reference standard prepared from a pure crystalline vitamin A alcohol. Tubes were prepared for antimony trichloride colorimetric assay according to the scheme outlined in table 4.

Table 4. Scheme for vitamin A assay of unsaponifiable material

Tube No.	1	2	3	4
$CHCl_3$ soln. of unsaps.	---	1 ml.	1/2 ml.	---
$CHCl_3$ soln. of vit. A	---	---	1/2 ml.	1 ml.
$CHCl_3$	1 ml.	---	---	---
$SbCl_3$	2 ml.	2 ml.	2 ml.	2 ml.

Tube 1, which contained 1 ml. of chloroform, was placed in the Bausch and Lomb Spectronic 20 colorimeter, 2 ml. of antimony trichloride was added and the instrument set for zero absorbance (100% transmittance) at a wavelength of 620 mu. Tube 2, which contained the sample solution, was placed in the colorimeter and 2 ml. of antimony trichloride added. The antimony

trichloride solution was added very rapidly and the absorbance read at a wavelength of 620 mu. within 5 seconds. This same technique was applied to tubes 3 and 4. Vitamin A content was calculated as follows:

$$\frac{(N_u)\ (N_s)}{N_{(u+s)} - N_u \quad (W)} = \text{Units of vitamin A per g. of unsaponifiable matter.}$$

Units of vitamin A x .3 = ug. of vitamin A per g. of unsaponifiable matter.

N_u = units of vitamin A alcohol in each ml. of chloroform solution of sample for assay. This is read from the standard curve, which will be described subsequently.

N_s = Units of vitamin A in each ml. of chloroform solution of vitamin A reference standard, calculated from the weight of the standard.

$N_{(u+s)}$ = Units of vitamin A in each ml. of the chloroform solution, corresponding to the absorbance reading of tube 3 above, read from the standard curve.

W = Amount of unsaponifiable material, expressed as grams, represented in each ml. of final chloroform dilution of sample under assay.

The standard curve (figure 3) was prepared by accurately weighing a sample of crystalline vitamin A alcohol and dissolving it in chloroform. A sample of 11.1 mg. of crystalline vitamin A alcohol was made up to a total volume of 25 ml. This stock solution was next diluted according to the scheme outlined in table 5. Absorbance readings were recorded for each dilution after the color was developed as described above. The absorbance readings were then plotted opposite the concentration of vitamin A on rectilinear paper.

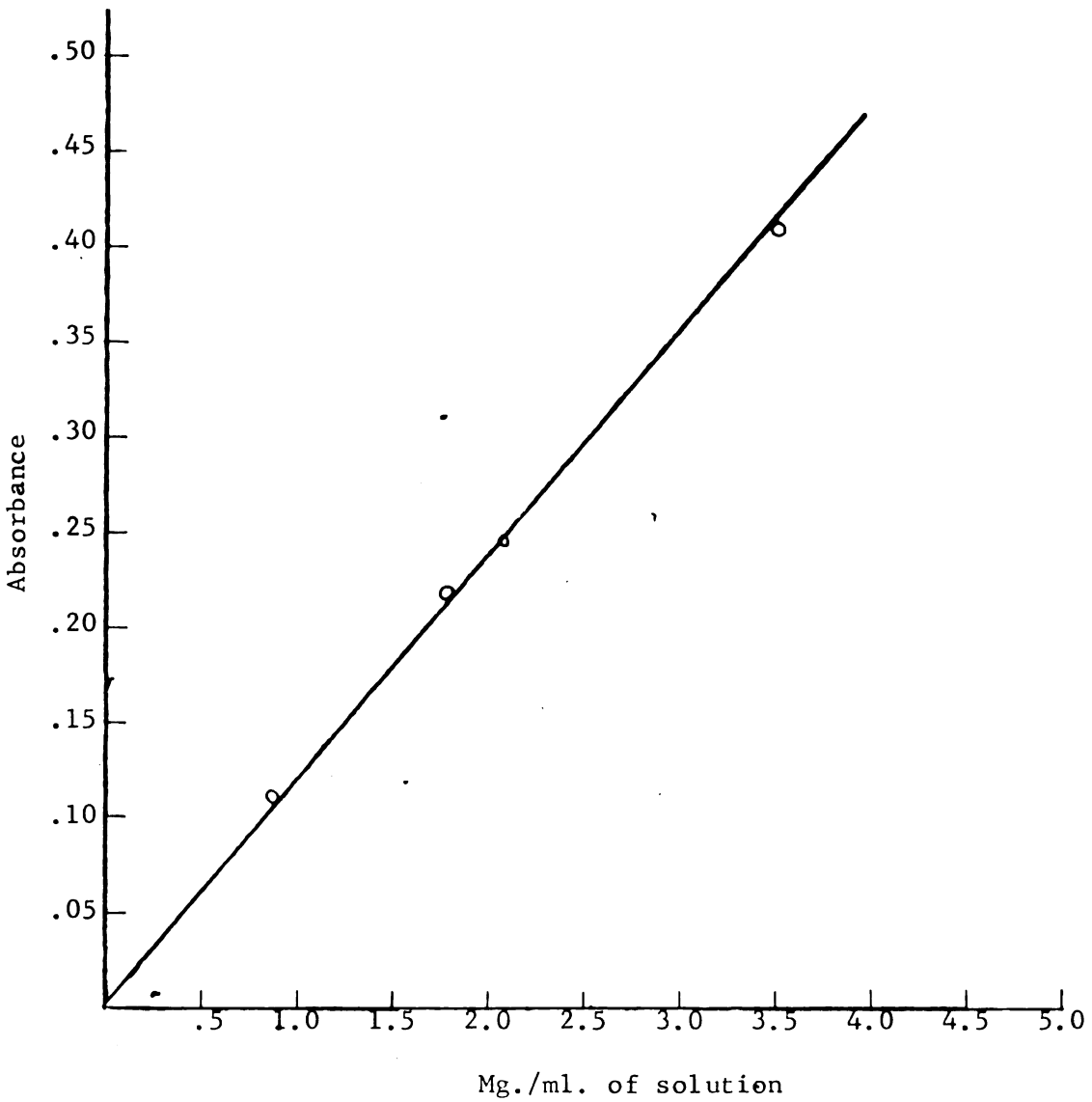

Figure 3. Vitamin A standard curve.

Table 5. Dilution scheme and absorbance values for vitamin A standards

Tube No.	1	2	3	4
Vit. A, std. soln. (ml.)	1	1	30*	2
$CHCl_3$ (ml.)	500	250	20	250
Vit. A/ml. (ug.)	0.888	1.776	2.131	3.552
Absorbance (620 mu)	0.111	0.220	0.243	0.410

*Thirty ml. of solution from tube 4 was used as the vitamin A standard solution and diluted up to a total volume of 50 ml.

Chromatography of Unsaponifiable Material

Removal of free fatty acids: The free fatty acids remaining in the unsaponifiable material were removed by the method of Capellas et al., (1960). About 1 g. of unsaponifiable material was dissolved in a minimum of chloroform and added to a 19 mm. chromatographic column containing 10 g. of basic copper carbonate. The copper carbonate was previously washed with 100 ml. of chloroform.

After the initial charge had almost passed through the column the sides of the column were washed down with 2 ml. of chloroform. The unsaponifiables were next eluted from the column with 450 ml. of chloroform. The solvent containing the unsaponifiable material was reduced to dryness under vacuum at room temperature. The residue was then ready for further fractionation.

Silicic acid chromatography: A 100 mg. sample of unsaponifiable material, which had been previously prepared was dissolved in a minimum amount of redistilled hexane. A 5 ml. charge was added to the top of an 11 mm. chromatographic column fitted with a stopcock and containing 3 g.

of Mallinckrodt's 100 mesh silicic acid. After the charge had almost passed through the column, the sides of the column were washed twice with 1-2 ml. of redistilled hexane. Elution was then began with the first solvent. Fractions of 4 ml. each were collected in small weighed test tubes, and the solvent was removed under vacuum in a stream of nitrogen at room temperature. When successive fractions contained no residue, the next solvent of increasing polarity was added according to the scheme suggested by Capellas et al., (1960) and shown in table 6.

Table 6. Elution scheme for separation of unsaponifiables

Fraction	Eluent
1	Hexane (redistilled)
2	Carbon disulfide
3	Hexane-5% benzene
4	Hexane-10% benzene
5	Hexane-20% benzene
6	Hexane-30% benzene
7	Hexane-2% ethyl ether
8	Hexane-8% ethyl ether
9	Chloroform-5% methanol

The weight of each fraction was determined by weighing the test tubes plus the residue after the solvent had been removed. Identification was attempted by the qualitative methods already described and/or by comparisons with known standards utilizing gas and thin-layer chromatography.

Ultraviolet and infrared absorption spectra were also determined on the various fractions.

Gas chromatography: This technique was used in an attempt to fractionate and identify the components of the total unsaponifiables and in the unsaponifiable material previously separated by silicic acid column chromatography. A Barber-Colman Model 20 gas chromatograph equipped with a radium ionization detector and a Barber-Colman recorder was used. Various column temperatures and argon carrier gas flow rates were used. Scavage and split flow rates were adjusted to 50 ml. per minute for all investigations. Systematic experimentation as well as information reported by Craig et al., (1962) aided in the selection of the proper column packing. For all gas chromatography, a 4 1/2 or 7 1/2 ft. section of copper tubing 1/4 inch in diameter was used. Columns were prepared with 60/80 mesh Chromasorb "W" coated with 2% silicone SE-30 purchased from Wilkens Instrument & Research Inc. The columns were packed using an electric vibrating needle and then coiled to a diameter of approximately 5 inches. The prepared column was preconditioned at 285°C and an argon flow rate of 100 ml. per minute for at least 24 hours.

Samples were dissolved in a small amount of diethyl ether or N,N-dimethylformamide and injected into the flash heater with a 10 ml. Hamilton Microsyringe. Various known compounds suspected of being present were chromatographed and their retention volumes compared to the retention volumes of unknown peaks from the chromatographed unsaponifiable material.

An attempt was made to collect fractionated components from the effluent stream of the gas chromatograph. For this attempt, a Barber-Colman

splitting tee was installed between the effluent end of the column and the detector. The splitting tee divided the effluent stream, with half of the components going to the detector and the other half going to the collecting apparatus. Collections were attempted into various qualitative functional group reagents, which have already been described.

Collections for infrared analysis were also carried out utilizing a modification of the procedure described by Gray et al., (1963). The modified collecting apparatus consisted of a short length (1 1/2 to 2 inches) of 1/4 inch glass tubing packed with 350 mg. of dry spectro-grade potassium bromide. Collection of components was attempted by holding the small potassium bromide column over the effluent stream, when a component was emerging from the column as indicated on the recorder chart. The potassium bromide was next pressed into a pellet and analyzed by infrared spectroscopy.

Thin-layer chromatography: This technique was employed to aid in identification of the compounds in the total unsaponifiable material, by comparing the R_f values of unknown compounds to those of known standards. Chromatography was carried out using Camag equipment purchased from the Arthur H. Thomas Company. It consisted of 20 X 20 in. glass plates, spotting template, applicator and a chromatographic developing tank. Special thin-layer chromatography absorbants, silica gel S, containing no binder, and silica gel B, containing a calcium sulfate binder, were purchased from Analabs, Inc. The absorbants were slurried with distilled water in a ratio of 1:2 (W/V) and applied to the glass plates in a uniform layer about 300 microns in thickness.

The plates were allowed to dry at room temperature for 15 minutes and then activated in an oven at 100°C for 30 minutes. The activated plates were stored in a desiccator until used. Chromatoplates were spotted with a 10 ul. Hamilton Microsyringe and developed in an equilibrated chromatographic tank utilizing 100 ml. of solvent. The solvent systems contained various combinations of diethyl ether and hexane. In addition, 1-2 ml. of concentrated acetic acid was added to the solvent to prevent excessive tailing. After development, the chromatoplates were allowed to dry at room temperature. Visualization of the developed plates was accomplished by utilizing an ultraviolet lamp and various spray reagents. After spraying, the plates were recorded on acetate tracing paper.

Spectral Analysis of Unsaponifiable Material

Ultraviolet: A Beckman DK-2 recording spectrophotometer was employed for the analysis of fractions obtained from silicic acid column chromatography. Samples investigated were dissolved in spectro-grade chloroform and their absorbance spectra determined using a chloroform blank. The instrument was set at a transmittance of 0% or an abosrbance of 100%, and the spectra from 340 mu. to 220 mu. was scanned. Known compounds suspected of being present were also scanned by the same procedure.

Infrared: This analysis was carried out on a Beckman IR-5 double-beam, recording spectrophotometer. Liquid samples from silicic acid column chromatography were analyzed in spectro-grade chloroform, using only chloroform in the reference cell. Samples from gas chromatographic fractionations were collected, as previously described on spectro-grade potassium

bromide. The potassium bromide powder was removed from the collecting tube and mixed thoroughly. The pellet was then pressed in the usual manner. The potassium bromide reference pellet had been subjected to the gas chromatographic effluent stream for an extended period of time. During this time no samples were injected, therefore, any column bleed could be accounted for. The instrument was set at approximately 100% transmittance and the samples were scanned in a range from 2 to 16 u.

RESULTS AND DISCUSSION

Incidence and utilization studies

Incidence

The data obtained from the odor evaluation of fat taken from the flank area or ham facing of 108 boars, 78 sows, 86 barrows and 96 gilts are presented in tables 7, 8 and 9. In subjecting these data to the Chi-square test, results indicated a significant difference ($P < 0.01$) between the incidence of sex odor in boars and all of the other sex classes. These data are not in agreement with the findings of Self (1957). However, the disagreement may possibly be explained by the fact that Self (1957) used a sample of diaphram muscle for testing, which is relatively low in fat and more likely to be contaminated with foreign material during the dressing operation. Fat was tested for boar odor in the present study because Craig and Pearson (1959), Dutt et al., (1959) and Craig et al., (1962) reported that the component(s) responsible for "boar odor" or "boar flavor" was/were located in the fatty tissue.

Although there was a real difference in the prevalence of sex odor between boars and all other sex groups, the differences between gilts, barrows and sows were not significant. These results are in contrast to those of Self (1957), who reported that 17% of all hogs possessed boar odor regardless of sex.

There was occasional disagreement among panel members on the presence or absence of sex odor in a particular sample. The disagreement between judges was more common for the samples tested early in the experiment.

Later it was observed that some of the disagreement was due to an ammonical odor that occurred not too infrequently in some samples, and which was confused with boar odor. This odor was distinctly objectionable when the samples were heated in the Erlenmeyer flasks, but was absent when the samples were heated in an open skillet. Although the ammonical odor is distinctly different from boar odor, the inexperienced tester might possibly confuse the two. This may account for small errors in the ratings.

Boars: Sex odor was found to be present in 64% of all fat samples taken from boar carcasses. This is in contrast to the findings of Self (1957), as he reported that only 25% of the samples from boar carcasses showed any trace of sex odor. The present study (table 7) showed that 36% of the samples tested had slight odor, whereas, 28% exhibited strong odor. The remaining 36% of the boars were free from boar odor. The fact that some boars do not exhibit sex odor is in agreement with the findings reported by Self (1957), but is in contrast to the statement of Lerche (1936), who reported that all sexually mature boars have a definite sex odor. While testing the fat samples from boar carcasses, the panel noted various objectionable odors arising from the heated fat that could not be classified as the typical boar odor. The inconsistency of results from different workers might possibly be accounted for through these unpleasant odors, which are definitely different from boar odor.

A supplementary study involving only 29 boars, indicated that the incidence of sex odor was somewhat lower in young, light-weight boars. The data are presented in table 8. In this study, 44.8% of the young boars were recorded as having no sex odor, whereas, 55.2% exhibited sex

Table 7. Incidence of boar odor in boars and sows

Sex	Boars					Sows				
Degree of odor	Number of animals					Number of animals				
	Panel member			Average	% incidence	Panel member			Average	% incidence
	A	B	C			A	B	C		
None	31	26	28	28.3	35.7	76	78	77	77.0	98.8
Slight	25	35	25	28.3	35.8	2	0	1	1.0	1.3
Strong	23	18	26	22.4	28.4	0	0	0	0.0	0.0
Total	79	79	79	79.0	100.0	78	78	78	78.0	100.0

Table 8. Incidence of sex odor in young, light-weight boars

Weight pounds	Number of animals			
	Total	Degree of odor		
		None	Slight	Strong
<175	10	7	2	1
175-200	7	1	5	1
200-250	8	4	3	1
>250	4	1	1	2

odor. Included in the 55.2% that exhibited boar odor, 37.9% had only slight odor and 17.3% were rated as having strong odor. With the limited number of animals tested in this trial, it is difficult to draw any definite conclusions. However, there seems to be a trend toward a lower incidence in the young, light-weight boars.

Sows: Sex odor could be detected in approximately 1% of the fat samples taken from sow carcasses. Samples where sex odor was apparent

were all scored as only slight. In many cases the fat samples from the sows exhibited an off odor, but were not scored as having boar odor. As in the case of boars, this off odor was distinctly different from sex odor.

Barrows: For 150 to 220 lb. market barrows, only 5% had sex odor. Included in the 5%, 2.7% were rated as exhibiting slight boar odor and 2.3% as having strong sex odor. One of the two barrows having strong boar odor was known to be a cryptorchid. Only one other known cryptorchid was included in the 86 barrows studied, and it was scored as having no boar odor. In both cases the testes were not atrophied. Since one of the cryptorchids had non-atrophied testes but did not exhibit boar odor, these results are contrary to the report of Lerche (1936), who stated that "sex odor" or "boar flavor" was present in all cryptorchids unless the testes were atrophied.

Gilts: About 5% of all 150-220 lb. market gilts were found to contain sex odor. Only 1% were scored as exhibiting strong sex odor, whereas, 4% were scored as having slight sex odor. Very few off flavors were noted in the remaining 95% of the samples taken from gilt carcasses.

Table 9. Incidence of boar odor in barrows and gilts

Sex	Barrows					Gilts				
	Number of animals					Number of animals				
Degree of odor	Panel member A	B	C	Average	% incidence	Panel member A	B	C	Average	% incidence
None	83	80	82	81.7*	95.0	92	90	91	91.0	94.8
Slight	1	4	2	2.3	2.7	3	5	4	4.0	4.1
Strong	2	2	2	2.0*	2.3	1	1	1	1.0	1.0
Total	86	86	86	86.0	100.0	96	96	96	96.0	100.0

*One barrow in the group was a known cryptorchid

Utilization of pork tissue containing boar odor

Cooked salami: In four trials, utilizing the 9-point hedonic scale of Peryam and Pilgrim (1957), the average taste panel score was 6.69 for the control, which contained pork free from sex odor, and 6.47 for the samples where pork having strong boar odor was used. When the data were subjected to an analysis of variance test, no significant differences were observed between the control samples and the experimental batches containing pork with strong boar odor. These results indicate that pork exhibiting boar odor can be used successfully in making cooked salami. However, if the salami prepared with pork containing sex odor was heated and tested by the trained odor panel, the pungent boar odor was readily apparent.

Braunschweiger: The braunschweiger was prepared with and without pork trim exhibiting strong boar odor, and then evaluated utilizing the 9-point hedonic scale if Peryam and Pilgrim (1957). The average taste panel scores were 6.67 for the control and 6.64 for the batch that contained pork exhibiting strong boar odor. These taste panel scores were averaged from two different trials. No significant differences were observed between the control and the batches containing pork with sex odor.

When the braunschweiger was subjected to the heat test, only the strong odor of liver could be detected. This was true for both the control and the sample containing pork exhibiting strong boar odor. Apparently the odor of the liver masked the boar odor. In a supplementary study, it was found that 40% boar meat containing strong sex odor could be added to boar liver before any sex odor could be detected upon heating.

These data indicate that boar meat can be successfully incorporated into sausages that are eaten cold. Since heating the fat volatilizes the component(s) responsible for boar flavor (Craig et al., 1962), the use of boar meat in products that are heated before serving would be an unwise practice. Considering the fact that the component(s) responsible for boar flavor is/are volatilized upon heating the fat, it would appear that heating might prove to be an efficient method for removing the objectionable odor. However, earlier studies (Craig, unpublished data, 1961) showed that the off odor was present after boiling continually for periods up to 24 hours.

Identification of pork fat volatiles

Sulfides

The unpleasant odors associated with sulfur containing compounds make these substances prime suspects for any off flavors or odors associated with meat products. The lipophilic nature of the component(s) responsible for boar odor would suggest that this possibility is somewhat minute. However, the presence of connective tissue in the fat prohibits the researcher from completely ruling out the possibility of such compounds as hydrogen sulfide, mercaptans or disulfides.

The volatiles arising from heated pork fat were carried by a stream of high purity nitrogen through a cold water condenser and then into a 2% lead acetate trap. Hydrogen sulfide was indicated by the formation of a black precipitate in the trap. This precipitate was believed to be due to the formation of insoluble lead sulfide from volatile hydrogen sulfide.

When the methylene blue test for hydrogen sulfide was carried out on a 25 ml. aliquot of the trapping solution, the appearance of a blue color further substantiated this hypothesis. With the knowledge that such compounds as mercaptans and disulfides do not interfere with the methylene blue test, it can be concluded that the black precipitate in the lead acetate solution was lead sulfide. The mercaptans can be ruled out since they form a red colored complex with the methylene blue reagent. The red complex absorbs at a maximum at 490 mu. and will not interfere with the absorption of the blue complex. The blue complex has a maximum absorption at 665 mu.

As hydrogen sulfide was shown to be evolved from both boar and barrow adipose tissue, it was decided to quantitatively compare the amount present. This determination was carried out utilizing a modification of the methylene blue technique described by Marbach and Doty (1956). The boar fat utilized for the quantitative test was known to exhibit a strong sex odor, whereas, all barrow fat was completely devoid of boar odor. During collection as the trapping solution became turbid, it was replaced with fresh lead acetate because of the low solubility of lead sulfide in aqueous solutions.

The colorimeter readings from each sample were converted into their equivalent hydrogen sulfide values by interpolation on a standard curve (figure 2) prepared with known quantities of sodium sulfide. These data are reported in table 10. Hydrogen sulfide values reported in table 10 were determined on 20 g. of fat and then converted to a kg. basis. All fat was heated at 100°C for 3 1/2 hours in this study. The absorbance

Table 10. Hydrogen sulfide liberated from boar and barrow fat

Sample No.	Boar		Barrow	
	Absorbance	H_2S (ug./kg)*	Absorbance	H_2S (ug./kg)*
1	0.075	51.0	0.056	40.5
2	0.077	52.0	0.060	42.5
3	0.099	63.0	0.067	46.2
4	0.099	63.0	0.061	43.5
5	0.073	49.0	0.066	46.0
6	0.070	48.0	0.070	48.0
Average	-----	54.3	-----	44.4

*These values are expressed as ug. of hydrogen sulfide per kg. of adipose tissue.

readings represent the color developed when the volatiles were collected and made up to a total volume of 1 liter with 2% lead acetate. Aliquots of 25 ml. were used for the methylene blue determination. The determinations show that the boar adipose tissue contained about 54 ug. of hydrogen sulfide per kg. of fat, whereas, the barrow fat contained about 44 ug. of hydrogen sulfide per kg. of fat.

Statistical analysis of the hydrogen sulfide data (table 10) is presented in table 11. Although the average hydrogen sulfide content was somewhat higher for the boars than the barrows, results show that the difference was not statistically significant. The failure of the data to meet requirements for statistical significance may be explained by the highly significant difference noted between animals. The difference between animals is easily seen when the data reported in table 10 are

Table 11. Analysis of variance for hydrogen sulfide data

Source	D.F.	S.S.	M.S.	"F"	Tabular F*
Between boars and barrows	1	293.49	293.49	4.44	7.71
Animals within sexes	4	264.61	66.15	45.94**	9.15
Within animals	6	8.65	1.44		
Total	11	566.75			

*From Snedecor (1956)

closely observed. The data show that the variance in hydrogen sulfide values between boars and barrows is lower than the variance within sexes. Results of this experiment indicate that the amount of hydrogen sulfide in both boars and barrows are essentially the same. Therefore, it seems unlikely that hydrogen sulfide is associated with boar odor.

The presence of mercaptans is readily indicated by their ability to form insoluble complexes in the presence of aqueous lead acetate. The mercaptan-lead complex has a yellowish appearance. When pork fat was heated at 100°C for 3 1/2 hours and the volatiles passed through a 2% lead acetate trap, the presence of mercaptans was not indicated. Since the trapping solution became turbid due to the black lead-sulfide complex, it is doubtful whether small amounts of the mercaptan-lead complex could be observed. Therefore, an attempt was made to hydrolyze the mercaptan-lead complex. This was done by heating a 25 ml. aliquot of the trapping solution with dilute hydrochloric acid. The volatiles arising from this solution were passed through a fresh solution of lead acetate. Since a

precipitate failed to appear it was concluded that mercaptans were not present in pork fat volatiles. To further substantiate these findings, the methylene blue test was conducted on the trapping solution and was found to be negative. If mercaptans had been present a red color would have appeared on testing with methylene blue.

Carbonyls

The appearance of a precipitate in trap 2 of the scheme presented in figure 1, indicated the presence of carbonyl compounds in the volatiles arising from heated pork fat. Trap 3, which also contained a saturated solution of 2,4-dinitrophenylhydrazine, did not contain any visual signs of a precipitate. This was probably because of the small amount of carbonyls present in the volatile stream. Only a very small amount of precipitate was observed in trap 2, when 1200 g. of fat were heated for 3 1/2 hours. Since no differences could be observed between boar and barrow fat, further studies were not initiated on these carbonyl precipitates.

Volatile fatty acids

The volatiles arising from heated pork fat were passed through two chloroform-water traps submerged in ice. The contents of the traps were first checked for acidity with Micro Essential Laboratory Hydrin Paper. From this test it appeared that the solutions were neutral. Next a 50 ml. aliquot of the trapping solution was titrated with 0.0272 N. methanolic sodium hydroxide, using m-cresol purple as an indicator. This test indicated that fatty acids were present in very minute quantities. Although the first trap contained about 0.05 millimoles of fatty acids, the second

trap was completely devoid of acidity. The amount of fatty acids present in a 50 ml. aliquot was so small that an accurate determination was difficult. No differences in the quantity of volatile fatty acids could be found between boar and barrow fat. Therefore, it is doubtful whether volatile fatty acids are involved as contributors to boar odor.

Volatile ammonia

When the aqueous layer of the above trapping solution was heated with potassium hydroxide and the vapors exposed to litmus paper, the paper indicated a basic reaction. Therefore, ammonia test paper was prepared and held in the volatiles arising from the heated solution. The appearance of a grayish-black color on the test paper indicated the presence of ammonia in the volatile material. It is believed that the presence of ammonia can be attributed to the breakdown of amino acids contained in the connective tissue. Boar and barrow adipose tissue were tested for volatile ammonia, but no differences were observed.

Saponification Study

Craig et al., (1962) reported that the component(s) responsible for sex odor was/were located in the fatty tissue, more specifically, in the unsaponifiable fraction. These authors also concluded that the component(s) responsible for sex odor was/were present in very small amounts. After considering these findings, it was decided to investigate the cold method of saponification and compare its efficiency with a standard hot saponification technique. In addition, the yield of unsaponifiable material for boar and barrow fat was determined as well as for beef fat.

Comparison of saponification methods

For this study, 15 g. of pork or beef fat was saponified by both the hot and cold methods. When utilizing the hot method, all fat was saponified for 1 1/2 hours with 12% potassium hydroxide. Cold saponification was carried out using sodium ethylate after the fat had been extracted from the connective tissue with redistilled diethyl ether. The fat-ether solution was mixed with the sodium ethylate and allowed to stand at room temperature for 12, 24, 36 or 48 hours. The unsaponifiable material was extracted with diethyl ether. Material saponified by the hot method was extracted from an aqueous solution, whereas, material saponified by the cold method was extracted directly from the soap.

Saponification data for pork fat are presented in table 12. All values reported in table 12 are the mean from 5 trials, with each trial being run in triplicate. After the unsaponifiable material was extracted, the solvent was removed under vacuum at 30-35°C. The drying procedure was continued until a constant weight was obtained. Good repeatability for the yield of unsaponifiable material was obtained by the hot method, whereas, yields obtained by the cold method were somewhat variable. On using the cold saponification method, considerable variation was also observed between triplicate samples that were run simultaneously. The variation is believed to be due to difficulty in extracting the unsaponifiable material directly from the solid soap, rather than from an aqueous solution as was done in the hot procedure. An attempt was made to dissolve the soap in distilled water and then extract with ether, but this was unsuccessful.

Table 12. Results of hot and cold saponification of pork fat

Sample No.	Time hr.	Method	Yield (g/100 g fat)	Hydroxylamine test (OD/g)[a]	Saponification efficiency
1	1.5	hot	0.531	0.182[b]	1.00
2	12.0	cold	0.308	0.201	0.91
3	24.0	cold	0.460	0.189	0.96
4	36.0	cold	0.465	0.148	1.23
5	48.0	cold	0.478	0.134	1.36

[a]Absorbance per gram of unsaponifiable material
[b]Absorbance readings were taken at 520 mu.

The hydroxylamine test for esters was used to measure the efficiency of saponfication. Without using a standard curve for this test, it is possible to ascertain the relative concentration of ester linkages in different samples. Utilizing the test in this way, an absolute quantitative measurement is not required. The efficiency values reported in tables 12 and 13 were calculated from absorbance values recorded for 15 g of fat and converted to absorbance per g of unsaponifiable material. Variations in yield due to efficiency of extraction, was accounted for by calculating in this manner. The absorbance of 1 g of unsaponifiable material obtained by hot saponification was assigned a value of one. All saponification efficiencies are expressed relative to this arbitrary value.

Data reported in table 12 show that cold saponification of pork fat was essentially as efficient as the hot method, if the reaction was allowed to proceed at room temperature for at least 24 hours. When the sodium

ethylate was allowed to react with the extracted fat for periods of 36 hours or longer, there appeared to be a lower concentration of ester linkages. This indicated that the cold procedure was more efficient if the reaction was allowed to proceed at least 36 hours.

Table 13 contains the results for the saponification of beef fat by the hot and cold methods. Procedures and calculations for the beef fat study were identical to those used for pork fat. The saponification efficiency recorded for the cold procedure approached the efficiency obtained by the hot method. Upon employing the cold procedure for beef unsaponifiable material, hydroxylamine absorbance values were very similar to those recorded for pork. However, the hot method was more efficient for saponifying beef fat than pork fat.

Table 13. Results of hot and cold saponification of beef fat

Sample No.	Time Hr.	Method	Yield (g/100 g fat)	Hydroxylamine test (OD/g)[a]	Saponification efficiency
1	1.5	hot	0.455	0.099[b]	1.00
2	12.0	cold	0.363	0.259	0.38
3	24.0	cold	0.384	0.182	0.54
4	36.0	cold	0.434	0.113	0.88
5	48.0	cold	0.397	0.108	0.91

[a]Absorbance per gram of unsaponifiable material
[b]Absorbance readings were taken at 520 mu.

These results indicate that pork fat can be saponified efficiently by the cold procedure, if a concentrated solution of sodium ethylate is allowed to react with the fat for 36 hours or longer. However, the ex-

traction of unsaponifiable material from the soap appeared to be less difficult if the hot method was employed. Component(s) responsible for sex odor was/were found to be present in unsaponifiable material obtained by the hot procedure as well as the cold method. Results indicate that beef fat must be allowed to react with the alkali for at least 48 hours, if the saponification efficiency is to approach that obtained by the hot method.

Yield of unsaponifiable material

For this study, boar fat exhibiting strong sex odor, and barrow fat, which was known to be free from sex odor, were used. The fat was saponified by the hot method discussed in the previous section.

Results show that boar fat contains approximately 0.53 g of unsaponifiable material per 100 g of fat. Barrow fat was found to contain approximately 0.50 g of unsaponifiable material per 100 g of fat. Beef fat was found to contain about 0.46 g of unsaponifiable material per 100 g of fat. From these data, it can be concluded that the yield of unsaponifiable material obtained from boar and barrow fat, was essentially the same.

Composition Studies on the Unsaponifiable Matter from Pork Fat

Qualitative tests on total unsaponifiable matter

Alcohols: The nitrochromic acid test for primary and secondary alcohols was carried out by adding a drop of unsaponifiable material to a solution of nitric acid and potassium dichromate. The solution's failure

to change from yellow to blue-gray indicated that alcohol compounds of this type were not present. Lanosterol and cholesterol were suspected of being present in the unsaponifiable material and would theoretically contain an alcoholic group. Thus, they were also tested by the nitrochromic acid test, but results were negative.

Carbonyls: A small amount of volatile and steam distillable carbonyls were reported in whole pork fat by Hornstein and Crowe (1960). The occurrence of these compounds in the unsaponifiable fraction of pork fat was indicated by O'Daniel and Parsons (1943), who also reported that the yellow color formed during the saponification process was due to an aldol condensation of carbonyl compounds. If the findings of these authors are valid, only minute quantities of carbonyls would be expected in the unsaponifiable material. This is because the aldol condensation process would tie up half of the available carbonyl groups.

When a sample of unsaponifiable material was added to a saturated solution of 2,4-dinitrophenylhydrazine, there was no evidence of a precipitate. However, on adding a small amount of alkali to the solution a deep wine color appeared indicating the presence of a small amount of carbonyl compounds.

Aromatic nucleus and aliphatic unsaturation: Ten drops of Le Rosen reagent were added to a 10 ml beaker. Next, one drop of unsaponifiable material was added. A deep wine color appeared immediately, indicating the presence of completely unsaturated ring structures and/or aliphatic unsaturation. This determination does not verify the presence or absence of a particular compound, however, it helps to account for the instability

of extracted unsaponifiable material. Therefore, the results of this test seem to indicate a high degree of unsaturation in at least some of the compounds in the unsaponifiable material.

Cholesterol: On dissolving the unsaponifiable material in chloroform and reacting with the Liebermann-Burchard reagent, the presence of cholesterol was indicated by a positive test for Δ-5 sterols. An orange-pink color first appeared and then changed to blue-green.

Nitrogen: The presence of ammonia in the volatiles arising from heated pork fat was established earlier in this study. Therefore, it seemed worthwhile to test a sample of unsaponifiable material for nitrogen containing compounds. This was attempted first by heating a sample of unsaponifiable material and exposing ammonia test paper to the volatiles. When this test proved to be negative, a nitrogen determination was made using the micro-Kjeldahl procedure. Results again indicated that the unsaponifiable material was devoid of nitrogen containing compounds.

Sulfur: Lead acetate paper indicated that hydrogen sulfide was absent in the unsaponifiable material. Therefore, it was decided to employ the sodium formate technique for the determination of non-volatile sulfur containing compounds. When sodium formate is heated above its melting point, it decomposes according to the following formula:

$$2HCOONa \text{ --------} > 2H + Na_2C_2O_4$$

consequently, the melted sodium formate acts as a hydrogen donor and therefore a strong reducing agent. If non-volatile sulfur-bearing organic

compounds are heated with sodium formate, hydrogen sulfide is formed.

When unsaponifiable material was heated in the presence of sodium formate, no color was noted on the lead acetate paper covering the mouth of the micro-test tube. A black color would have appeared on the test paper, if hydrogen sulfide had been present in the vapors. Results of this study indicate, that neither volatile nor non-volatile sulfur-bearing compounds are present in the unsaponifiable fraction of pork fat.

Separation of unsaponifiable material by silicic acid chromatography

Capellas et al., (1960) separated unsaponifiable material into 9 fractions using 9 eluents varying in polarity from hexane to a mxiture of 5% methanol in chloroform. This same procedure was used in an attempt to separate 100 mg of unsaponifiable material on a 3 g silicic acid column. The Liebermann-Burchard test for cholesterol, the hydroxylamine test for esters, the Le Rosen test for unsaturation and the antimony trichloride test for vitamin A were applied to each of the fractions.

Fractions 1 (hexane), 3 (95% hexane-5% benzene) and 6 (70% hexane-30% benzene) contained a very small amount of material. Each of these fractions gave a positive test for unsaturation and a negative test for sterols, esters and vitamin A. However, the limited amount of sample available prevented any further determinations.

Fraction 2 (carbon disulfide) was eluted from the column and contained a yellow, viscous material. When this material was subjected to the Liebermann-Burchard test for cholesterol and the hydroxylamine test

for esters, results were negative. Only a faint blue color was obtained with the antimony trichloride test indicating that vitamin A was present but only in minute quantities. A positive Le Rosen test for unsaturation was indicated by the formation of a deep wine color. The positive Le Rosen test would be expected, since a high concentration of double bonds would occur due to the vitamin A in this fraction. Capellas et al., (1960) reported the presence of squalene in this fraction, which would also account for a great deal of unsaturation.

In the present study, squalene was indicated by thin-layer chromatography (figure 4). The color of the spots and R_f values for synthetic squalene (0.97) and spot 4 (0.98) of fraction 2 were almost identical. Spot 1 of fraction 2 turned blue when the sulfuric acid spray reagent was applied to the chromatoplate. Mangold (1961) reported that both vitamin A and its esters could easily be recognized by the blue color they yield when heated with sulfuric acid. Thus, this observation complements the antimony trichloride test and further indicates the presence of vitamin A in fraction 2. A standard vitamin A sample was not available at this time for comparing the R_f values by thin-layer chromatography. Although spot 1 may have been vitamin A, a positive identification was not made. No attempt was made to identify spots 2 and 3 of this fraction.

Capellas et al., (1960) reported the presence of esters in fractions 4 (90% hexane, 10% benzene) and 5 (80% hexane, 20% benzene). Since only a very small amount of material was obtained in these fractions, they were combined for testing. The hydroxylamine test verified the presence

Solvent System - 80% hexane - 20% ether - 2 ml. acetic acid

Chromatoplate - S_iO_2 with 15% calcium sulfate binder

Spray Reagent - 50% aqueous H_2SO_4

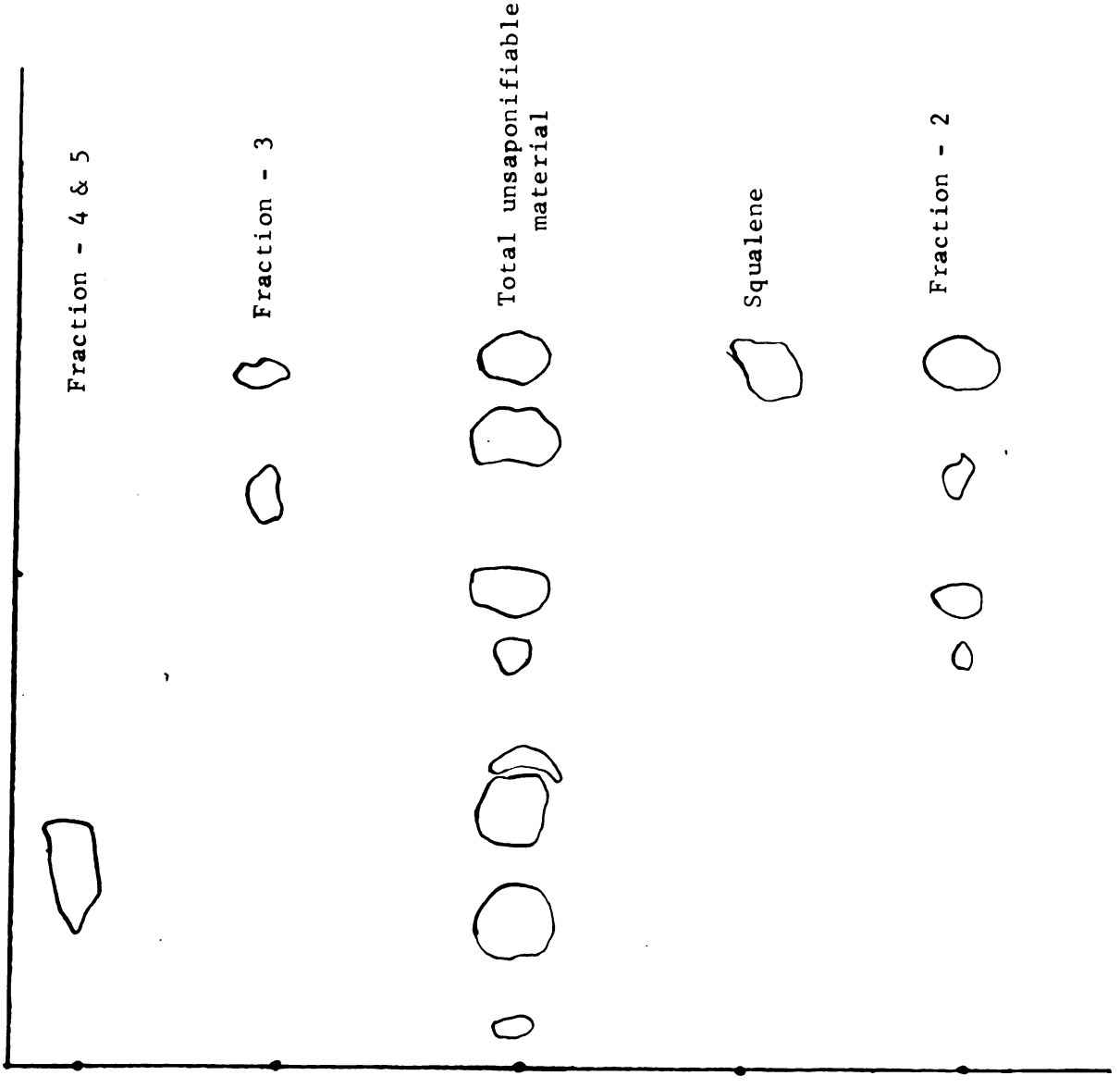

Figure 4. Thin-layer chromatogram of squalene, fractionated and unfractionated unsaponifiable material.

of esters in the combined fractions. A positive Liebermann-Burchard test indicated the presence of sterols. However, when this fraction was subjected to thin-layer chromatography (figure 4), no evidence of cholesterol was seen. Since the Liebermann-Burchard test also gives a positive reaction in the presence of cholesterol esters and the chromatographic results show only one spot, it can be hypothesized that the major components of this fraction are sterol esters. Thin-layer chromatography indicated that cholesteryl acetate was not one of the esters present. Tests for vitamin A were negative when conducted on this fraction. However, a slight wine color was observed when the material was subjected to the Le Rosen test for unsaturation.

Fraction 7 (98% hexane, 2% ethyl ether) yielded a yellow, waxy substance. This material was negative to the hydroxylamine test for esters and the antimony trichloride test for vitamin A. The Le Rosen test gave a faint wine color, indicating the presence of a small amount of unsaturation. When this material was subjected to the Liebermann-Burchard test, a stable yellow color was formed. According to Capellas et al., (1960), this is not indicative of cholesterol, but indicates the presence of triterpenoid alcohols. This finding is not surprising since the triterpenoid alcohol, lanosterol, is known to be an intermediate in the biosynthesis of cholesterol. When a commercial sample of lanosterol was subjected to the Liebermann-Burchard test a stable yellow color also appeared. This color seemed to be identical to the color obtained from fraction 7.

Figure 5 shows a thin-layer chromatogram where material from fraction 7 was developed along with a standard sample of lanosterol. The standard

sample of lanosterol produced 2 spots. The second lanosterol spot was very dim. Spot 2 from lanosterol (figure 5) had the same color and R_f value as spot 1 of fraction 7. It is interesting to note (figure 5) that the smaller spots of both fraction 7 and the lanosterol are almost identical, whereas, the major spots shown from these materials are quite different. The smaller spots might be accounted for as either degradation products or intermediates in the synthesis of lanosterol. Assuming this hypothesis to be valid, the presence of lanosterol in fraction 7 is further substantiated. However, the quantity present appears to be very small.

Fraction 8 (92% hexane, 8% ethyl ether) was one of the larger fractions obtained. The bulk of this material was a white crystalline substance, however, some yellow, waxy material was also present. This yellow material appeared to be the same as that observed in other fractions. Neither ester linkages nor vitamin A could be detected in this fraction. The Le Rosen test indicated that some unsaturation was present. A very strong test for cholesterol was observed when a sample of this material was subjected to the Liebermann-Burchard test. This material was then subjected to thin-layer chromatography. Results are reported in figure 5. Two spots were obtained when a standard cholesterol was chromatographed. Three distinct spots were obtained from fraction 8, two of which seemed to be attributable to cholesterol and a degradation product of cholesterol. R_f values of 0.06 and 0.19 were recorded for spots 1 and 2, respectively, in both the known and unknown materials. Spot 1 in both cases is believed to be a 7 ketosterol, since Bergstrom

Solvent system - 80% hexane - 20% ether - 2 ml. acetic acid

Chromatoplate - S_iO_2 with 15% calcium sulfate binder

Spray reagent - 50% aqueous H_2SO_4

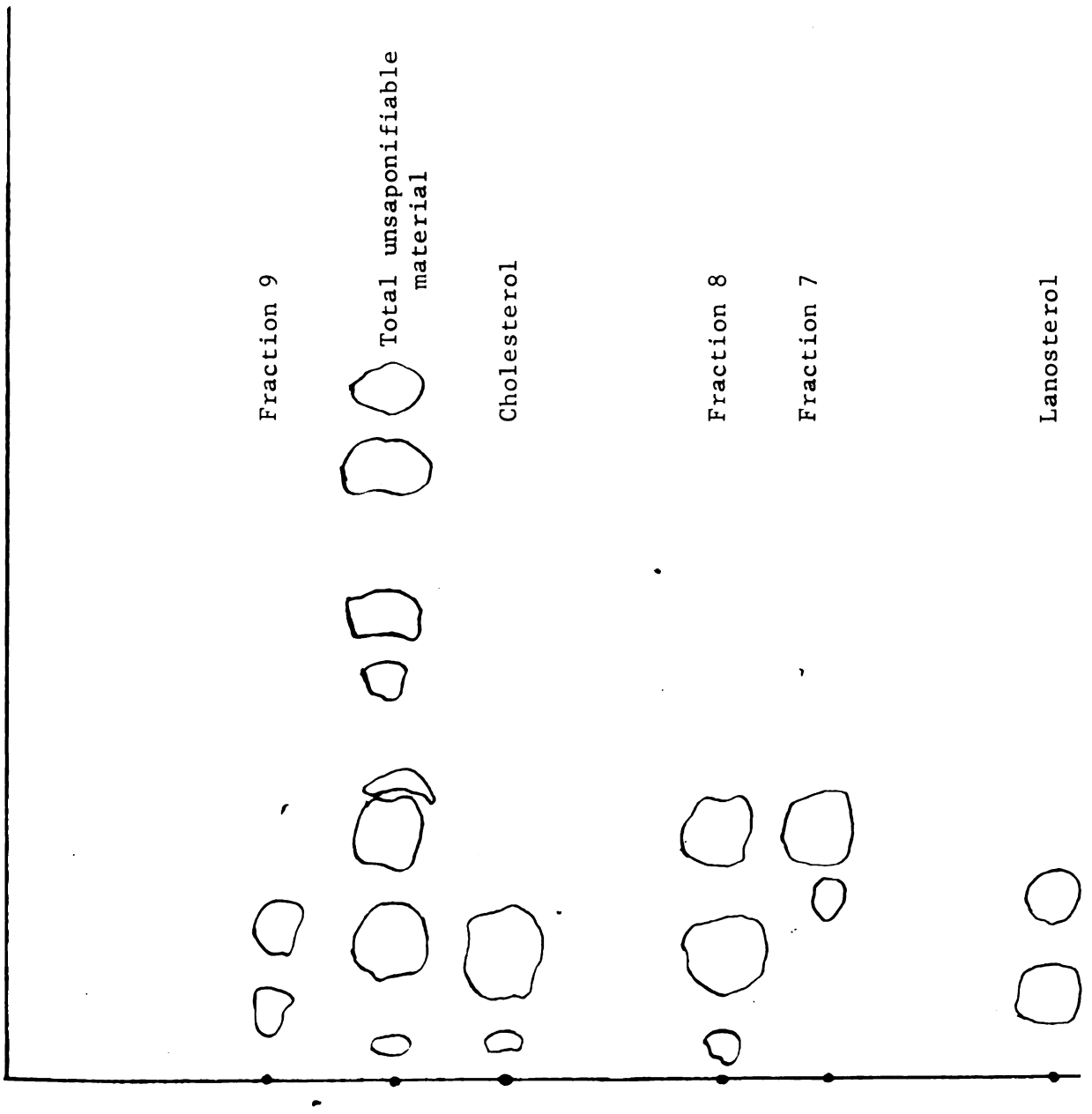

Figure 5. Thin-layer chromatogram of fractionated and unfractionated total unsaponifiable material.

and Wintersteiner (1941) reported this compound to be the chief reaction product when cholesterol was subjected to oxidation in air. Some oxidation could have taken place in the standard cholesterol since it had been in the laboratory for about 2 years. In addition, spot 3 seemed to be the result of poor separation since an identical spot was noted in the material from fraction 7.

The column was stripped with 5% methanol in chloroform to obtain fraction 9. This yielded a brown-yellow material, which represents most of the compounds that were not eluted by the previous solvents. The material in fraction 9 gave a negative test for vitamin A and ester linkages. However, a small amount of color was formed when the Liebermann-Burchard and Le Rosen tests were conducted. Figure 5 shows a thin-layer chromatogram of fraction 9. A spot similar to cholesterol and two slower moving spots can be seen. In addition, a great deal of material remained at the point of origin, and could not be separated by the solvents used in this chromatogram.

The separation of unsaponifiable material by silicic acid chromatography was not complete. Spots with identical colors and R_f values were observed when consecutive fractions were subjected to thin-layer chromatography (figures 4 and 5). However, much information was gained. The Le Rosen test indicated the presence of unsaturation in all fractions, especially in fraction 2. Cholesterol, vitamin A and squalene were identified by thin-layer chromatography and various qualitative tests. In addition, cholesterol esters, a 7 ketosterol and a triterpenoid alcohol were indicated. Thin-layer chromatography indicated that

an intermediate or degradation product of lanosterol was probably responsible for the presence of the triterpenoid alcohol.

Gas chromatographic investigation of unsaponifiable material

Separation by gas chromatography depends largely on the boiling point and polarity of the individual compounds. A complex mixture, such as the one found in the unsaponifiable fraction of pork fat, is very difficult to separate. This difficulty arises due to the presence of compounds with extreme differences in boiling point and polarity. For this separation, a 1/4 inch column packed with 2% Silicone SE-30 (dimethylsilicone) coated on Chromasorb "W" was used. The liquid support was chosen because of its thermal stability and polarity. Only 2% SE-30 was used because retention time is a function of the amount of liquid phase. Larger concentrations of liquid support would cause high boiling compounds such as the sterols to be held on the column for hours or even days. Retention time and efficiency of separation are also functions of the column length. Columns 4 1/2, 5 1/2 and 7 1/2 ft. in length were tested. Results indicated that the 7 1/2 ft. column gave the best combination of an efficient separation and practical retention times for total unsaponifiable material.

After the variable parameters, such as temperature, carrier gas flow rate and etc., were adjusted for maximum efficiency, a 1 ul sample of unsaponifiable material was analyzed utilizing the Barber-Colman Model 20 gas chromatograph. Examination of the resulting chromatogram (figure 6) reveals 20 different peaks. The first peaks to emerge from the column were not separated as well as one would like to see. However,

considering that peak 20 required about 75 minutes for elution, whereas, peak 1 only required 2.1 minutes, the separation was quite practical. The retention times of the peaks obtained in figure 6 were compared with the retention times obtained from various known compounds suspected of being present. Cholesterol and cholesterol acetate were subjected to this analysis. Cholesterol (figure 7) was eluted from the column in 75 minutes, which was identical to the retention time recorded for peak number 20 from the unsaponifiable material. Thus, results indicate that cholesterol was responsible for peak number 20 from the unsaponifiable material.

Thin-layer chromatography (figure 5) revealed the presence of a second component in the cholesterol standard. However, under the conditions of this experiment, the second component could not be detected. The retention time for cholesterol acetate was in excess of 110 minutes, and was therefore eliminated as a possible component of the unsaponifiable material. Thin-layer chromatography also revealed that lanosterol or a closely related compound was present in the unsaponifiable matter. This finding could not be substantiated by gas chromatography. Various other sterols were subjected to gas chromatography but were not found in detectable amounts.

Under these conditions, a sample of synthetic squalene was eluted in 35 minutes (figure 8). Peak 19 of the unsaponifiable material (figure 6) also had a retention time of 35 minutes. This indicates the presence of squalene and complements the data reported in the previous section. Because of the nature of the molecular structure of squalene it was de-

cided to expose a sample to oxidation in air. This sample was allowed to set at room temperature for about 2 weeks and was then subjected to gas chromatography again (figure 9). In this chromatogram, 2 peaks were observed in addition to the squalene peak. Peak 1 had retention time of 2.1 minutes and corresponds exactly to peak 1 observed in the unsaponifiable material. Peak 2 in figure 9 had a retention time of 26 minutes, which was the same as the retention time of peak 18 in the unsaponifiable material. The appearance of extra peaks from oxidized squalene was no surprise when the unsaturated nature of the squalene molecule was considered. According to the current theories, autoxidation would have taken place due to the unsaturation, and carbonyl compounds would result. When the squalene was tested with 2,4-dinitrophenylhydrazine, a precipitate appeared. When this same test was conducted on a fresh squalene sample, no precipitate was observed. These results indicate that either peak 1 or 2 shown in figure 9 is a carbonyl compound. Although not proven, the peak which is not a carbonyl is probably due to a free radical. Gas chromatographic data reported in figures 6, 8, and 9 indicate that squalene as well as the 2 products formed by the autoxidation of squalene are present in unsaponifiable material.

When a standard sample of vitamin A was chromatographed, 2 peaks were obtained (figure 10). Peak 1 had a retention time of 4 minutes, which corresponds to the retention time for peak 5 in the unsaponifiable material (figure 6). Peak 2 of figure 10 had a retention time of 5 minutes, which is the same as the retention time calculated for peak 7 in the unsaponifiable material.

In an attempt to explain the presence of the 2 peaks from a fresh sample of vitamin A, the material was subjected to thin-layer chromatography. These results are reported in figure 11. Figure 11 shows only 1 spot for vitamin A. Therefore, it is assumed that when vitamin A is subjected to high temperature gas chromatography, it is changed in some way. These results indicate that peaks 5 and 7 from the total unsaponifiable material can be accounted for by the breakdown of vitamin A. Figure 11 also indicates the presence of vitamin A in the unsaponifiable fraction of pork fat.

To further elucidate the classes of compounds responsible for the peaks obtained when unsaponifiable matter was gas chromatographed (figure 6), an infrared analysis was conducted. The absorption spectra of the unsaponifiable material dissolved in spectro-grade chloroform was scanned from 2 to 16 u. (figure 12). The presence of carbonyls was further substantiated by the absorption noted in the range of 5.5 to 5.9 u. The absence of alcohols was indicated since little absorption occurred below 3.5 u. The absorption spectra of the unsaponifiable material (figure 12) also indicates the absence of fatty acids, which complements a previously conducted negative qualitative test.

It is well known that such compounds as saturated hydrocarbons and various halogenated compounds are insoluble in cold concentrated sulfuric acid. Halogenated compounds can be eliminated as possibilities because they are easily detected by gas chromatography, if the argon ionization system of detection is employed. This is because the energy available from the metastable argon atom is 11.6 electron volts and

12.8 to 17.3 electron volts are required to ionize the halogens. Therefore, instead of getting a positive response on the recorder chart, a negative peak occurs. Since figure 6 shows no negative peaks, it can be concluded that unsaponifiable material was devoid of halogen containing compounds. Therefore, any material that was not soluble in cold concentrated sulfuric acid can be classified as saturated hydrocarbons.

Since hexane is not soluble in cold sulfuric acid, the unsaponifiable material was dissolved in hexane and extracted. After one extraction the material was again subjected to gas chromatography and the results are presented in figure 13. A second extraction was made, and these results are shown in figure 14. The amount of unsaponifiable material represented in figures 13 and 14 are approximately the same as that represented in figure 6. However, the material in figure 6 was not extracted with sulfuric acid. After one extraction with sulfuric acid, all peaks observed in figure 6 were reduced except peaks 10, 11, 13, 15 and 17. Results can be compared in figures 6 and 13. These observations were even more pronounced on comparing figures 6 and 14. It is difficult to compare the exact peak height for peak 10 because it is partially covered by peak 9. However, the peak height measurements appear to be about the same for this peak in both the extracted and unextracted material. Thus, results indicate that peaks 11, 13, 15, 17 and possibly 10 (figure 6) are due to compounds, which are not soluble in cold concentrated sulfuric acid. Since it is known that halogenated compounds are absent, it can be assumed that the compounds responsible for these peaks are saturated hydrocarbons.

Saturated hydrocarbons containing less than 10 carbon atoms were subjected to gas chromatography. These compounds emerged with the solvent

peak, and were undetectable. Therefore, it can be concluded that peaks 11, 13, 15, 17 and possibly 10 are due to saturated hydrocarbons containing 10 or more carbon atoms.

Various other extractions failed to aid in the chemical classification of the remaining 9 peaks. The possibility that these peaks are due to sterols is very minute, because the retention time of the unknown material was too short. Sulfur and nitrogen containing compounds were also eliminated by qualitative tests reported in a previous section. Infrared and qualitative analysis on unsaponifiable material revealed that fatty acids and alcohols were also absent. Saturated hydrocarbons have also been accounted for by cold sulfuric acid extraction. After considering these findings, the remaining 9 peaks can be assumed to be unsaturated hydrocarbons and/or carbonyl compounds. It is doubtful whether peak number 9 (figure 6) could be a carbonyl, since only a small amount of carbonyls was indicated in the unsaponifiable fraction of pork fat, and yet peak 9 was relatively large.

Attempts to collect gas chromatographic fractions from the effluent stream of the Barber-Colman model 20 gas chromatograph were unsuccessful.

Quantitative determinations on total unsaponifiable material

Cholesterol and triterpene alcohols: Since cholesterol was identified as being present in the unsaponifiable fraction of pork fat, a quantitative determination was conducted. The procedure of Luddy et al., (1953) was used, since triterpene alcohols as well as cholesterol can be determined by this procedure. Recovery experiments were conducted to verify the accuracy of the method. Data are reported in table 14.

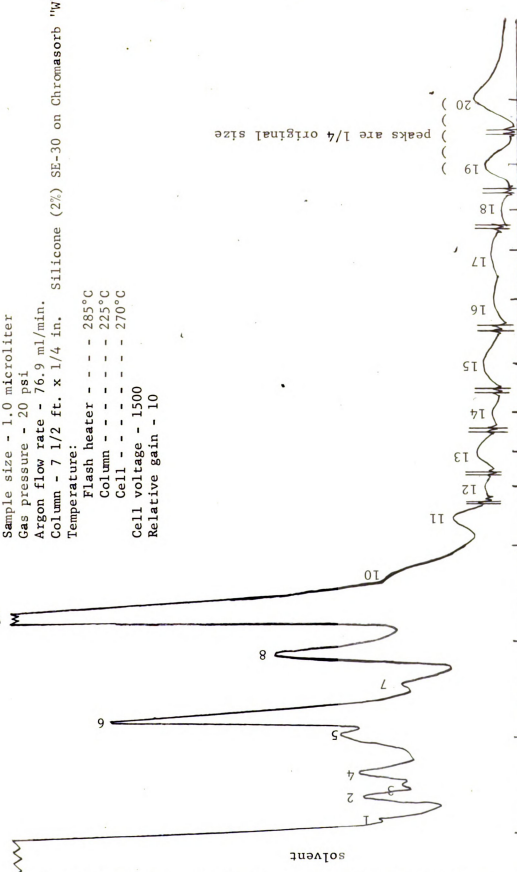

Figure 6. Gas chromatogram of total unsaponifiable material in ether.

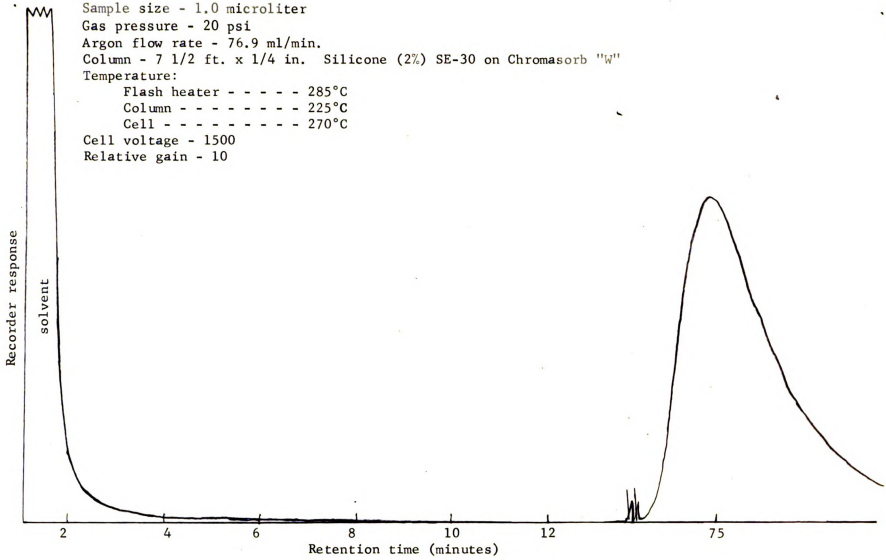

Figure 7. Gas chromatogram of cholesterol in ether.

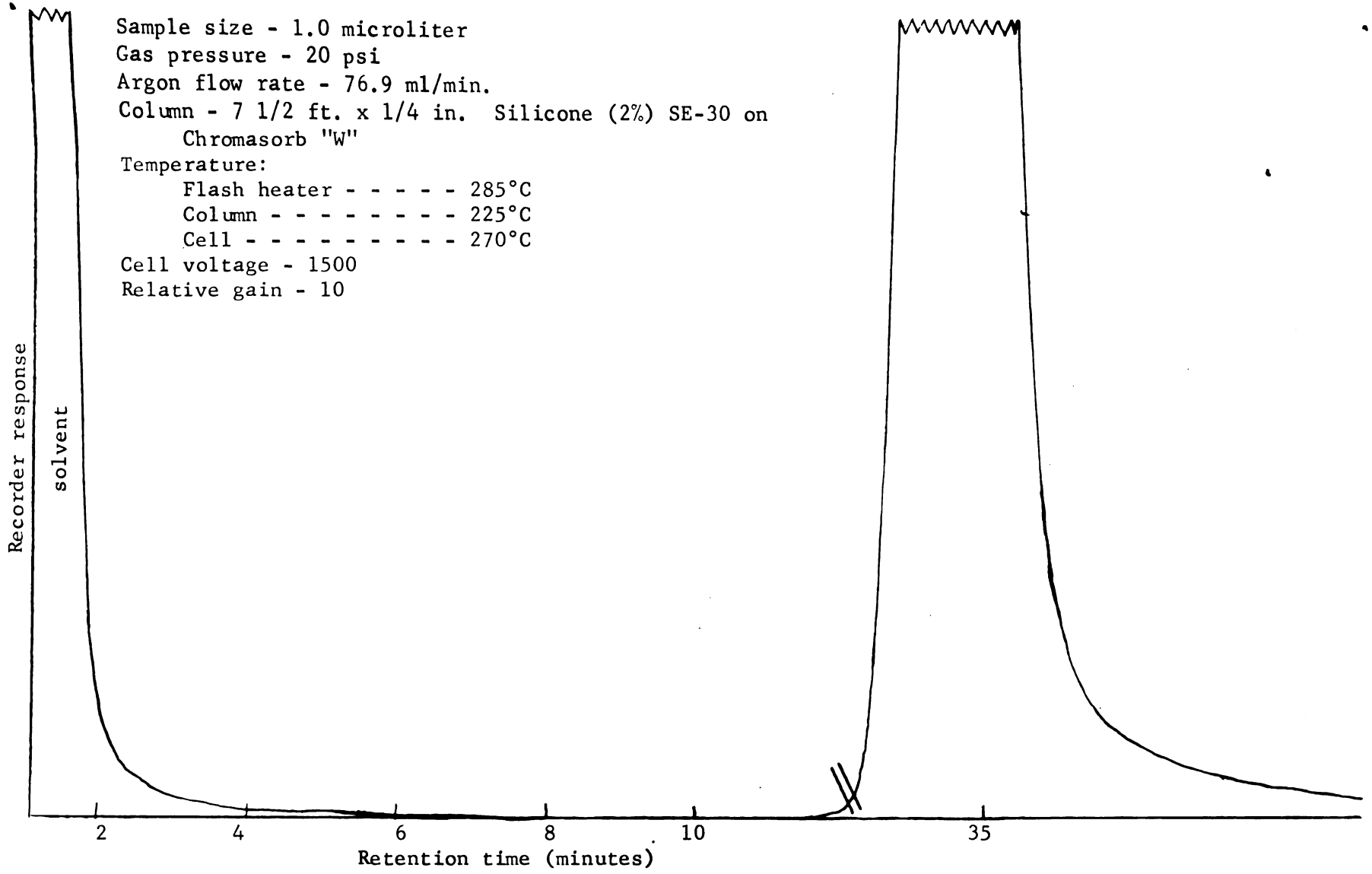

Figure 8. Gas chromatogram of squalene in ether.

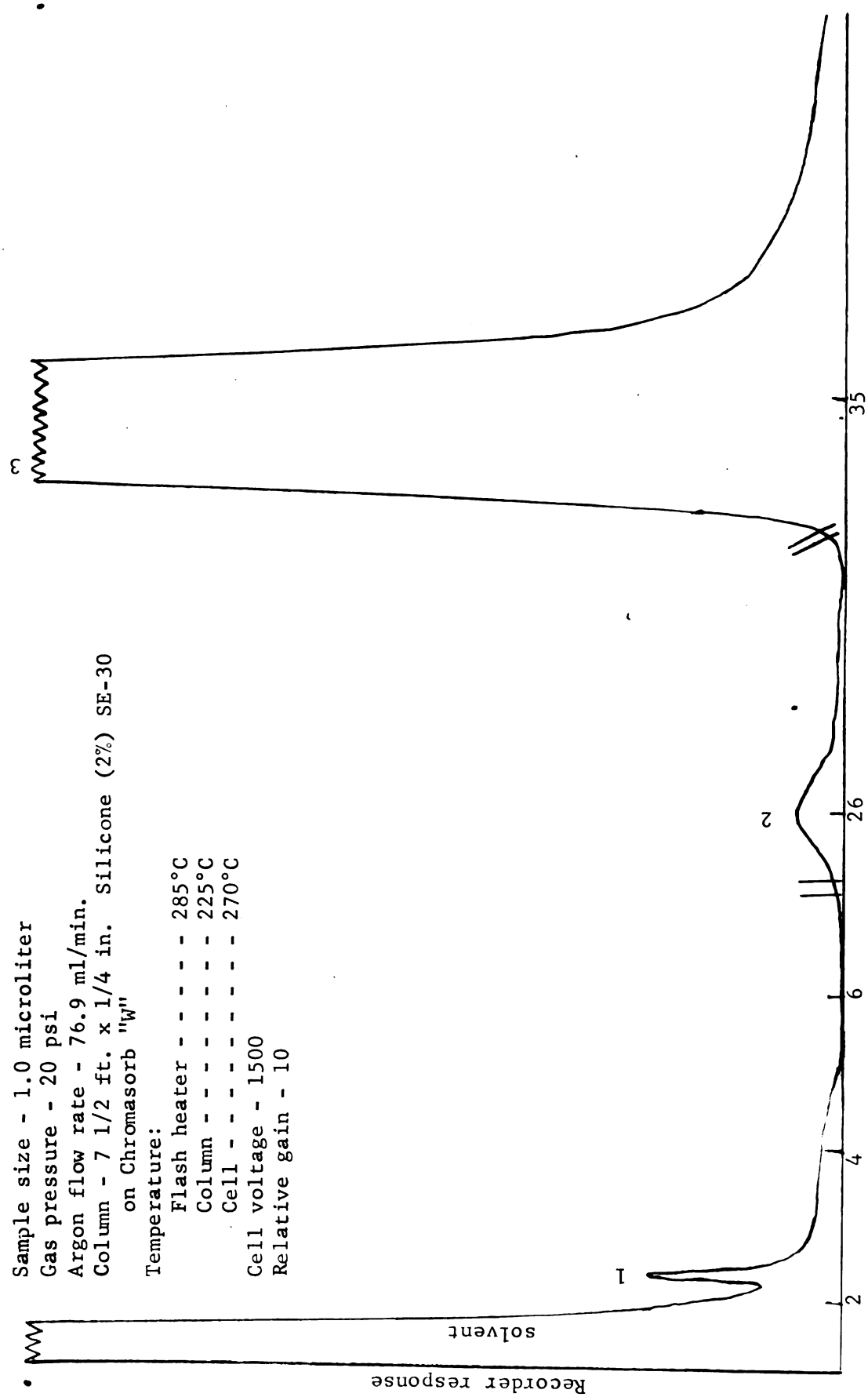

Figure 9. Gas chromatogram of oxidized squalene in ether.

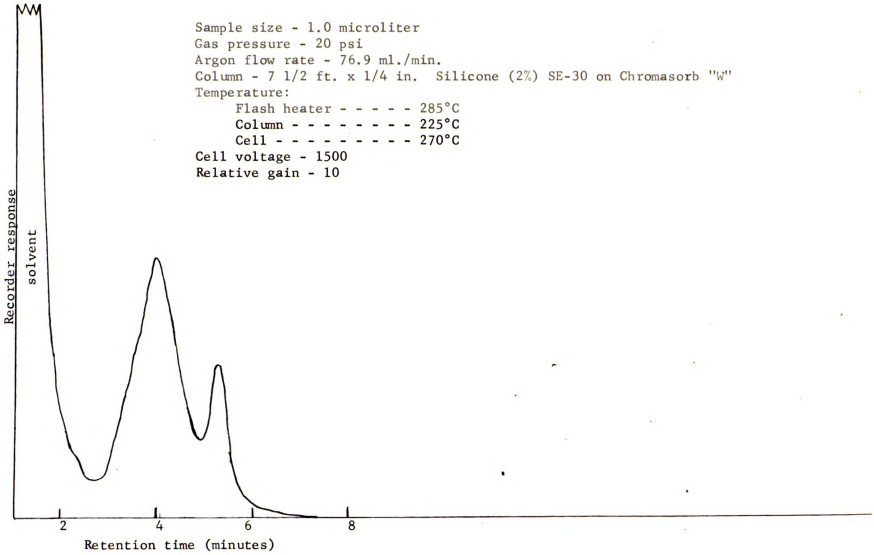

Figure 10. Gas chromatogram of vitamin A in ether.

Solvent system - 80% hexane - 20% ether - 2 ml. acetic acid

Chromatoplate - S_iO_2

Spray reagent - 50% aqueous H_2SO_4

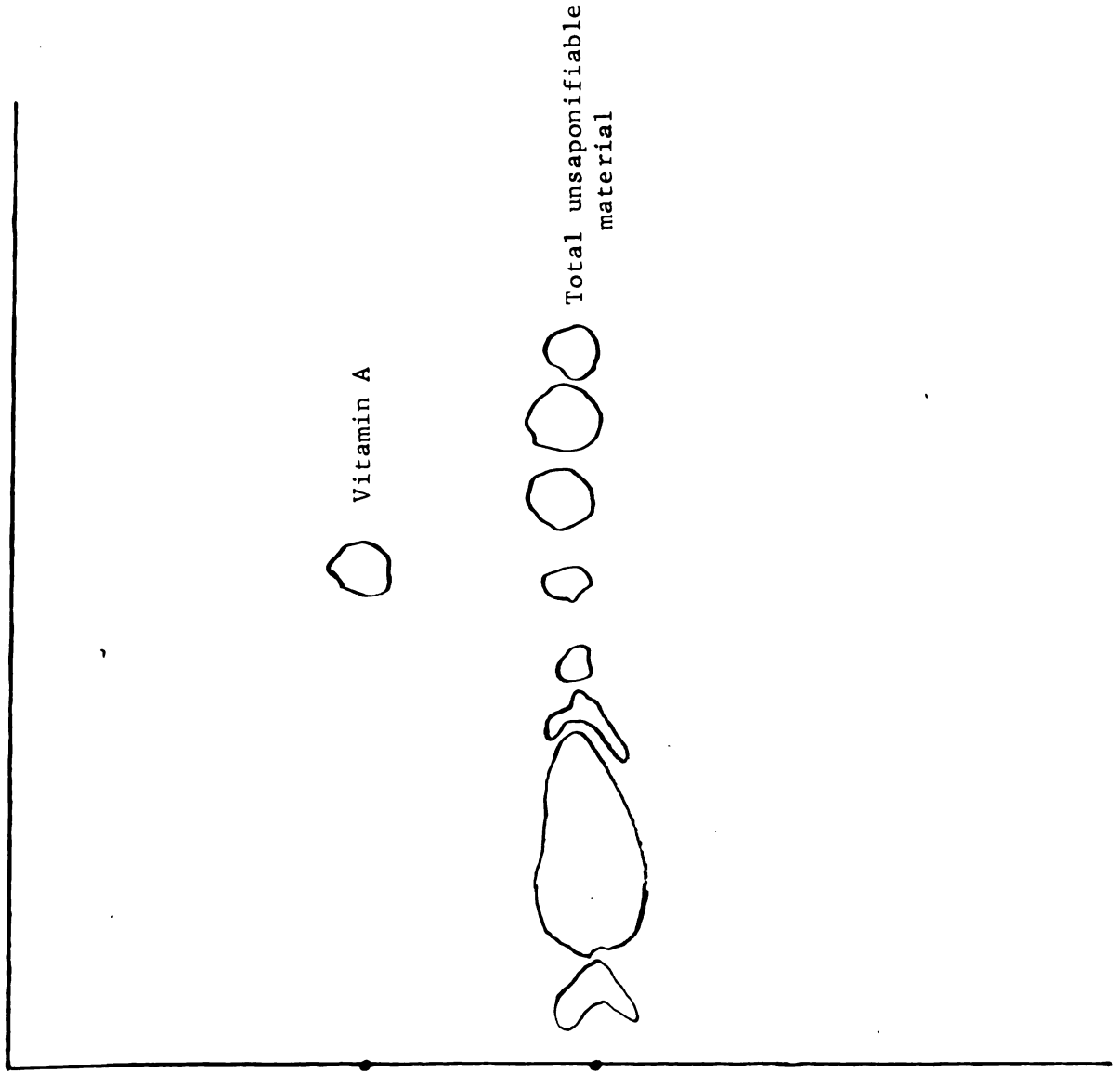

Figure 11. Thin-layer chromatogram of total unsaponifiable material and a standard sample of vitamin A.

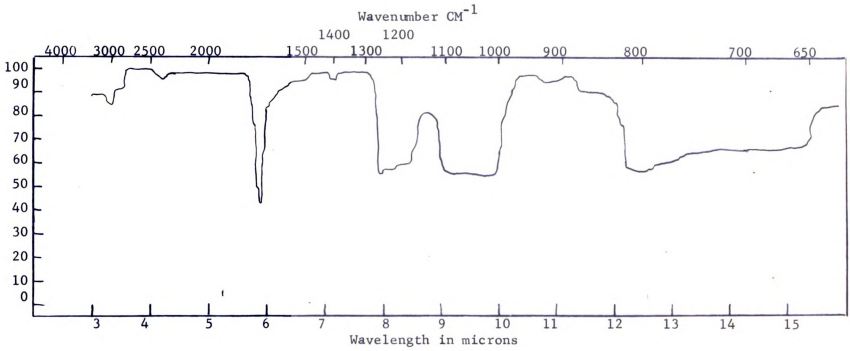

Figure 12. Infrared spectra of total unsaponifiable material in chloroform.

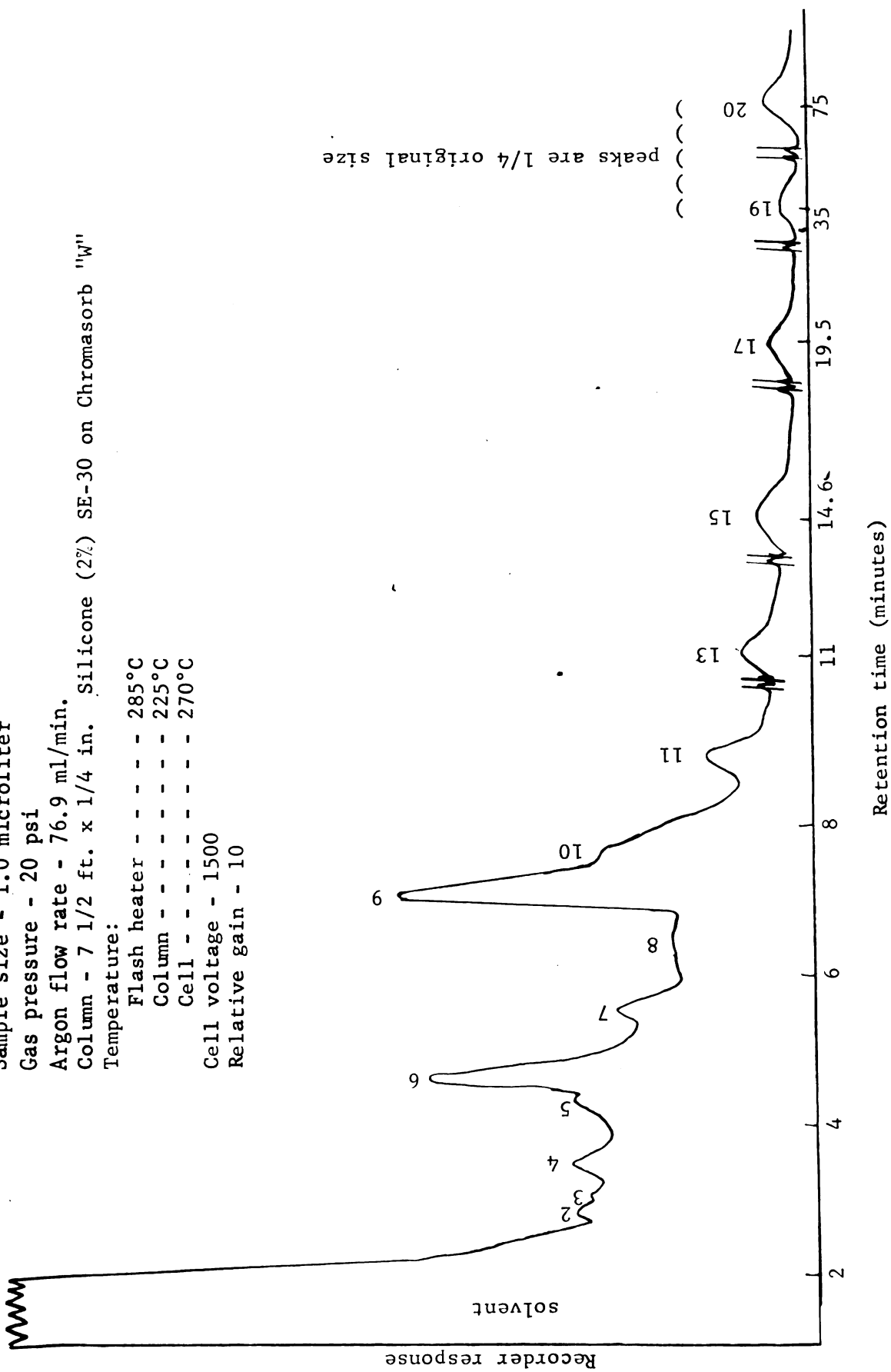

Figure 13. Gas chromatogram of total unsaponifiable extracted once with cold concentrated sulfuric acid.

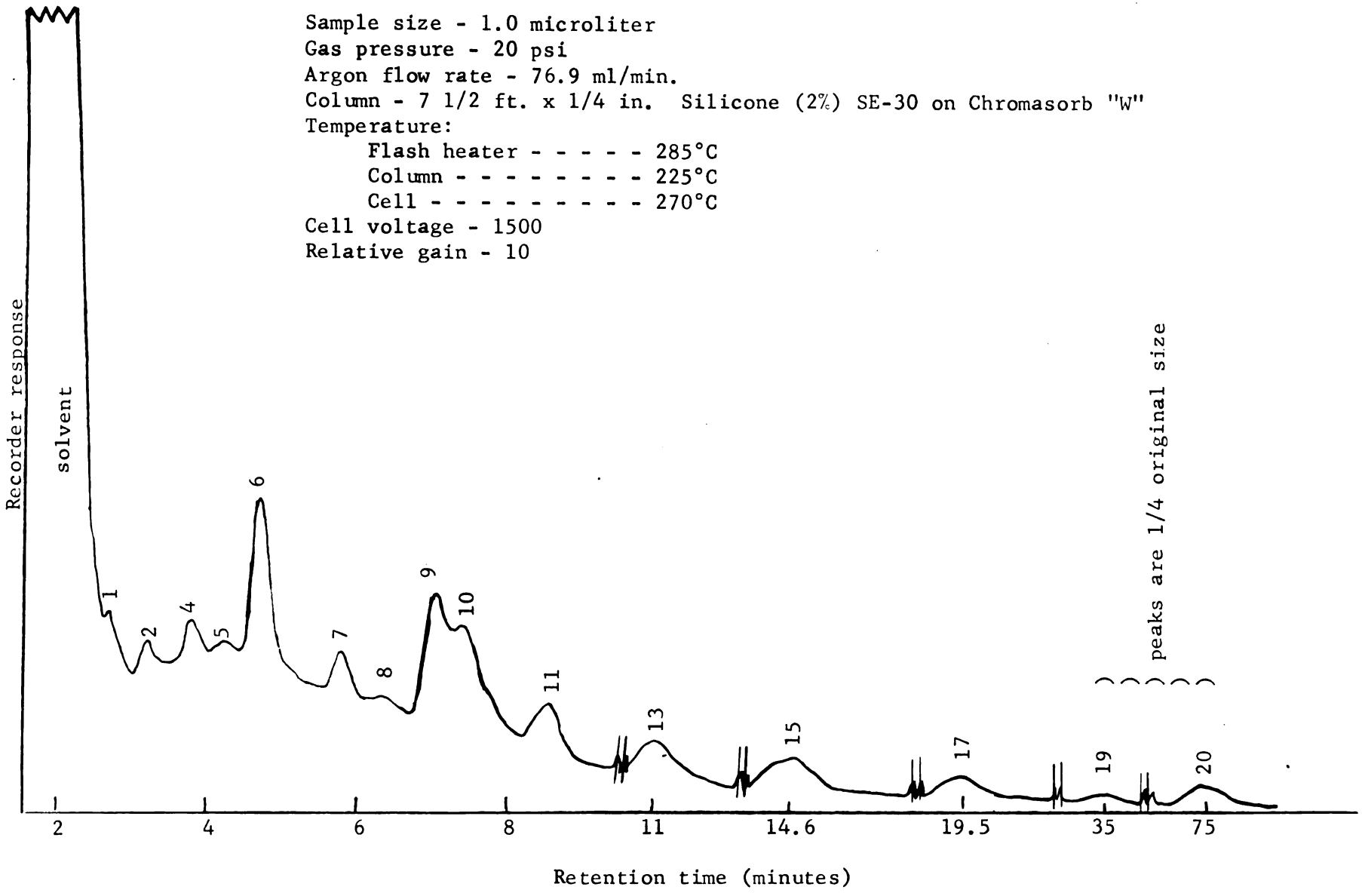

Figure 14. Gas chromatogram of total unsaponifiable extracted twice with cold concentrated sulfuric acid.

Table 14. Cholesterol and triterpene alcohol recovery experiment

Cholesterol		Triterpene alcohol[a]	
Mg. added	Mg. found	Mg. added	Mg. found
15.0	15.05	10.9	10.93
15.0	14.94	10.9	10.93
40.0	40.12	44.8	44.58
40.0	39.84	44.8	44.58

[a]Lanosterol was used to represent the triterpene alcohols.

Unsaponifiable material obtained from pork fat was found to contain 8.71% cholesterol. This is equivalent to 46.16 mg. of cholesterol per 100 g of fat. These values are somewhat lower than those reported by Lange (1950), who reported 50-122 mg. of cholesterol per 100 g of pork fat. Considering the wide range of cholesterol in fat reported by Lange (1950), and the accuracy reported above in the recovery experiment, a value of 46.16 mg/100 g fat appears to be realistic. Boar and barrow fat were found to contain essentially the same amount of cholesterol with values of 47.0 and 44.6, respectively.

Vitamin A: Utilizing a standard curve (figure 3), pork fat was found to contain 1.65 units of vitamin A per gram. These data are in agreement with the results of the biological assays of Herb et al., (1953) and the colorimetric results of Ames and Harris (1954), who reported a range of 0.4 to 2.5 units of vitamin A per gram of lard. However, the range reported by these authors is somewhat larger than the range of 1.45 to 1.98 units of vitamin A per gram of fat found in the present study. Results

reported in this study are based on 5 animals, tested in triplicate twice. No differences in the vitamin A content of boar and barrow fat were observed in the present study, where corresponding values of 1.75 and 1.83 were found.

SUMMARY AND CONCLUSIONS

The first part of this investigation was undertaken to determine the incidence of "sex odor" or "boar odor" in swine of various sex classes, and to study the effects of incorporating boar meat having strong sex odor into some comminuted meats. From these studies, the following conclusions were drawn:

1. The incidence of sex odor in boars was 64%, which was significantly higher than any other sex group. The difference in incidence between sows, barrows and gilts was not statistically significant. The incidence of sex odor was approximately 1%, 5% and 5% for sows, barrows and gilts, respectively. These results indicate that "sex odor" or "boar odor" is largely dependent upon the sex of the animal.

2. The taste panel scores of cooked salami and braunschweiger, which contained boar meat exhibiting strong sex odor, were essentially the same as the panel scores obtained from the control preparations made from pork free of sex odor. Results indicated that boar meat possessing strong sex odor can be incorporated into some comminuted meats without detection. This was found to be true only for comminuted meats that are consumed without heating, or in those products containing liver. Apparently in the latter case, the strong odor of the liver masked the sex odor.

The second phase of this study dealt with the fractionation and identification of volatile and non-volatile components in the fatty tissue of boars and barrows, with particular emphasis on the unsaponifiable fraction. The Liebermann-Burchard test for Δ -5 sterols, the nitrochronic

acid test for alcohols, the Le Rosen test for unsaturation, the hydroxylamine test for esters and the 2,4-dinitrophenylhydrazine test for carbonyls were employed as qualitative tests. In addition, ammonia, nitrogen- and sulfur-containing compounds were qualitatively determined utilizing ammonia test paper, the Kjeldahl procedure and the sodium formate reduction methods, respectively. The methylene blue test for hydrogen sulfide and the antimony trichloride test for vitamin A were utilized for both qualitative and quantitative determinations. The following observations were made on this portion of the study:

1. Hydrogen sulfide was shown to be present in the volatiles arising from both boar and barrow fat. The amount varied from 44 to 54 ug per Kg of fat. Subsequent statistical analysis indicated that there was no significant difference in the amount present in either boar or barrow fat.

2. Lead acetate precipitation of mercaptans from the volatile fraction was attempted, but no evidence for the presence of these compounds was observed. However, qualitative tests indicated that ammonia, carbonyls and fatty acids were present in the volatiles arising from heated boar and barrow fat.

3. Cold saponification of pork fat was shown to be as efficient as hot saponification, if the cold reaction was allowed to proceed for 36 hours or more at room temperature. For beef fat, cold saponification must be allowed to proceed for at least 48 hours in order to approach the efficiency of the hot method.

4. Boar fat was found to contain 0.53 g of unsaponifiable material

per 100 g of fat while barrow fat contained 0.50 g. Beef fat was shown to contain 0.46 g of unsaponifiable material per 100 g of fat.

5. Qualitative tests indicated that carbonyls, Δ -5 sterols, ester linkages and unsaturated compounds were present in total unsaponifiable material. However, qualitative tests for alcohols, fatty acids, sulfur- and nitrogen-containing compounds were negative.

6. Unsaponifiable material was separated into fractions using silicic acid chromatography. The presence of vitamin A (fraction 2) and cholesterol (fraction 8) was further substantiated by thin-layer chromatography and qualitative tests. Squalene was identified in fraction 2 by thin-layer chromatography. In addition, cholesterol esters (fractions 4 and 5), a 7-ketosterol (fraction 8) and a triterpene alcohol (fraction 6) were indicated.

7. Gas chromatographic analysis verified the presence of cholesterol, vitamin A and squalene in the unsaponifiable material. Sulfuric acid extraction and subsequent gas chromatography indicated that at least 4 saturated hydrocarbons were present. In addition, a carbonyl and possibly a free radical were indicated as a result of the autoxidation of squalene.

8. Infrared analysis further verified the presence of carbonyls and the absence of alcohols and fatty acids in unsaponifiable material.

9. Colorimtric quantitative determinations showed 8.71% of the unsaponifiable material to be cholesterol. This is equivalent to 46.16 mg of cholesterol per 100 g of fat. No differences were observed between boar and barrow fat.

10. Pork fat was found to yield 1.45 to 1.98 units of vitamin A per gram. The content of boar and barrow fat was essentially the same.

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APPENDIX

Table 1. Panel scores for incidence of sex odor in boars[a]

Pig.No.	Panel member A	B	C	Pig No.	Panel member A	B	C	Pig No.	Panel member A	B	C
1	1	1	2	28	1	1	1	55	0	0	0
2	2	2	2	29	2	0	1	56	1	1	1
3	0	1	0	30	0	1	0	57	0	0	1
4	2	2	2	31	2	2	2	58	0	2	1
5	2	1	1	32	2	2	2	59	0	0	0
6	1	1	0	33	2	2	2	60	0	0	0
7	0	1	0	34	0	1	0	61	1	1	1
8	1	2	1	35	2	1	2	62	0	1	0
9	1	2	2	36	0	0	2	63	0	0	1
10	1	2	1	37	1	1	1	64	2	1	2
11	2	1	0	38	2	2	2	65	0	1	1
12	1	1	2	39	0	1	1	66	0	1	1
13	2	2	1	40	0	0	0	67	2	1	2
14	1	2	1	41	0	0	0	68	0	1	0
15	1	1	2	42	0	0	2	69	1	0	1
16	2	1	2	43	0	0	1	70	1	0	1
17	1	1	0	44	2	2	2	71	1	1	0
18	2	2	2	45	0	0	1	72	I	1	0
19	1	1	1	46	0	1	0	73	1	0	0
20	2	2	2	47	0	0	0	74	2	0	2
21	1	1	0	48	0	0	0	75	2	2	2
22	2	2	2	49	2	2	2	76	1	0	1
23	0	0	0	50	0	0	0	77	2	0	2
24	1	1	2	51	1	1	1	78	2	1	1
25	0	0	0	52	1	0	2	79	0	0	0
26	1	1	0	53	0	0	1				
27	0	1	0	54	0	1	0				

[a]Scores were as follows: 0 - none, 1 - slight, 2 - strong.

Table 2. Panel scores for incidence of sex odor in sows[a]

	Panel member				Panel member				Panel member		
Pig No.	A	B	C	Pig No.	A	B	C	Pig No.	A	B	C
80	0	0	0	107	0	0	0	134	0	0	0
81	0	0	0	108	0	0	0	135	0	0	0
82	0	0	0	109	0	0	0	136	0	0	0
83	0	0	0	110	0	0	0	137	0	0	0
84	0	0	0	111	0	0	0	138	0	0	0
85	0	0	0	112	0	0	0	139	0	0	0
86	0	0	0	113	0	0	0	140	0	0	0
87	1	0	0	114	0	0	0	141	0	0	0
88	1	0	0	115	0	0	0	142	0	0	0
89	0	0	0	116	0	0	0	143	0	0	0
90	0	0	0	117	0	0	0	144	0	0	0
91	0	0	0	118	0	0	0	145	0	0	0
92	0	0	0	119	0	0	0	146	0	0	0
93	0	0	0	120	0	0	0	147	0	0	0
94	0	0	0	121	0	0	0	148	0	0	0
95	0	0	0	122	0	0	0	149	0	0	0
96	0	0	0	123	0	0	0	150	0	0	0
97	0	0	0	124	0	0	0	151	0	0	0
98	0	0	1	125	0	0	0	152	0	0	0
99	0	0	0	126	0	0	0	153	0	0	0
100	0	0	0	127	0	0	0	154	0	0	0
101	0	0	0	128	0	0	0	155	0	0	0
102	0	0	0	129	0	0	0	156	0	0	0
103	0	0	0	130	0	0	0	157	0	0	0
104	0	0	0	131	0	0	0				
105	0	0	0	132	0	0	0				
106	0	0	0	133	0	0	0				

[a]Scores were as follows: 0 - none, 1 - slight, 2 - strong.

Table 3. Panel scores for incidence of sex odor in barrows[a]

Pig No.	Panel member A	B	C	Pig No.	Panel member A	B	C	Pig No.	Panel member A	B	C
158	0	0	0	185	0	0	0	212	0	0	0
159	0	0	0	186	0	0	0	213	0	0	0
160	0	0	0	187	0	0	0	214	0	0	0
161	0	0	0	188	0	0	0	215	0	0	0
162	0	0	0	189	0	0	0	216	0	0	0
163	0	0	0	190	0	0	0	217	0	0	0
164	0	0	0	191	0	0	0	218	0	0	0
165	0	0	0	192	0	0	0	219	0	0	0
166	0	0	0	193	0	1	0	220	0	0	0
167	0	0	0	194	0	0	0	221	0	0	0
168	0	0	0	195	0	0	0	222	0	0	0
169	0	0	0	196	0	0	0	223	0	0	0
170	0	0	0	197	0	0	0	224	0	0	0
171	0	0	0	198	0	0	0	225	0	0	0
172	0	0	0	199	0	0	0	226	0	0	0
173	0	0	0	200	0	0	0	227	0	0	0
174	2	2	2	201	0	1	0	228	0	1	0
175	0	0	0	202	0	0	0	229	0	1	0
176	0	0	0	203	0	0	0	230	0	0	0
177	1	0	1	204	0	0	0	231	0	0	0
178	0	0	0	205	0	0	0	232	0	0	0
179	0	0	0	206	0	0	0	233	0	0	0
180	0	0	0	207	0	0	0	234	0	0	0
181	0	0	0	208	0	0	0	235	0	0	0
182	0	0	0	209	0	0	0	236	0	0	0
183	0	0	0	210	0	0	0	237	0	0	1
184	0	0	0	211	0	0	0	238	0	0	0
								239	0	0	0
								240	0	0	0

[a]Scores were as follows: 0 - none, 1 - slight, 2 - strong.

Table 4. Panel scores for incidence of sex odor in gilts[a]

Pig No.	Panel member A	B	C	Pig No.	Panel member A	B	C	Pig No.	Panel member A	B	C
241	0	0	0	273	0	0	0	305	0	1	0
242	0	1	0	274	0	0	0	306	0	0	0
243	0	0	0	275	0	0	0	307	0	0	0
244	0	0	0	276	0	0	0	308	0	0	0
245	0	1	0	277	0	0	0	309	0	0	1
246	0	0	0	278	0	0	0	310	0	0	2
247	0	0	0	279	0	0	0	311	0	0	0
248	0	0	0	280	0	0	0	312	2	2	1
249	0	1	0	281	0	0	0	313	1	0	0
250	0	0	0	282	0	0	0	314	0	0	0
251	0	0	0	283	0	0	0	315	0	0	0
252	0	0	0	284	0	0	0	316	0	0	0
253	0	0	0	285	0	0	0	317	0	0	0
254	0	0	0	286	0	0	0	318	0	0	0
255	0	0	0	287	0	0	0	319	0	0	0
256	0	0	0	288	0	0	0	320	0	0	0
257	0	0	0	289	0	0	0	321	0	0	0
258	0	0	0	290	0	0	0	322	0	0	0
259	0	0	0	291	0	0	0	323	0	0	0
260	0	0	0	292	0	0	0	324	0	0	0
261	0	0	0	293	0	0	0	325	0	0	0
262	0	0	0	294	0	0	0	326	0	0	0
263	0	0	0	295	0	0	0	327	0	0	0
264	0	0	0	296	0	0	0	328	0	0	0
265	0	0	0	297	0	0	0	329	0	0	0
266	0	0	0	298	0	0	0	330	1	0	1
267	0	0	0	299	0	0	0	331	1	1	1
268	0	0	0	300	0	0	0	332	0	0	0
269	0	0	0	301	0	0	0	333	0	0	0
270	0	0	0	302	0	1	0	334	0	0	0
271	0	0	0	303	0	0	0	335	0	0	0
272	0	0	0	304	0	0	0				

[a]Scores were as follows: 0 - none, 1 - slight, 2 - strong.

Table 5. Panel scores incidence of sex odor in young, lightweight boars[a]

Pig No.	Weight	Panel member A	B	C	Pig No.	Weight	Panel member A	B	C
1	206	1	1	1	16	159	0	0	1
2	220	2	2	2	17	148	0	0	0
3	230	0	0	0	18	177	0	1	2
4	207	0	1	0	19	152	0	0	0
5	188	1	2	1	20	157	0	1	0
6	182	0	1	1	21	310	1	2	2
7	187	1	2	2	22	283	2	2	2
8	176	0	1	2	23	300	0	1	2
9	173	1	2	0	24	206	0	0	0
10	251	0	0	0	25	197	0	0	0
11	168	2	2	2	26	202	1	1	1
12	164	1	1	0	27	196	1	1	2
13	160	1	0	0	28	203	1	1	1
14	165	0	0	0	29	204	0	0	0
15	167	0	0	0					

[a]Scores were as follows: 0 - none, 1 - slight, 2 - strong.